10 YEARS OF ETHNOPHARMACOLOGY

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10 YEARS OF ETHNOPHARMACOLOGY

Topic Editor:
Michael Heinrich, UCL School of Pharmacy London, United Kingdom

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Certainly, Frontiers' mission[1] is highly pertinent to the fast evolving field of ethnopharmacology, which is a transdisciplinary field of research focusing on locally and traditionally used medicines integrating approaches from social and natural sciences, especially pharmacology, plant sciences and phytochemistry (and occasionally medicine) (e.g. Etkin et al 1998, Schultes and Raffauf 1991). Importantly, a main the goal is to contribute to a better and safer use of these medicines (Heinrich and Jaeger 2015).

Even though we have made progress, achieving longer and healthier lives remains a challenge for many people, especially in the poorer and poorest regions of the world. Medicinal plants and other locally available resources are a core resource to the majority of the world's population. At the same time, in many countries there is a rising interest in the study and advancement of 'traditional medicine'. In many ways

[1] Research is the foundation of modern society and it's thanks to advances in science that we enjoy longer, healthier and more prosperous lives than ever before in human history. We want to build on successful science and make it even more powerful by ensur ing it is openly available. This way, society will be able to generate more knowledge and accelerate innovation, health and prosperity for all. (https://www.frontiersin.org/about/about- frontiers)

China is today leading with the enormous efforts to assess potential health benefits and risks of Traditional Chinese Medicine. However, similar efforts are made in many countries on all continents and especially in the fast-evolving economies of the global South. Local and traditional medicines remain an inspiration in drug discovery and development, as exemplified in the wonderful research and development leading to the antimalarial medicine artemisinin from *Artemisa annua* L. (Tu 2016). There remain many gaps in our knowledge of such plants and fungi, but at the same time a recent bibliometric analysis showed that from 1958 to November 2018, about 60,000 publications -linked to 'ethnopharmacology' were indexed in the Web of Science contributed by over 20,600 institutions from practically all countries/ regions of the world (Yeung et al 2018).

The section 'Ethnopharmacology' in the journal *Frontiers in Pharmacology* took – as other sections - a while to develop, but today it is a highly active branch within the journal, representing the enormous interest in this field of research. This volume provides a showcase of a small selection of particularly relevant Review articles, highlighting both the breadths and quality of the research. Core areas of interest include:

- The treatment of specific diseases and syndromes like:
  - Dermatological conditions including ophthalmology and otorhinolaryngology
  - Respiratory, gastro-intestinal, cardiovascular, endocrine and central nervous systems disorders
  - Obstetrics, gynaecology, and urinary-tract disorders
  - Infections including viral, bacterial, protozoal and parasitic diseases
  - Chronic and acute inflammatory diseases, musculoskeletal and joint diseases
  - Malignancies and immunosuppression
  - Metabolic disorders
- Anthropological and historical studies
- Quality and composition traditional medicines, metabolomics and genomics
- The interface of food and medicine, and nutrition in ethnopharmacology
- Clinical studies
- Pharmacokinetics
- Public health aspects of medicinal plant use

The contributions selected for this ebook span the entire ten-year period and we have selected examples which have had a particular impact on the debates in the field. Broadly speaking, they fall into four main areas:

- Overarching reviews within ethnopharmacology
- Reviews of specific species or other taxa regarding their pharmacology; phytochemistry and local / traditional use
- Assessments of the pharmacological evidence for specific active compounds or classes of compounds
- Assessments of the safety and potential risks of herbal substances.

With these themes, this eBook contributes to the debate about the evidence- base of such practices incorporating both the scientific evidence available and the local / traditional concepts associated with their use. By definition, ethnopharmacology must be a transdisciplinary or at the very least interdisciplinary field of research. As Graham Jones (University of New England Armidale, Australia) put it beautifully, one can see 'Ethnopharmacology [as] constituting a respectful marriage between modern science and ancient wisdom with much to be gained in both directions' (Heinrich and

Jaeger 2015). These contributions set the scene for the scientific developments of the next decade and ethnopharmacology's changing frontiers, including the translation of our scientific understanding into better practice. Simultaneous, I must highlight that there is a need to ascertain best practice in our research. This is clearly stated in our Four Pillars of best practice and it was also recently addressed in a joint perspective paper of the editors in chief of the seven leading journals in the field (Heinrich et al 2020). Importantly the unique requirements based on the obligations resulting from the international treaties on biodiversity, its equitable use and associated benefit sharing (Secretariat of the Convention on Biological Diversity 2001) are also covered .

It is a great pleasure to acknowledge the support of everyone involved, first and foremost the authors who also give their time as reviewers, as well as the hard work of all Associate Editors, who handle manuscripts on a regular basis. Special thanks are also due to Dr. Javier Echeverria for his contributions to the development of the section. Thank you also to the Frontiers Editorial Office teams, both in Lausanne and London, for their wonderful initiatives, hard work and all the tremendous input. Frontiers is a true community-driven endeavour.

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Traditional medicines and globalization: current and future perspectives in ethnopharmacology

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The ethnopharmacological approach toward the understanding and appraisal of traditional and herbal medicines is characterized by the inclusions of the social as well as the natural sciences. Anthropological field-observations describing the local use of nature-derived medicines are the basis for ethnopharmacological enquiries. The multidisciplinary scientific validation of indigenous drugs is of relevance to modern societies at large and helps to sustain local health care practices. Especially with respect to therapies related to aging related, chronic and infectious diseases traditional medicines offer promising alternatives to biomedicine. Bioassays applied in ethnopharmacology represent the molecular characteristics and complexities of the disease or symptoms for which an indigenous drug is used in "traditional" medicine to variable depth and extent. One-dimensional *in vitro* approaches rarely cope with the complexity of human diseases and ignore the concept of polypharmacological synergies. The recent focus on holistic approaches and systems biology in medicinal plant research represents the trend toward the description and the understanding of complex multi-parameter systems. Ethnopharmacopoeias are non-static cultural constructs shaped by belief and knowledge systems. Intensified globalization and economic liberalism currently accelerates the interchange between local and global pharmacopoeias via international trade, television, the World Wide Web and print media. The increased infiltration of newly generated biomedical knowledge and introduction of "foreign" medicines into local pharmacopoeias leads to syncretic developments and generates a feedback loop. While modern and post-modern cultures and knowledge systems adapt and transform the global impact, they become more relevant for ethnopharmacology. Moreover, what is traditional, alternative or complementary medicine depends on the adopted historic-cultural perspective.

Keywords: knowledge transmission, globalization, medical pluralism, global economy, traditional medicines, syncretism

INTRODUCTION TO ETHNOPHARMACOLOGY

Ethnopharmacology is a multidisciplinary field of inquiry investigating the anthropological rationale and the pharmacological basis of the medicinal use of plants, animals, fungi, micro-organisms, and minerals by human cultures. An "interdisciplinary approach is essential"... in "the attempts to present an equilibrated appraisal of the therapeutic potential of an indigenous drug" (Rivier and Bruhn, 1979). Richard Evans Schultes and Tony Swain reasoned: "Peoples whom we have chosen to consider members of less-advanced societies have consistently looked to the Plant Kingdom"... Should we as chemists, pharmacologists, and botanists – with so many and varied means at our disposal – not take a lesson from them?" (Rivier and Bruhn, 1979). While commercial interests have always been a driving force behind the search for new therapies, the global economy is accelerating the commodification of indigenous and local knowledge (Posey, 2002). But today, in a more globalized world, the concern has matured that meanwhile "our goal should not be to freeze people in time. Instead we must find ways to ensure that in a pluralistic interconnected world all peoples may benefit from modernity without that engagement demanding the sacrifice of their ethnicity." (Davis, 2010).

Traditional medicine and medicinal plants are frequently used in urban settings as alternatives in daily health care and self-medication against minor and chronic ailments but especially relied upon in less wealthy rural areas or times of economic crises. Common to most systems of "traditional" or local medicine is the adoption of a holistic perspective on human health. The evaluation of descriptive field-data, as well as the identification of anthropological aspects in traditional medicines, benefit from hypothesis-driven approaches. Well-defined and testable research questions in medical anthropology not only restrain from arbitrary conclusions but also consolidate and foster the bond between the social and the natural sciences. The focus of ethnopharmacology centers on the bio-cultural investigation and description of "traditional," indigenous or local pharmacopoeias and the experimental evaluation of *materia medica* through biological test systems. The molecular characteristics and complexities of the disease or symptoms for which a medicinal agent is used in "traditional" medicine are represented to

variable depth and extent in the bioassays used to corroborate the observed health claims. The ultimate goal of ethnopharmacology is contributing to evidence-based medicine, and to this aim, conclusive negative results may be as important as positive data.

CHALLENGES IN ETHNOPHARMACOLOGY

"Traditional" systems of medicine often recommend complex herbal mixtures and multi-compound extracts. This polypharmacological approach is closer to the treatment of diseases related to multifactorial causes such as chronic and degenerative ailments, than biomedicine since here the concept of "one disease – one target – one drug" does not hold (Ulrich-Merzenich et al., 2009; Verpoorte et al., 2009). A mixture of moderately active metabolites present in an extract are potentially able to interfere and down regulate different proteins of the same signaling network leading to synergistic pharmacological effects (Wagner and Ulrich-Merzenich, 2009). In this respect it was also pointed out that reductionistic *in vitro* approaches are scarcely suited for the detection of synergistic properties or pro-drugs and how important it is to follow a more holistic *in vivo* approach either via clinical trials or animal experiments (Verpoorte et al., 2005). Since *in vivo* studies are not always feasible it has furthermore been outlined how -omics approaches and systems biology are conceptually nearer to the holistic perspective of ethnopharmacology (Verpoorte et al., 2005; Zhang et al., 2010a). Metabonomics for instance tries to measure as many metabolic factors as possible in order to asses and better understand the alteration, perturbation and functioning of complex biological systems upon determined exogenous stimuli such as medicinal plant extracts. Especially with respect to Chinese medicine (TCM) -omic techniques are increasingly gaining importance (for a review, see Buriani et al., 2012). Adopting a systems approach for the analysis of quality and safety (Wang et al., 2009) and rationalization of polypharmacological mechanisms or network pharmacology (Hopkins, 2007) exerted by complex mixtures of compounds and extracts complies best with the holistic standard of Chinese and Ayurvedic medicine (Joshi et al., 2010; Zhang et al., 2010a).

The enormous amount and diversity of therapeutic uses of traditional medicines described in literature, correlates all too well with their activities assessed in one-dimensional bioassays. Frequently exceedingly high – until the desired effect can be observed – and therapeutically questionable concentrations of extracts or natural products are applied to test systems. This is also the point where the story usually ends as only few claims observed *in vitro* can be repeated *in vivo* (Gertsch, 2009). The matching of ethnomedical indications with biological test systems is not always straightforward and many plant species and crude drugs are indicated and applied for different diseases and symptoms at the same time within one culture and even to a greater extend between different cultures (Spjut, 2005; Gyllenhaal et al., 2012). Moreover, ethnopharmacopoeias are cultural constructs and in constant exchange and transformation. Local use of medicinal plants and associated cultural healing concepts are able to co-exist with, and complement, the local commercialization and use of pharmaceuticals. Such co-existence frequently leads to syncretic developments as shown for rural indigenous communities in Mexico and Peru (Giovannini et al., 2011; Monigatti et al., 2012). Intensified globalization currently accelerates the interchange between local and global pharmacopoeias not only through international business interests but also via print media, television and the World Wide Web generating a feedback loop. The infiltration of global phytotherapeutical and biomedical knowledge into local pharmacopoeias may lead to circular argumentations in debates related to medical anthropology and drug discovery (Leonti, 2011). The global commercialization of herbal medicine and the emergence of what is called Complementary and Alternative Medicine as a social phenomenon during the past decades is also due to the perception among Western societies, that the consumption of such products would be devoid of health risks, especially in comparison to pharmaceuticals (Etkin, 2006, p. 207). However, such a perception is, for example, not compliant with the philosophy and criteria of Chinese medicine, which experimentally exploits the boundaries between toxic and non-toxic applications of herbal formulas and medicine in therapy (Wang et al., 2012a). Also, deliberate or accidental adulterations or misidentified herbal drugs constitute a continuous problem and latent health risk (Ouarghidi et al., 2012; Walker and Applequist, 2012).

In this perspective review, we highlight the historical legacy and development of ethnopharmacology, the relevance of human diet and the importance of allelochemicals. The interface between phytotherapy and drug discovery is evidenced with a discussion on bioprospecting ancient texts, phylogenetic approaches in ethnopharmacology, and evolutionary ecology. Through a discourse touching on aspects of knowledge transmission, integration of medicinal systems, globalization, and medicinal pluralism we tag economy and commercialism as a driving force in ethnopharmacology.

THE GEARWHEEL OF ETHNOPHARMACOLOGY

In the introduction to the chapter of "Pharmacohistoria" to his fundamental work "Handbuch der Pharmakognosie" (Handbook of Pharmacognosy) Tschirch (1910, p. 446) quotes a colleague: "...in its true sense no discovery is really independent, each [discovery] is conditioned and most often also evocated by the actual spirit of its time – in any case, however, primed by previous works." In the following I shall give some historical sketches and examples of how this quote may be interpreted.

Ergot, for example, the sclerotium of *Claviceps purpurea* (Fr.) Tul. (Clavicipitaceae) was used as an abortifacient in Central European folk medicine when Adam Lonicer (Lonicerus 1528–1586) described its application for the first time in 1582 (Ringrose, 1962). Women made use of repeated doses of three sclerotia against pains in the uterus (Lonicerus, 1962, p. 525). Prior to its acceptance as a birth-accelerating drug (thanks to the study by Stearns, 1808) authorities tried to ban ergot's use (Ginzburg, 1990, p. 297). Indeed, ergot did not stand the test of time as a labor-inducing agent since the risks for the unborn were too high and, therefore, continued to be used only to stop the bleeding after parturition (Hofmann, 2002). The hemostatic principle of ergot was finally isolated, elucidated and named ergometrine in 1935 (Stoll, 1935). In an attempt to optimize the pharmacological characteristics of ergometrine Albert Hofmann (1906–2008) derivatized

lysergic acid, the pharmacophore common to all ergot alkaloids, with butanolamine (instead of propanolamine as in ergometrine) obtaining methylergometrine, a compound with more favorable qualities. Still today the 5-hydroxytryptamine (5-HT) antagonist Methergin® is prescribed worldwide in midwifery for its reliable uterus contracting and hemostatic action. Methysergide, another ergometrine derivative interacting with 5-HT receptors was introduced to the market as a prophylaxis of migraine headaches in 1961 (von Bruckhausen et al., 1993, p. 970).

After the success with methylergometrine and in search of a circulation and respiration activator, Hofmann synthesized one of the most controversial compounds ever produced by humans: lysergic acid diethylamide (LSD-25). LSD was introduced as a research tool in psychiatry and sold under the name of Delysid by Sandoz who propagated this strong entheogen as a psyche-decentralizing agent in analytical psychotherapy (Hofmann, 2002, pp. 52–55). During the late 1950s and the 1960s LSD was widely used in psychotherapy alongside insulin shock therapy and with its psyche opening quality served as a therapeutic alternative to the tranquilizers frequently used at that time (Sandison, 1959a,b). Despite of the controversy accompanying the recreational use of LSD, which is generally hold responsible for the decline of therapeutic research with this psychedelic, it was recently pointed out how the difficulties in establishing its efficacy through controlled clinical trials additionally contributed to LSD's demise in experimental psychotherapy (Oram, 2012).

The next example focuses on the same compound class but on a completely different ethnological background. In the Florentine Codex (a description of cultural practices in post-conquest Mexico) Bernardino de Sahagún (1499–1590) refers to the indigenous use of mind-altering mushrooms. The Florentine Codex is also referred to as "The Historia General de las Cosas de Nueva España" (General History of the Things of New Spain; ca. 1545–1590) and considered being the first truly ethnographic study due to the adopted methodological approach. In book 11 p. 130 Sahagún reports on inebriating mushrooms: "It is called *teonanacatl*." It grows on the plains, in the grass. The head is small and round, the stem ling and slender. . .it makes one besotted; it deranges one, troubles one. It is a remedy for fever and gout. Only two or three can be eaten. . .. He who eats many of them sees many things, which make him afraid, or make him laugh. He flees, hangs himself, hurls himself from a cliff, cries out, takes fright. One eats it in honey. . .of one who is haughty, presumptuous, vain, of him it is said: "he mushrooms himself" (de Sahagún, 1961, Book 11, p. 130). Although Sahagún's descriptions are quite precise it was not until July 1938 when the anthropologists Bassett Johnson (1915–1944) together with his wife and two colleagues were invited to attend a ceremony in Huautla de Jimenez (Oaxaca, Mexico) that the mushroom rite was rediscovered (Johnson, 1939; Wasson and Wasson, 1957, p. 237). In the same year Richard Evans Schultes (1915–2001) obtained fresh mushroom samples in Huautla, which he identified as *Panaeolus campanulatus* L. var. *sphinctrinus* (Fr.) Bresadola (Wasson and Wasson, 1957, p. 237). The first outsider to experience the effect of psilocybin containing mushrooms was Gordon Wasson (1898–1986) who in 1953 participated in a mushroom ceremony held by the *curandera* Maria Sabina in Huautla. His subsequent report "Seeking the Magic Mushrooms" published in Life Magazine brought this ancient and local knowledge to a broad North-American audience and assured that the mushroom rite received world-wide publicity. It was again Albert Hofmann who in the late 1950s isolated and elucidated the psychoactive principles of *teonanacatl*, the serotonin mimicking psilocin and its pro-drug psilocybin, disenchanting the magic mushrooms (Hofmann, 2002, pp. 118–126).

Both psilocybin containing mushrooms and LSD were soon experimented with outside the scientific community and became globally used recreational drugs influencing subcultures and propelling countercultural movements. The triggering of overwhelming psychedelic experiences led to the theory that the administration of the classical hallucinogens would result in an overall enhancement of brain activity in humans. This theory was relatively well accepted until recently although no thorough pharmaco-physiological studies of hallucinogen action have taken place since the 1960s (Lee and Roth, 2012). Intriguingly, a new study by Carhart-Harris et al. (2012) provide evidence that psilocybin leads to a decrease of cerebral blood flow and oxygen levels suggesting that the subjective cognition is caused by a reduced activity and decreased connectivity of the brain's connector hubs (Carhart-Harris et al., 2012). In an interesting survey subjective case studies on cluster headache self-treatment with psilocybin and LSD were gathered (Sewell et al., 2006). Cluster headache is an extremely painful condition for which currently no medications terminating cluster periods or extending remission periods are available. Although the survey's limitation due to non-clinical settings and an intrinsic selection bias, the results are intriguing, also because the apparent effectiveness of sub-hallucinogenic doses. A prophylactic effect of psilocybin perceived as complete cessation of attacks was reported by 52% of psilocybin users, while a further 41% reported a decreasing intensity and frequency of cluster headache attacks. Respondents also reported extension of remission period upon LSD and psilocybin consumption (see Sewell et al., 2006). New insights into the pharmaco-physiological interactions of hallucinogens and surveys conducted with individual users might help for a more directed and concerted use of psychedelic compounds in psychotherapy and pain therapy.

In the biogeographic study "On the trail of the ancient opium poppy" Merlin (1984) traces the cultural expansion of one of the most important and potent drug plants. Today opium alkaloids are a worldwide therapeutic commodity, while their molecular scaffolds inspired scores of pharmaceutical chemists and pharmacologists alike. One of the major concerns of medical opioid administration is the fast development of tolerance and withdrawal symptoms as well as minor complications such as constipation, emesis, and drowsiness (Darvishzadeh-Mahani et al., 2012). Recent experimental research on the therapeutic value of opioids (remifentanil) shows that single but highly dosed opioid administration reverses hyperalgesia in rats (Drdla-Schutting et al., 2012). This finding suggests that in case of hyperalgesia, instead of chronic low dose opioid administration, brief but highly dosed opioids might erase chronic pain persistently (Drdla-Schutting et al., 2012).

Also ginger (*Zingiber officinale* Roscoe, Zingiberaceae) besides its status as a spice and food item, has become a global medicinal commodity used against rheumatism, headache and above all

against digestive and respiratory problems showing potent anti-inflammatory properties *in vitro* and *in vivo* (Chevallier, 2000; Williamson, 2002; van Breemen et al., 2011). While paradol, was found to inhibit COX-1 (Nurtjahja-Tjendraputra et al., 2003) 10-gingerol, 8-shogaol and 10-shogaol were shown to inhibit COX-2 (van Breemen et al., 2011) but shogaols and gingerols furthermore concurrently inhibit IL-1β and prostanoid secretion leading to strong upstream anti-inflammatory effects (Nievergelt et al., 2011). Murine *in vivo* data suggest that ginger extract administration is able to prevent morphine tolerance as well as naloxone-induced withdrawal signs (Darvishzadeh-Mahani et al., 2012). As an underlying mechanism L-type channel blocking properties by ginger constituents leading to an attenuation of calcium channel over expression induced by morphine has been suggested (Darvishzadeh-Mahani et al., 2012). The therapeutic benefit of ginger in this context might include its anti-inflammatory potential as it was shown that morphine triggers neuroinflammation in the CNS mediated via binding to the Toll-like receptor 4 (Wang et al., 2012b). Well-conducted clinical trials on the therapeutic efficacy and effectiveness of ginger preparations in painful inflammatory conditions are, however, still lacking (Terry et al., 2011).

Bacopa monnieri (L.) Pennell (Scrophulariaceae) is an important Ayurvedic medicinal plant used for a variety of health problems but above all to treat mental problems (Williamson, 2002; Russo and Borrelli, 2005). Since its Hindu name "Brahmi" is also valid for the Apiaceae species *Centella asiatica* L. (Sanskrit: Mandukaparni) the first written accounts are difficult to assign to either of the two species (see Hoernle, 2011, p. 301). Phytochemical analyses with *B. monnieri* started as early as 1931 and two saponins, bacoside A and B, are considered to be responsible for the neuropharmacological effects, while clinical trials with standardized extracts have shown promising results (Russo and Borrelli, 2005). Recent experimental data obtained with rats suggests that *B. monnieri* leaf extract up-regulates expression of tryptophan hydroxylase and serotonin transporters (Charles et al., 2011). A randomized controlled trial over a long-term period with a standardized *B. monnieri* extract is currently assessing the cognitive performance of subjects and tries to find correlations with and between inflammation, oxidative stress and cardiovascular health (Stough et al., 2012).

Semisynthetic derivatives of artemisinin, obtained from the ancient Chinese medicinal herb "qing hao" (*Artemisia annua* L., Asteraceae) are used in combination therapies and are a mainstay in the treatment of malaria (Kumar and Clark, 2001, pp. 80–81; Hsu, 2006). Artemisinin is a highly bioactive sesquiterpene lactone containing a 1,2,4-trioxane pharmacophore and its possible molecular mode of action has caused some debate (O'Neill et al., 2010). Besides artemisinin's anti-plasmodial activity also its anti-tumor activity has come into focus (Woerdenbag et al., 1993; Efferth, 2007). The same rationale proposed for artemisinin's anti-plasmodial activity may explain the sensitivity of cancer cells toward artemisinin (Mercer et al., 2011). Recent evidence suggests that hemoglobin degradation products such as heme are activating artemisinin leading to carbon-centered radicals exerting oxidative stress in the parasite (Klonis et al., 2011; Mercer et al., 2011). Artemisinin derived anti-malarial drugs are, however, not easily accessible or affordable to a large portion of the malaria struck population (Kokwaro, 2009; Wright et al., 2010). Therefore, *A. annua* has been introduced to rural areas afflicted by malaria epidemics around the globe for self-medication purposes. Locally grown and carefully prepared and applied herbal tea of *A. annua* could present a reasonable and affordable alternative (Wright et al., 2010; van der Kooy and Verpoorte, 2011). When adopting the preparation methods described in ancient Chinese medicinal texts it was shown that only a fraction of pounded juice is needed with respect to the quantity of aqueous infusion in order to provide the required amount of artemisinin (Wright et al., 2010). However, also by applying the correct infusion method artemisinin extraction efficiencies up to 90% can be reached (van der Kooy and Verpoorte, 2011).

Intriguingly, a survey about the anti-malarial use of *A. annua* in Kenya and Uganda revealed that in the meantime a considerable part of the respondents use the herb also against HIV/AIDS (Willcox et al., 2011). Encouraged by this report Lubbe et al. (2012) showed that *A. annua* as well as *A. afra* Jacq ex Willd., which does, however, not contain artemisinin, exhibit potent anti-HIV activity *in vitro*. Although the identification of the responsible compound(s) as well as *in vivo* studies are still due, Lubbe et al. (2012) have pointed out how people adapt their pharmacopoeia to the emergence of new diseases and epidemics. The use of "magic mushrooms" and LSD to treat cluster headaches and the use of *A. annua* in rural areas of Africa to combat HIV infection highlights that serendipity and the perception of collateral properties in the search of new medical therapies still plays an important role. Especially during the appearance of new diseases, lack of financial resources and in cases where no effective treatment methods exist (Sewell et al., 2006; Willcox et al., 2011) ethnopharmacology may lead us to effective therapies. These cases also highlight that ethnopharmacological research is not dependent on "traditional" knowledge systems in the narrower sense but that there exist feedback loops wherein modern and post-modern knowledge systems are becoming more relevant.

DIET AND ETHNOPHARMACOLOGY

Etkin and Ross (1991) were referring to the "extra-nutritive" aspect of food phytochemistry and associated pharmacology when they asked whether we should "set a place for diet in ethnopharmacology". The evolutionary perspective of health and nutrition comprises the co-evolution of the food-medicine continuum of wild gathered and cultivated vegetables (Etkin, 2006; Lindeberg, 2010; Leonti, 2012). Especially wild gathered food plants are often reported in local and popular traditions to have pharmacologic activities and are frequently associated with beneficial effects on the gastrointestinal tract, the cardiovascular system and with diuretic properties (e.g., Pardo-de-Santayana et al., 2005; Rivera et al., 2005; Guarrera and Savo, 2013). But although domestication of staple crops has led to a reduction of allelochemicals (Johns, 1990, pp. 102–159; Etkin and Ross, 1991), regularly consumed food products may provide relevant concentrations of pharmacologically active plant metabolites.

The focus thus falls on pharmacological properties of allelochemicals present in food, i.e., the bioactivity of secondary metabolites that evolved upon ecological constraints such as

herbivory and not on our physiologic requirement for vitamins, pigments (carotenoids, lycopene) and fatty acids, which is considered in nutrition sciences (cf. Lindeberg, 2010; Carrera-Bastos et al., 2011). Also, antioxidant *in vitro* activities exerted by polyphenol-rich extracts and polyphenolic compounds such as flavonoids are hardly translatable into *in vivo* conditions and thus fall not in the narrower focus of ethnopharmacology. Following evolutionary concepts it was pointed out that flavonoids did not evolve for the purpose of scavenging free radicals (Zhang et al., 2010b).

The human dependence on salt in regions far from the seashore is occasionally satisfied by the production of vegetable salts from plant ashes. The salt obtained from the Witoto "woman-plant" (*Bactris humilis* (Wallace) Buret, Arecaceae) showed a remarkably high content of zinc and sodium with respect to salts obtained from other plant ashes. Zinc is a microelement required by pregnant and lactating women, while sodium is important for the retention and formation of amniotic liquid and might explain why the Witoto refer to *B. humilis* as "woman-plant" (Echeverri and Román-Jitdutjaaño, 2011).

A more regular example is the use of highly toxic *Aconitum* as root vegetable in the region of Shaanxi (China; Kang et al., 2012). Before being consumed, tubers of cultivated *Aconitum carmichaelii* Debeaux are boiled for up to 10 h, leading to degradation products of the diterpene alkaloids, which are far less toxic (Kang et al., 2012). The input for this dish, which is only consumed during the cold season and considered a "functional food" is in any case remarkable and warrants an ethnopharmacological analysis. Apart from the nutritional factors the ancient Romans highly appreciated the medicinal properties of cabbage, which they used for interior and external ailments (Matthioli et al., 1967–1970, book 2, p. 498). The claim that the juice of kale leaves has gastroprotective effects, preventing the formation of ulcers has been experimentally assessed (Carvalho et al., 2011; Lemos et al., 2011). Aqueous and hydroalcoholic *Brassica oleracea* L. (Brassicaceae) extracts (50–100 mg/kg) were found to be as effective as the positive controls in preventing experimentally induced gastric lesions in murines.

Insufficiently validated health claims and aggressive marketing strategies may, however, lead to negative socio-economic impacts on the local level. It was for instance not possible to date to scientifically confirm the claimed health benefits of açai (*Euterpe oleracea* Mart., Arecaceae) that range from rapid weight loss, prevention of cardiovascular diseases and aging in general (Heinrich et al., 2011). After an initial boost of worldwide açai commercialization triggered by local and global media coverage (Heinrich et al., 2011) the global sales of açai berries are expected to drop leaving trade volume to other, scientifically better corroborated nutraceuticals. In such a scenario local Brazilian farmers would be left empty handed. Another fruit enjoying growing popularity on the global health food market is the Goji berry (*Lycium* spp., Solanaceae; Potterat, 2010). While Goji berry products are considered a tonic and the juice a longevity drink, no such claims have hitherto been corroborated by scientific data not to mention by clinical studies (Potterat, 2010).

A closer cooperation of ethnopharmacologists with the agriculture sectors in areas such as food production, nutrition and health could counteract such scientifically deregulated developments (Heywood, 2011). It remains to be seen, however, what the priorities of the agriculture sector and the nutraceuticals industry are; easy money or controlled and rational health claims?

Turmeric (*Curcuma longa* L., Zingiberaceae) is an important ingredient in Indian and Chinese cuisine and medicine. India is the biggest producer and consumer of turmeric, while the US imports of turmeric stem to the largest extent from India. With respect to crude botanical materials, where the product price increases dramatically along the value chain (producer to consumer) without receiving additional value (final product is unprocessed) products including turmeric generally receive added value through different processing methods (Booker et al., 2012). In the case of mere encapsulated turmeric, which confers a medicinal appearance to the end product a 10-fold price increment has been observed (Booker et al., 2012). The active metabolite, curcumin, is actually a mixture of the three preponderant diferuloylmethanes also used as a colouring agent in food industry and labeled "E 100." A large body of *in vitro* and *in vivo* evidence has been accumulated confirming curcumin's anti-inflammatory properties (Goel et al., 2008). Clinical studies evidenced curcumin's therapeutic relevance with rheumatoid arthritis, psoriasis, inflammatory bowel disease, inflammatory eye disease, and kidney transplantation (White and Judkins, 2011). In practice, however, curcumin administration suffers from the molecule's hydrophobic nature, which determines its poor absorption, rapid metabolism, and limited tissue distribution. Also therefore, curcumin is well tolerated and regarded as safe, while doses as high as 12.000 mg per day have resulted in no significant adverse effects (Lao et al., 2006; Anand et al., 2007; Hsu and Cheng, 2007). Bioavailability of curcumin is, however, not so much an issue with the gastrointestinal tissues, mucose membranes in general and skin lesions, which can be exposed directly to biologically active concentrations (Hsu and Cheng, 2007). But curcumin has been shown to act as an iron chelator able to interfere with iron metabolism (Jiao et al., 2009). In patients with marginal iron stores curcumin supplementation may therefore lead to or aggravate anemia (Jiao et al., 2009). A possible solution with the aim at overcoming curcumin's poor bioavailability and enhancing its aqueous dispersion is nanoparticle-encapsulation (Bisht et al., 2007). A mixture of curcumin with piperine, an alkaloid obtained from pepper (*Piper nigrum* L. and *P. longum* L., Piperaceae) able to enhance curcumin's bioavailability via the inhibition of hepatic and intestinal glucuronidation (Shoba et al., 1998) has already been released to the market. Other brands contain mixtures of curcumin and broccoli (*Brassica oleracea* var. *italica* Plenck, Brassicaceae) seed extract with up to 6% of detoxifying sulfhoraphane glucosinolates and are currently sold for 39.95 USD (end user price) a bottle containing 30 servings (see also: http://www.youtube.com/watch?v=ZuvhiiD1qAk).

By nature of its assignment a completely different perspective regarding curcumin's use as a food dye has been adopted by the European Food Safety Authority (EFSA; EFSA, 2010). Even though several previous safety evaluations by a joint FAO/WHO expert committee on food additives from 1974 to 2004 have established that curcumin was acceptable for use in food, EFSA re-evaluated the use of curcumin eliminating concerns over genotoxicity and concluding that consumption up to an average daily intake of

3 mg/kg bodyweight is safe (EFSA, 2010). This example highlights how different aims and perspectives generate disparate hypothesis, although EFSA's concerns were primarily the chronic exposure to curcumin.

Recent commercial endeavors by the nutraceutical industry reveal a trend toward sophisticated formulations adopting the concept of polypharmacology or "network pharmacology." Instead of approaching pharmacology with the concept of "one lock" (target) and "one key" (drug) Hopkins (2007) argued that one should consider that there are drug target networks and not just single disconnected drug targets. In herbal combination formulae practiced, for example in Ayurvedic and in Chinese medicine the pharmacokinetics and – dynamics of a single or a couple of compounds may be influenced by the rest of the metabolites present in the extract (Aggarwal et al., 2011; He et al., 2012, see Challenges in Ethnopharmacology). Medicinal plants have, however, not been "designed" to cure human diseases or alleviating their symptoms and undesired antagonistic effects are equally realistic. Re-engineer botanical drugs by designing extracts driven by therapy success could be a way forward toward "more intelligent mixtures" (Gertsch, 2011). The strategy of polypharmacology is as old as medicine itself and the food supplement industry provides several examples where phytochemicals of different vegetables, fruits or spices are blended to provide synergistic servings appearing as "molecular novel cuisine." One should, however, keep in mind, that a variegated and balanced diet not only tastes better than pills and capsules, but is also healthier.

DIACHRONIC STUDIES AND BIOPROSPECTING ANCIENT TEXTS

Since culture is not static but in a constant flux the importance of *materia medica* is exposed to different socio-economic factors and may change over time. These factors comprise cultural interaction (e.g., migration), change in diseases prevalence, lack or prove of efficacy and effectiveness, introduction of therapeutic alternatives (e.g., biomedicine), change in availability of new species and products and ecology (e.g., agricultural practices). Changes in medicinal plant use can be perceived as "continuity" (maintenance), "disjunction" (same species but new context or conceptual background), "discontinuity" (abandonment) and synchronism (substitution and adoption; see Bye et al., 1995). Odonne et al. (2011) found only one out of five previously described plant species used by the Wayãpi against leishmaniasis still in use, while previously not reported species are now relied upon. Generally field studies on medical ethnobotany show "snapshots," i.e., the *status quo* of medicinal plant use for a certain community and point in time. Writing has enabled physicians, explorers, historians, and merchants to document, conserve and pass on gathered information and knowledge on medical plant use in codices, manuscripts and books. Thanks to these written sources plant uses can be traced diachronically through ages and over centuries up to the present usage (e.g., Leonti et al., 2010; Luczaj, 2010; Sõukand and Kalle, 2011; Obón et al., 2012; Pieroni et al., 2013) and even causal influences of herbal texts on modern local plant use can be observed (Leonti et al., 2010). Consequently, filtering down to the popular level, scientific literature influences local plant use and thereby shapes the results of current ethnobotanical field-studies. Quantitative diachronic evaluations of plant use additionally provide historic depth and information on the therapeutic rationales but of course would not be possible without "snapshots." In the words of Steve Jobs (1955–2011) this approach reads "You can't connect the dots looking forward; you can only connect them looking backwards" (Jobs, 2005).

Today, information regarding the use of medicinal and food plants is readily accessible via the WWW. While until recently global migration and urban environments were in the focus of changing and persisting plant use, the WWW increasingly strengthens such dynamics and renders them even more complex (Casselman and Heinrich, 2011). How the video-sharing platform YouTube™ is disseminating and influencing global use patterns regarding the hallucinogenic application of *Salvia divinorum* Epling and Játiva (Lamiaceae) through user generated contents has been highlighted recently (Casselman and Heinrich, 2011).

In more economically developed countries and regions herbal medicine and phytotherapy is used as a therapeutic alternative to biomedicine for the treatment of mild and chronic health problems. Complementary and alternative therapies are also used to sustain conventional medicine and to treat their side effects such as the collateral symptoms frequently occurring during cancer therapy (Kronenberg et al., 2005). Moreover, today, a part of the world's population has the choice of using herbal medicine (and also nutraceuticals) also as a fashionable life-style treatment. In many parts of the world conditions and opportunities for experimenting with herbal medicines in case of sever and acute health problems are not given anymore. Therefore, one question arising here relates to the differences in the application and function of for instance TCM or Ayurvedic medicine in rural and deprived societies with respect to a more prospering population.

During the pre-biomedical era, the use of herbal medicine and *materia medica*, together with primordial surgery and healing ceremonies were the only form of medical treatment. Historical texts documenting the ancient use of *materia medica* contain information, which is not present anymore in local knowledge of communities pertaining to industrialized nations. Therefore, ancient herbals may reveal interesting information for bioprospecting attempts. Buenz et al. (2004) provide some practical and theoretical considerations for approaching historic texts on *materia medica* as a source for drug discovery. Plant and drug identification in ancient texts can be challenging especially when a wide range of names is used for the same product and when no images are available (Lev, 2007). Another crucial point relates to the correct interpretation of the description of diseases, ailments, and the respective etiologies. When iconographical studies together with textual interpretations are possible (e.g., the case for most Renaissance herbals) the identification of most *materia medica*, is relatively straightforward, especially to the genus level. More complicated may be the correct interpretation of descriptions of amorphous products such as plant exudates (Lardos et al., 2011).

Malaria, for instance, was widespread in Europe and only eradicated toward the mid-twentieth century. The European Renaissance herbals make clear distinctions between remedies against *malaria tertiana* (*Plasmodium vivax*), and *malaria quartana* (*Plasmodium malariae*; Adams et al., 2011a). However, published data regarding their anti-plasmodial *in vitro* activity exist only for the

minority of the taxa indicated against malaria in eight different German Renaissance herbals (Zimmermann et al., 2012). A comparative screening involving extracts obtained from species indicated against malaria and a control group of European medicinal species with indications other than malaria showed nine out of the ten most active extracts deriving from species with a documented anti-malarial use (Zimmermann et al., 2012). From frog's spoon (*Alisma plantago-aquatica* L., Alismataceae), which is described in different sixteenth and seventeenth century herbals against "three" and "four day" fevers, protostane triterpenoids with moderate anti-plasmodial *in vitro* activity were isolated (Adams et al., 2011b).

EVOLUTIONARY ASPECTS OF PHARMACOPOEIAS AND BIOPROSPECTING WITH PHYLOGENETIC APPROACHES

While drug discovery from natural products focuses on pure bioactive and bio-available, "drug-able" chemical entities, herbal medicine and phytotherapy research pursues the characterization of more complex pharmacological interactions exerted by an extract containing diverse chemical entities. The historical legacy testifies that ethnopharmacology has contributed to both domains, drug discovery and the development of herbal medicines (Elvin-Lewis, 2001; Fabricant and Farnsworth, 2001). Both domains focus on secondary metabolites, adaptive traits that evolved in response to ecological pressures. The fact that closely related taxa show more similar patterns of secondary metabolites than phylogenetically distant taxa, can be exploited for chemotaxonomical approaches in drug discovery.

A recent census regarding the taxonomic source of FDA approved and clinical-trial natural product drugs has provided evidence for a non-random distribution with clustered and disjunct taxonomic affiliations (Zhu et al., 2011). From a total of 740 existing Viridiplantae families (comprising ca. 450 Angiosperm families) only 59 families of the Angiospermae, four of the Gymnospermae, three of the mosses and one of the algae family provide all FDA approved and clinical-trial natural product drugs derived from plants (Zhu et al., 2011). Many of the Angio- and Gymnosperm species censed by Zhu et al. (2011; S5–S7) are also elements of local ethnopharmacopoeias and used in global herbal medicine or are used as spices and staple food and derive from an intimate human-plant relationship and have a history of an ethnopharmacological use. The "traditional" indications of the herbal medicines are, however, far more diverse and not always congruent with the biomedical applications of the purified and FDA approved compounds (Leonti et al., 2013). Similarly to FDA approved drugs, also the compositions of ethnopharmacopoeias show disproportionate selections of the available flora. Several statistical studies have pointed out that certain plant families (e.g., Asteraceae, Lamiaceae) are generally over proportionally relied upon for a selection within indigenous and local pharmacopoeias, while others (e.g., Poaceae and Orchidaceae) are normally a neglected source of medicine (e.g., Moerman et al., 1999; Saslis-Lagoudakis et al., 2011; Thomas et al., 2011; Weckerle et al., 2011; Weidenhammer et al., 2011; Turi and Murch, 2013). Most of these studies focus on ecological and universal aspects of herbal medicine and the different factors influencing plant selection, or advocate such statistical approaches as bioprospecting tools. A more sophisticated approach toward bioprospecting with comparisons of medicinal floras uses reconstructed phylogenies of ethnopharmacopoeias (Saslis-Lagoudakis et al., 2012). Exactly which practical instructions for bioprospecting purposes might be obtained from cross-cultural comparisons, or the analysis of disproportionate inclusion of plant taxa in pharmacopoeias, without considering the influence of other parameters, have both been put up for discussion (Gertsch, 2012).

Herbal medicines usually work with their entire bio-available chemical profile in synergistic and antagonistic relationships, which exacerbates bioprospecting attempts. Moreover, factors influencing medicinal plant selection by human communities are manifold ranging from ecology, cultural history, human cognition, and medical anthropology. Recently the first author characterized the prototypical medicinal plant as "an abundant Euasterid weedy species, possibly utilizable as food, routinely attracting pollinators, rich in pharmacologically active and bio-available metabolites, endowed with pronounced organoleptic properties and ideally, with a bit of fantasy, the signature of the organoleptic virtues could be matched with the plant's medicinal indication, which thereby would facilitate its transmission and through the belief in its semiotic appearance at the same time enhance the meaning response of its medical application" (Leonti, 2011). But one of the crucial points with respect to drug discovery is that most medicinal species are used for a wide range of therapeutic applications, which renders the formulation of tangible instructions for bioprospecting attempts difficult. It has also been shown that when indigenous medicines are evaluated across a range of bioassays, ethnomedical indications rarely correspond with meaningful screening results (Gyllenhaal et al., 2012). A particularly interesting fact is, however, that plant species used as medicine by human societies achieve higher number of hits with respect to random collections when screened in cell based and mechanistic bioassays (Spjut and Perdue, 1976; Gyllenhaal et al., 2012).

This may be due to the overrepresentation of weeds and widespread taxa in local and official pharmacopoeias (Stepp and Moerman, 2001; Stepp, 2004); it has been proposed that taxa with wide biogeographical distributions have encoded a broader range of ecological responses in their genes with respect to more locally occurring taxa (Leonti et al., 2013). These responses include the synthesis of allelochemicals with broad-spectrum biological interactions, comprising the targeting of proteins of mammals and primates.

MEANING RESPONSE, EFFICACY, AND EFFECTIVENESS

With respect to biomedicine, traditional systems of medicine base diagnostics, perception of illness and healing traditions on elaborate, and from a Western scientific point of view, challenging (if not unacceptable) philosophy (e.g., Jiang et al., 2012; Mukherjee et al., 2012; see Foster and Anderson, 1978). Often these philosophies are grounded on physiologic equilibrium models, which are also expressed through curing rites and psychosocial support (Foster and Anderson, 1978). Such philosophies and holistic approaches comfort the end-user and thereby impart a beneficial and self-affirming "placebo effect" also called "meaning response" (cf. Moerman and Jonas, 2002). Higher expectation on treatment outcome by patients with chronic pain had a significant impact on

the effectiveness of acupuncture therapy (Linde et al., 2007). It has been suggested that psychological factors such as expectation and behavioral conditioning are able to induce neurobiological mechanisms that interact with pharmacological pathways (Bingel et al., 2011; Rief et al., 2011). Although the pharmacological assessment and evaluation of the meaning response goes beyond capabilities of standard pharmacology laboratories, the relevance of the placebo and the nocebo effect may not be recognized enough: the therapeutic context, in which an optimal drug efficiency may be achieved should be included in the treatment recommendations (Rief et al., 2011). Even though 25 years ago Etkin (1988) already pointed out how drug efficacy depends on the specific socio-cultural context, this insight has not been incorporated widely into clinical studies to the extent it might deserve (Witt, 2013). Last et al. (2011) defined "efficacy" as treatment outcomes obtained under ideal and standardized conditions (e.g., clinical study setting) as opposed to "effectiveness," which is used to describe the therapeutic success in routine and non-experimental circumstances (see also Witt, 2013). As globalization changes socio-cultural contexts one can expect that the current development will have an impact on the meaning response of a specific therapy. For example in Western societies "more" is regarded as "better" above all in relation to monetary affairs. One plausible hypothesis would therefore be that more sophisticated and costly a (herbal) preparations would achieve more pronounced placebo effects, and hence effectiveness, also on the global scale.

TRADITIONAL MEDICINES: GLOBALIZATION, KNOWLEDGE TRANSMISSION, INTEGRATION, AND MEDICAL PLURALISM

For the largest part of human history information and knowledge has been transmitted orally and by observation and direct copying. With the advent of writing systems a more precise copying and knowledge transmission was possible and thanks to scripts the transmission of knowledge related to *material medica* over wide ranges of space and time was possible. Oral and written knowledge transmission is in constant exchange and written knowledge may result in changes to traditions, which are then passed on orally – and *vice versa* – and from that point of view a dichotomization makes no sense (Totelin, 2009). However, there exists a clear quantitative difference in knowledge transmission emanated by texts used as guides and references over a long period of time, over broad geographical extensions and diverse cultural backgrounds. Generally, scripts allow a more conservative knowledge transmission and may lead to a homogenization of knowledge (Leonti, 2011).

That goods and associated knowledge from Indian and Chinese *materia medica* were exchanged along the Silk Road reaching and influencing early Mediterranean medical traditions can be traced in scripts dealing with *materia medica* at least from the fifth to the fourth century BC onward (Touwaide and Appetiti, 2013). On the other hand received the Hindu system of medicine at the same time apparently little external influences. A collection of 51 birch-bark leaves known as the Bower Manuscript is regarded as the oldest still existing script dealing with Hindu medicine (Winternitz, 1902). It was discovered in Kucha (Chinese Turkestan) situated on the Silk Road in 1889, translated by the German-British orientalist Rudolf Hoernle (1841–1918) and dated to the fifth century AD. Not even opium, which at that time was one of the most important drugs in the Mediterranean and the Near East, is mentioned in the Bower Manuscript (Tschirch, 1910, p. 508; Hoernle, 2011). Although earlier, and now lost, medicinal scripts dealing with ancient Hindu medicine existed, such medical knowledge within India has mainly been passed on by practitioners and through the individual training of pupils (Srivastava, 1954; Foster and Anderson, 1978, p. 62; Mukherjee et al., 2012). Medicinal practices on the Indian subcontinent began to intermingle with the Arab system of medicine (Unani Tibb) from the twelfth century onward and toward the end of the eighteenth century the British began to introduce the Western system of medicine (Srivastava, 1954). The first European medical school in India, the Calcutta Medical College, opened in 1835 (Anonymous, 1879). This historical development led to the current situation that, while the Sanskrit classics altogether describe some 700 medicinal plant species, depending on the source, more than 10,000 plant species are associated with Ayurvedic medicine (Padma, 2005).

Thanks to global commercialization and intercultural knowledge and information exchange, patients increasingly have the choice between different medicinal systems for their health care needs with access to physicians specialized within different medical systems (e.g., Miles, 1998; Khan, 2006; Stranieri and Vaughan, 2010). Based on the historical and economic development in a country or region a nation's traditional system(s) of medicine is often complemented with biomedicine. Conversely, in other regions, alongside biomedicine different traditional systems of medicine and alternatives (complementary medicine) are established. If and how alternative therapies should be included and covered by national health services and what is a fair price for individual therapies, medicine and health care in general is the matter of continuous debates (**Figure 1**).

However, Khan (2006) argues that although medical pluralism is already a historic reality in many societies there is a need to bring the discussion beyond the pluralist and liberal arguments

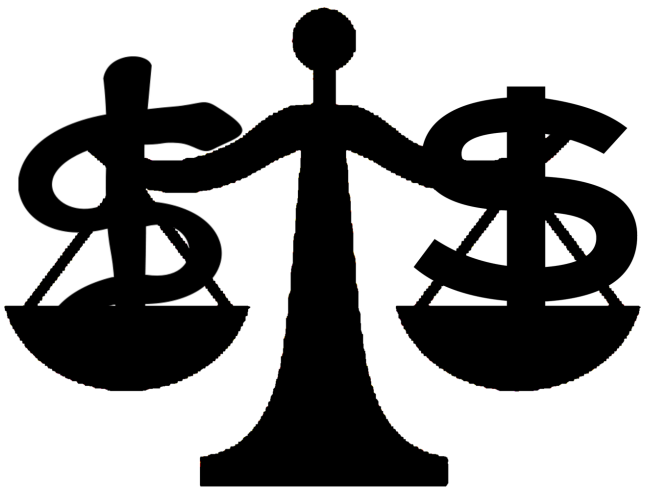

FIGURE 1 | A fair price for medicine – does it exist?

including factors such as "domination and hegemony" widening the historical, social and political context of the discussion. With respect to India, Khan (2006) specifies, that while Western culture and medicine exerted authority over indigenous science and medicine during the colonial phase it were the national leaderships and governments, which consolidated this development. On the other hand did the demand for indigenous alternatives, according to Khan (2006), although effective, not get enough response from the national authorities. Likewise, local health care and medicine is subjected to global commercialism, which progressively influences individual therapeutic choices and preferences (cf. Miles, 1998). But while patients may choose between different medicinal systems, therapeutic settings, where specialists from different medicinal systems collaborate, are rare. One recently highlighted example stems from a modern community hospital in Thailand, where Thai Traditional Medicine is integrated alongside biomedicine into the modern healthcare service (Chotchoungchatchai et al., 2012). The identified key factors for the successful integration are knowledge transmission of Royal Thai Traditional Medicine and locally practiced herbal medicine to the hospital practitioners and a healthcare team consisting of members with different backgrounds, together with a well organized supply of herbal medicine (Chotchoungchatchai et al., 2012). Integrating medical systems is not only a challenge for hospital facilities but also in health informatics (Health Information Systems), which is currently closely linked to the dimensions of Western biomedicine (Stranieri and Vaughan, 2010). Since the trend toward co-existence of different medicinal systems is bound to augment it is appropriate to design health informatics applications compliant with the characteristics of different medicinal systems. The visualization of arguments *pro* and *contra* for choosing a certain medicinal system in a particular health condition is put forward by Stranieri and Vaughan (2010) as an example of how the coalescing of medicinal systems into health informatics may support patients' informed decisions making. In this respect Witt (2013) argues that there is a lack of clinical studies focusing on comparative effectiveness of traditional herbal medicine and food products.

Traditional medicines will only be taken seriously and accepted by primary health care systems when experimental and clinical data establish an evidence base (Cordell, 2011; Verpoorte, 2012). High standard studies confirming the safety and the effectiveness of traditional medicines are, therefore, required (Cordell, 2011; Verpoorte, 2012). Since co-medication of medicinal plants and biomedicine is frequently observed, practitioners and clinicians are encouraged to watch out for adverse as well as beneficial interactions (Giovannini et al., 2011; Nagata et al., 2011; Odonne et al., 2011). Clinical studies instead of focusing exclusively on efficacy could focus also on effectiveness by including a more heterogeneous sample of participants and adopting research settings that reflect usual heath care situations as far as possible (Witt, 2013). According to Verpoorte (2012) and others (see Weidenhammer et al., 2011 and Xu et al., 2013), the changing global economic scene and changing paradigm in drug development might present an advantage in the attempt to officially register traditional medicines with governmental agencies. However, as pronounced by Etkin (2006, p. 212), "aggressive marketing of both pharmaceuticals and supplements blurs the line between scientific observation and product advertisement."

CONCLUSION

The use of indigenous drugs, herbal medicine and traditional *materia medica* can only be understood through a combination of historical, ecological, economic, cognitive, and pharmacological approaches, while anecdotal references are lost in space and time. Ongoing globalization driven by neo-liberalism increases medical plurality through intercultural knowledge and information exchange. Although there is a trend toward the development of global pharmacopoeias, local pharmacopoeias, despite being influenced by global economy, and politics will continue to keep their role and identity. While knowledge transmission along trade and value chains tend to secure hegemonic and financial interests over local economies and pharmacopoeias, the local availability of biomedicine and access to research data on traditional and herbal medicine may lead to a syncretic development with reconciling effects. Not only herbal products but also food supplements and nutraceuticals have considerably raised their market share in the last few years. Since sound scientific data for many commercialized health products is still lacking, this calls for a much more rigorous multidisciplinary science-driven approach to local and traditional medicines, which also empowers the local keepers of this knowledge and their users.

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REVIEW
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Medicinal Plant Analysis: A Historical and Regional Discussion of Emergent Complex Techniques

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The analysis of medicinal plants has had a long history, and especially with regard to assessing a plant's quality. The first techniques were organoleptic using the physical senses of taste, smell, and appearance. Then gradually these led on to more advanced instrumental techniques. Though different countries have their own traditional medicines China currently leads the way in terms of the number of publications focused on medicinal plant analysis and number of inclusions in their Pharmacopoeia. The monographs contained within these publications give directions on the type of analysis that should be performed, and for manufacturers, this typically means that they need access to more and more advanced instrumentation. We have seen developments in many areas of analytical analysis and particularly the development of chromatographic and spectroscopic methods and the hyphenation of these techniques. The ability to process data using multivariate analysis software has opened the door to metabolomics giving us greater capacity to understand the many variations of chemical compounds occurring within medicinal plants, allowing us to have greater certainty of not only the quality of the plants and medicines but also of their suitability for clinical research. Refinements in technology have resulted in the ability to analyze and categorize plants effectively and be able to detect contaminants and adulterants occurring at very low levels. However, advances in technology cannot provide us with all the answers we need in order to deliver high-quality herbal medicines and the more traditional techniques of assessing quality remain as important today.

Keywords: herbal medicine, medicinal plant, analysis, quality, pharmacopoeia, complexity, advances

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INTRODUCTION

Medicinal plants have been a resource for healing in local communities around the world for thousands of years. Still it remains of contemporary importance as a primary healthcare mode for approximately 85% of the world's population (Pešić, 2015), and as a resource for drug discovery, with 80% of all synthetic drugs deriving from them (Bauer and Brönstrup, 2014). Concurrently, the last few hundred years has seen a prolific rise in the introduction, development, and advancement of herbal substances analysis. Humans have been identifying and selecting medicinal plants and foods based on organoleptic assessment of suitability and quality for thousands of years, but it is only in

the span of the last seven decades since the invention of basic analytical techniques, e.g., paper chromatography, that has seen rapid development from sight, touch, and smell to using sophisticated instrumentation. Though this mechanization of the senses has appeared relatively recently, historically conceptual expansion has been building throughout the scientific revolution, outwards toward the universe and inwards to a scale below recognition capable with a human eye, leading to development of some of the earliest analytical tools assisting the senses, the telescope and microscope. From the initial discovery of new microscopic worlds, through structural, chemical, and atomic levels, the sensitivity and range of human perception has been extended and enhanced.

Rapid progress is especially evident considering that the concept of a laboratory was only formally formed in Europe during the early 1600s. First as an extension of philosophers', doctors', and scientists' workrooms, it becomes a space to study nature and gather empirical evidence (Wilson, 1997), where studies could be conducted at the analyst's convenience rather than at specific times when daylight or weather permitted. This was a small but important step towards more formalized analytical investigations.

In modern analysis, single techniques such as paper chromatography and much earlier colorimetry appeared. It was followed by a greater range and wider application of these techniques until early hyphenations such as LC-UV emerged, culminating more recently in multiple combinations of multi-hyphenated instrumentation, availing of the analytical advantages inherent in each individual technique. The emergence of hyphenated analytical techniques in many aspects is analogous to the organoleptic synthesis that occurs when selecting a medicinal plant; viewing, smelling and tasting it to use combinations of different senses, increasing the points of reference/statistical degrees of freedom to improve the probability of correctly identifying and assessing its quality. The emergence and application of these hyphenated techniques only became possible and useful as computer systems and data management tools developed, enabling rapid and selective synthesis of information from the large amount of instrumental and analytical data signals generated.

Probably the single greatest influence in recent times in the advancement of the analysis of herbal materials (and arguably analysis generally) is, though, how large amounts of data can be collected, assimilated, and used more meaningfully in human readable forms. Similar to the historical advancements in combinatorial hyphenated instrumentation, now combinatorial data processing techniques like fingerprinting, metabolomic profiling, and pattern recognition algorithms have emerged, further increasing analytical capabilities, while reducing operator time and expertise required. This trend has further accelerated the pace and rate of advancement of analytical techniques and has led to an increase in the pace and capability of the associated research. In this paper, we analyze publication trends and pharmacopoeial developments in order to better understand the role and progression of analytical techniques. Since their initial discovery and development, with a particular focus on China, an Asian country with both deep cultural and long-term historical roots in plant medicine, to more modern day developments and applications.

PUBLICATION TRENDS

Increasing interest in medicinal plant research and analysis is reflected in the number of recent publications, with more than a three-fold increase from 4,686 publications during the year 2008 to 14,884 in 2018. Output published during the 8 years of the present decade alone outnumbered all those combined before 2000, since the included database records began in 1800 (**Figure 1**).

The largest proportion of publications cited in current databases over the last 10 years for medicinal plant analysis reports are in the disciplines of pharmacology and pharmacy (**Figure 2**). With plant sciences, biochemical molecular biology and agriculture research following closely behind, together comprising almost 70% of the total publications.

REGIONAL TRENDS—LAST 10 YEARS

The majority (about 58%) of medicinal plant analysis publications in the last 10 years have collectively emerged from mainland China, India, USA, and South Korea (**Figure 3**). This may be an expression of the strong medicinal plant traditions in Asia in addition to the USA's dominant presence as an international user of herbal products (Hu et al., 2013). The major East Asian regions, in particular, China, Japan, South Korea, together with Taiwan, contribute more than half of the total citations (55%). This may be indicative of the rapid economic progress and technological capability of these countries. China is the major contributor, with a 15% increase in its dominance of research outputs in the last 10 years. This influence has also been seen in the effect of China's growing involvement in aiding the development of pharmacopoeias around the world and as a leader in the analysis of Chinese medicinal plants (**Figure 3**).

REGULATION AND A CHANGING ANALYTICAL LANDSCAPE

From a regulatory perspective, the pharmacopoeial requirements are the central reference point for the analysis of medicinal plants. Though internationally many pharmacopoeias exist, the most comprehensive of these relating to herbal medicinal materials is the Chinese Pharmacopoeia (ChP). The current ChP introduced in 2015 is the 10th iteration presented in three volumes and includes 5,608 drugs, a 10-fold increase from its first edition in 1953. More than half of the current monographs (Hamid-Reza et al., 2013, 598) relate to CHM specifically including raw plants, slices, herbal mixtures, and oils. A noticeable inclusion in the current version compared with the previous version is the addition of 400 herbal mixtures (Qian et al., 2010).

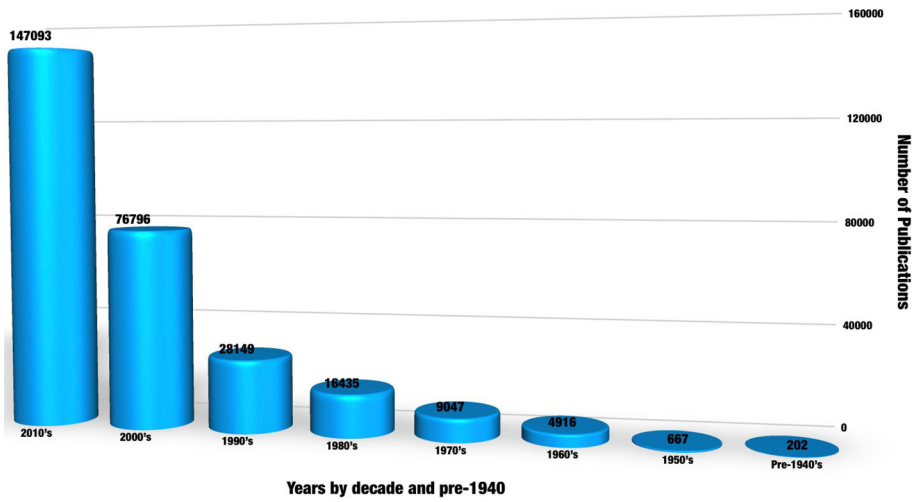

FIGURE 1 | The herbal substance analysis publications trend since search records began in 1800. A keyword search was conducted using the combination "medicinal plant" OR "herbal medicine" AND "analysis" chosen for the maximum retuned records after exploring a list of similar topic and combination of keywords such as "photochemical analysis," "traditional medicine," and "herbal." The Web of Science or collection, KCI- Korean Journal database, MEDLINE®, Russian Science Citation index, and SciELO Citation index databases were included in the search.

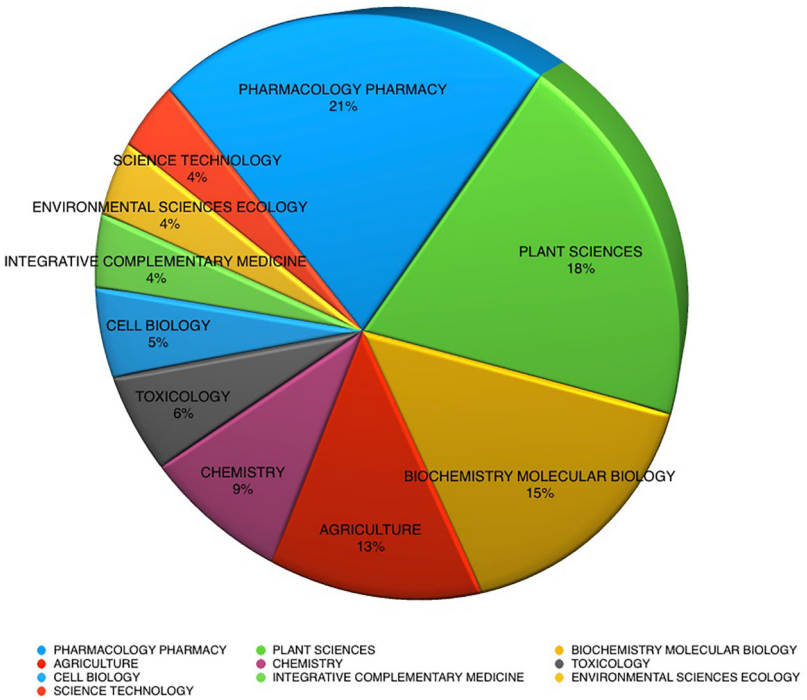

FIGURE 2 | Herbal substance analysis publications by discipline, 2008–2018 (169,917 records).

PHARMACOPOEIA MONOGRAPHS—THEIR INFLUENCES AND CHALLENGES

Though more recently the ChP is playing an increasing role in influencing medicinal plant analysis, the development of the ChP has been heavily influenced by Western pharmacopoeias. Historically the identification, preparation, and analysis of medicinal plants were based on classic texts such as the Shengnong Bencao Jing (Shengnong Materia Medica, 25–220 CE), where the category and quality of 365 plants and 113 prescriptions were assessed by taste. Organoleptic sensing of bitterness, sweetness, saltiness, and even neutral tastes were

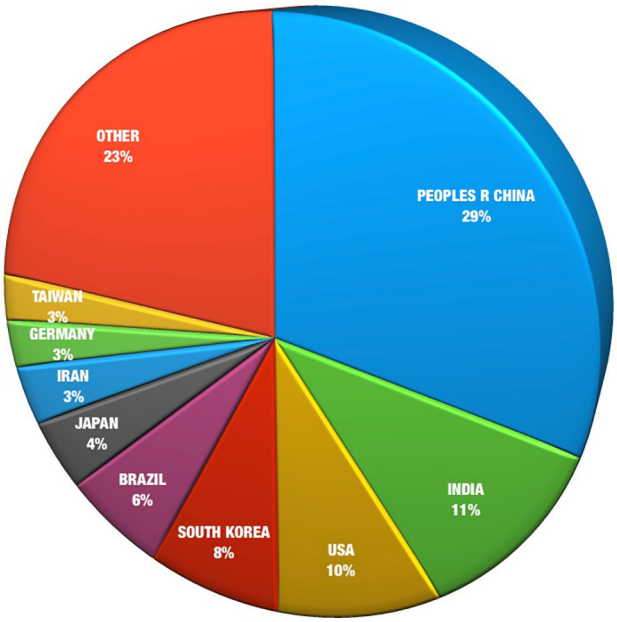

FIGURE 3 | Herbal analysis publications by region, 2008–2018.

thought to indicate the function and application of the medicine. Arguably, the most influential Chinese pharmacy monograph is the Bencao Gangmu (Compendium of Materia Medica, 1368–1644 CE) containing 1,892 plant descriptions and 11,096 prescriptions sorted in 16 divisions and 60 orders, emphasizing appearance, taste, and odor as a key to authentication and quality.

However, the main precursor to the modern format of the current Chinese Pharmacopoeia was printed in the 1930s with 670 drugs. Even at this early stage, the then dominant Western powers such as Britain, Germany, America, and Japan found challenges in understanding and forming consensus for recognizing, categorizing, and assuring the quality of Chinese medical materials. At this time a difficulty emerged in securing materials for the more Western styled "scientifically run" hospitals. Initially it was though that as Japan had adopted a translation of the German pharmacopoeia in 1886, the Chinese could follow suit using the British Pharmacopoeia, which in 1927 had been translated into Chinese as a joint effort by the London and British Chambers of Commerce. However, some differences in opinion between the four occupiers had to be first resolved.

Many of the technological demands necessary to produce and maintain the pharmacopoeial standards required by the Americans was beyond the ability and technological capability of the Chinese at that time. America had recently just printed a Chinese translation of its United States Pharmacopeia (10th edition) published in 1926. The strict American standards for aconite, digitalis, adrenalin, and insulin were purported to be managed by new or foreign trained pharmacists (Read, 1930). Preparations such as liniments found in the British and U.S. Pharmacopoeias were included in the Chinese version. Syrups such as those of codeine and glucose and tinctures of cannabis were from the British influence. Foreign residents in China found it difficult to ingest local food and stated an "extensive need for bowel remedies." Therefore, drugs of the time, albuminis, aspidium, and emetin, were included. Vaccines for diphtheria, tetanus, and smallpox were maintained through the instruction of the USP.

German chemists had already gained a reputation for the isolation of chemical compounds, many of which were used medicinally and were already included in the Japanese Pharmacopoeia such as oxalic acid, pyrogallic acid, and bromine. Therefore, the existing German-Japanese analytical methods were generally utilized for these areas, which comprised about 25% of the new Chinese Pharmacopoeia. Whereas more British and American derived analytical methods and preparations were included for vegetable- and animal-based materials.

Agreement over the correct translation and naming of chemical compounds also proved problematic, e.g. when attempting to resolve disagreement between German-Latin and Anglo-American descriptions such as "natrium chloratum" and "sodii chloridum." The shared Latin common language elements aided European and American common understanding; however, translation into Chinese was troublesome. A potentially easier route would have been to adopt the Japanese Pharmacopoeia names and descriptions, often possessing the same Asian (Hanzi) character as that in China, however, this was resisted due to the strong nationalistic sentiment at the time in mainland China (Read, 1930).

Though the Japanese favored direct foreign phonetic transliterated terms for drugs, about 60 original Chinese *materia medica* entries had persisted in the Japanese Pharmacopoeia including entries for camphor, ginger, aloes, cardamom, and star anise.

Difficulty in plant identification and common naming was not confined to Asia. During the early 1900s period of European and American political expansion, attempts were being made in Europe to catalogue multilingual terms for similar plants such as the publication of "the illustrated polyglot dictionary of plants names" in Latin, Arabic, Armenian, English, French, German, Italian, and Turkish languages (Bedevian, 1936), cataloguing 3,657 plants in eight languages.

CHRONOLOGY OF PHARMACOPOEIAL DEVELOPMENTS IN CHINA

1900–1949

Medicinal plant publications during the early 1900s, before the formation of the People's Republic of China in 1949, were greatly influenced by the previous "age of exploration." Many scientific societies were set up by explorers, their peers, and investors as forums to communicate knowledge and acknowledge ownership of findings and discoveries (Fyfe and Moxham, 2016). The rise in fashion of the "gentleman scholar" engaging in academic pursuits supported the occupation of writing. During this time, many publications focused on the identification and

classification of ethnic/indigenous medical plants, such as Aztec medicinal plants still in use in modern Mexico (Braubach, 1925; Heinrich et al., 2014), Algonquians from nowadays, Canada, (Speck, 1917), Micronesians (St John, 1948), Babylonians and Assyrians, (Jastrow, 1914), Native American Indian tribes (Castetter et al., 1935), Persia, (Garrison, 1933) and India, (Chopra, 1933). Publications in English describing the history and use of Chinese medicine in the context of Western orthodox also appeared (Chan, 1939).

Post-1949

Periods of advancements in TCM research after 1949 to the present day have been described as occurring in three defined phases lasting about 20 years each. The first was 1950–1970, springing from the rapid development of TCM in universities, research, and hospitals in China during this time. The second phase took place during 1980–2000s, where we see the construction of legal, economic, and scientific networks. The third phase, from 2000 to date, is defined by a focus on elucidating the scientific basis and scientific clinical practice of TCM using cross-disciplinary and global collaborations (Xu et al., 2013).

1950–1969

Political Context

This period immediately followed the formation of the People's Republic of China and saw a rise in nationalism and political introspection. International relationships cooled and a closer connection with the Soviet Union was officially forged with the Sino-Soviet Treaty of Friendship, Alliance, and Mutual Assistance in 1950.

Regulatory and Pharmacopoeial Developments

This period saw the launch of the first edition of the People's Republic of China Pharmacopoeia (ChP) in Chinese launched in 1953. It contains 531 monographs and mainly retains the information of the previous precursor published in the 1930s, compiled from foreign influences. It guided both identification and quantification of synthetic drugs and medicines together in one issue. Some crude herbal materials were listed, but not in analytical detail. Internationally post-World War II, good-will fostered a sense of cooperation and collaboration. This was also reflected by the World Health Organization's release of the international pharmacopoeia (Ph. Int) issued by the World Health Organization in 1951, produced in two volumes. It contained 344 monographs and 84 tests, with an aim to provide a harmonized international reference for pharmacopoeial methods. The first European Pharmacopoeia Ph. Eur. was produced in 1967, with a more European focus, but combining many common elements of the long-existing British Pharmacopoeia and the United States Pharmacopeia.

Medicinal Plant Research and Analytical Development

Research publication output during the 1950s was varied but the most cited publication trends concerned identification of plant species using electron microscopy (Watson, 1958), the use of plant tissue staining methods (Bergeron and Singer, 1958; Fernstrom, 1958), and use of plant extracts for colorimetric analysis (Holt and Withers, 1958; Lillie, 1958). Though originating in the 19th century, the analytical tradition of extraction, purification, and separation of chemical plant components, e.g., the alkaloids, became increasingly sophisticated during this period (Svoboda et al., 1959). Toxicity studies during this time were still basic, exposing mainly mice to plant extracts and using mortality rate counting and organ biopsy and cell station techniques, e.g., quercetin, podophyllotoxin, and podophyllin extract toxicity studies (Leiter et al., 1950) and induced liver lesions with Pyrrolizidine alkaloid extracts (Schoental, 1959).

Chemical screening of plants for their medicinal effects in various chemical and clinical trials is featured (Farnsworth, 1966) as did their use in derivatized forms for the treatment of nerve inflammation (Jancso et al., 1967) and in human metabolism studies (Pletscher, 1968). Studies into the use of medicinal plants for their potential use in cancer treatments were encouraged by the first isolation of paclitaxel from the pacific yew, *Taxus brevifolia* Nutt.

Older basic chromatographic techniques that had been already in use remained commonly used analytical techniques, e.g., paper chromatography applied to the analysis of common broom [*Cytisus scoparius* (L.) Link.] (Jaminet, 1959) and in medicinal plant quality control (Paris and Viejo, 1955). Separation of alkaloids e.g. in *Duboisia myoporoides* R. Br. (Hills and Rodwell, 1951) remained a common interest and the analysis of other important metabolites including scilliroside in red squill, *Drimia maritima.* (L.) Stearn (Dybing et al., 1954). An investigation of *Cannabis sativa* L. for its antibacterial activity was also conducted during this timeframe (Krejci, 1958).

Much of the medicinal plant research of this period concerned the extraction and isolation of single compounds from plants. Basic colorimetric tests, UV-visible and infrared spectroscopy, and paper chromatography had previously supported this type of analysis. Spectroscopic techniques such as UV-Vis spectrometry with chart recorders had been in use since the 1920s (Hardy, 1938). These were being increasingly used for quantitative applications, such as in the analysis of glucoside in walnuts and monitoring the chemical composition of plants in relation to seasonal variations (Daglish, 1950).

However, the 1950–1970s was a golden period for the development of analytical technology. A time when the techniques of mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, and gas chromatography (GC) techniques had come of age. Mass spectrometry, which had been invented in the late 1800s and used in a more analytical form during the 1910s, had now come into a relatively more advanced era. It was during the period 1950–1970 that the ion trap technique was developed, for which Dehmelt and Paul later received a Noble prize. The Purcell and Bloch groups at Harvard and Stanford University, respectively, developed NMR techniques and in 1952 also received a Nobel Prize (in Physics). In 1952, Archer John Porter Martin and Richard Synge also shared a Nobel Prize (in chemistry) for inventing partition chromatography, the basis of modern GC. Gas–liquid separations solved the problem of separating sugar-based

molecules, which tended to bond with traditional stationery phases such as silica and volatile compounds, such as volatile oils, which are lost through evaporation during collection, preparation, and analysis. GC was applied for the first time to resolve 17 difficult to separate plant glycosides from a broad range of chemical classes, including phenolic, coumarin, isocoumarin, isoflavone, anthraquinone, cyanogenic, isothiocyanate, and monoterpene (Furuya, 1965), 15 kinds of valerian sesquiterpenoids in valerianaceous plant oils (Furuya and Kojima, 1967), and the extraction and analysis of rose oil (Minkov and Trandafilov, 1969).

Publications included well-applied examples where visible, ultra-violet (UV), and infrared (IR) spectral data were combined to elucidate structural characteristics of plants while undergoing chemical degradation, e.g., the stereochemical discrimination of lignin components paulownin and isopaulownin from *Paulownia tomentosa* Steud. (Takahashi and Nakagawa, 1966), the alkaloids of the Orchidaceae (Lüning et al., 1967), and terpenoids of *Zanthoxylum rhetsa* DC (Mathur et al., 1967).

MS was also used side-by-side with NMR, resulting in the structural elucidation of key metabolites, e.g., the characterization of the opium papaverrubine alkaloids and their *N*-methyl derivatives in the genus *Papaver* (Brochmann-Hanssen et al., 1968), the analysis of three new coumestan derivatives from the root of licorice, *Glycyrrhiza* spp., (Shibata and Saitoh, 1968), and the isolation and purification of polyprenols from the leaves of *Aesculus hippocastanum* L. (horse chestnut) (Wellburn et al., 1967).

Up to this time, China had played a very marginal role in international research and development activities, a situation that was to change significantly in the following period.

1970–1989

Political Context

1971 saw China's introspection from the Mao era revert to more external international engagement with the "People's Republic of China" (PRC) elected as a permanent member of the United Nations' General Assembly. This followed the American government's extension of political relations with PRC after the Richard Nixon presidential visit that catalyzed an "Opening up to the West" phase in Chinese history. This opening began in 1978, orchestrated by the interim leader Deng Xiaoping, who initiated support for wide sweeping economic reforms. On a local level this manifested as individuals within China being allowed to make personal economic decisions, with the tightly governed communes being dissolved. Rural markets were replaced by open markets, resulting in a dramatic increase in international trade, supporting Xiaoping's wish to fund economic growth from foreign investment. In the context of medicine, China's ambition to look outward was highlighted over a decade earlier by a University College London anatomy Professor, Derrick James, when a British delegation visited China in 1954 and in his subsequent Lancet article outlined China's intention to introduce a more scientific, modernized TCM (James, 1955).

As international trade from China expanded, so did the trade in medicinal plants from Asia and with it, increased access for Chinese scientists to modern analytical instrumentation. Internally by the mid-1980s, 25 Chinese medicine colleges were formed in a reportedly scientific and modern style with an almost 30-fold increase of TCM hospital beds to 2.5 million since the formation of the state in 1949 (Cai, 1988).

Regulatory and Pharmacopoeial Developments

The establishment in 1985 of the China State Administration of Traditional Chinese Medicine began the formal organization of TCM research and development nationally and internationally, sowing the seeds for the formal cooperative global links that would provide the backbone for the future of international Chinese medicinal plant research. China's motivation to secure international links was also manifest in the publication of the PRC's first dual Chinese and English language Pharmacopoeia, ChP, 4th edition in 1997, which began its new 5-year publication cycle trend.

Medicinal Plant Research and Analytical Developments

The newly fostered R&D investment and cooperation during this period globally is represented by the leap in sophistication and complexity of the research published, with a shift from basic to more advanced biochemical investigations and more emphasis focused on disease and diagnosis strategies such as in cancer and infectious disease. The most widely cited articles of this time include advanced biomedical research on Forskolin, from the roots of *Plectranthus barbatus* Andrews as a diterpene activator in nucleotide metabolism. Even though basic biochemical equipment and colorimetric methods and spectrometric enzymatic assays were used, a more complex understanding of plant metabolites is apparent (Seamon et al., 1981).

This is also evident in the investigation of lectins as cell recognition molecules and their involvement in a wide range of molecular processes and potential pathologies, e.g., in metabolic regulation, viral, and bacterial infection processes (Sharon and Lis, 1989). In addition to plants playing a role as phytochelants in complexing heavy metals (Grill et al., 1985 and Grill et al., 1987), licorice was studied in greater depth using a conceptually new approach of assessing the mineral-corticoid activity of licorice and its role in sodium retention (Stewart et al., 1987) and the radical scavenging properties of its flavonoids (Hatano et al., 1988).

Awareness of plants having a role in cancer with both causative and curative effects emerged, with a highly cited review of potential causes of esophageal cancer in China. Particular concerns were linked to effects of fungal growth and associated nitrosamines due to poor storage conditions (Mingxin et al., 1980). This was a precursor to later studies on aflatoxins, which are now acknowledged as causing serious health problem linked to poor storage and processing. From a therapeutic perspective, the interest in antileukemia and anti-tumor agents, e.g., in *Taxus brevifolia* Nutt. stem bark, first investigated some decades before, continued and ultimately resulted in the introduction of a completely new therapeutic approach (Wani et al., 1971).

One of the landmark discoveries in medicinal plant history was reported to the west during this period. The antimalaria effect of artemisinin, derived from *Artemisia annua* L., for which the Chinese scientist Youyou Tu later received a Nobel Prize in Medicine (Klayman, 1985), described a conceptual shift in the approach to treating malaria, illustrating both a change in approach from using quinoline-based drugs, which parasites were showing increasing resistance to, and paving the way for the development of new classes of drugs e.g. with potential in antiviral and anticancer treatment (Su and Miller, 2015).

1990–2008

Political Context

This period in China was characterized largely by economic, political, and academic success delivering on the earlier aspirations of Deng Xiaoping through focused planning and the tight administrative grip of three successive presidents (Chairpersons) and state administration. An unusually high-performing economy producing more than a 10% sustained gross domestic profit (GDP) created a stable base for China to successfully join the world trade organization in 2001, marking its arrival on the world stage as a competent economic power and its transition to a market economy (Morrison, 2013). This, however, came with challenges to families and the environment.

On a local level as communes of the last decades had dissolved, a system of "household responsibility" was adapted as a kind of contract that guaranteed agricultural family holdings to provide a certain level of food (and herb) output (Ash, 1988). This ensured that levels of agricultural production were optimized for the land available. Because families were now allowed to sell grown products in an open market that mirrored the economic national trend, food and medicinal herbs began to take on more distinct financial attributes. This combined with mass migration of rural workers to rapidly developing industrialized cities away from countryside homes without sufficient locally produced food in urban surrounds created a situation of widespread supply and demand, leading to new value chains for food and medicinal plant products, along with potential motivation for the substitution or adulteration of these products.

Regulatory and Pharmacopoeial Developments

As industrialization occurred so too did environmental pollution, with increased volume and concentration of raw materials and waste presenting greater potential for pollution of medicinal plant material. The PRC at this stage had gone through a period of prolonged political stability. Economic policy became more flexible and governance developed an increasingly regulatory role compared with that of previous, more rigid enforcement. Regulation and safety testing of medical products saw further guidance through the production of four further volumes of the ChP in both Chinese and English culminating in the 8th edition in 2005, listing 3,217 monographs, almost double that of the 1990 edition. This period saw China's confidence increase and extend to regulatory and guidance aspects, with the ChP undergoing the greatest leap in analytical sophistication and rate of change to date. The 1990 edition was a significant step in the acceptance and introduction of modern instrumental analytical techniques for standard herbal substance testing. Since the 1985 edition, specific identification tests were introduced using mainly thin layer chromatography (TLC). Now chromatogram images of the crude and test samples were included and required for testing. Basic identification was expanded to require quantitation where high-performance liquid chromatography (HPLC) and GC were now included for the first time and TLC extended for content analysis. More instrumental techniques replaced older ones such as the introduction of spectrophotometric determination of the alkaloid content of berberine, which had been gravimetrically analyzed in previous editions. Quantification moved from measuring simpler marker components to more specific active compounds like anthroquinone from He Shou Wu, *Polygonum multiflorum* Thunb [now *Reynoutria multiflora* (Thunb.) Moldenke]. The 2000 edition introduced assays for residues of organic chlorine pesticides for Gan Cao, *Glycyrrhiza uralensis* Fisch. ex DC. and Huang Qi, *Astragalus membranaceus* Fisch. ex Bunge (Kwee, 2002). Another leap occurred in the 2005 edition with an expansion of the acceptance of HPLC-MS, LC-MS-MS, and DNA molecular markers and chemical fingerprinting, setting the stage for 21st century pharmacopoeial trends and the ChP as a central global influence for the analysis of medicinal plants.

Medicinal Plant Research and Analytical Developments

The fruition of investment in external academic relations from the "opening up" phase and internal support for the now formed TCM structures of the previous decades state initiatives were borne out by the publication output in this period, with a six-fold increase in output compared with that of the previous equivalent 20-year period. Much of the output from this time demonstrated a refinement of thought around the effect of plant compounds on humans as a holistic system rather than the more singular metabolic pathway thinking of previous years. It also shows a tremendous emphasis on obtaining large datasets especially of the known metabolites and a wide exploration of acclaimed effects. Whole plant extracts and combinations of metabolites rather than single ones became a core theme, as became a medicinal plant's effect on longer term health and preventative medicine. This ignited a resurgence of interest in the analysis of medicinal plants as a source of lead compounds for drug discovery.

The role of medical plants in coronary disease analysis becomes topical during this phase, e.g., long-term studies on elderly demonstrating the reduced risk of death from sustained flavonoids intake *via* inhibition of the oxidation of low-density lipoprotein (Hertog et al., 1993). More sophisticated quantitative analysis and differentiation appeared during this time such as HPLC of mulberry leaves containing four varieties of flavonoids (including rutin and quercetin), and their antioxidant properties (Zhishen et al., 1999). Flavonoid coronary disease risk prevention and cancer roles were advanced by the characterization and analysis studied in a wide range of fruits, seeds, oils, wines, and tea (Middleton et al., 2000). A greater

awareness of the potency and efficacy of drugs and medicinal plants became evident as in the studies and analysis of the effect of fluorine on drug binding and potency (Purser et al., 2008). Cancer research also demonstrated further advances through combining previous findings on receptor binding with advancements in DNA extraction, amplification techniques, and cloning techniques. Resveratrol became a key area of interest for its chemoprotective effects (Jang et al., 1997).

Many of the most cited publications of these two decades were detailed reviews, which brought together the findings of previous research on individual plant research.

21st Century

China's growing influence was marked in 2011 with the Chinese State Administration of TCM (SATCM) forming an official relationship with the European Directive on the Quality of Medicines (EDQM) to share expertise and knowledge in addition to raising the standards of testing in China and Europe through cooperation. These include translation of historical TCM documents, information relating to preparation of products, process, and sourcing. Europe, seen as an aggregate, has an approximately 16% representation in the last decades' research output, higher than the USA. The European Pharmacopoeia (Ph Eur) manages CHM's by allowing importation of CHM's to countries who have signed up to the European Pharmacopoeia convention. Currently there are 43 CHMs included in the Ph Eur, 8th edition, 34 from the Ph Eur TCM Working Party, 21 of which have been included as full monographs (Wang and Franz, 2015). New Ph Eur CHM monographs are being developed based, in part, on the ChP. This was facilitated by a working party on TCM (Ph Eur WP) and was officially introduced in 2005. It included 38 member states with a delegation from the EU (a representative from DG Health & Food Safety and the European Medicines Agency). Additional observers are composed of 27 countries/regions/organizations [which include 7 European countries, the Taiwan Food and Drug Administration (TFDA), and World Health Organization (WHO)] (EDQM, 2017). The WHO, through participation in the PhEur, additionally has led efforts to develop a harmonized international pharmacopoeia (WHO, 2018).

The monographs for medicinal plants in Ph Eur have developed from standard western drug monographs with an emphasis on chemical and physical testing, while those in the ChP have formed from revisions of older traditional texts.

As pharmacopoeial monographs expand and develop, so too does the range and complexity of analytical methods and analytical hardware needed to meet the regulatory demands and expectations of quality.

These emerging research trends and pharmacopoeial directives have paved the way for the development of a broad range of analytical techniques, mainly centering around the use of liquid chromatography (LC), GC, MS, and established UV/visible spectrophotometric techniques.

We present a selection of these analytical techniques and give examples of their applications in the analysis of medicinal plants and medicinal plant products.

Analytical Hardware, Attested and Emerging Methods

High-Performance Liquid Chromatography

HPLC is one of the most developed and widely used analytical techniques. It is built on a historical knowledge base amassed from TLC and optical chemistry experience. HPLC chromatography elements rely on similar principles of TLC/HPTLC, where separation of components is dependent on selective affinities to stationary supports and liquid phases.

Detection employs a photomultiplier system able to detect individual wavelengths of light, a range (spectrum) and/or multiple simultaneous wavelengths in its different iterations, combined in an enclosed automated instrument system with sample injectors; this has significantly increased the precision and reproducibility of the chromatography when compared with older chromatographic methods. The widespread use of HPLC has made it more affordable for laboratories. High operator skill level is not required; it is robust and sensitive to low level detection and is particularly used for the quantification of components (active substances and adulterants).

HPLC applied to herbal products is well developed, and it has been successfully applied to the analysis of complex mixtures of similar compounds, both for the separation of individual compounds and for the differentiation of medicinal plant species. The high resolution of the technique has supported the development of the concept of a characteristic "fingerprint" developed for medicinal plants and herbal products to aid identification and authentication, e.g., Li et al. (2010) demonstrated differentiation of the same type of medicinal plant product from 40 different manufacturers, while simultaneously separating nine marker chemical compounds (berberine, aloe-emodin, rhein, emodin, chryso- phanol, baicalin, baicalein, wogonoside, and wogonin).

High-Performance Thin Layer Chromatography

HPTLC has become a common addition to the method section of new monographs, replacing the widely used TLC tests; it has shown to be a reliable and reproducible method of analysis that provides essential information regarding the compositional quality of an herbal substance.

Some advantages of this technique include low cost and a relatively simple test method. It does not require advanced sample preparation methods or high levels of expertise. Sample amounts are relatively small, and it is a more sensitive technique compared with HPLC, well suited to detecting contaminants. However, some disadvantages are that the reproducibility is dependent on a variety of external factors, and although more sensitive than HPLC, it is not able to sufficiently detect compounds at very low concentrations (PPB) where LC-MS (or HPTLC-MS) may be more suitable. HPTLC relies on the same principle as TLC and uses similar TLC plates and mobile phases, although relatively small amounts of solvents are required compared with standard TLC. The process of adding the sample to plates (spotting) has been made more reproducible and precise by spraying the sample onto the plate to form a band of compound rather than a spot. Retention factors for individual

compounds are more reproducible due to controlled humidity during development. Derivatizing the analysis plates is completed mainly by machine and the visualization is captured by modern camera systems connected to powerful software. The software allows further manipulation of images to optimize visualization in a way that would be very difficult chemically. Another advantage is that the HPTLC system can be easily linked to a scanning densitometer; this not only allows for more precise quantitative work to be carried out but also the data can be exported for multivariate analysis. It is likely that more of the monographs with TLC requirements will be upgraded to HPTLC in the future.

Gas Chromatography

GC in respect to medicinal plant analysis is mainly used for the analysis of compounds with higher volatility, e.g., compounds found within essential oils, and more volatile adulterants, e.g., pesticides. While single GC column chromatography and its hyphenated derivatives have been use for many years, 1991 saw the introduction of 2D-GC or GC x GC, where the eluents of a standard separation are trapped and recirculated for another round of separation. This allows not only greater resolution and better separation but also the ability to purge undesired or interfering compounds so that more specific areas of the separation can be targeted (Liu and Philips, 1991). This led the way for multidimensional gas chromatography (MDGC) and the advances of the modules and valve systems that trap, control, and divert sample streams. These improvements extend to the thermal control and valve systems allowing greater thermal flow and split streaming (Bahaghighat et al., 2019). One key problem with GC is the introduction of sample into a gas stream. Historically squeezing, boiling, and later distillation of herbal materials were used for the collection and production of volatile compounds such as oils. However, the inherent instability of volatile components and losses as well as the poor recovery of these substances presented difficulties. This situation has somewhat been overcome by advances in extraction techniques such a solvent-free microwave extraction, e.g., for citrus peel oils [Citrus sinensis (L.) Osbeck]. No solvents or water are necessary for high recoveries with this method, and it allows for highly efficient, compatible sample introduction without the need for interfering solvents (Aboudaou et al., 2018). This sample extraction method commonly known as headspace analysis for GC has undergone many iterations (Gerhardt et al., 2018). It has now developed to the stage where it is increasingly used for bacterial and microorganism detection such as in *Commiphora* species (Rubegeta et al., 2018).

Microextraction techniques are essential for the introduction of small sample volumes into the GC gas stream. Needle-based extraction techniques have the advantage of automation, ease of interface to other instruments, and compatibility with miniaturization. Advances in solid phase dynamic extraction (SPDE), In-tube extraction (ITEX), and needle trap extraction (NTE) have refined the use of these techniques for natural and herbal compounds (Kędziora-Koch and Wasiak, 2018), e.g., SPDE and ITEX for pesticide residues in dried herbs (Rutkowska et al., 2018), herbal mint aromas compounds in commercial wine (Picard et al., 2018), and volatiles in Chinese herbal formula Baizhu Shaoyao San (Xu et al., 2018).

Supercritical Fluid Chromatography

Another liquid-based chromatographic technique based on pressurized low viscosity (supercritical) fluids, often carbon dioxide, is supercritical fluid chromatography (SFC). Since its introduction by Klesper in 1962, it has made large advances mainly due to improvements in its initially troublesome instrumentation (Desfontaine et al., 2015). Its main advantage over other techniques is in its usefulness for separating complex components characteristic of natural compounds. Selection of the correct conditions of SFC mobiles phases and modifiers can be finely tuned across a wide range of polarities from non-polar to polar allowing a broad selection of separations (Gao et al., 2010). Early analysis of natural products with SFC was when it was first hyphenated with gas chromatography (King, 1990). Recently, it has been more fully developed to analyze a range of natural compounds in herbal substances, notably, focusing on terpenes, phenolics, flavonoids, alkaloids, and saponins. This has been achieved with hyphenation to MS, diode array detectors, SFC-ELSD, in addition to the development of novel stationary phases such as cyanopropyl, pentaflouro phenyl (PFP), and imidazolyl. An example of this is with the separation of coumarins in *Angelica dahurica* (Hoffm.) Benth. & Hook.f. ex Franch. & Sav. roots and anthraquinones in rhubarb root (Pfeifer et al., 2016).

Near-Infrared Spectroscopy

Although commonly used within industry since the 1990's, near-infrared (NIR) spectroscopy was not the method of choice for medicinal plant analysis mainly due to overlapping peaks making interpretation of data problematic, and consequently, it never became the instrumentation of choice within the quality control laboratory in the same way that HPLC and TLC developed. However, with the addition of new computational software, NIR is re-emerging as an affordable and useful analytical technique used in the analysis of medicinal plants and has been particularly favored by Chinese companies in routine quality control analysis due to its ability to both rapidly differentiate between species and provide quantitative information on metabolite content (Li et al., 2013; Zhang and Su, 2014).

As with HPTLC and NMR data, NIR also provides an opportunity for multivariate analysis and it appears capable of resolving very small variations in metabolite content. It is argued that more traditional TLC or HPLC techniques can be more subjective in the data interpretation stage and require a high degree of operator skill and that NIR is more suitable for high volume analysis in the routine quality control laboratory (Wang and Yu, 2015). However, this has partly been addressed by the introduction of the fully automated systems available for HPTLC analysis and the inclusion of scanning densitometry equipment that reduce the need for operator interpretation. The main advantages of NIR appear to be the preservation of sample integrity, little sample preparation needed, and no need for solvents, and it has shown to perform well comparable to HPLC

for species differentiation and quantification of metabolites (Chan et al., 2007). Probably the main drawback in NIR compared with other methods, and especially, TLC, HPTC, LC-MS is in its sensitivity and some reports suggest that this technique may only be suitable for detecting compounds that exist at a concentration above 0.1% (Lau et al., 2009). Another consideration is that variation in NIR data is dependent both on the chemical and physical properties of the sample, with the physical properties, e.g., particle size, having greater effects on the variation than the chemical. Therefore, before multivariate analysis can take place some pre-treatment of the spectral data is necessary, e.g., to reduce baseline noise, light scattering, and consequently enhance any chemical variation in the sample set (Chen et al., 2008). Some advantages of NIR certainly are apparent, although it may not be appropriate for all situations and all types of samples. The technology has made a huge leap forward since its first introduction and now it needs to establish itself more widely as a useful tool in the quality analysis of medicinal plants.

Hyphenated Techniques

Combinations of techniques with modern developments in metabolomic analysis and computational pattern recognition programs open up a wider scope of applications to medicinal plant analysis. Tandem combinations of analytical instrumentation such as MS with HPLC has proved a productive route to expanding analytical medicinal plant applications. Not only in identification and fingerprinting but further chemical characterization of individual compounds e.g., Liu et al. (2011), characterized a spectrum of alkaloid components in the Chinese herb Ku Shen (*Sophora flavescens* Aiton). Further combinations and permutations of MS and NMR in combination with HPTLC have been demonstrated, such as the detection of acetylcholinesterase inhibitors in galbanum in a search for natural product drug candidates (Hamid-Reza et al., 2013), and mass spectroscopy (MS) HPTLC-MS shown for *Ilex vomitoria* Aiton with the use of a sampling probe following HPTLC combined with MS with Electrospray Ion Trap (Ford and Van Berkel., 2004) and *Hydrastis canadensis* L., with HPLTLC-MS atmospheric pressure chemical ionization (Van Berkel et al., 2007).

Analytical combinations including ESI-IT-TOF/MS-HPLC-DAD-ESI-MS have been demonstrated for the analysis of coumarin patterns in Angelica polymorpha Maxim. roots (Liu et al, 2011) and multihyphenated techniques such as SPE-LC-MS/MS-ABI quadrupole trap have been used for the analysis of six major flavones in *Scutellaria baicalensis* Georgi (Fong et al., 2014) and 38 saponins in the roots *of Helleborus niger* L. by LC-ESI-IT-MS (Duckstein et al., 2014).

Merging the separation ability of HPTLC or HPLC with the analysis power of NMR and MS has significant benefits for analyzing complex samples in complex matrices such a blood, soil, and plants. However, each technique also possesses its inherent disadvantages. MS being complex, expensive, and time-consuming, requiring high analytical skill levels, it may not be suitable for a general quality assurance laboratory. Though powerful, extensive method development and post analysis data processing is required when applied to natural compounds with broad complex compositions in contrast to simpler synthesized pharmaceutical ingredients. Similarly, NMR is also expensive and sensitive to variations in sample preparation and composition. It is not fully applicable to all natural compound samples and signals generated from NMR analysis often overlap making data analysis for individual compounds problematic. However, the relative speed, rich information output, and insight into the overall composition of medicinal plants from both MS and NMR far outweigh the disadvantages. These techniques allow the detection of compounds into the parts per billion analytical range (MS) and allow a detailed fingerprint of metabolites across differing polarities (NMR) and so for research and for larger companies they are highly applicable analytical hardware.

METABOLOMICS

Pharmacopoeial methods focus on authentication and quality of herbal materials; however, metabolomics allow us to go a step beyond authentication and look in more detail at a broad range of secondary metabolites. By coupling analytical data to multivariate software, this allows us to develop statistical models to firstly differentiate between species but also to get a better idea of a typical metabolite composition for a particular species. The advantage of this is that it can help to inform any laboratory test or clinical intervention. There has been great emphasis on making sure that any experiment or intervention uses plant material that is authenticated, with a herbarium specimen deposited. However, the requirements do not stipulate that a good representative of the species should be used. This is where metabolomics can provide essential information—by collecting a wide range of samples from different geographical locations, altitudes, growing conditions, it allows us to map their metabolite differences and highlight how diverse or how similar metabolite composition is. When an experiment is performed, we have the choice to use a specimen that may be typical, i.e., contains an average composition or we can look at compositions that are atypical, containing greater amounts of specific metabolites or even different metabolites. Moreover, if a particular experiment produces positive results and we want to reproduce the data, a metabolomic model allows us to choose species that have a similar composition.

This approach has important economic implications as a detailed understanding of metabolomic analysis allows us to inform industry as to how to grow plants that will be of the best composition and so help to support local livelihoods of farmers and primary processors in developing economies, e.g., Chachacoma (*Senecio nutans Sch. Bip.*) cultivation in the high altitude regions of Chile where metabolomics has helped to establish the best altitude for growing plants with the highest content of the anti-inflammatory acetophenone (Lopez et al., 2015).

This strategy also has applications in product development, where metabolomics can help to determine the quality of products based on their metabolite content, e.g., *Curcuma longa* L. (Turmeric products) (Booker et al., 2014), and also

help to provide evidence that can lead to value addition of a product and greater confidence in its quality and safety.

NANOPARTICLES

Nanoparticles 1–100 nm sized ions or organic/inorganic molecules have proven to be important in the development of new analytical testing (Tao et al., 2018), occupying the analytical regions of space between the ionic dimensions and small molecules.

Recent developments in nanoparticle research has led to an increased focus on chemo-bio sensing, as DNA has become the most used biological molecule to functionalize nanoparticles. Nanoparticles have provided many advantages to more consistent and specific testing including providing a more reproducible stable matrix for research and development, more controllable and reliable basis for designing and conjugating to functional molecules, and a wide rebate of flexibility for purification, selection, and modification of analytes. Nanoparticles have been used in creating a biological bar code for trace analysis of mycotoxins in Chinese herbs e.g. conjugated nanoparticles with DNA fragments to bind and target Chinese medicinal plants, e.g., Jue Ming Zi [Cassia seeds—*Senna obtusifolia* (L.) H.S.Irwin & Barneby], Yuan Zhi (*Polygala tenuifolia* Willd.), and Bai Zi Ren [*Platycladus orientalis* (L.) Franco] (Yu et al., 2018).

THE FUTURE

The next steps in analytical advancement in combination with technological improvements will most likely occur in the realm of artificial intelligence. Neural networks have already shown promise in consumer electronics and online search engine optimization. Self-learning algorithms have been in development for decades, with great potential for the application of self-synthesizing, auto-creating, and auto-adapting algorithms, which can optimally recognize and synthesize analytical data into meaningful and useful patterns. This goes beyond what a single human mind could hope to achieve in lifetimes, now possible in seconds with current and more so with future technology. This extends not only the human potential of thinking and observation but also prediction and design. This could potentially play a role in self-design of analytical instrumentation and its modules, self-optimizing of methods in real-time, saving time that would perhaps take an analyst weeks or months of human work-hours to complete.

The greatest challenge with AI is its opacity and computational complexity. With self-learning systems already self-generating codes and pathways that would take decades for a single human to decode and understand, if ever possible. This presents a great challenge for use in reproducible, validated quality-driven, audit-trailed regulated orientated environments. This is where natural compounds such as herbal substances can play a significant role i.e. data from the same plants species with variable composition can help verify the input and outputs of complex analysis and recognition software. In AI-driven systems, natural substances are ideal candidates for testing the analytical attributes such as accuracy, precision, and robustness of whole AI-instrumentation systems.

CONCLUSIONS

As pharmacopoeial requirements continue to develop and instrumental technology advances, it is clear that we will be able to delve further and further into the chemical composition of medicinal plants and develop more advanced techniques for the detection and quantification of adulterants and contaminants. However, it should be considered that although these technological advances give us this opportunity, more traditional organoleptic analysis also provides us with essential sensory information regarding medicinal plant quality.

We have shown the emergence and historical importance of complex analytical techniques used in medicinal plant analysis. However, any analytical approach, can only provide a partial perspective on complex multicomponent preparations. So future improvements in this area may not entirely rely on developing ever more complex analytical techniques, but in implementing best practice throughout all stages of the production and supply of herbal medicines.

AUTHOR CONTRIBUTIONS

AB wrote the sections on applications of metabolomics, NIR, parts of the introduction, and conclusions. MF wrote most of the instrumentation, trends in publications and history, part of the introduction and conclusions. MH contributed towards the methodological design of the study and assisted with the data analysis.

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frontiers in Pharmacology

REVIEW
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Traditional and Current Food Use of Wild Plants Listed in the Russian Pharmacopoeia

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Historically Russia can be regarded as a "herbophilious" society. For centuries the multinational population of Russia has used plants in daily diet and for self-medication. The specificity of dietary uptake of medicinal plants (especially those in the unique and highly developed Russian herbal medical tradition) has remained mostly unknown in other regions. Based on 11th edition of the State Pharmacopoeia of the USSR, we selected 70 wild plant species which have been used in food by local Russian populations. Empirical searches were conducted via the Russian-wide applied online database E-library.ru, library catalogs of public libraries in St-Petersburg, the databases Scopus, Web of Science, PubMed, and search engine Google Scholar. The large majority of species included in Russian Pharmacopoeia are used as food by local population, however, aerial parts are more widely used for food. In this review, we summarize data on medicinal species published in Russia and other countries that are included in the Russian Pharmacopoeia and have being used in food for a long time. Consequently, the Russian Pharmacopoeia is an important source of information on plant species used traditionally at the interface of food and medicine. At the same time, there are the so-called "functional foods", which denotes foods that not only serves to provide nutrition but also can be a source for prevention and cure of various diseases. This review highlights the potential of wild species of Russia monographed in its pharmacopeia for further developing new functional foods and—through the lens of their incorporation into the pharmacopeia—showcases the species' importance in Russia.

Keywords: medicinal plants, traditional use, functional food, health benefits, Russian Federation

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INTRODUCTION

Until today over 7000 species have been recorded to be edible (Lim, 2012) and for many of these specific health benefits have been claimed, with, however, generally only limited pharmacological evidence for such claims. Only a small share of these is used on a large, extensive scale. Wild and cultivated food plants may have medicinal, nutraceutical, pharmaceutical, and health benefits. Plants are excellent sources of active phytochemicals with importance in the prevention of different diseases. Herbal medicinal products are going recognition for the treatment and prophylaxis of different diseases. Direct nutritional benefits of such plants are often known

only to a limited extend. Herbal infusions will add small quantities of essential micronutrients and vitamins to the daily intake. For sick or malnourished individuals this may contribute to restoring the balance between nutrients and thereby improve or sustain health (Lim, 2012).

Blurring of the food and medicine interface is a common theme across multiple contexts and cultures (Heinrich, 2016). The association between food and disease is widely recognized as the bedrock of preventive nutrition. Throughout the ages, and at least since the proclamation "let food be your medicine, let medicine be your food" which has been attributed to Hippocrates' (ca. 480–377 BC), physicians recognized the impact of food on human health. The question of the continuum between food and medicine is of great interest to (ethno-)pharmacologists (Etkin and Ross, 1982; Leonti, 2012; Valussi and Scire, 2012; Jennings et al., 2014; Alarcón et al., 2015).

In many countries medicinal plants are widely used as dietary supplements, in daily foods and as functional foods, with the aim of promoting health. In Eastern cultures food and medicine are seen to come from the same source. Their use is based on the same fundamental theories, and they are equally important in maintaining and improving health and for preventing and curing diseases, as well as facilitating rehabilitation. In China, Japan, Korea, and Southeast Asia, medicinal plants are widely used both daily foods (cereals, vegetables, and fruits) and functional foods, for replacement and medical purposes (Shi et al., 2011). The last few decades has seen the introduction to Europe of a wide range of species used in other continents, including from South Africa e.g. rooibos (*Aspalathus linearis* (Burm.f.) R.Dahlgren) and to a lesser extent honey bush tea (*Cyclopia* sp.) or *Hoodia gordonii* (Masson) Sweet ex Decne. to regulate the appetite. Also some exotic fruits, such as acerola (*Malpighia glabra* L.) from South America, rich in vitamin C are now marketed as food supplements (Franz et al., 2011).

In the early history of Western biomedicine, diet figured prominently in the prevention of diseases and therapeutics. The history of biomedicine customarily is traced to Greece and the Hyppocratic Corpus, some 60 medical texts dating to the fifth to fourth centuries BCE. Diet regulation was the most prominent element in all the Hippocratic texts (Etkin, 2006). Due to its location between East and West Russian tradition of medicine and diet has accumulated and adopted approaches that originated in Europe and Asia. The Greek influence on Russian medicine is of particular relevance in this context (Shikov et al., 2014a).

Russia in the past and present can be regarded as a "herbophilious" society. The term "herbophilia" was used by Łuczaj (2008) for such cultures, in which medicinal and food plant species are often used and highly prized. Approximately 58% of the Russian population was reported to use phytopharmaceuticals as a form of treatment (Shikov et al., 2011) including wild plants. Plants are an integral part of Russian everyday life. A large number of species of green vegetables are used in agricultural communities, particularly those in which food shortages are frequent. Due to the harsh climatic conditions coupled with poor crop harvests Russian peasants were educated on how to survive by relying on wild plants (Kovaleva, 1972).

Wild food plants became highest priority in the USSR during the Second World War most notably during the siege of Leningrad (1941–1944). In 1942 a special manual about most important wild food plants of the Leningrad region was published and distributed to the army and civilians. The scientists from V.L. Komarov Botanical Institute described thirty seven common herbs and two edible lichens, their nutritional and functional properties, specificity of harvesting, and food processing (Tikhomirov, 1942).

As a result of socio-economic changes after the Second World War, the active role of wild plants gradually diminished in regular diet. However, some species are still gathered and made into food products by small companies. Wild berry harvesting is very popular especial among of rural populations. The practice of preparing jam, compotes, and home made liqueurs from wild berries has been passed down by housewives from generation to generation (Grigorieva, 2008).

A wide choice of herbal medicines or botanicals has provided increasing opportunities for both the development and marketing of herbal food products, dietary supplements, and functional foods.

Wild species were selected for food application by humans not only because of their pleasant taste and aroma, but some pharmacological effects were considered as well (Gammerman and Grom, 1976). Herbal medicine products have become popular over the past decade and they are widely used for the treatment and prevention of various diseases. However, safety and efficacy of herbal medicine should be confirmed. Utilization of knowledge about positive pharmacological effects of edible plants included in in a pharmacopeia is one of probably safe and effective way for development of functional products with new beneficial effects (Kunakova et al., 2011).

Searching for new food supplements is essential. Systematic explorations of pharmacopoeial plants are one particularly relevant opportunity in Eastern Europe, especially in those areas which, for historical reasons, remain relatively isolated. The use and importance of Russian medicinal plants remains largely unknown in the West. The aim of this review is to summarize data about species that are referenced in the Russian Pharmacopoeia and about their health food value with a primary focus on literature published in Russian.

OVERVIEW OF SPECIES FROM THE RUSSIAN PHARMACOPOEIA USED AS FOODS

For centuries the multinational population of Russia has used plants in daily diet and for self-medication. The beneficial effects of plants were carefully collected by "knowledgeable experts" (znahar = "знахарь"in Russian) and recorded in chronicles and manuscripts. In 1778, Russia was among the first countries to implement a national pharmacopeia: The Pharmacopoeia Rossica in St. Petersburg. This work contains 770 monographs, of which 316 texts are on herbal medicinal preparations (Shikov et al., 2014a).

Since 1990, the Russian Federation has followed the State Pharmacopoeia of the USSR (1990) 11th edition. This Pharmacopeia includes 83 individual monographs for plants describing 119 species. There are 26 Rosaceae, 12 Compositae (syn. Asteraceae), eight Lamiaceae, six Polygonaceae, four each of the Apiaceae, Betulaceae, Ericaceae, and Malvaceae species, three representatives each of the Solanaceae, Leguminosae (Fabaceae), and Plantaginaceae, two species each of the Adoxaceae (Caprifoliaceae), Asparagaceae (Liliaceae, s.l), Araliaceae Fagaceae, Gentianaceae, Hypericaceae, Laminariaceae, Pinaceae, Rhamnaceae, and Violaceae, and one member each of the Acoraceae (Araceae), Brassicaceae, Caprifoliaceae (Valerianaceae), Crassulaceae, Cucurbitaceae, Cupressaceae, Equisetaceae, Hymenochaetaceae, Linaceae, Menyanthaceae, Myrtaceae, Papaveraceae, poaceae, Polemoniaceae, Ranunculaceae, Rubiaceae, Saxifragaceae, Schisandraceae, and Urticaceae, respectively.

In the most of monographs, just one species is described as a source of plant material. However, 13 species are approved for Fructus rosae, 11 species are approved for Fructus crataegi, two species are approved for Herba hyperici, Fructus alni, Herba violae etc. Different parts are described for Viburnum opulus L. (Cortex viburni as diuretic and Fructus viburni as diaphoretic and anti-inflammatory) and Crataegus (Flos crataegi—13 species and Fructus crataegi—11 species for cardiovascular problems).

The large majority of species included in the Russian Pharmacopoeia (98 out of 119) are known to have been used as food by local population in Russia. For a majority of species, the part of plant used in food and as a medicine is the same. For example, the aerial parts of *Origanum vulgare* L., *Thymus serpyllum* L., *Leonurus cardiaca* L., are used as a medicine and as a food. Acorns and leaves of *Quercus robur* L. and Q. petraea (Matt.) Liebl. are used in food, while oak bark is used as a medicine. Fruits and bark of *Viburnum opulus* L. are used as medicine, with only the fruits being edible. Roots and rhizomes of *Acorus calamus* L., *Taraxacum campylodes* G.E.Haglund (syn. *Taraxacum officinale* L.), and *Aralia elata* (Miq.) Seem. are used as a medicine while young sprouts, leaves, flowers and fruits are used in food. The aerial parts of *Equisetum arvense* L. are used in medicine, while its cone-bearing shoots and black nodules attached to the roots are eaten raw in spring.

In general, considering all 98 edible plant species in the Russian Pharmacopoeia 11th edition, a higher number of plant parts are used in nutrition as compared to medicine. In the context of this article, the term "wild medicinal plants" referrings mainly to non-cultivated species. Twenty one plants including *Calendula officinalis* L., *Eucalyptus viminalis* Labill., *Pimpinella anisum* L., *Zea mays* L., and some others do not occur in Russia outside of cultivation and are excluded from this review. All poisonous and toxic species are not used in food as such as the species belonging to the Solanaceae (*Atropa belladonna* L., *Datura stramonium* L., *Hyoscyamus niger* L.), Asparagaceae (*Convallaria majalis* L., *Convallaria keiskei* Miq.), Rhamnaceae (*Frangula alnus* Mill., *Rhamnus cathartica* L.), Ranunculaceae (*Adonis vernalis* L.), Papaveraceae (*Chelidonium majus* L.) and some species from the newly circumscribed Plantaginaceae (*Digitalis purpurea* L., *Digitalis grandiflora* Mill.).

Consumer and marketing studies invariably showed that taste, as opposed to perceived nutrition or health value is the key influence on food selection (Drewnowski and Gomez-Carneros, 2000). Sensitized to the astringent taste of tannins or some bitter poisons, humans reject foods that are perceived to be excessively astringent or bitter. *Polemonium caeruleum* L. (Polemoniaceae), *Rubia tinctorum* L. (Rubiaceae), *Alnus incana* (L.) Moench and Alnus glutinosa (L.) Gaertn. (Betulaceae) are not considered to be edible due to their taste, as well as *Gnaphalium uliginosum* L. and *Bidens tripartita* L. (Asteraceae) which, however, are without any strong taste.

Edible wild species included in the Russian Pharmacopoeia have a wide range of uses: expectorant, diuretic, astringent, haemostatic, bitterness and choleretic, anti-inflammatory, diaphoretic, tonic, sedative, spasmolytic, polyvitamin, cardiovascular, and antihelminthic (**Table 1**).

Plants with expectorant properties alleviate the symptoms of patients in certain stages of bronchitis or tracheitis. Beneficial expectorant effects are mentioned for nine edible wild species. Certain edible species (nine species) are natural diuretics. Astringent species (eight species) provide not only specific taste for wine, tea, and other beverages due to high tannins content, but are effective in stopping the flow of blood or other secretions, and resolve diarrhea. Bitterness and choleretic herbs (seven species) are useful for development of functional foods with digestion-enhancing properties. The haemostatic effects of herbs (six species) are often due to mechanisms such as tannin astringency rather than enhancement of coagulation, although there are a few haemostatic herbs have been shown to reduce clotting times and have inhibitory effects on the Platelet Aggregation Factor. Four species included in the Russian Pharmacopoeia are used for their anti-inflammatory action. In the last decades, so-called natural anti-inflammatory diets have gained popularity (Sears, 2015). Diaphoretic herbs (four species) induce involuntary perspiration and thus reduce fever, promote circulation, relieve muscle tension, aching joints, and inflammatory skin conditions. These herbs are used as well to relieve diarrhea, dysentery, kidney nephritis, liver, urinary, and gall bladder. Tonic herbs (four species) as supplement to food are consumed throughout the world on a daily basis to promote radiant health and are included in many beverages. Motherwort and valerian provide mild sedative effects. The species with spasmolytic effects (two species) are noticeable as components of diets for treatment of gastro-intestinal ailments.

Hawthorn berries and flowers are useful for the creation of functional products with cardiovascular properties. Antihelmintic properties of tansy are complementary to its choleretic effects. The well documented anxiolytic effect of *L. cardiaca* L. may lead to the development of antistress functional food products. Increased access to information through systematic evaluation of medicinal plants and its nutritional properties will support the generation of ideas for functional food with new value added properties.

Underground parts like roots, shoots and leafy greens, berries and other fleshy fruits, seeds, and other species commonly yield wild-harvested foods, a practice which is based on specialized

TABLE 1 | Wild medicinal plants and their applications in food preparations.

Species, family	Pharmacological group according Pharmacopoeia	Part described in Pharmacopoeia	Form and dose as medicine**	Part used in food	Application in food	References
Achillea millefolium L., Asteraceae	Haemostatic*, anti-inflammatory*	Aerial part	Infusion (15 g in 200 ml), 65–100 ml, 2–3 times a day	Aerial part, leaves, flowers	Fresh herb added to meat and fish dishes, used as a seasoning in salads and vinaigrettes. Decoctions are added to the pastry and compotes. Dry aerial part with flowers used for liqueurs, table wines, kvass, cheese, jellies and mousses, as a spice in cooking. As a surrogate for tea. Inflorescence added to soups.	Gubanov et al., 1976; Koscheev, 1981; Grisjuk et al., 1989; Sokolov, 1993; Pieroni, 2000; Budantsev and Lesiovskaya, 2001; Soenov, 2002; Khan and Abourashed, 2010; Moerman, 2010; Kalle and Sõukand, 2012; Svanberg, 2012
Acorus calamus L., Acoraceae	Bitterness (appetite stimulant) and choleretic	Rhizome	Infusion (10 g in 200 ml), 25 ml, 3–4 times a day	Rhizome, leaves	Rhizome edible fresh as snack, candied as sweetmeats. Used for jam, candied peel, in confectionery, pasteurized compotes, syrup, kvass, liquors, brandy, beer, for the aromatic vinegar, as spice, for flavoring in preserves, food concentrates, for bakery products and ice cream. Rhizomes are source of starch. Leaves are edible fresh.	Annenkov, 1878; Vul'f and Maleeva, 1969; Gubanov et al., 1976; Koscheev, 1981; Kibala, 1986; Tcherepnin, 1987; Grisjuk et al., 1989; Sokolov, 1994; Budantsev and Lesiovskaya, 2001; Soenov, 2002; Moerman, 2010; Kalle and Sõukand, 2012
Althaea officinalis L., *A. armeniaca* Ten., Malvaceae	Expectorant	Roots	Decoction (6 g in 200 ml), 65–100 ml 3–4 times a day	Roots	Eaten cooked or roasted as vegetable. Used as additive to bread, in manufacture of sweetmeats, in home made alcohol, in soup. Infusion is added to pie and meringues. As tea substitute.	Keller, 1941; Ipatiev, 1966; Sokolov, 1985; Budantsev and Lesiovskaya, 2001; Parada et al., 2011
Aralia elata (Miq.)Seem., Araliaceae	Tonic*	Roots	EtOH (70%) tincture, 1.5–2.0 ml, 2–3 times a day	Roots	In beer, tonic alcoholic and non-alcoholic beverages.	Budantsev and Lesiovskaya, 2001; Palagina et al., 2011; Palagina and Bogoutdinova, 2012.
				Leaves, shoots	Fried and cooked as garnish.	Izmodenov, 1989; Zhuravlev and Kolyada, 1996
Arctostaphylos uva-ursi (L.) Spreng., Ericaceae	Diuretic	Leaves	Infusion (10 g in 200 ml), 65–100 ml, 3–5 times a day.	Berries	Eaten fresh or cooked with fish.	Holloway and Alexander, 1990; Turner et al., 2011
			Decoction (10 g in 200 ml), 15 ml 3–5 times a day		Dry in flour for bakery.	Molchanov et al., 1989
Artemisia absinthium L., Asteraceae	Bitterness (appetite stimulant) and choleretic	Aerial part & leaves	EtOH (70%) tincture, 0.75–1.0 ml, 3 times a day. Infusion (10 g in 200 ml), 50 ml, 3 times a day	Aerial part & leaves, inflorescences	Spicy seasoning to food, in the alcoholic (flavoring of liqueur, absinthe, wine, grappa) and non-alcoholic beverages, substitute of hop in home brewing. Essential oil is used for flavoring of ice cream, puddings, jellies, candies, and bakery. Inflorescences are used as a substitute for tea.	Ipatiev, 1966; Vul'f and Maleeva, 1969; Gubanov et al., 1976; Koscheev, 1981; Sokolov, 1993; Pieroni, 2000; Budantsev and Lesiovskaya, 2001; Khan and Abourashed, 2010; Parada et al., 2011; Kalle and Sõukand, 2012.
Bergenia crassifolia (L.) Fritsch, Saxifragaceae	Astringent, for external use	Rhizome	Decoction (10 g in 200 ml), 15–30 ml 3 times a day	Rhizome Leaves	Rhizomes eaten as a substitute for bread. Dry leaves ground into flour for bakery. Black leaves as tea substitute.	Vereschagin et al., 1959; Gubanov et al., 1976; Sokolov, 1987; Tcherepnin, 1987; Bugdaeva et al., 2005; Shikov et al., 2014b.
Betula pendula Roth, *B. pubescens* Ehrh., Betulaceae	Diuretic	Buds	EtOH (90%) tincture, 5–15 ml.	Buds	Essential oil from buds used as a flavoring in alcoholic beverage.	Gubanov et al., 1976; Fedorov, 1984; Budantsev and Lesiovskaya, 2001.
			Decoction (10 g in 200 ml), 15 ml 3–4 times a day. Infusion (10 g in 200 ml), 65–100 ml, 2–3 times a day	Sap	Birch sap is used as a fresh drink and for preparation of kvass.	Tikhomirov, 1942; Svanberg et al., 2012.

(Continued)

TABLE 1 | Continued

Species, family	Pharmacological group according Pharmacopoeia	Part described in Pharmacopoeia	Form and dose as medicine**	Part used in food	Application in food	References
Capsella bursa-pastoris (L.) Medik., Brassicaceae	Haemostatic*	Aerial part	Infusion (10 g in 200 ml), 15 ml, 4–5 times a day	Aerial part, leaves	Eaten fresh as a vegetable in salads, added to soups, borsch, schi, seasoning for meat dishes, vegetable purees and pastes, omelets, filling for pies, as a spice.	Annenkov, 1878; Tikhomirov, 1942; Klobukova-Alisova, 1958; Ipatiev, 1966; Gubanov et al., 1976; Koscheev, 1981; Reid, 1982; Sokolov, 1985; Grisjuk et al., 1989; Budantsev and Lesiovskaya, 2001; Hu, 2005; Moerman, 2010; Parada et al., 2011; Turner et al., 2011; Kalle and Sõukand, 2012; Łuczaj et al., 2013
Carum carvi L., Apiaceae	Spasmolytic*	Fruits	Infusion (20 g in 200 ml), 65–100 ml, 2–3 times a day	Fruits, roots, shoots	Spice for pickling of cabbage and cucumbers, in the production of home made cheeses, sausages, added in bread, cakes, omelets, puddings, cookies, muffins, as stuffing for dumplings. Added to soups, sauce, tea, as substitute of flour for bread. Oil from the fruits is used for flavoring of ice cream, candy, pickles, and soft drinks. Roots eaten fresh as vegetable. Young shoots cooked in soup and schi.	Keller, 1941; Tikhomirov, 1942; Gubanov et al., 1976; Koscheev, 1981; Markova et al., 1985; Tcherepnin, 1987; Sokolov, 1988; Budantsev and Lesiovskaya, 2001; Hu, 2005; Łuczaj, 2008; Moerman, 2010; Turner et al., 2011; Kalle and Sõukand, 2012; Svanberg, 2012; Sõukand et al., 2013
Cyanus segetum Hill (*Centaurea cyanus* L.), Asteraceae	Diuretic	Flowers	Infusion (5 g in 200 ml), 15 ml, 3 times a day	Flowers	Eaten fresh as vegetable, for side dishes decoration, for making of jelly, added to beer, as a coloring for sugar, gelatin and confectioneries, in soft drinks and alcoholic beverages.	Gubanov et al., 1976; Łuczaj, 2008; Turner et al., 2011; Łuczaj et al., 2012
Centaurium erythraea Rafn., *C.pulchellum* (Sw.) Druce., Gentianaceae	Bitterness (appetite stimulant)	Aerial part	Infusion (10 g in 200 ml), 65–100 ml, 2–3 times a day	Aerial part	Spice and bitterness for vermouth, aperitifs, tonic, non-alcoholic beverages. Used as substitute of hop.	Sokolov, 1990; Khan and Abourashed, 2010
Crataegus sanquinea Pall., *C. dahurica* Koehne ex Schneid.,	Cardiovascular	Flowers	Infusion (5 g in 200 ml), 100 ml, 3 times a day	Flowers	Eaten fresh	Sokolov, 1987
C. chlorocarpa Lenn, & K.Koch, *C. monogyna* Jacq., *C. rhipidophylla* Gand. *(C. curvisepala* Lindm.), *C. laevigata* (Poir.) DC., *C. pentagyna* Waldst.& Kit ex Willd, Rosaceae	Cardiovascular	Fruits	EtOH (70%) tincture, 0.5–0.75 ml. Infusion (15 g in 200 ml), 65–100 ml, 2–3 times a day.	Fruits	Eaten fresh, used for pie filling, in jelly, comfiture. Dry ground into flour for pie filling together with sugar and honey, flour added to the pastry for baking sweet bakery products, bread and pancakes, for jam, candy, sweets, biscuits. For soft drinks: kissel, compote, jelly (cooked with milk), and for wine. Whole fruits as substitute of tea and roasted as substitute of coffee.	Klobukova-Alisova, 1958; Vereschagin et al., 1959; Gubanov et al., 1976; Koscheev, 1981; Sokolov, 1987; Tcherepnin, 1987; Grisjuk et al., 1989; Efremova, 1992; Budantsev and Lesiovskaya, 2001; Pieroni et al., 2005; Khan and Abourashed, 2010; Łuczaj et al., 2012
Equisetum arvense L., Equisetaceae	Diuretic	Aerial part	Decoction (20 g in 200 ml), 65–100 ml, 2–3 times a day.	Aerial part, nodules	Young shoots and aerial part are eaten raw or cooked, for filling of pie, casseroles, okroshka and sauces. Nodules eaten fresh.	Berson, 1991; Moerman, 2010; Turner et al., 2011
Helichrysum arenarium (L.) Moench, Asteraceae	Choleretic	Flowers	Decoction (10 g in 200 ml), 100 ml, 2–3 times a day.	Flowers	Spice, flavoring additive to tea.	Facciola, 1998

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TABLE 1 | Continued

Species, family	Pharmacological group according Pharmacopoeia	Part described in Pharmacopoeia	Form and dose as medicine**	Part used in food	Application in food	References
Hypericum perforatum L., *Hypericum maculatum* Crantz., Hypericaceae	Astringent, antiseptic	Aerial part	Decoction (10 g in 200 ml), 65 ml, 3 times a day. EtOH (40%) tincture, 1.0–1.5 ml 3–4 times a day.	Aerial part	Spice seasoning for fish dishes, and sausages. In home made liquors, in alcoholic beverage industry as flavoring for bitterness brandy "Zveroboy," "Erofeevich." As tea substitute.	Timoshenko, 1940; Klobukova-Alisova, 1958; Makarova et al., 1960; Gubanov et al., 1976; Koscheev, 1981; Grisjuk et al., 1989; Mashanov and Pokrovsky, 1991; Budantsev and Lesiovskaya, 2001; Parada et al., 2011
Inula helenium L., Asteraceae	Expectorant	Roots and rhizome	Decoction (16 g in 200 ml), 100 ml, 2–3 times a day.	Roots and rhizome	Added to puddings, candy, to make jam, for soups, compotes, jelly, boiled in sugar and as substitute of ginger. To flavor vermouth, French and Swedish absinthe. Essential oil for flavoring of alcoholic beverages (spirits, beer), soft drinks, candy, cakes, ice cream, jelly, sausage, fish sauce.	Klobukova-Alisova, 1958; Ipatiev, 1966; Vul'f and Maleeva, 1969; Gubanov et al., 1976; Koscheev, 1981; Grisjuk et al., 1989; Sokolov, 1993; Budantsev and Lesiovskaya, 2001; Khan and Abourashed, 2010; Parada et al., 2011
Juniperus communis L., Cupressaceae	Diuretic	Fruits	Infusion (10 g in 200 ml), 15 ml, 3–4 times a day.	Fruits	Eaten fresh, used for preparation of juniper kvass, morse, beer, gin, liquors. Added as flavor and condiment in soup, pickles, sour cabbage, mincemeat, pate, wildfowl and meat dishes. In production of molasses, honey cake, candy, as coffee substitute. Extracts and essential oil added to ice cream, sausages, meat products, jelly, candy, bakery, alcoholic and non-alcoholic beverages.	Keller, 1941; Klobukova-Alisova, 1958; Gubanov et al., 1976; Koscheev, 1981; Tcherepnin, 1987; Grisjuk et al., 1989; Budantsev, 1996; Pieroni, 2000; Budantsev and Lesiovskaya, 2001; Khan and Abourashed, 2010; Moerman, 2010; Parada et al., 2011; Turner et al., 2011; Łuczaj et al., 2012; Svanberg, 2012
Ledum palustre L., Ericaceae	Expectorant	Shoots	Infusion (10 g in 200 ml), 50 ml, 2–3 times a day.	Shoots Leaves	Substitute of hop for beer. Substitute of tea.	Sokolov, 1985; Budantsev and Lesiovskaya, 2001.
Leonurus cardiaca L., *L. quinquelobatus* Gilib., Lamiaceae	Sedative	Aerial part	Infusion (15 g in 200 ml), 100 ml, 2 times a day. EtOH (70%) tincture, 0.75–1.25 ml 3–4 times a day.	Flowers, aerial part	Fresh or dry flowers as condiment in soup with legumes especial in lentil or split peas, for flavoring of beer and tea. Aerial part is used in schi.	Annenkov, 1878.
Matricaria chamomilla L. (*Matricaria recutita* L.), Asteraceae	Anti-inflammatory, spasmolytic	Flowers	Decoction (10 g in 200 ml), 65–100 ml, 2–3 times a day.	Flowers	Substitute of tea, added to liquor and bitter brandy, vermouth. Essential oil for enhance of fruity flavor of ice cream, candies, bakery, chewing gum, puddings, jellies.	Vul'f and Maleeva, 1969; Rubtsov, 1971; Gubanov et al., 1976; Sokolov, 1993; Pieroni, 2000; Budantsev and Lesiovskaya, 2001; Khan and Abourashed, 2010; Parada et al., 2011; Kalle and Sõukand, 2012; Svanberg, 2012
Menyanthes trifoliata L., Menyanthaceae	Bitterness (appetite stimulant) and choleretic	Leaves	Infusion (10 g in 200 ml), 65–100 ml, 3 times a day.	Leaves	Substitute of hop for beer. As substitute of tea.	Kalle and Sõukand, 2012
Origanum vulgare L., Lamiaceae	Expectorant	Aerial part	Infusion (10 g in 200 ml), 100 ml, 2 times a day.	Aerial part	Fresh leaves and tender shoots are boiled and eaten as green vegetables; fresh and dry leaves and flowers as spice and condiment in salads, soup, stews meat, sausages, casseroles, sauces, toppings and egg dishes, pickling olives, cucumber, mushrooms, and tomatoes, bakery. In soft drinks, kvass, beer, vine, brandy and liquors. As tea substitute.	Annenkov, 1878; Klobukova-Alisova, 1958; Ipatiev, 1966; Vul'f and Maleeva, 1969; Rubtsov, 1971; Gubanov et al., 1976; Koscheev, 1981; Boeva et al., 1984; Kibala, 1986; Grisjuk et al., 1989; Sokolov, 1991; Pieroni, 2000; Budantsev and Lesiovskaya, 2001; Khan and Abourashed, 2010; Parada et al., 2011; Kalle and Sõukand, 2012; Svanberg, 2012; Sõukand et al., 2013

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TABLE 1 | Continued

Species, family	Pharmacological group according Pharmacopoeia	Part described in Pharmacopoeia	Form and dose as medicine**	Part used in food	Application in food	References
Panax ginseng C.A.Mey., Araliaceae	Tonic	Rhizome	EtOH (70%) tincture, 0.375–0.625 ml 3 times a day	Roots	Added to candy, jam, chewing gum, blended coffee, bakery, canned chicken, soups, porridge, snacks, sweets, honey, punch, soft and alcoholic beverages.	Grushvitsky, 1961; Budantsev and Lesiovskaya, 2001; Khan and Abourashed, 2010; FAO/WHO, 2012b; CODEX STAN 321-2015, 2015
Persicaria bistorta (L.) Samp. (*Polygonum bistorta* L.), Polygonaceae	Astringent	Rhizome	Infusion (10 g in 200 ml), 100 ml, 2–3 times a day.	Rhizome	Eaten roasted, baked, cooked, as additive to rye flour, in alcoholic beverages.	Gubanov et al., 1976; Fedorov, 1984; Tcherepnin, 1987; Moerman, 2010
Persicaria hydropiper (L.) Delabre. (*Polygonum hydropiper* L.), Polygonaceae	Haemostatic	Aerial part	Infusion (20 g in 200 ml), 65 ml, 3–4 times a day.	Aerial part	Spice and condiment to meat, ragout. Young shoots cooked, roasted and eaten as vegetable.	Ipatiev, 1966; Vul'f and Maleeva, 1969; Reid, 1982; Fedorov, 1984; Budantsev and Lesiovskaya, 2001; Hu, 2005; Moerman, 2010
Persicaria maculosa Gray. (*Polygonum persicaria* L.), Polygonaceae	Astringent, hemostatic, diuretic*	Aerial part	Infusion (20 g in 200 ml), 15 ml, 3 times a day.	Aerial part	Eaten fresh as snack, added to salad, soup, stews, casseroles, roasted or cooked and served with oil or vinegar; in alcoholic beverages.	Reid, 1982; Fedorov, 1984; Turner et al., 2011
Picea abies (L.) H.Karst., Pinaceae	Anti-inflammatory	Cones	Infusion (30 g in 200 ml), external application.	Cones	As tea surrogate.	Budantsev, 1996; Budantsev and Lesiovskaya, 2001
Pinus sylvestris L., Pinaceae	Expectorant	Buds	Decoction (10 g in 200 ml), 65–100 ml, 2–3 times a day.	Buds, needles, sprouts	Essential oil from needles for flavoring of soft and alcoholic beverages, ice cream, jelly, pudding. Buds for beer. Sprouts in infusion as source of vitamine C. Fresh sapwood is edible and added in flour.	Vereschagin et al., 1959; Berson, 1991; Khan and Abourashed, 2010
Plantago major L., Plantaginaceae	Expectorant	Leaves	Decoction (10 g in 200 ml), 65–100 ml, 3–4 times a day.	Leaves	Fresh young leaves are eaten as a green vegetable in salads, added to soup, borsch, omelets, quiches, porridge, mashed potatoes, and cutlet. Dry leaves as surrogate of tea.	Tikhomirov, 1942; Klobukova-Alisova, 1958; Efremova, 1967; Rubtsov, 1971; Gubanov et al., 1976; Reid, 1982; Tcherepnin, 1987; Grisjuk et al., 1989; Sokolov, 1990; Pieroni, 2000; Budantsev and Lesiovskaya, 2001; Hu, 2005; Moerman, 2010; Parada et al., 2011; Turner et al., 2011; Kalle and Sõukand, 2012
Polygonum aviculare L., Polygonaceae	Diuretic*	Aerial part	Infusion (15 g in 200 ml), 65-100 ml, 2-3 times a day.	Aerial part	Fresh young leaves eaten as a green vegetable in salads, added to soup, pottage, condiment for meat and fish. Leaves as substitute of tea.	Reid, 1982; Fedorov, 1984; Tcherepnin, 1987; Grisjuk et al., 1989
Prunus padus L. (*Padus racemosa* (Lam.) Gilib., *Padus avium* Mill., *P. asiatica* Kom.), Rosaceae	Astringent	Fruits	Infusion (10 g in 200 ml), 100 ml, once a day.	Fruits	Eaten fresh as snack; dry fruits as substitute of tea. Flour from dried fruit is used in confectionery and bakery, cheesecakes, cookies, pancakes, bread (added to the grain flour), and for pie filling. Fresh and dry fruits used in kissel, jelly, jam, compotes, liquors and brandy.	Klobukova-Alisova, 1958; Vul'f and Maleeva, 1969; Gubanov et al., 1976; Koscheev, 1981; Markova et al., 1985; Sokolov, 1987; Tcherepnin, 1987; Grisjuk et al., 1989; Toren, 1996; Budantsev and Lesiovskaya, 2001; Soenov, 2002; Łuczaj, 2008; Turner et al., 2011; Kalle and Sõukand, 2012; Svanberg, 2012

(Continued)

TABLE 1 | Continued

Species, family	Pharmacological group according Pharmacopoeia	Part described in Pharmacopoeia	Form and dose as medicine**	Part used in food	Application in food	References
Sedum roseum (L.) Scop. (*Rhodiola rosea* L.), Crassulaceae	Tonic	Rhizome & roots	EtOH (70%) extract, 0.125–0.25 ml 2–3 times a day.	Rhizome & roots.	Cooked and used wit butter as side dishes to meat and fish; in tonic beverages. As additive to tea.	Sokolov, 1990; Soenov, 2002
				Leaves	Young shoots and leaves in salad.	Saratikov, 1974
Rosa majalis Herrm., *R. acicularis* Lindl., *R. laxa* Retz., *R. davurica* Pall., *R. rugosa* Thunb., *R. canina* L., *R. corymbifera* Borkh., *R. tomentosa* Smith., *R. micrantha* Smith., Rosaceae	Polyvitamin	Fruits	Infusion (10 g in 200 ml), 100 ml, 2–3 times a day.	Fruits	Eaten fresh as snack, for pie and dessert filling, added to salads, used in puree and syrup preparation. Used in jam, marmalade, paste, comfiture, kissel, compote, and for vinegar, wine and brandy. Dry added to soup and in flour for bakery. Whole fruits as substitute of tea and roasted as substitute of coffee.	Tikhomirov, 1942; Klobukova-Alisova, 1958; Baikov, 1968; Vul'f and Maleeva, 1969; Gubanov et al., 1976; Koscheev, 1981; Markova et al., 1985; Sokolov, 1987; Tcherepnin, 1987; Grisjuk et al., 1989; Efremova, 1992; Pieroni, 2000; Budantsev and Lesiovskaya, 2001; Soenov, 2002; Hu, 2005; Pieroni et al., 2005; Khan and Abourashed, 2010; Turner et al., 2011; Kalle and Sõukand, 2012; Sõukand et al., 2013; Novruzov, 2014
Sambucus nigra L., Adoxaceae	Diaphoretic (sudorific)	Flowers	Infusion (5 g in 200 ml), 65–100 ml, 2–3 times a day.	Flowers	Eaten fresh, in salads. As flavoring in jelly, jam, bread, bakery, pudding, ice cream, for soft drinks and in wine, liquors, rafatia, and brandy. Young inflorescences fried in batter eaten as a dessert or as a side dish to meat. As tea substitute. Fruits for kissel, syrup, jelly, jam, and soft drinks.	Nekrasova, 1958; Pojarkova, 1958; Vul'f and Maleeva, 1969; Koscheev, 1981; Khan and Abourashed, 2010; Vallès et al., 2010; Turner et al., 2011; Lim, 2012; Svanberg, 2012; Sõukand et al., 2013 Berson, 1991
Schisandra chinensis (Turcz.) Baill., Schisandraceae	Tonic	Seeds	EtOH (95%) tincture, 0.5–0.75 ml 2–3 times a day.	Fruits	Eaten fresh or dry. Dry fruits and juice for sweets, soft drinks, kissel, jams, syrups, and flavoring extracts. In wine industry; as additive to tea. Dry fruits in bakery products.	Gubanov et al., 1976; Fedorov, 1984; Budantsev and Lesiovskaya, 2001; Hu, 2005; Panossian and Wikman, 2008; Khan and Abourashed, 2010; Smertina et al., 2011
Sorbus aucuparia L., Rosaceae	Polyvitamin	Fruits	Decoction (10 g in 200 ml), 100 ml, 3 times a day.	Fruits	Eaten fresh. Used in confiture, paste, marmalade, jam, sweet, kissel, jelly, juice, kvass, compote, candy, for pie filling. Flour from dried fruits added in bread. Added in wine, brandy. Dry as tea substitute.	Klobukova-Alisova, 1958; Vul'f and Maleeva, 1969; Koscheev, 1981; Sokolov, 1987; Grisjuk et al., 1989; Toren, 1996; Budantsev and Lesiovskaya, 2001; Soenov, 2002; Łuczaj, 2008; FAO/WHO, 2012a; Kalle and Sõukand, 2012
Tanacetum vulgare L., Asteraceae	Antihelminthic and choleretic	Flowers	Infusion (10 g in 200 ml), 15 ml, 3 times a day.	Flowers	Dry flowers as a garnish, as ginger and cinnamon substitute, added to meat and fish, for meat preservation; for flavoring of beverages. Flowering parts and leaves as tea substitute, in liquor, brandy, as hop substitute in beer.	Annenkov, 1878; Nekrasova, 1958; Koscheev, 1981; Berson, 1991; Sokolov, 1993; Budantsev and Lesiovskaya, 2001; Parada et al., 2011; Kalle and Sõukand, 2012

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TABLE 1 | Continued

Species, family	Pharmacological group according Pharmacopoeia	Part described in Pharmacopoeia	Form and dose as medicine**	Part used in food	Application in food	References
Taraxacum campylodes G.E. Haglund. (*Taraxacum officinale* (L.) Weber ex F.H.Wigg.), Asteraceae	Bitterness (appetite stimulant) and choleretic	Roots	Infusion (10 g in 200 ml), 65 ml, 3–4 times a day.	Roots, leaves, buds	Eaten fresh in salads, milled root added to the flour for bread baking. Extract for flavoring of ice cream, sweet, cheese, jelly, pudding, bakery, and beverages. Roasted as substitute of coffee and tea. Fresh leaves in salads, buds for pickling.	Keller, 1941; Tikhomirov, 1942; Klobukova-Alisova, 1958; Vul'f and Maleeva, 1969; Rubtsov, 1971; Gubanov et al., 1976; Koscheev, 1981; Tcherepnin, 1987; Litvintsev and Koscheev, 1988; Berson, 1991; Sokolov, 1993; Budantsev and Lesiovskaya, 2001; Khan and Abourashed, 2010; Kalle and Sõukand, 2012
Thymus serpyllum L., Lamiaceae	Expectorant	Aerial part	Infusion (10 g in 200 ml), 15 ml, 2–3 times a day. Decoction (20 g in 200 ml), 15–30 ml, 3–5 times a day.	Aerial part	Fresh or dry as condiment in salads, sauce, for vegetarian, fish, and meat dishes; for flavoring of vinegar, cocktails, sausages, cheese, confectionery, in beverages. For pickling of cucumber. As tea substitute.	Klobukova-Alisova, 1958; Vul'f and Maleeva, 1969; Gubanov et al., 1976; Koscheev, 1981; Grisjuk et al., 1989; Sokolov, 1991; Budantsev and Lesiovskaya, 2001; Hu, 2005; Pieroni et al., 2005; Parada et al., 2011; Kalle and Sõukand, 2012; Svanberg, 2012
Tilia cordata Mill., *T. platyphyllos* Scop., Malvaceae	Diaphoretic (sudorific)	Flowers	Infusion (10 g in 200 ml), 200–400 ml, 2–3 times a day.	Flowers, young leaves, bast	Flowers, leaves and bast eaten fresh. Flowers for flavoring of wine, liquor, cognac. As tea substitute.	Klobukova-Alisova, 1958; Nekrasova, 1958; Gubanov et al., 1976; Sokolov, 1985; Tcherepnin, 1987; Grisjuk et al., 1989; Budantsev and Lesiovskaya, 2001; Parada et al., 2011; Kalle and Sõukand, 2012
Tussilago farfara L., Asteraceae	Expectorant	Leaves	Infusion (5 g in 200 ml), 65–100 ml, 2–3 times a day.	Leaves	Young leaves eaten fresh in salads, soup and as vegetables. Dry as tea substitute.	Soenov, 2002; Kalle and Sõukand, 2012
Urtica dioica L., Urticaceae	Haemostatic	Leaves	Infusion (10 g in 200 ml), 50–100 ml, 3–5 times a day. EtOH (70%) extract, 0.625–0.75 ml 3 times a day.	Leaves	Leaves eaten fresh in salads, cooked as vegetables; in soup, schi, borsch, pottage, omelet, porridge, puree, filling for tortellini, component of sauces, and condiments. Milled added in flour for bakery. For juice, syrup, balsam, cocktail. Dry as tea substitute.	Tikhomirov, 1942; Klobukova-Alisova, 1958; Nekrasova, 1958; Ipatiev, 1966; Gubanov et al., 1976; Koscheev, 1981; Fedorov, 1984; Litvintsev and Koscheev, 1988; Toren, 1996; Pieroni, 2000; Budantsev and Lesiovskaya, 2001; Khan and Abourashed, 2010; Moerman, 2010; Parada et al., 2011; Turner et al., 2011; Kalle and Sõukand, 2012; Svanberg, 2012; Łuczaj et al., 2013
Vaccinium myrtillus L., Ericaceae	Astringent	Fruits	Eaten fresh and dry	Fruits	Eaten fresh and dry, as side dishes. Used in jam, confiture, compote, marmalade, extracts, syrup, morse, kissel, juice, compote, wine, brandy. Milled added in flour for pancakes, pie, jelly, confectionery.	Gubanov et al., 1976; Koscheev, 1981; Sokolov, 1985; Grisjuk et al., 1989; Pieroni, 2000; Budantsev and Lesiovskaya, 2001; Łuczaj, 2008; Khan and Abourashed, 2010; Kalle and Sõukand, 2012; Łuczaj et al., 2012; Svanberg, 2012
Vaccinium vitis-idaea L., Ericaceae	Diuretic	Leaves	Decoction (6 g in 200 ml), 65–100 ml, 2–3 times a day.	Leaves	As tea surrogate.	Klobukova-Alisova, 1958; Efremova, 1967, 1992; Krylov, 1972; Sokolov, 1985; Grisjuk et al., 1989; Toren, 1996; Budantsev and Lesiovskaya, 2001;
				Fruits	As tea surrogate, in juice.	Stryamets et al., 2015

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TABLE 1 | Continued

Species, family	Pharmacological group according Pharmacopoeia	Part described in Pharmacopoeia	Form and dose as medicine**	Part used in food	Application in food	References
Valeriana officinalis L., Caprifiolaceae	Sedative	Rhizome & roots	Infusion (20 g in 200 ml), 30–45 ml, 3–4 times a day. EtOH (70%) tincture, 0.625–0.75 ml 3–4 times a day.	Rhizome & roots	Added to soup; for pie filling, as condiment to stew meat. Extracts and essential oil as flavoring in ice cream, sweets, bakery, jelly, pudding, soft drinks, liquor, beer.	Wustenfeld and Gesler, 1959; Mashanov and Pokrovsky, 1991; Khan and Abourashed, 2010
Viburnum opulus L., Adoxaceae	Hemostatic	Bark	Decoction (10 g in 200 ml), 15–30 ml, 3–4 times a day.	Fruits	Eaten fresh (after freezing). Used in porridge, bakery, confiture, jam, compotes, kissel, marmalade, paste, juice, morse, mousse, vinegar, for filling of pie and sweets. As condiment to meat. As substitute of tea, and roasted as substitute of coffee.	Baikov, 1968; Gubanov et al., 1976; Tcherepnin, 1987; Grisjuk et al., 1989; Sokolov, 1990; Toren, 1996; Budantsev and Lesiovskaya, 2001; Smertina et al., 2011
	Diaphoretic, anti-inflammatory	Fruits	Infusion (10 g in 200 ml), 65 ml, 3–4 times a day.			
Viola tricolor L., *V. arvensis* Murray, Violaceae	Expectorant	Aerial part	Infusion (5 g in 200 ml), 100 ml, 3–4 times a day.	Flowers, leaves	Small fragrant flowers eaten fresh in salads, for dishes decoration. Cooked leaves as vegetables.	Łuczaj et al., 2013

**Pharmacological group according to the online State Register of Medicinal preparations by the Ministry of Public Health of the Russian Federation*[1].
***Formulation and dose as described in Sokolov (2000).*
Compote – a beverage with fresh, dried or frozen fruit (whole or cut into pieces) and/or berries that's slowly cooked in a sweet water.
Kvass – national beverage, from sour rye flour or from baked rye bread with malt.
Okroshka – cold soup with kvass, meat, green vegetables and boiled egg.
Schi – a soup with meat or meatless, from cabbage (chopped or sour); sometimes cabbage is replaced with sorrel.
Borsch – a schi with beetroot, beef or pork, or lard.

[1]Online State Register of Medicinal preparations by the Ministry of Public Health of the Russian Federation, http://grls.rosminzdrav.ru/GRLS.aspx (accessed Aug 2017).

cultural knowledge of their harvesting, preparation, cooking and other forms of processing.

EDIBLE WILD PLANTS–APPROACHES AND METHODS FOR ASSESSING USES AND EVIDENCE

Based on the State Pharmacopoeia of the USSR, we selected seventy wild species that are used as food in Russia and systematically searched the scientific literature (published between 1878-2016) for data using the Russia-wide applied online database E-library.ru, library catalogs of public libraries in St-Petersburg, the databases Scopus, Web of Science, PubMed, and search engine Google Scholar. The primary search criterion was a food application of medicinal plants.

Edible wild plants include food categories familiar to everyone: green vegetables and potherbs, wild berries and fruits, beverages, tea and coffee substitutes, seasonings and spices, sweets, bread surrogates, plants used for preserves. Family names of the species are based on www.theplantlist.org database with the names in the Russian Pharmacopoeia given in brackets (**Table 1**). Also included are the applications in food.

Green Vegetables and Potherbs

This category includes the majority of examined plants. Especially in the spring or at the beginning of their growing season, many wild species produce tender, edible leaves and shoots, and flowers at the beginning of flowering. Some, like shepherd's-purse (Capsella bursa-pastoris (L.) Medik.), cornflower (*Cyanus segetum Hill*., syn. *Centaurea cyanus* L.), and broadleaf plantain (*Plantago major* L.) can be eaten raw, after being peeled from soil (Sokolov, 1985, 1990; Tcherepnin, 1987; Grisjuk et al., 1989; Budantsev and Lesiovskaya, 2001; Turner et al., 2011), whereas others, like stinging nettles (*Urtica dioica* L.) must be steamed or cooked in some way (Litvintsev and Koscheev, 1988; Fedorov, 1984; Toren, 1996). These plants are used for salads, added to soups, borsch, omelets, cooked and used as a garnish.

Many edible greens emerging right after the melting of snow are particularly important for their vitamin C content in the spring and traditionally have been used to prevent and alleviate scurvy. In particularly, soup and borsch with nettles are popular not only among rural but also many urban people (Gubanov et al., 1976; Koscheev, 1981; Tcherepnin, 1987; Litvintsev and Koscheev, 1988).

Tonic properties of shoots of aralia (*Aralia elata* (Miq.)Seem.) were recognized by Ussuri aborigines (Far East). Young shots no more than 20 cm are cooked and served as garnish (Izmodenov, 1989).

Wild Berries and Fruits

Presumably, wild berries and fruits are the most favored group of edible medicinal plants in Russia, and today probably used most frequently. The wild fruits most commonly collected in Russia include bilberry (*Vaccinium myrtillus* L.), hawthorn (*Crataegus* spp.), and rose hips (*Rosa* spp.) (Gubanov et al., 1976; Koscheev, 1981; Sokolov, 1987; Tcherepnin, 1987; Grisjuk et al., 1989; Efremova, 1992; Budantsev and Lesiovskaya, 2001; Soenov, 2002; Łuczaj et al., 2012; Sõukand et al., 2013; Novruzov, 2014). All of them are consumed fresh, but some are also preserved for the winter by making jams, marmalade or pasteurized compotes (Turova and Sapozhnikova, 1989). Furthermore, they are used to make beverages. Some berries are popular among all groups of the population (like bilberry), other species that once were used as a valuable food source in some regions of Russia have seen that food use diminished because of the bitter or astringent taste as such as guelder rose (*Viburnum opulus* L.), bird cherry (*Prunus padus* L.), and rowan (*Sorbus aucuparia* L.) (Baikov, 1968; Rubtsov, 1971; Gubanov et al., 1976; Sokolov, 1985, 1990; Tcherepnin, 1987; Grisjuk et al., 1989; Toren, 1996; Smertina et al., 2011). The berries and seeds of *Schisandra chinensis* (Turcz.) Bail. were eaten by Nanai (Goldes or Samagir) hunters from Far Eastern Russia since "it gives forces to follow a sable all the day without food" and were acclaimed as a tonic, to improve night vision and to reduce hunger, thirst and exhaustion (Panossian and Wikman, 2008).

Beverage

Aerial parts, leaves or flowers are used mainly for making beverages, while the underground parts are used rarely. Aromatic plants belonging to the Asteraceae and Lamiaceae are used to sweeten or to flavor alcoholic and non-alcoholic beverages: yarrow (*Achillea millefolium* L.), tansy (*Tanacetum vulgare* L.), and oregano (*Origanum vulgare* L.) (Timoshenko, 1940; Wustenfeld and Gesler, 1959; Koscheev, 1981; Berson, 1991; Sokolov, 1991; Budantsev and Lesiovskaya, 2001; Soenov, 2002; Kalle and Sõukand, 2012; Sõukand et al., 2013). Juicy fruits of species belonging to the Rosaceae (rowan and bird cherry) are used often for coloring of alcoholic beverages and giving a more pronounced flavor. The fruits of these plants are used both in home made alcoholic beverages as well as in wine- and brewing industry for the production of liqueurs, bitters, wines, and beers (Wustenfeld and Gesler, 1959; Gubanov et al., 1976; Koscheev, 1981; Tcherepnin, 1987; Toren, 1996; Budantsev and Lesiovskaya, 2001). Making liqueurs out of rowan berries seems to be an old habit that has been in vogue in Russia (Timoshenko, 1940). The aroma is an important attribute of refreshing soft drinks (kvass, lemonade, and fruit morse), which are popular in the summer season.

Tonic properties of ginseng and aralia were recorded in the Far Eastern Russia by hunters and their roots were regularly added to alcoholic and nonalcoholic beverages, and balsams (Grushvitsky, 1961; Zhuravlev and Kolyada, 1996; Budantsev and Lesiovskaya, 2001; Palagina et al., 2011; Palagina and Bogoutdinova, 2012).

Birch (*Betula pendula* Roth., and *B. pubescens* Ehrh.) sap has been gathered all over Russia and was usually seen as a refreshing drink (Fedorov, 1984; Tcherepnin, 1987; Budantsev and Lesiovskaya, 2001). Today, Russia, Estonia, Latvia, Lithuania, Ukraine and Belarus are the only countries where the gathering and use of birch sap has remained an important. Large birch forests, low population density and the incorporation of sap into the former Soviet economic system facilitated this (Svanberg et al., 2012). Birch sap was utilized the diverse ways as described

by the Russian ethnographer Zelenin (1927). It was drunk fresh, but also fermented by adding malt, wax, beans or rye bread.

Tea and Coffee Substitutes

After water, tea is the second most-consumed beverage worldwide (Keating et al., 2015). Although the English term "tea" generally denotes an infusion made of the leaves of *Camellia sinensis* (L.) Kuntze, many herbal teas, which usually are mono- or polyherbal formulations made from (medicinal) plant(s), are available worldwide. Historically in Russia such herbal teas often made from local species were widespread. Samovar and tea-drinking are an indispensable element of Russian culture. With *C. sinensis* not being available or affordable to the vast majority of population, local surrogates/substitutes of tea have been used for centuries. The best known tea surrogates were prepared from fireweed (*Epilobium angustifolium* L.) and, in Siberia - *Bergenia* spp. (Tcherepnin, 1987; Pohlebkin, 1998; Shikov et al., 2006, 2014b; Sõukand et al., 2013). Prior to the introduction of oriental (black or green) tea in Europe, *E. angustifolium* was esteemed as the "original Russian tea" and used widely throughout Russia and beyond (Litvintsev and Koscheev, 1988; Sõukand et al., 2013). However, this botanical drug is not in the Russian Pharmacopoeia. Extracts of black and fermented leaves of *B. crassifolia* are reported as appetite and energy intake suppressants (Shikov et al., 2012). Other species that are used as substitutes for tea in Russia belong to different families. First of all they are expected to bring delicious flavor, taste and nice color to the tea. Marsh Labrador tea (*Ledum palustre* L.), thyme (*Thymus serpyllum* L.), wormwood (*Artemisia absinthium* L.), are highly aromatic. Aerial parts of Saint John's wort (*Hypericum perforatum* L.), oregano, thyme, leaves of lingonberry (*Vaccinium vitis-idaea* L.), flowers of linden (*Tilia cordata* Mill. and T. platyphyllos Scop.) are among the most popular components of herbal teas in Russia (Krylov, 1972; Koscheev, 1981; Sokolov, 1985; Grisjuk et al., 1989; Efremova, 1992; Kalle and Sõukand, 2012). Dry fruits of rose hips, hawthorn, and rowan are used as substitutes of tea (Gubanov et al., 1976; Tcherepnin, 1987; Budantsev and Lesiovskaya, 2001). Ethnobotanical data also lists dried and roasted rhizomes of dandelion (*Taraxacum campylodes* G.E. Haglund), dry seeds of guelder rose, roasted fruits of hawthorn, and fruits of *R. canina* L.as coffee substitutes (Klobukova-Alisova, 1958; Vul'f and Maleeva, 1969; Koscheev, 1981; Sokolov, 1990, 1993).

Seasoning, Spices

Aromatic herbs are very important part of local gastronomy, especial for seasoning and as spices in southern Russia. In particular, sweet flag (A. calamus L.), valerian (*Valeriana officinalis* L.), elecampane (*Inula helenium* L.) roots and rhizomes, juniper (*Juniperus communis* L.), and caraway (*Carum carvi* L.) fruits, dwarf everlast (*Helichrysum arenarium* (L.) Moench) and tansy flowers, yarrow, oregano, thyme, and artemisia aerial parts are normally used as aroma and flavor enhancer or digestives (Sokolov, 1988, 1994; Berson, 1991; Mashanov and Pokrovsky, 1991). These species are used both in culinary preparations at home: they are added to the soups and main dishes, salads, meats, bakery, as well as in the food industry in the production of sausages, confectionery and bakery products.

Sweets

The Russian make a kind of sweet flag and elecampane candied peel by boiling the transverse root slices in syrup, and drying (Gubanov et al., 1976; Koscheev, 1981; Berson, 1991). Candied fruits of hawthorn, bird cherry, guelder rose are used as sweetness (Gubanov et al., 1976; Koscheev, 1981; Tcherepnin, 1987; Budantsev and Lesiovskaya, 2001).

Bread Surrogates

In the eighteenth and the first half of the nineteenth century flour and products like bread derived from it were the main foods for Russian peasants. Consequently, every an extended period of bad weather resulting in crop failure resulted in large-scale famines. This explains why a vast variety of plant parts was used for filling bread, including roots, fruits, leaves, and barks. Roots and rhizomes of some plants are known to be good sources of starch and carbohydrates. Powdered roots of *Persicaria bistorta* (L.) Samp. were added to the rye flour for baking bread (Rubtsov, 1971; Fedorov, 1984; Tcherepnin, 1987). Bergenia rhizomes were eaten as a substitute for bread (Vereschagin et al., 1959; Tcherepnin, 1987). Flour from black leaves of bergenia is used for cookies (Bugdaeva et al., 2005). Roots of sweet flag were used as source of starch (Berson, 1991). Whole or crushed caraway seeds, dried and ground into flour fruits of hawthorn, bird cherry, rowan, guelder rose were added to the pastry when baking bread and sweet bakery (cakes and pancakes) (Gubanov et al., 1976; Koscheev, 1981; Tcherepnin, 1987; Budantsev and Lesiovskaya, 2001).

In the middle of nineteenth century, fruits of guelder rose (*Viburnum opulus*) were popular in Tver province of Russia as ingredients for making "kulaga," a porridge based on malt flour. Guelder rose fruits tenderized with flour and honey were "a tasty cuisine and refined taste for urban residents, not excluding the nobility" (Toren, 1996). Residents of Pskov province received the nickname "kalinniki" because they cooked a delicious porridge with guelder rose ("kalina" in Russian) berries and licorice flour, which was sold at the bazaar (Toren, 1996).

Despite the fact that today wild bread additives have almost completely disappeared from the European diet (Łuczaj et al., 2012), in Russia some local bakeries produce bread and buns with nettle, hawthorn, and dog rose.

Species Used in Preserves

Home made preserves have a long tradition in Russia because of the long and severe cold season. There are many canning recipes in every household for foods which have been handed down from generation to generation. Plants used for preserves can be divided into two large groups. Fresh juicy berries of rose hips, guelder rose, rowan, bird cherry, and bilberry are widely used in jam, comfiture, and marmalade. All these berries are useful for filling pies (Klobukova-Alisova, 1958; Baikov, 1968; Gubanov et al., 1976; Sokolov, 1985, 1990; Budantsev and Lesiovskaya, 2001; Soenov, 2002). The second group includes species used as spices and condiments for pickling and salting of cabbage, cucumbers,

tomatoes and other vegetables, as well as mushrooms: caraway, juniper, and thyme (Rubtsov, 1971; Gubanov et al., 1976; Koscheev, 1981; Tcherepnin, 1987; Litvintsev and Koscheev, 1988; Grisjuk et al., 1989; Sõukand et al., 2013).

CONCLUSIONS

This review focuses on botanical drugs with a monograph in the Russian Pharmacopoeia 11th edition and their potential as untapped resources beyond their strictly medical uses. In general, the Soviet period was characterized by the country being closed not only from a political point of view, but also scientifically. Most commonly, research output was only available in Russian and not translated into English dramatically restricting its availability to the international community. Here such information published in Russian was evaluated focusing on medicinal species from the Russian (Soviet) Pharmacopoeia and used as a food. This highlights the importance of the Russian Pharmacopoeia as a source of information on plant species used traditionally at the interface of food and medicine.

Clearly our approach has the limitation of only focusing on species included in the pharmacopeia and, consequently, local or endemic species are mostly excluded. However, in the context of developing high value products with potential health benefits priority needs to be given to species which are widely available and not at risk of being overexploited if the demand increases.

The evidence for the individual species varies, and while we do not claim that their use could be, based on the current data, evidence-based, the review provides a basis for further research and development.

"Functional food" are foods that not only serve to provide nutrition but also can be a source for prevention and cure of various diseases. This review highlights the potential of wild Russian species monographed in its pharmacopeia for further developing new functional foods and—through the lens of their incorporation into the pharmacopeia—showcases the species' importance in Russia.

AUTHOR CONTRIBUTIONS

AS, AT, OP, and VM have written the first draft of the manuscript. AS, OP, and MH revised and improved the various drafts and revisions. All authors have seen and agreed on the final version of the manuscript.

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frontiers in Pharmacology

REVIEW
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Cornus mas and *Cornus Officinalis*—Analogies and Differences of Two Medicinal Plants Traditionally Used

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Among 65 species belonging to the genus *Cornus* only two, *Cornus mas* L. and *Cornus officinalis* Sieb. et Zucc. (Cornaceae), have been traditionally used since ancient times. *Cornus mas* (cornelian cherry) is native to southern Europe and southwest Asia, whereas *C. officinalis* (Asiatic dogwood, cornel dogwood) is a deciduous tree distributed in eastern Asia, mainly in China, as well as Korea and Japan. Based on the different geographic distribution of the closely related species but clearly distinct taxa, the ethnopharmacological use of *C. mas* and *C. officinalis* seems to be independently originated. Many reports on the quality of *C. mas* fruits were performed due to their value as edible fruits, and few reports compared their physicochemical properties with other edible fruits. However, the detailed phytochemical profiles of *C. mas* and *C. officinalis*, in particular fruits, have never been compared. The aim of this review was highlighting the similarities and differences of phytochemicals found in fruits of *C. mas* and *C. officinalis* in relation to their biological effects as well as compare the therapeutic use of fruits from both traditional species. The fruits of *C. mas* and *C. officinalis* are characterized by the presence of secondary metabolites, in particular iridoids, anthocyanins, phenolic acids and flavonoids. However, much more not widely known iridoids, such as morroniside, as well as tannins were detected particularly in fruits of *C. officinalis*. The referred studies of biological activity of both species indicate their antidiabetic and hepatoprotective properties. Based on the available reports antihyperlipidemic and anticoagulant activity seems to be unique for extracts of *C. mas* fruits, whereas antiosteoporotic and immunomodulatory activities were assigned to preparations of *C. officinalis* fruits. In conclusion, the comparison of phytochemical composition of fruits from both species revealed a wide range of similarities as well as some constituents unique for cornelian cherry or Asiatic dogwood. Thus, these phytochemicals are considered the important factor determining the biological activity and justifying the use of *C. mas* and *C. officinalis* in the traditional European and Asiatic medicine.

Keywords: ***Cornus mas*, *Cornus officinalis*, cornelian cherry, cornel dogwood, traditional Chinese medicine**

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INTRODUCTION

Recently published ethnopharmacological studies regarding the genus *Cornus* (Cornaceae) have shown that among 65 species only two of them have a long tradition in a medicinal use. *Cornus mas* L. native to southern Europe and southwest Asia, known as cornelian cherry, is used since ancient times (Dragendorff, 1898). The fruits of this plant were used for a broad variety of diseases and complaints in all areas of its geographical distribution. The preparations from *C. mas* (European cornel, cornelian cherry) were considered as astringent, tonic and antipyretic remedies (Fournier, 1948). *Cornus officinalis* Sieb. et Zucc., also named Asiatic dogwood (cornel dogwood, Japanese cornel), is a deciduous tree distributed in eastern Asia, mainly in China, as well as Korea and Japan (Ma et al., 2014). It was used as a traditional Chinese medicine for more than 2,000 years to promote a healthy liver and kidney, and is a common ingredient of many TCM prescriptions (Liu X. et al., 2011).

The dogwood genus *Cornus* L. is a member of Cornales in the Asterids clade (The Angiosperm Phylogeny Group; APG IV, 2016). The dogwood genus is morphologically heterogenous, variable in inflorescences, fruits and chromosomes (Eyde, 1988; Xiang et al., 2005; Ma et al., 2017). Four major lineages of the dogwood genus *Cornus* L., such as the blue- or white-fruited group, the cornelian cherry group, the big-bracted group and the dwarf dogwood group, have been drawn (Xiang et al., 2006, 2008). However, the systematical classification of flowering plants from Cornales clade and relationships within this taxon seems to be problematic for many years (Xiang et al., 1996, 1998, 2002, 2006; Fan and Xiang, 2001; APG IV, 2016). Despite these controversies, phylogenetic analyses of this clade based on four DNA regions, such as *rbcL* and *matK* from chloroplast DNA genome as well as 26S rDNA and ITS (internal transcribed spacer) 1–5. 8S rDNA from the nuclear genome, classified both *C. mas* and *C. officinalis* in the group of cornelian cherries (Xiang et al., 1998, 2002, 2006; Fan and Xiang, 2001). In addition, the subgenera *Afrocarnia* (Harms) Wangerin, *Cornus* L. and *Sinocornus* Q.Y. Xiang were included into the group of cornelian cherries. *Cornus mas* and *C. officinalis* were classified in the subgenus *Cornus* L. (Fan and Xiang, 2001; Xiang et al., 2006). Both of them are temperate deciduous small shrubs or trees blooming before leaf development. The morphological characters, such as umbellate cymes terminal subtended by four not showy scale-like bracts, red or purple black fruits and the wall of fruit stone riddled with cavities, supported the membership of these species in the monophyletic group of cornelian cherries. All these data strongly supported the sister phylogenetic relationship of *C. mas* and *C. officinalis* (Xiang et al., 2005). The genus *Cornus* is widely distributed in the northern hemisphere. Fossil fruit stones similar to the contemporary fruit stones of *C. mas* are likely to derive from the late Miocene of Poland, as well as from Pliocene and Pleistocene of France, Germany and Netherlands. Evidence from analyses of fossils, phylogeny and biogeographic distribution support an origin and early diversification of *Cornus* likely in Europe. It is suggested that the ancestor of the living cornelian cherries spread from Europe to eastern Asia around the mid-Oligocene and the mid-Miocene. *Cornus mas* is a single representative species of cornelian cherry in Europe. On the other hand, *C. officinalis* in eastern Asia represents the Miocene migrant from Europe (Xiang et al., 2005). Based on the different geographic distribution of the closely related species but clearly distinct taxa, the ethnopharmacological use of *C. mas* and *C. officinalis* seems to be independently originated. In the traditional medicine of Europe and Middle East only fruits of *C. mas* were used and in the traditional medicine of China reports describe only preparations of fruits from *C. officinalis* for medicinal application.

Plant materials belonging to the genus *Cornus* are characterized by the presence of secondary metabolites, in particular iridoids, anthocyanins, tannins and flavonoids. Although many reports on the quality of fruits of *C. mas* were performed due to the fact of their value as edible fruits, as well as few studies compared their properties with other edible fruits used for a production of wines, liquors or jams (Tarko et al., 2014; Cosmulescu et al., 2017), the detailed phytochemical profiles of *C. mas* and *C. officinalis*, in particular fruits, have never been compared. The chemical composition of plant materials obtained from *C. mas* and *C. officinalis* can be significantly different depending on the geographic location, climatic factors, season, as well as cultivation practice. For this reason, the phytochemical composition is considered an important factor determining the biological activity and justifying the traditional use of these species.

All these facts taking together make interesting to compare the therapeutic use of both traditional species based on their chemical composition. This review particularly highlights the similarities and differences in phytochemicals found in fruits of *C. mas* and *C. officinalis* in relation to their biological effects based on reports available in this field.

SIMILARITIES AND DIFFERENCES IN THE CHEMICAL COMPOSITION OF FRUITS FROM *C. MAS* AND *C. OFFICINALIS*

General

The main secondary metabolites found in dogwoods seem to be iridoid and non-flavonoid glucosides. Even though the blue-fruited dogwoods contain non-flavonoid glucoside salidroside derived from shikimic acid, iridoid glucosides were found to be unique in all red-fruited dogwoods, and they are derived from mevalonic acid (Bate-Smith et al., 1975; Jensen et al., 1975). The dogwoods iridoids were classified into three groups, including cornin and its precursor dihydrocornin, monotropein and its precursor geniposide as well as secoiridoids, secologanin and morroniside (Bate-Smith et al., 1975). Thus, the presence of iridoids (**Figures 1**, **2**) in red-fruited dogwoods is considered a symplesiomorphy (Xiang et al., 1996), and confirms the close taxonomical relationship of *C. mas* and *C. officinalis*. The representative compounds of this group, such as loganin, loganic acid, cornin, sweroside, and cornuside, have been found in both species.

Furthermore, the polyphenolic compounds are represented by anthocyanins, flavonoids and phenolic acids in fruits of *C. mas* and *C. officinalis*. Pelargonidin 3-*O*-galactoside followed by

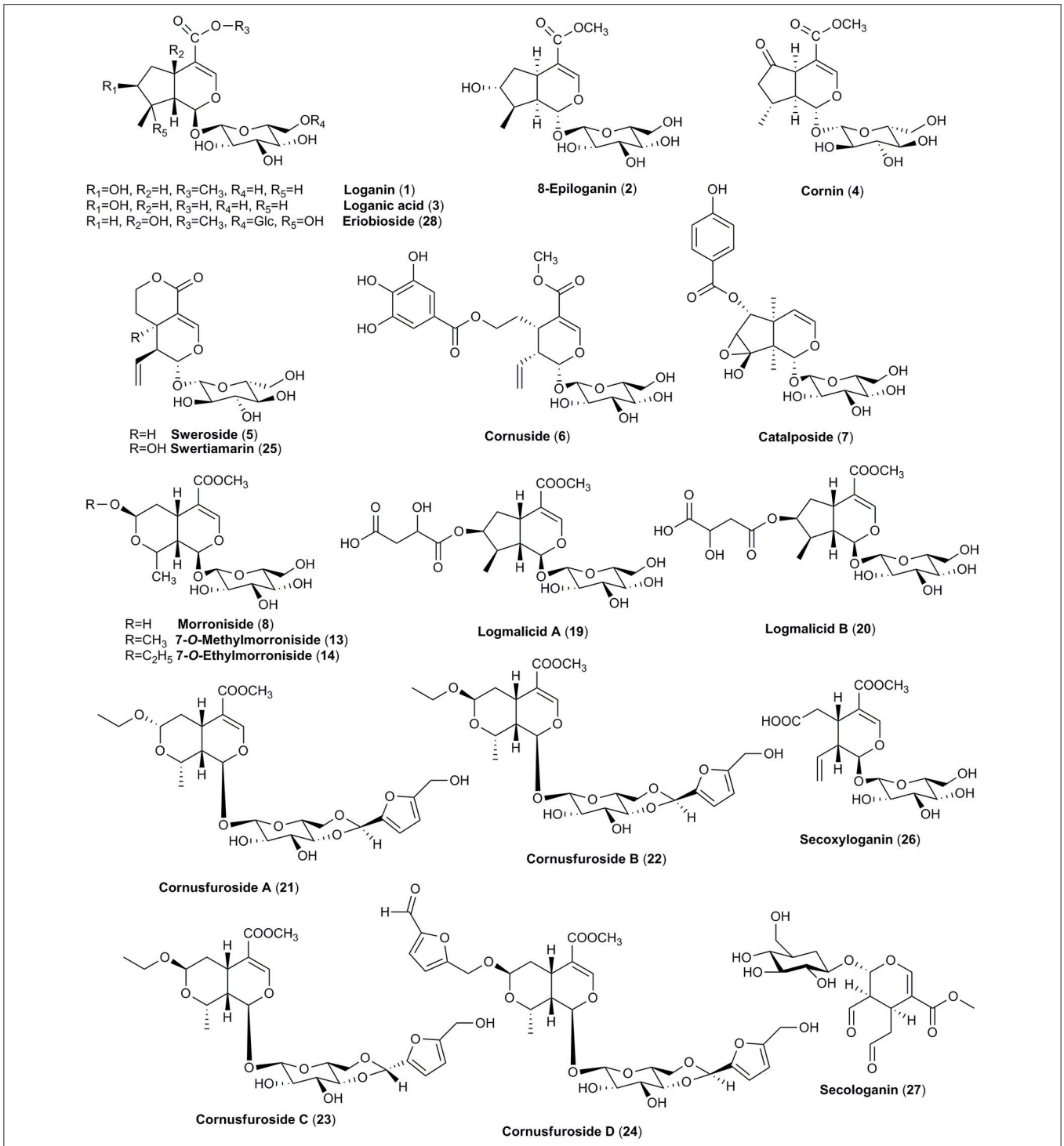

FIGURE 1 | The chemical structures of iridoids identified in fruits of *C. mas* and/or *C. officinalis*.

cyanidin 3-*O*-galactoside and delphinidin 3-*O*-galactoside were the most abundant anthocyanins in fruits of both species. Among flavonoids, quercetin, and kaempferol glycosides were detected in both species. Taking into consideration that flavonoids are widely distributed in plants, their occurrence was considered the similar characteristic for both species. Nevertheless, some discrepancy in the flavonoid composition occurs. Even more flavonoids have been referred by Huang et al. (2018) but full availability of the studies concerning their identification or isolation is rather limited.

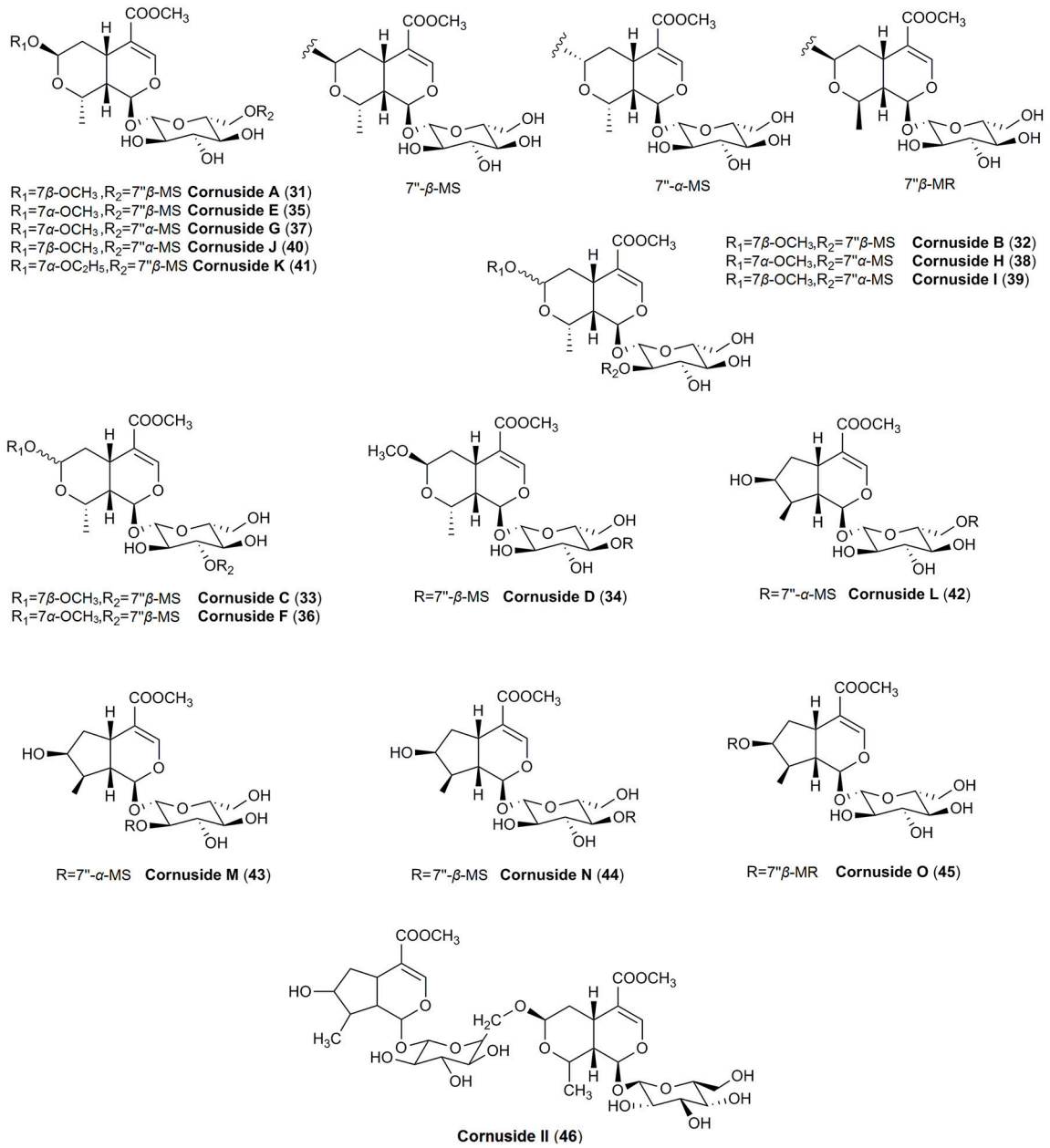

FIGURE 2 | The chemical structures of iridoids identified in fruits of *C. officinalis*.

Last but not least, the similar constituents of *C. mas* and *C. officinalis* are considered triterpenoids, in particular represented by ursolic acid detected in both species, and carbohydrates. It is worth to note that neither in fruits of *C. mas* nor in fruits of *C. officinalis* phenylpropanoid esters have been reported to date, even though they usually co-exist with iridoids as it is observed in some plants from Oleaceae, Scrophulariaceae, Verbenaceae, Plantaginaceae, and Lamiaceae. This might be justified by the less significant role of shikimate pathway in biosynthesis of compounds in red-fruited dogwoods. **Table 1** summarizes the key phytochemicals of fruits from *C. mas* and *C. officinalis* that can be found in the literature. The names of compounds have been provided as they were described in the referred literature. Due to the fact that HPLC-MS is currently one of the most popular method for elucidation of chemical composition of plant preparations, the molecular weights of phytochemicals were also listed (**Table 1**) to help reader with comparing experimental data in order to preliminary identification detected constituents.

Similarities of Chemical Composition

Iridoids

It is believed that particularly loganin (**1**) plays a crucial role in the formation sequences of highly oxidized glucosides of iridoids

TABLE 1 | The comparison of phytochemical constituents from fruits of *C. mas* and *C. officinalis*.

Compound no.	Compound name	Molecular weight	*C. mas*	*C. officinalis*	References
IRIDOIDS					
1	Loganin	390	X	X	Du et al., 2008; Yokozawa et al., 2010; Cao et al., 2011, 2012; Lin et al., 2011; Lee A. Y. et al., 2012; Lee N. H. et al., 2012; Xie et al., 2012; Cai et al., 2013; Deng et al., 2013; Ma et al., 2014; Perova et al., 2014; He et al., 2016; Jiang et al., 2016; Ye et al., 2017
2	8-Epiloganin	390		X	Ye et al., 2017
3	Loganic acid	376	X	X	Yokozawa et al., 2010; Cao et al., 2011, 2012; West et al., 2012b; Deng et al., 2013; Ma et al., 2014; Sozanski et al., 2014, 2016; Kucharska et al., 2015; Antolak et al., 2017; Ji et al., 2017
4	Cornin (verbenalin)	388	X	X	Cao et al., 2011; Liu Z. et al., 2011; Cai et al., 2013; Perova et al., 2014; Jiang et al., 2016
5	Sweroside	358	X	X	Du et al., 2008; Cao et al., 2011, 2012; Liu Z. et al., 2011; Cai et al., 2013; Deng et al., 2013; Perova et al., 2014; Jiang et al., 2016; Ye et al., 2017
6	Cornuside	542	X	X	Hatano et al., 1990; Du et al., 2008; Lin et al., 2011; Liu Z. et al., 2011; Lee A. Y. et al., 2012; Deng et al., 2013; Ma et al., 2014; Perova et al., 2014; Sozanski et al., 2014; Kucharska et al., 2015; Jiang et al., 2016; Antolak et al., 2017
7	Catalposide	482	X		Sochor et al., 2014
8	Morroniside	406		X	Du et al., 2008; Yokozawa et al., 2010; Cao et al., 2011, 2012; Lin et al., 2011; Lee A. Y. et al., 2012; Lee N. H. et al., 2012; Cai et al., 2013; He et al., 2016; Jiang et al., 2016; Ji et al., 2017
9	7-α-*O*-Methylmorroniside	420		X	Ma et al., 2014; Jiang et al., 2016; Ye et al., 2017
10	7-β-*O*-Methylmorroniside	420		X	Xie et al., 2012; Ma et al., 2014; Jiang et al., 2016; Ye et al., 2017
11	7-α-*O*-Ethylmorroniside	434		X	Cao et al., 2012; Ma et al., 2014; Jiang et al., 2016; Ye et al., 2017
12	7-β-*O*-Ethylmorroniside	434		X	Cao et al., 2012; Ma et al., 2014; Jiang et al., 2016; Ye et al., 2017
13	7-*O*-Methylmorroniside	420		X	Liu Z. et al., 2011; Cai et al., 2013; Ji et al., 2017
14	7-*O*-Ethylmorroniside	434		X	Cao et al., 2011; Ji et al., 2017
15	7-β-*O*-Dimethyl butanedioate morroniside	550		X	Park et al., 2016
16	(7*R*)-*n* butyl morroniside	462		X	Lin et al., 2011
17	7-α-Morroniside	406		X	Xie et al., 2012; Ma et al., 2014; Ye et al., 2017
18	7-β-Morroniside	406		X	Xie et al., 2012; Ma et al., 2014; Ye et al., 2017
19	Logmalicid A	506		X	Ma et al., 2014
20	Logmalicid B	506		X	Ma et al., 2014
21	Cornusfuroside A	542		X	He et al., 2017
22	Cornusfuroside B	542		X	He et al., 2017
23	Cornusfuroside C	542		X	He et al., 2017
24	Cornusfuroside D	622		X	He et al., 2017
25	Swertiamarin	374		X	Cao et al., 2011; Liu Z. et al., 2011
26	Secoxyloganin	404		X	Ma et al., 2014
27	Secologanin (loniceroside)	388		X	Jensen et al., 1973
28	Eriobioside	568		X	Liu Z. et al., 2011
29	7-Dehymorroniside	404		X	Cao et al., 2012
30	7-*O*-methyloganic acid	390		X	Cao et al., 2012
31	Cornuside A	809		X	Ye et al., 2017
32	Cornuside B	809		X	Ye et al., 2017

(Continued)

TABLE 1 | Continued

Compound no.	Compound name	Molecular weight	*C. mas*	*C. officinalis*	References
33	Cornuside C	809		X	Ye et al., 2017
34	Cornuside D	809		X	Ye et al., 2017
35	Cornuside E	809		X	Ye et al., 2017
36	Cornuside F	809		X	Ye et al., 2017
37	Cornuside G	809		X	Ye et al., 2017
38	Cornuside H	809		X	Ye et al., 2017
39	Cornuside I	809		X	Ye et al., 2017
40	Cornuside J	809		X	Ye et al., 2017
41	Cornuside K	823		X	Ye et al., 2017
42	Cornuside L	779		X	Ye et al., 2017
43	Cornuside M	779		X	Ye et al., 2017
44	Cornuside N	779		X	Ye et al., 2017
45	Cornuside O	779		X	Ye et al., 2017
46	Cornuside II	779		X	Liu Z. et al., 2011
47	Cornuside III	506		X	Wang et al., 2017
48	Cornuside IV	506		X	Wang et al., 2017
49	Corniside I	779		X	Cao et al., 2012
50	Corniside II	542		X	Cao et al., 2011, 2012
51	Williamsoside D	822		X	Ye et al., 2017
ANTHOCYANINS					
52	Pelargonidin	271	X		Sozanski et al., 2016
53	Pelargonidin 3-*O*-galactoside	433	X	X	Du and Francis, 1973; Seeram et al., 2002; Jayaprakasam et al., 2006; Vareed et al., 2006; Sozanski et al., 2014, 2016; Kucharska et al., 2015
54	Pelargonidin 3-*O*-glucoside	433	X		Jayaprakasam et al., 2005; Tural and Koca, 2008; Pawlowska et al., 2010; Capanoglu et al., 2011; Milenković-Andjelković et al., 2015; Antolak et al., 2017
55	Pelargonidin 3-*O*-robinobioside (pelargonidin 3-*O*-rhamnogalactoside)	579	X		Du and Francis, 1973; Sozanski et al., 2014, 2016; Kucharska et al., 2015; Antolak et al., 2017
56	Pelargonidin 3-*O*-rutinoside	579	X		Pawlowska et al., 2010
57	Cyanidin	287	X		Sozanski et al., 2016
58	Cyanidin 3-*O*-galactoside	449	X	X	Du and Francis, 1973; Seeram et al., 2002; Jayaprakasam et al., 2005, 2006; Vareed et al., 2006; Pawlowska et al., 2010; Begic-Akagic et al., 2013; Drkenda et al., 2014; Sozanski et al., 2014, 2016; Kucharska et al., 2015; Milenković-Andjelković et al., 2015
59	Cyanidin 3-*O*-glucoside	449	X		Tural and Koca, 2008; Capanoglu et al., 2011; Antolak et al., 2017
60	Cyanidin 3-*O*-robinobioside (cyanidin 3-*O*-rhamnogalactoside)	595	X		Du and Francis, 1973; Sozanski et al., 2014, 2016; Kucharska et al., 2015; Antolak et al., 2017
61	Cyanidin 3-*O*-rutinoside	595	X		Du and Francis, 1973; Tural and Koca, 2008; Capanoglu et al., 2011; Begic-Akagic et al., 2013; Drkenda et al., 2014
62	Peonidin 3-*O*-glucoside	464	X		Begic-Akagic et al., 2013; Drkenda et al., 2014
63	Delphinidin 3-*O*-glucoside	465		X	Jayaprakasam et al., 2005
64	Delphinidin 3-*O*-galactoside	465	X	X	Du and Francis, 1973; Seeram et al., 2002; Jayaprakasam et al., 2006; Vareed et al., 2006; Sozanski et al., 2014, 2016; Kucharska et al., 2015; Milenković-Andjelković et al., 2015
65	Petunidin 3-glucoside	479	X		Antolak et al., 2017

(Continued)

TABLE 1 | Continued

Compound no.	Compound name	Molecular weight	*C. mas*	*C. officinalis*	References
FLAVONOIDS					
Flavonols					
66	Quercetin	302	X	X	Kim and Kwak, 1998; Xie et al., 2012; Radovanović et al., 2013; Drkenda et al., 2014; Ma et al., 2014; Sochor et al., 2014; Antolak et al., 2017; Cosmulescu et al., 2017
67	Quercetin 3-*O*-β-D-xyloside (avicularin)	434	X		Pawlowska et al., 2010; Begic-Akagic et al., 2013; Drkenda et al., 2014
68	Quercetin 3-*O*-α-L-rhamnoside (quercitrin)	448	X		Pawlowska et al., 2010; Begic-Akagic et al., 2013; Drkenda et al., 2014; Sochor et al., 2014; Popović et al., 2017; Bajić-Ljubičić et al., 2018
69	Quercetin 3-*O*-β-D-glucoside (isoquercetin, isoquercitrin)	464	X	X	Pawlowska et al., 2010; Liu Z. et al., 2011; Xie et al., 2012; Begic-Akagic et al., 2013; Drkenda et al., 2014; Ma et al., 2014; Milenković-Andjelković et al., 2015; Antolak et al., 2017; Popović et al., 2017; Bajić-Ljubičić et al., 2018
70	Quercetin 3-*O*-β-D-galactoside (hyperoside)	464	X	X	Pawlowska et al., 2010; Xie et al., 2012; Begic-Akagic et al., 2013; Drkenda et al., 2014; Ma et al., 2014; Milenković-Andjelković et al., 2015; Popović et al., 2017; Bajić-Ljubičić et al., 2018
71	Quercetin 3-*O*-β-D-glucuronide (querciturone)	478	X	X	Pawlowska et al., 2010; Lin et al., 2011; Liu Z. et al., 2011; Popović et al., 2017; Bajić-Ljubičić et al., 2018
72	Quercetin 3-*O*-rutinoside (rutin)	610	X		Pawlowska et al., 2010; Begic-Akagic et al., 2013; Deng et al., 2013; Radovanović et al., 2013; Drkenda et al., 2014; Sochor et al., 2014; Milenković-Andjelković et al., 2015; Rudrapaul et al., 2015; Cosmulescu et al., 2017; Popović et al., 2017; Bajić-Ljubičić et al., 2018
73	Quercetin 3-*O*-robinobioside	610	X		Begic-Akagic et al., 2013; Drkenda et al., 2014
74	Quercetin 3-*O*-β-D-glucuronide methyl ester	492		X	Ma et al., 2014
75	Quercetin 3-*O*-β-D-(6″-*n*-butyl glucuronide)	534		X	Lin et al., 2011
76	Kaempferol	286		X	Kim and Kwak, 1998; Xie et al., 2012
77	Kaempferol 3-*O*-β-D-galactoside (trifolin)	448	X	X	Pawlowska et al., 2010; Ma et al., 2014
78	Kaempferol 3-glucoside	448	X		Begic-Akagic et al., 2013; Drkenda et al., 2014; Milenković-Andjelković et al., 2015
79	Kaempferol 3-*O*-β-D-rutinoside	594		X	Ma et al., 2014
80	4′-Methylkaempferol (kaempferide)	300		X	Xie et al., 2012
81	Myricetin	318	X		Rudrapaul et al., 2015; Cosmulescu et al., 2017
82	Myricetin 3-galactoside	480	X		Antolak et al., 2017
83	2*R*, 3*R*-*trans*-Aromadendrin	288	X		Rudrapaul et al., 2015
84	Aromadendrin 7-*O*-β-D-glucoside (sinensin)	450	X		Pawlowska et al., 2010
85	Naringenin 7-*O*-methylether	286	X		Rudrapaul et al., 2015
86	7,3′-Dihydroxy-5,4′-dimethoxyflavanone	316	X		Rudrapaul et al., 2015
87	4-Acetoxy-5,2′,4′,6′-β-pentahydroxy-3-methoxychalcone	376	X		Rudrapaul et al., 2015
Flavan-3-ols					
88	(+) Catechin	290	X		Radovanović et al., 2013; Milenković-Andjelković et al., 2015

(Continued)

TABLE 1 | Continued

Compound no.	Compound name	Molecular weight	*C. mas*	*C. officinalis*	References
89	(-) Epicatechin	290	X		Radovanović et al., 2013; Milenković-Andjelković et al., 2015; Cosmulescu et al., 2017
90	(-) Epicatechin-3-*O*-gallate	442		X	Lin et al., 2011
91	Procyanidin B1	578	X		Begic-Akagic et al., 2013; Drkenda et al., 2014
92	Procyanidin B2	578	X		Begic-Akagic et al., 2013; Radovanović et al., 2013; Drkenda et al., 2014; Milenković-Andjelković et al., 2015
PHENOLIC ACIDS					
93	Gallic acid	170	X	X	Tian et al., 2000; Du et al., 2008; Cao et al., 2011; Lin et al., 2011; Liu Z. et al., 2011; Cao et al., 2012; Youn and Jun, 2012; Cai et al., 2013; Deng et al., 2013; Radovanović et al., 2013; Sochor et al., 2014; Milenković-Andjelković et al., 2015; Rudrapaul et al., 2015; Jiang et al., 2016; Antolak et al., 2017; Cosmulescu et al., 2017
94	Caffeic acid	180	X	X	Lin et al., 2011; Radovanović et al., 2013; Ma et al., 2014; Cosmulescu et al., 2017
95	*p*-Coumaric acid (coumaric acid)	164	X	X	Youn and Jun, 2012; Radovanović et al., 2013; Antolak et al., 2017; Cosmulescu et al., 2017
96	Ellagic acid	302	X	X	Cao et al., 2012; Milenković-Andjelković et al., 2015; Cosmulescu et al., 2017
97	Protocatechuic acid	154	X	X	Liu Z. et al., 2011; Antolak et al., 2017
98	Benzoic acid	122	X		Krivoruchko, 2014
99	Cinnamic acid (*trans*-cinnamic acid)	148	X		Antolak et al., 2017; Cosmulescu et al., 2017
100	Ferulic acid	194	X		Krivoruchko, 2014; Antolak et al., 2017; Cosmulescu et al., 2017
101	Sinapic acid	224	X		Cosmulescu et al., 2017
102	Salicylic acid	138	X		Krivoruchko, 2014; Cosmulescu et al., 2017
103	Syringic acid	198	X		Radovanović et al., 2013; Cosmulescu et al., 2017
104	Vanillic acid	168	X		Krivoruchko, 2014; Cosmulescu et al., 2017
105	Rosmarinic acid	360	X		Antolak et al., 2017
106	Chlorogenic acid	354	X		Begic-Akagic et al., 2013; Deng et al., 2013; Drkenda et al., 2014; Sochor et al., 2014; Milenković-Andjelković et al., 2015; Cosmulescu et al., 2017
107	Neochlorogenic acid	354	X		Popović et al., 2017; Bajić-Ljubičić et al., 2018
108	Tachioside	302		X	Ma et al., 2014
109	3,5-Dihydroxy-2-(2-methoxy-2-oxoethyl) phenyl 4-hydroxybenzoate	318		X	Ma et al., 2014
110	Caffeoyltartaric acid dimethyl ester	340		X	Park et al., 2016
111	Caftaric acid monomethyl ester	326		X	Lin et al., 2011
TRITERPENOIDS					
112	Ursolic acid	456	X	X	Jayaprakasam et al., 2006; Wang H. et al., 2008; Yu et al., 2009; Youn and Jun, 2012; He et al., 2016
113	Oleanolic acid	456		X	Wang H. et al., 2008; Lin et al., 2011
114	Arjunglucoside I	666		X	Liu Z. et al., 2011
115	Arjunglucoside II	650		X	Liu Z. et al., 2011
CARBOHYDRATES					
116	Calcium pectate	–	X		Bilejić et al., 2011; Jaćimović et al., 2015
117	FCAP1	–		X	Yang et al., 2010
118	PFCC-I	75,700		X	Li et al., 2012
119	PFCA-III	17,400		X	Li et al., 2012

(Continued)

TABLE 1 | Continued

Compound no.	Compound name	Molecular weight	*C. mas*	*C. officinalis*	References
TANNINS					
120	7-*O*-galloyl-D-sedoheptulose	362		X	Yamabe et al., 2009; Yokozawa et al., 2010; Liu Z. et al., 2011; Park et al., 2015
121	2,3-Di-*O*-galloyl-β-D-glucose	484		X	Okuda et al., 1984; Hatano et al., 1989a; Cao et al., 2011, 2012
122	1,7-Di-*O*-galloyl-D-glucose	484		X	Liu Z. et al., 2011
123	1,2,3-Tri-*O*-galloyl-β-D-glucose	636		X	Hatano et al., 1989a; Cao et al., 2011, 2012; Bhakta et al., 2017
124	1,2,6-Tri-*O*-galloyl-β-D-glucose	636		X	Okuda et al., 1984; Hatano et al., 1989a
125	1,2,3,6-Tetra-*O*-galloyl-β-D-glucose	788		X	Okuda et al., 1984; Hatano et al., 1989a, 1990; Cao et al., 2011, 2012; Bhakta et al., 2017
126	Tellimagrandin I	786		X	Okuda et al., 1984; Hatano et al., 1989a; Cao et al., 2012; Bhakta et al., 2017
127	Tellimagrandin II (tellimagrandin-α, eugeniin)	938		X	Okuda et al., 1984; Hatano et al., 1989a, 1990; Cao et al., 2011, 2012; Bhakta et al., 2017
128	Cornusiin A	1,571		X	Okuda et al., 1984; Hatano et al., 1989a
129	Cornusiin B	1,087		X	Okuda et al., 1984; Hatano et al., 1989a
130	Cornusiin C	2,572		X	Hatano et al., 1989a
131	Cornusiin D	1,722		X	Hatano et al., 1989b
132	Cornusiin E	1,875		X	Hatano et al., 1989b
133	Cornusiin F	2,202		X	Hatano et al., 1989b
134	Cornusiin G	1,725		X	Hatano et al., 1990
135	Camptothin A	1,419		X	Hatano et al., 1989b
136	Camptothin B	1,723		X	Hatano et al., 1989b
137	Gemin D (3-*O*-galloyl-4,6-(S)-hexahydroxydiphenoyl-D-glucose)	634		X	Okuda et al., 1984; Hatano et al., 1989a
138	Oenothein C	784		X	Okuda et al., 1984; Hatano et al., 1989a
139	Isoterchebin	955		X	Okuda et al., 1981, 1984; Hatano et al., 1989a; Bhakta et al., 2017
CAROTENOIDS					
140	(13*Z*)+(13′*Z*)-Lutein	568	X		Horváth et al., 2007
141	(9*Z*)+(9′*Z*)-Lutein	568	X		Horváth et al., 2007
142	(9′*Z*)-Neoxanthin	601	X		Horváth et al., 2007
143	(*E*)-Neoxanthin	601	X		Horváth et al., 2007
144	Lutein-5,6-epoxide	585	X		Horváth et al., 2007
145	Luteoxanthin (epimers)	601	X		Horváth et al., 2007
146	Neochrome (epimers)	553	X		Horváth et al., 2007
147	β-carotene	537	X		Horváth et al., 2007
148	β-carotene-5,6-monoepoxide	553	X		Horváth et al., 2007
149	β-cryptoxanthin	553	X		Horváth et al., 2007
FATTY ACIDS					
150	Behenic acid	340	X		Krivoruchko, 2014
151	Lauric acid	200	X		Brindza, 2009; Krivoruchko, 2014
152	Linoleic acid	280	X		Brindza, 2009; Krivoruchko, 2014
153	Linolenic acid	278	X		Brindza, 2009; Krivoruchko, 2014
154	Myristic acid	228	X		Brindza, 2009
155	Oleic acid	282	X		Brindza, 2009; Krivoruchko, 2014
156	Palmitic acid	256	X		Brindza, 2009; Krivoruchko, 2014
157	Plamitoleic acid	254	X		Brindza, 2009; Krivoruchko, 2014
158	Pentadecenic acid	240	X		Brindza, 2009
159	Pentadecanoic acid	242	X		Krivoruchko, 2014

(Continued)

TABLE 1 | Continued

Compound no.	Compound name	Molecular weight	*C. mas*	*C. officinalis*	References
160	Stearic acid	284	X		Brindza, 2009; Krivoruchko, 2014
161	Vaccenic acid	282	X		Brindza, 2009
162	*cis*-10,12-Octadecadienic acid	280	X		Brindza, 2009
OTHER CONSTITUENTS					
163	5-Hydroxymethylfurfural (5-Hydroxymethyl-2-furaldehyde)	126		X	Du et al., 2008; Yokozawa et al., 2010; Cao et al., 2011, 2012; Cai et al., 2013; Jiang et al., 2016
164	Dimethyltetrahydrofuran *cis*-2,5-dicarboxylate	188		X	Kim and Kwak, 1998
165	β-sitosterol	415		X	Lin et al., 2011
166	Daucosterol-6′-malate	692		X	Xie et al., 2012
167	(1′*S*, 2′*R*)-guaiacyl glycerol 3′-O-β-D-glucoside	376		X	Ma et al., 2014
168	Mevaloside	292		X	Yokozawa et al., 2010
169	2,7-Anhydro-D-sedoheptulose (sedoheptulosan)	192		X	Liu Z. et al., 2011
170	Resveratrol	228	X		Sochor et al., 2014
171	Malic acid	134	X	X	Cao et al., 2012; Begic-Akagic et al., 2013; Deng et al., 2013; Drkenda et al., 2014; Krivoruchko, 2014
172	Malic acid methyl ester	148		X	Cao et al., 2011, 2012
173	Dimethyl malate	162		X	Miyazawa et al., 2003
174	Tartaric acid	150	X	X	Cao et al., 2012; Begic-Akagic et al., 2013; Deng et al., 2013; Drkenda et al., 2014
175	Butoxysuccinic acid	190		X	Lin et al., 2011
176	Citric acid	192	X		Begic-Akagic et al., 2013; Deng et al., 2013; Drkenda et al., 2014
177	Succinic acid	118		X	Zhang Q. C. et al., 2013

as well as in the biosynthesis of secoiridoids glucosides and indole alkaloids. The cyclization to a hemiacetal is likely to take place through the incorporation of an oxygen function into the C-8 position of secologanin (**27**) derived from loganin (**1**) to generate further iridoid derivatives (Inouye et al., 1974). Secoiridoids are believed to be even more advanced due to the more complicated biostynthetic steps (Xiang et al., 1996). Thus, the most closely related in the iridoids transformation pathway compounds, such as loganin (**1**), loganic acid (**3**), cornin (**4**), sweroside (**5**), and representative of secoiridoids, cornuside (**6**), have been detected both in fruits of *C. mas* and *C. officinalis* (Deng et al., 2013; Ma et al., 2014; Perova et al., 2014; Sochor et al., 2014; Kucharska et al., 2015; Jiang et al., 2016). However, it should be noted that in the 26 cultivars of *C. mas* it was shown that the most abundant iridoid was loganic acid (**3**) (81.5–461.1 mg/100 g fw) constituting 88–96% of total iridoids (86.9–493.7 mg/100 g fw). Cornuside (**6**) has already followed loganic acid (**3**) but its content was much lower than loganic acid (**3**) and maximally reached 36.2 mg/100 g fw (West et al., 2012a; Kucharska et al., 2015). Only two reports describe the identification of loganin (**1**) and sweroside (**5**) in fruit of *C. mas* (Deng et al., 2013; Perova et al., 2014). Nevertheless, the reason of limited data on identification of loganin and sweroside in fruits of cornelian cherry is due to their low concentration compared to loganic acid. More detailed studies are needed in this field.

Anthocyanins

The mature fruits from both *C. mas* and *C. officinalis* are dark red colored and for this reason they might be easily confused. Thus, in addition to iridoids (**Figures 1**, **2**), anthocyanins (**Figure 3**), red colorant compounds, are widely distributed in fruits of these dogwoods. The content of anthocyanins increases when the fruits change their color from light yellow to dark red (Gunduz et al., 2013). Fruits of cornelian cherry and Asiatic dogwood contain in particular pelargonidin (**52**) and cyanidin (**57**) derivatives. Both in *C. mas* and *C. officinalis* pelargonidin 3-*O*-galactoside (**53**), cyanidin 3-*O*-galactoside (**58**), and delphinidin 3-*O*-galactoside (**64**) were detected and quantified. Pelargonidin 3-*O*-galactoside (**53**) (1.62 mg/g) and cyanidin 3-*O*-galactoside (**58**) (1.66 mg/g) have been noted as the most abundant anthocyanins of *C. mas* fresh fruit (Jayaprakasam et al., 2006; Vareed et al., 2006; Sozanski et al., 2014; Kucharska et al., 2015). The amount of pelargonidin 3-*O*-galactoside (**53**) in fruit of *C. officinalis* (0.78 mg/g) was approximately half of the concentration determined in fruit of *C. mas*. The other detected anthocyanins of *C. officinalis* fruit were present even at lower concentrations (0.15–0.21 mg/g; Vareed et al., 2006). In the literature, more attention has been focused on anthocyanins of *C. mas* (Pantelidis et al., 2007; Popović et al., 2012; Bilejić et al., 2015; Kucharska et al., 2015). These pigments were identified using methods such as HPLC, LC-ES/MS and NMR (Seeram et al., 2002; Kucharska

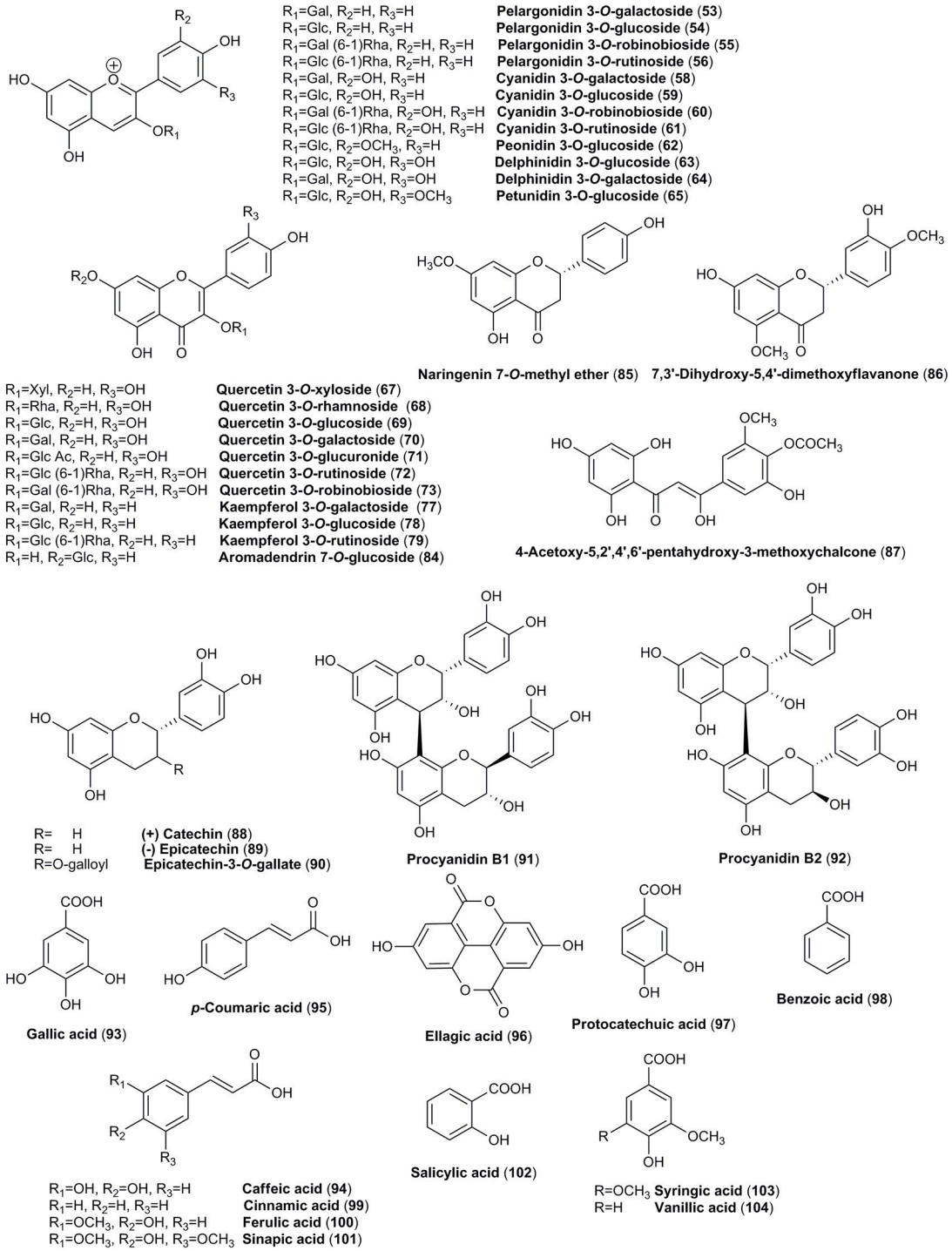

FIGURE 3 | The chemical structures of anthocyanins, flavonoids, and phenolic acids identified in fruits of *C. mas* and/or *C. officinalis*.

et al., 2015), HPLC (Tural and Koca, 2008; Capanoglu et al., 2011) and LC-PDA-MS (Pawlowska et al., 2010). In some cultivars of the southeastern Europe peonidin 3-*O*-glucoside (**62**) followed by cyanidin 3-*O*-galactoside (**58**) was established as the major constituent of *C. mas* fruits (Drkenda et al., 2014).

Flavonoids

Cornus species have been proven to contain flavonols mostly quercetin (**66**) and kaempferol (**76**) derivatives (**Figure 3**). Most of reported compounds were identified as monoglycosides containing glucose, galactose, rhamnose, and glucuronic acid as a sugar moiety. The quercetin glycosides (**69–71**) as well as

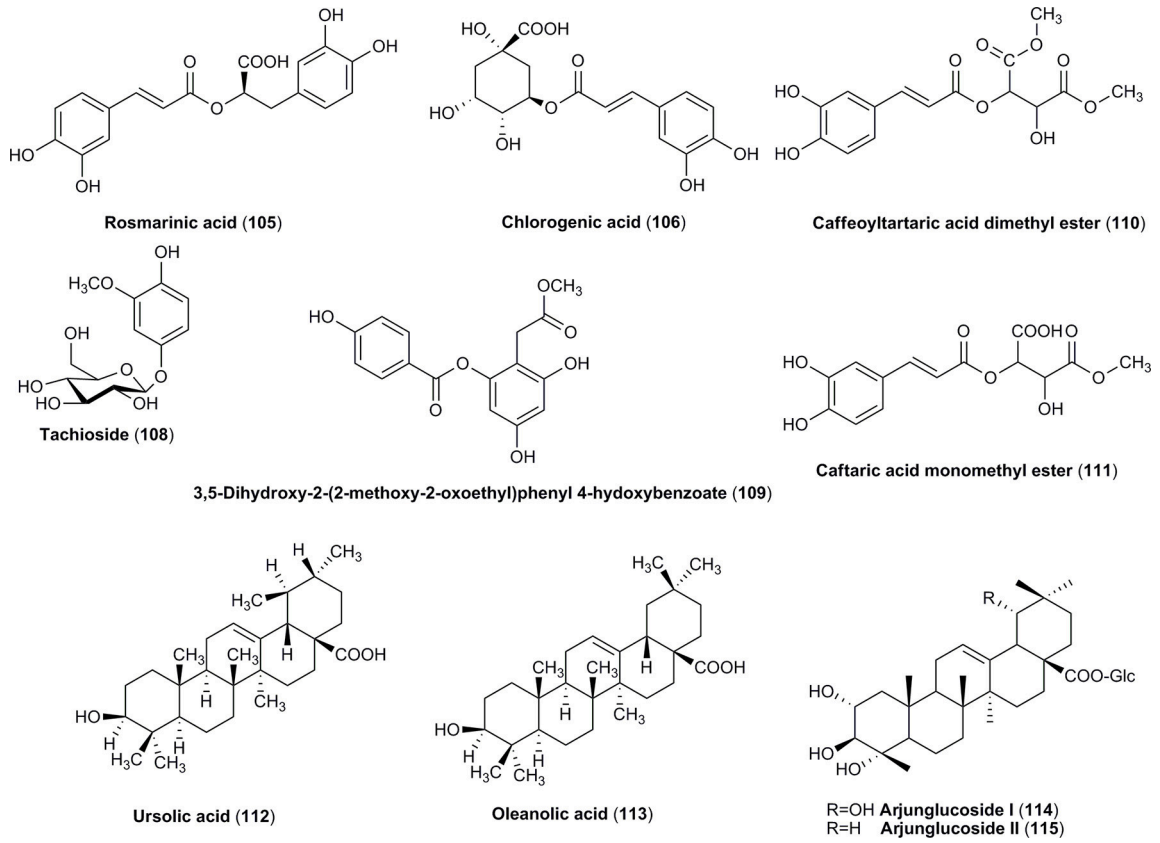

FIGURE 4 | The chemical structures of phenolic compounds and terpenoids identified in fruits of *C. mas* and/or *C. officinalis*.

kaempferol 3-*O*-β-D-galactoside (**77**) were determined in both species (Pawlowska et al., 2010; Xie et al., 2012; Ma et al., 2014). The trace amounts of aglycone of flavonols such as quercetin (**66**) was found in fruits of both *C. mas* and *C. officinalis* (Xie et al., 2012; Rudrapaul et al., 2015).

Phenolic Acids

Three different groups of phenolic acids (**Figure 3**), including benzoic acid, *trans*-cinnamic acid and ellagic acid derivatives have been described in the fruits of *C. mas* and *C. officinalis*. The typical representatives of each group such as gallic acid (**93**), caffeic acid (**94**), *p*-coumaric acid (**95**), ellagic acid (**96**), and protocatechuic acid (**97**) have been found in both dogwoods (Liu Z. et al., 2011; Cao et al., 2012; Radovanović et al., 2013; Milenković-Andjelković et al., 2015; Rudrapaul et al., 2015; Antolak et al., 2017; Cosmulescu et al., 2017). Ellagic acid (**96**), which is a marker of ellagitannins occurrence, detected in the fruits of cornelian cherry suggests the presence of ellagitannins in this plant material. However, this hypothesis requires further investigation.

Triterpenoids

Additionally, some other compounds such as triterpenoids (**Figure 4**) and carbohydrates have been identified in both species. Among terpenoids only ursolic acid (**112**) has been reported in the fruits of cornelian cherry and Asiatic dogwood (Jayaprakasam et al., 2006; Wang H. et al., 2008; Jang et al., 2014; He et al., 2016). This compound was often reported as an active constituent found in ethanolic and methanolic extracts from fruits of *C. officinalis* (Gao et al., 2008). In addition, other three triterpenoid acids (**113–115**), including oleanolic acid, have been identified in *C. officinalis* (Jayaprakasam et al., 2006; Wang H. et al., 2008; Jang et al., 2014; He et al., 2016).

Carbohydrates

Carbohydrates are the crucial compounds determining the quality of fruits due to the fact that the sugar content is a major parameter affecting a direct consumption of fruits in addition to food preservation aspects (Çopur et al., 2003; Brindza, 2007; Bilejić et al., 2011, 2015). Thus, monosaccharides and polysaccharides are present in the fruits of both species. The fruits of *C. mas* contain the atypical fibers such as calcium pectate (**116**) (1.31%; Bilejić et al., 2011; Jaćimović et al., 2015). The crude fiber (0.36–0.76%) and total pectin (0.26–1.04%) contents were evaluated in *C. mas* fruits grown in different regions of Europe (Çopur et al., 2003; Sochor et al., 2014). Therefore, the juice of *C. mas* is considered an alternative fermentation substrate for the production of functional beverage. The simultaneous decreasing total sugars content and increasing

contents of lactic and acetic acids were studied in the juice as well as unripe cornelian cherries fruits fermented by *Lactobacillus* sp. (Nouska et al., 2016; Czyzowska et al., 2017). It is worth to note that fermented cornelian cherry-derived products of iridoid glycosides and phenolic acids may complement gut microbiota and in this manner play a role of new probiotic functional products (Czyzowska et al., 2017). Additionally, the fibers and particularly oligosaccharides, which are generally found in fruits, are considered the non-digestible food ingredient stimulating the growth or selective activity of gut microbiota (Gibson, 2008). The dietary fiber in both cornelian cherries (Nawirska-Olszanska et al., 2010) and cornel dogwood fruits (Shao, 2011) is the important constituent of a diet determining human well-being due to a wide range of metabolic health benefits such as reduction of cholesterol level, blood pressure and body weight. Thus, the potential prebiotic activity of constituents such as dietary fiber from fruits of both dogwoods should not be excluded. On the other hand, a purification and isolation of bioactive polysaccharides of *C. officinalis* fruit (Yang et al., 2010; Li et al., 2012; You et al., 2013; Yin et al., 2016) have been also shown. The list of polysaccharides (**117–119**) identified in the fruits of *C. officinalis* is presented in **Table 1**. The most abundant monosaccharide components of these polysaccharides were glucose, rhamnose and arabinose (Yang et al., 2010; Li et al., 2012). The monosaccharide profile of *C. mas* fruits have been represented by glucose, fructose and sucrose (Bilejić et al., 2011; Perova et al., 2014; Tarko et al., 2014; Antolak et al., 2017).

Differences in Chemical Composition

General

Despite a wide range of similarities and close phylogenetic relationship of *C. mas* and *C. officinalis*, few differences in chemical composition of fruits can be found. The most valuable characteristic established only in fruits of *C. officinalis* seems to be the presence of tannins (**120–139**) as well as unique iridoids**.** Morroniside (**8**) is one of the most important iriodid glucoside reported in the fruits of *C. officinalis*. Additionally, 43 other iridoids (**9–51**) have been identified in this plant material. It should be underlined that co-existence of iridoids and tannins seems to be very rare in plants. Furthermore, 5-hydroxymethylfurfural (**163**) and steroids, such as β-sitosterol (**165**) and daucosterol-6′-malate (**166**) are unique for *C. officinalis*.

On the other hand, flavan-3-ols derivatives, such as procyanidin B1 (**91**) and B2 (**92**), carotenoids (**140–149**) and fatty acids (**150–162**) have been identified only in fruits of *C. mas*. It is worth to note that many reports provide data on ascorbic acid or pectin content in fruits of *C. mas* due to their dietary role. As far as the Asiatic dogwood is concerned, these data are completely missed.

The one comparative study on quantities of secondary metabolites in aqueous extracts from leaves of *C. mas* and *C. officinalis* have revealed that extracts of *C. mas* are characterized by lower content of flavonoids, hydroxycinnamic acids, total polyphenols and tannins than extracts of *C. officinalis* (Forman et al., 2015). On the other hand, no comparative quantitation of major secondary metabolites, except for anthocyanins (Seeram et al., 2002; Vareed et al., 2006) of dogwood fruits is available to date. Thus, further comparative elucidation of chemical composition as well as quantification of phytochemicals in fruits of both species still seem to be a challenge.

Unique Iridoids of *C. officinalis*

Although iridoids were classified as similar characteristic for both species, much more not widely known iridoids have been found only in fruits of *C. officinalis*. In particular, in contrary to the cornelian cherry fruits the major iridoid constituents of Asiatic dogwood fruits were isomers of α and β of morroniside (**8**) as well as their methyl and ethyl derivatives (**9–15**) (Du et al., 2008; West et al., 2012a,b; Xie et al., 2012; Jiang et al., 2016; Park et al., 2016; Ji et al., 2017). Additionally, apart from typical iridoids described previously, less-known compounds such as logmalicids A and B (**19–20**) as well as cornusfurosides A-D (**22–24**) were found in fruits of *C. officinalis* (Ma et al., 2014; He et al., 2017). Fifteen rare iridoid glucoside dimers, named cornusides A-O (**31–45**) (**Figure 2**), have been isolated from an aqueous extract of *C. officinalis* fruits (Ye et al., 2017).

Tannins of *C. officinalis*

Similarly, tannins are attributed only to the fruits of *C. officinalis* and have been widely described. Therefore, tannins (**Figures 5**, **6**) in the fruits of *C. officinalis* constitute the most important difference between both plant materials. Despite the mean content of tannins in fruits of *C. mas* was established as 0.34% (Gunduz et al., 2013), the detailed reports on the identification of tannins in the fruits of *C. mas* have been limited in contrary to *C. officinalis*. The Asiatic dogwood is particularly considered a rich source of hydrolyzable tannins (Okuda et al., 1981, 1984). Twenty tannins (**120–139**), including ellagitannins and gallotannins, have been described in *C. officinalis*. The derivatives of galloylglucose (**121–125**), particularly digalloylglucose, have been identified to date. Tellimagrandin I (**126**) and II (**127**) as well as gemin D (**137**) and oenothein C (**138**) are representative of ellagitannins (Okuda et al., 1981, 1984; Lee et al., 1989; Cao et al., 2012; Bhakta et al., 2017). Most of unique dimeric and trimeric hydrolyzable tannins named cornusiins A–G (**128–134**) have been found only in the fruits of *C. officinalis* to date (Okuda et al., 1984; Hatano et al., 1989a,b, 1990). One of the bioactive constituents which should be mentioned due to the fact of potential biological activity is also 7-*O*-galloyl-D-sedoheptulose (**120**) (Yamabe et al., 2009; Yokozawa et al., 2010; Liu Z. et al., 2011; Park et al., 2015). It is worth to note that some tannins were detected in the fruits of cornelian cherry (Kucharska, 2012). However, their detailed identification was not conducted, and in fact the data concerning tannins in *C. mas* are not available.

Carotenoids and Fatty Acids of *C. mas*

Some lipophilic compounds such as carotenoids as well as fatty acids have been identified in the fruits of *C. mas* in contrast to *C. officinalis*. One report showed the determination of ten

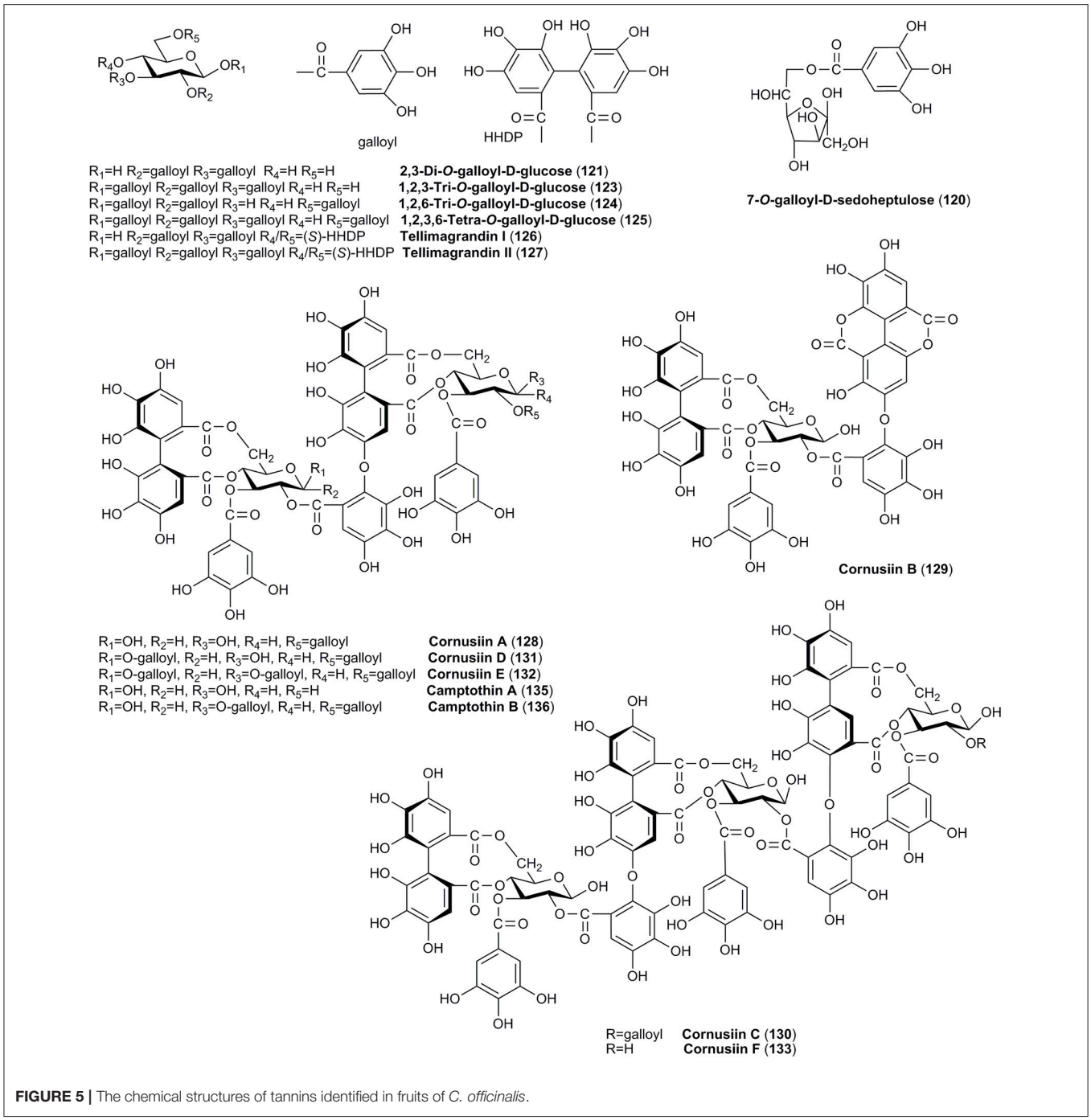

FIGURE 5 | The chemical structures of tannins identified in fruits of *C. officinalis*.

carotenoids (**140–149**) in the fruits of *C. mas* (Horváth et al., 2007), in addition to their quantitation in fresh and dried fruits (Rosu et al., 2011; Lofti et al., 2014). Carotenoids are believed to be regulators of lipid metabolism and antioxidants. Among carotenoids the most abundant in a diet is β-carotene (**147**), known singlet oxygen quencher and precursor for vitamin A (Grune et al., 2010). Despite limited data on this group of compounds in fruits of *C. mas*, their potential role in biological effectiveness of *C. mas* fruits should be considered. To the best of our knowledge there is no report on carotenoids in *C. officinalis*. Similarly, the available data on fatty acids in the fruits of Asiatic dogwood have been limited and the detailed analysis seems to be necessary. Fatty acids (**150–162**) such as linoleic acid (**152**), oleic acid (**155**) and palmitic acid (**156**) have been the most abundant in the fruits of *C. mas* (Krivoruchko, 2014).

Diversity of Phenolic Compounds in *C. mas* and *C. officinalis*

Nine quercetin derivatives (**67–75**) have been identified in the fruits of *C. mas* (Pawlowska et al., 2010). Simultaneously,

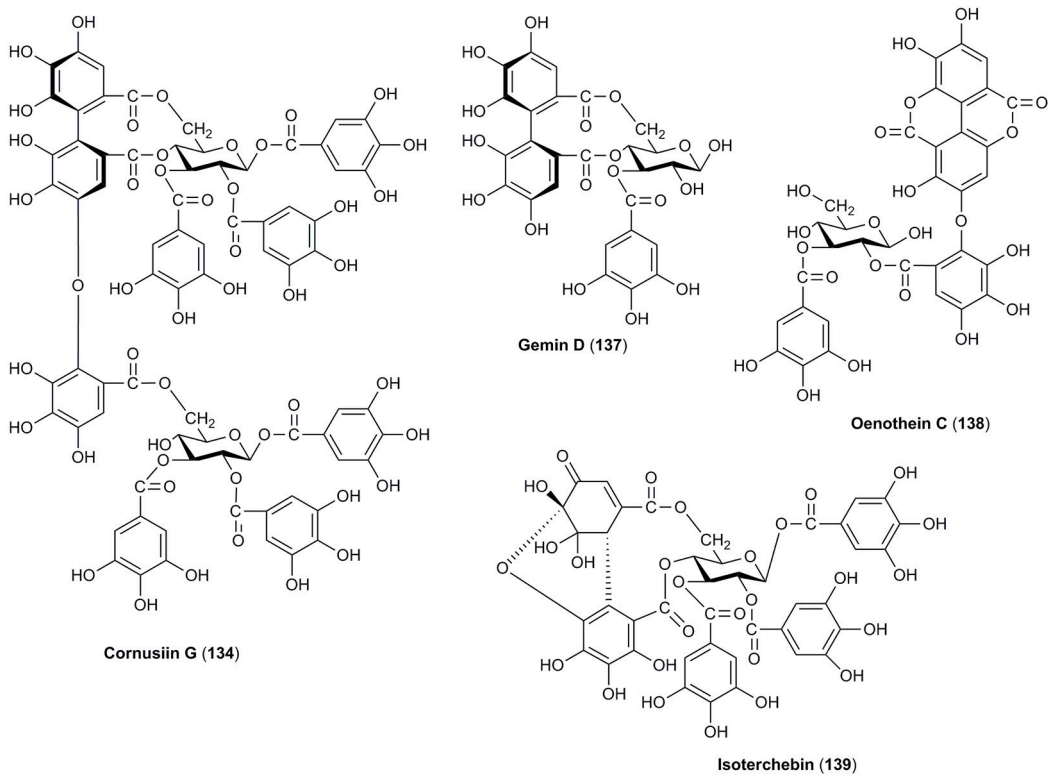

FIGURE 6 | The chemical structures of tannins (continued) identified in fruits of *C. officinalis*.

kaempferol 3-*O*-β-D-galactoside (**77**), myricetin (**81**), and naringenin 7-*O*-methylether (**85**) have been detected in the fruits of *C. mas* along with 7,3′-dihydroxy-5,4′-dimethoxyflavanone (**86**) (Pawlowska et al., 2010; Rudrapaul et al., 2015). It is worth to note that 2*R*, 3*S*-*trans*-aromadendrin (**83**) and its 7-*O*-β-D-glucoside (**84**), which are not so widely distributed in plants compared to quercetin and kaempferol glycosides, were determined in the fruits of *C. mas* (Pawlowska et al., 2010; Rudrapaul et al., 2015). On the other hand, some glycosides, such as quercetin 3-*O*-β-D-glucuronide methyl ester (**74**), quercetin 3-*O*-β-D-(6″-*n*-butyl glucuronide) (**75**), kaempferol 3-*O*-β-D-rutinoside (**79**) as well as kaempferide **(80)** have been reported in fruits of *C. officinalis* according to the available literature (Lin et al., 2011; Xie et al., 2012; Ma et al., 2014).

Flavan-3-ols such as catechin (**88**) and epicatechin (**89**) have been identified and quantified in the fruits of *C. mas* (Capanoglu et al., 2011; Milenković-Andjelković et al., 2015). In the study of Milenković-Andjelković et al. (2015), catechin (**88**) was the predominant favan-3-ol in the fruit extracts of *C. mas* (**Figure 3**). Additionally, procyanidin B2 (**92**) was found only in the fruit extracts of *C. mas* (Radovanović et al., 2013; Milenković-Andjelković et al., 2015). The degree of polymerization in the fruits of cornelian cherry was assessed at the level of 63 total bound (epi)catechin units/end units (Capanoglu et al., 2011).

The mixture of benzoic acid (**98**) and cinnamic acid (**99**) derived phenolic acids, including ferulic acid (**100**), sinapic acid (**101**) as well as vanillic acid (**104**), was identified in the fruits of *C. mas* (Radovanović et al., 2013; Milenković-Andjelković et al., 2015; Rudrapaul et al., 2015; Cosmulescu et al., 2017). In addition, the esters formed between phenolic acids' particles, such as rosmarinic acid (**105**) and chlorogenic acid (**106**) have been found only in the fruits of cornelian cherry (**Figure 4**; Deng et al., 2013; Antolak et al., 2017; Popović et al., 2017; Bajić-Ljubičić et al., 2018). The unique derivatives of phenolic acids such as tachioside (**108**), caffeoyltartaric acid dimethyl ester (**110**), and caftaric acid monomethyl ester (**111**) are characteristic for Asiatic dogwood's fruits (**Figure 4**; Lin et al., 2011; Ma et al., 2014; Park et al., 2016).

Other Constituents

Due to the fact of the usage of *C. mas* for a production of concentrates, jams, and even more often fruit juice and alcoholic beverages, rheological and physicochemical properties related to the quality of liquid foods have been reported (Güleryüz et al., 1998; Brindza, 2009; Kalyoncu et al., 2009; Karadeniz et al., 2009; Yilmaz et al., 2009; Hassanpour et al., 2012; Gastoł et al., 2013; Bilejić et al., 2015; Rad et al., 2016; Bozdogan, 2017). In particular, apart from titrable acidity, soluble solid content as well as total sugars and pectin content, vitamin C was often quantified in the fruits of *C. mas*. They were characterized by the high content of ascorbic acid ranging from 43.6 to 76.7 mg/100 g, or even reaching 112 mg/100 g in Turkish cultivars (Güleryüz et al., 1998; Çopur et al., 2003; Demir and Kalyoncu, 2003; Brindza, 2007,

2009; Yalcinkaya et al., 2009; Yilmaz et al., 2009; Rop et al., 2010; Dokoupil and Rezníček, 2012; Hassanpour et al., 2012; Bilejić et al., 2015; Kostecka et al., 2017). However, it was noted that cornelian cherry fruits from Greece were characterized by higher content of vitamin C than these ones from Poland (Kostecka et al., 2017). On the other hand, some physicochemical properties of cornel dogwood wine during its fermentation and storage were studied once (Zhang Q. -A. et al., 2013).

Few reports considered the potential bioactivity of 5-hydroxymethylfurfural (**163**) isolated from fruits of *C. officinalis* (**Figure 7**; Du et al., 2008; Cao et al., 2011, 2012; Jiang et al., 2016). Additionally, steroid compounds including β-sitosterol (**165**) and daucosterol-6′-malate (**166**) have been identified only in fruits of *C. officinalis* (**Figure 7**; Lee et al., 1989; Lin et al., 2011; Xie et al., 2012).

SIMILARITIES AND DIFFERENCES OF PHARMACOLOGICAL EFFECTS OR BIOLOGICAL ACTIVITIES

General

Taking into account different medical approaches in the traditional medicine in ancient Europe and Asia, in particular China, it is rather interesting that there are some similar indications reported for both species. They have been traditionally used to improve liver and kidney functions and are considered therapeutically usefull due to their antidiabetic and antimicrobial properties. Considering the phytochemical similarities between both species the comparable use of both species is scientifically reasonable. For this reason, the antidiabetic effect as well as renal and hepatic protective activities of both species were particularly considered in the reported studies. In addition, the positive effects on central nervous and cardiovascular systems of both species have been noted. The background of these effects seems to be the antioxidant activity as well as an amelioration of inflammatory processes through regulation of NF-κB and MAPK signaling pathways by constituents of extracts from fruits of both species. The isolated constituents of fruits, such as cornuside (**6**), loganin (**1**), ursolic acid (**112**), anthocyanins, and more specific for *C. officinalis* morroniside (**8**), and 7-*O*-galloyl-D-seduheptulose (**120**), demonstrated the biological impact. However, the pharmacological effect of other phytochemicals found in fruits of both species should not be excluded in the provided reports. Thus, the accurate qualitative and quantitative characterization of phytochemicals in extracts is required in the studies, and the beneficial role of complex phytochemical formulation providing the synergistic or additive effect should be underlined.

The antimicrobial and antitumor activities of both species seem to be mostly related to the presence of polyphenolic compounds in fruit extracts. However, these activities of both species do not seem to be well-established.

Table 2 demonstrates similarities and differences of the biological or pharmacological activities between the preparations from fruits of both species. The biologically or pharmacologically active groups of compounds or isolated constituents of fruit extracts were included in **Table 2**.

Similar Biological or Pharmacological Activities of *C. mas* and *C. officinalis*

Antidiabetic Effect

Comparing the reported traditional therapeutic use of fruits from *C. mas* and *C. officinalis* it is obvious that the treatment of metabolic disorders played an important role. Based on different fundamentals of ancient medicine in China and Europe or western Asia and despite missing modern diagnostic tools for precise detection of diabetes, the physicians recognized definitely metabolic disease in the distant past and tried to treat their accompanying symptoms. Under this point of view the use of fruits from both species should be discussed in order to their potential usefulness in the treatment of metabolic diseases in different geographic regions. Due to the wide spectrum of natural compounds and modern investigations of their biological activity an antidiabetic effect of cornelian cherry seems to be proven. Based on some animal experiments investigating the effect of preparations made from *C. mas* fruits the blood glucose decreasing activity and enhancement of insulin sensitivity were demonstrated (Jayaprakasam et al., 2006; Mirbadalzadeh and Shirdel, 2012; Narimani-Rad et al., 2013; Asgary et al., 2014). The same findings were reported also for fruits of *C. officinalis* (Park et al., 2011; Gao et al., 2012; Liu et al., 2012; Han et al., 2014). Data from clinical studies evaluating the therapeutic effect of the herbal preparation are available only for *C. mas*. One study on the fruit extract showed its antidiabetic effect associated with the reduction of risk factors for secondary diseases (Soltani et al., 2015). Comparing the chemical constituents present in both species and their biochemical or pharmacological effects some similarities can be seen. In the extract of both species polyphenolic compounds (e.g., flavonoids, anthocyanins), which inhibit α-glucosidase activity combined with decreasing postprandial blood glucose level, are present (Park et al., 2011; Asgary et al., 2014; Shishehbor et al., 2016). Ursolic acid (**112**) also present in both species is capable to stimulate the glucose uptake through the phosphorylation of insulin receptor (Zhang et al., 2006). Specific antidiabetic effects were reported for 7-*O*-galloyl-D-seduheptulose (**120**) and iridoids [e.g., loganin (**1**), morroniside (**8**)] present in *C. officinalis* (Yamabe et al., 2009; Park et al., 2010, 2013; He et al., 2016) as well as for cyanidin 3-*O*-galactoside (**58**) and cyanidin 3-*O*-glucoside (**59**) present in *C. mas* (Jayaprakasam et al., 2005). On the other hand, loganin (**1**) and ursolic acid (**112**) were not able to affect an expression of phosphoenolpyruvate carboxykinase (PEPCK) in rat hepatoma H4IIE cell line on the contrary methanolic extract of *C. officinalis* fruit (50 μg/ml) which exerted insulin mimetic effect in this manner, and one of its fraction enhanced insulin secretion by rat insulin-secreting BRIN-BD11 cells in response to high glucose concentration (16.7 mM; Chen et al., 2008). Although there is only one published clinical study of the fruit extract from *C. mas* the antidiabetic effect seems to be proven (Soltani et al., 2015).

The prolonged high blood glucose concentration leads to progressive lesions in heart, kidney, blood vessels and

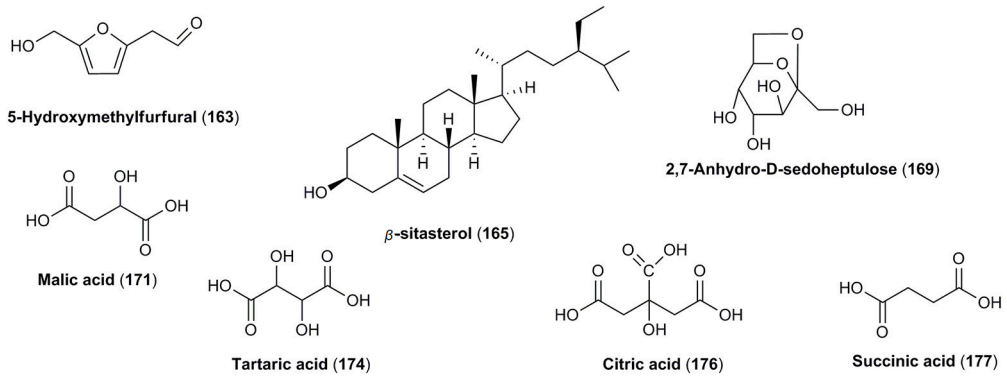

FIGURE 7 | The chemical structures of other compounds identified in fruits of *C. mas* and/or *C. officinalis*.

other organs. Moreover, increased intracellular reducing sugars and their derivatives may participate in glycation and formation of advanced glycation endproducts (AGEs) which play an important role in further development of chronic diabetic complications, atherosclerosis and aging process. The phytochemicals of *C. officinalis* fruits such as gallotannins, particularly 7-*O*-galloyl-D-sedopheptulose (**120**), and morroniside (**8**) inhibited AGE formation or inhibited expression of receptors for AGEs (Yamabe et al., 2009; Lee et al., 2011; Park et al., 2012b, 2015; Lv et al., 2016). Due to the series of diabetes type II complications, the inhibition of AGE formation seems to be a promising target for therapeutic intervention supporting the AGE-related disorders.

Antioxidant Activity

In addition to the studies of total phenolic, flavonoid, and anthocyanins content, antioxidant properties of the extracts from cornelian cherries were determined in a correlation with these phytochemicals. Apart from polyphenols, ascorbic acid and carotenoids in *C. mas* play a significant antioxidant role. Thus, both antioxidants content and antioxidant capacity of dogwoods fruits are factors determining the quality of cultivars and basic physicochemical properties. *In vitro* assays such as free radical scavenging with DPPH (2,2-diphenyl-1-picrylhydrazyl radical), ferric ion reducing antioxidant power (FRAP), total antioxidant capacity with $ABTS^+$ (2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation) and oxygen radical absorbance capacity (ORAC) were the most frequently used methods in a wide range of studies on *C. mas* (Dragović-Uzelac et al., 2007; Pantelidis et al., 2007; Hamid et al., 2011; Celep et al., 2012; Popović et al., 2012; Gunduz et al., 2013; Pyrkosz-Biardzka et al., 2014; Stankovic et al., 2014; Milenković-Andjelković et al., 2015; Hosu et al., 2016; Moldovan et al., 2016) and *C. officinalis* (Lee et al., 2006; West et al., 2012a; Hwang et al., 2016). Moreover, the hydromethanolic extracts of some cornelian cherry fruit cultivars from Turkey demonstrated significant H_2O_2 scavenging activity (79.1%; Ersoy et al., 2011). Galactosides of cyanidin (**58**) and pelargonidin (**53**) detected in both species inhibited lipid peroxidation by 70.2 and 40.3% in an iron-catalyzed liposomal model, respectively (Seeram et al., 2002). On the other hand, the antioxidant properties, including protection of catalase and glutathione peroxidase, of *C. officinalis* fruits are also assigned to polysaccharides (Li et al., 2012; Wu et al., 2013) and ursolic acid (Yu et al., 2009).

Anti-inflammatory Activity

Anti-inflammatory and analgesic activities of extracts are reported for both species. As mentioned before most of the results were obtained in cell culture or biochemical experiments. The beneficial effect of aqueous extract from fruits of *C. officinalis* and its iridoid constituent cornuside (**6**) on protein expression of COX-1 and COX-2 as well as PGE_2 production in LPS-induced macrophages RAW 264.7 was shown (Sung et al., 2009; Choi et al., 2011). Similarly, ursolic acid (**112**), which was isolated from an ethanolic extract of *C. officinalis* inhibited NF-κB activation in LPS-stimulated peritoneal macrophages. Oral administration of ursolic acid (**112**) significantly inhibited 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colon shortening and MPO activity in mice, and also suppressed TNBS-induced COX-2 and iNOS expression as well as NF-κB activation in colon tissues. Ursolic acid (**112**) may ameliorate colitis by regulating NF-κB and MAPK signaling pathways via the inhibition of LPS binding to toll-like receptors 4 (TLR4) on immune cells (Jang et al., 2014). Taking into consideration a wide range of ingredients and general mechanisms responsible for induction or prevention of inflammation, *in vitro* results fit to conclusions obtained from animal experiments and a clinical study (Sung et al., 2009; Sozanski et al., 2014, 2016; Moldovan et al., 2016). In particular, cornuside (**6**) was indicated as an immunomodulatory dogwood-derived compound in a model of sepsis in rats (Jiang et al., 2009). The link between chronic inflammation and cancer involves cytokines and mediators of inflammatory pathways. Plants contain numerous secondary metabolites shown to modulate inflammatory pathways. Studies have shown that these metabolites, among which some are used in traditional medicine, can reduce inflammation and carcinogenesis (Desai et al., 2018). Phenolic and other compounds present in the extracts of the dogwood fruits target various inflammation-related molecules and pathways

TABLE 2 | Similarities and differences of the biological or pharmacological activities of fruits from *C. mas* and *C. officinalis*.

Biological and pharmacological activities	Preparations or characterized natural products responsible for the activity of the fruits	
	***Cornus mas*[a]**	***Cornus officinalis*[b]**
Antidiabetic activity	Hydroalcoholic extracts Dried fruits (powder) Anthocyanins Ursolic acid	Hydromethanolic extract Ethanolic extract Aqueous extracts Dried fruit powder 7-*O*-galloyl-D-sedoheptulose Loganin Morroniside Ursolic Acid Oleanolic acid
Antihyperlipidemic activity	Dried fruits (powder) Anthocyanins Loganic acid Ursolic acid	Not reported
Anti-inflammatory activity	Dried powder Polyphenols-rich extract Loganic acid	Aqueous extract Cornuside
Neuroprotective Anti-amnesic activity	Dried fruits (powder)	Methanolic extract Iridoid glycosides *p*-Coumaric acid Ursolic acid Gallic acid
Hepatoprotective activity	Hydromethanolic extract	Aqueous extract
Antioxidant activity	Dried fruits (powder) Ethanolic extract	Ethanolic extract Aqueous extract Iridoid glycosides Polysaccharides
Anticoagulant activity	Dried fruits (powder) Hydromethanolic extract	Not reported
Cardioprotective activity	Hydromethanolic extract Aqueous extract	Aqueous extract Cornuside
Kidneyprotective activity	Hydromethanolic extract	Total triterpene acids
Antitumor activity	Hydromethanolic extract Ethanolic extract Aqueous extract	Hydromethanolic extract Ethanolic extract Aqueous extract
Antimicrobial activity	Hydromethanolic extract Ethanolic extract Aqueous extract Dried fruit powder	Aqueous extract
Insecticidal activity	Not reported	Methanolic extract
Antiosteoporotic activity	Not reported	Aqueous extract
Immunomodulatory activity	Not reported	Aqueous extract
Ophtalmic activity	Anthocyanin-iridoid fraction Loganic acid	Not reported

[a]*Modified according to Dinda et al. (2016) and Hosseinpour-Jaghdani et al. (2017).*
[b]*Modified according to Huang et al. (2018).*

associated with cancer (Choi et al., 2011). Polyphenols modulate important cellular signaling processes such as NF-κB (nuclear factor κ light-chain enhancer of activated B cells) activation, chromatin structure, glutathione biosynthesis, Nrf2 (nuclear redox factor) activation, direct ROS scavenging or antioxidant effect via glutathione peroxidase activity. Finally, they regulate inflammatory genes in macrophages and lung epithelial cells. Thus, recent data suggest that polyphenols can play a role of modifiers of signal transduction pathways to elicit their beneficial effects. However, to correlate this with beneficial effects in humans their metabolism and bioavailability need to be considered (Rahman et al., 2006).

Renal and Hepatic Protective Activities

The metabolism of xenobiotics is often linked with the production of harmful radicals or metabolites damaging such organs like liver and kidney resulting in inflammation or carcinogenic transformation. As mentioned before natural products present in fruits of both species are able to inhibit both damage generated by radicals and inflammatory processes

in vitro. Animal experiments performed with methanolic extracts of *C. mas* fruits are reported to show hepatoprotective activities against chemically induced hepatotoxicity after oral application. The dosage used in the investigations with rats ranging from 200 to 700 mg/kg body weight (Abbasi et al., 2014; Alavian et al., 2014; Saei et al., 2016). Similar reports concerning *C officinalis* are also published (Lee N. H. et al., 2012). The protective effect on kidneys is described for both species using comparable animal experimental settings (Yokozawa et al., 2008; Jiang et al., 2012; Abbasi et al., 2014; Es Haghi et al., 2014). In particular, the iridoids [loganin (**1**) and morroniside (**8**)] present mainly in the fruits of *C. officinalis* as well as 7-*O*-galloyl-D-sedoheptulose (**120**) are able to prevent renal damage in diabetic rats (Xu et al., 2006; Park et al., 2012a).

Antimicrobial Effect

Antibacterial effects are also reported for extracts of the fruits from both species without remarkable activity (Mau et al., 2001; Krzyściak et al., 2011; Turker et al., 2012; Radovanović et al., 2013; Kyriakopoulos and Dinda, 2015; Milenković-Andjelković et al., 2015; Hosseinpour-Jaghdani et al., 2017). Due to the presence of a complex of different phenolic compounds in dogwood fruits the antimicrobial activity is not surprising. All phenolic compounds to a certain extent express this activity (Radulović et al., 2013). In addition, it is worth to note that some phytochemicals of plant materials may reduce the adhesion of bacteria (*Asaia* spp.) found in food products/beverages, as it was shown in the case of juices of cornelian cherry (Antolak et al., 2017). Thanks to this, the antiadhesive activity of fruit-derived constituents prevents the formation of biofilm on the solid surface, which can be potential source of product contamination. Thus, they are likely to protect both food and plant preparations used in the phytotherapy against loss of organoleptic quality manifested by turbidity and flock formation.

Effects on Memory and Brain Functions

The phytotherapy of diseases or complaints related to the central nervous systems, including memory retention, has a long tradition in Asian and also in European medicine. The reported anti-amnestic and neuroprotective activities of both species seem to be based on similar constituents such as flavonoids and terpenoids which express antioxidant or more precisely radical scavenging effects (Dinda et al., 2016; Cooper and Ma, 2017; Huang et al., 2018). Animal experiments as well as biochemical investigations showed that the dried fruit powder and fruit extracts were able to increase level of physiological antioxidants such as glutathione and antioxidative enzymes, including superoxide dismutase or catalase, as well as to prevent cells and tissues against free radicals in this manner (Lee et al., 2006; Kim et al., 2011; Celep et al., 2012; Lee N. H. et al., 2012; Sozanski et al., 2014). In particular, morroniside (**8**) has been proved to protect human neuroblastoma cells against H_2O_2-induced stress, inhibit the apoptosis and accumulation of intracellular Ca^{2+} as well as restore the mitochondrial potential in SH-SY5Y cell line (Wang W. et al., 2008; Wang et al., 2009). The results of *in vitro* studies in cellular models were confirmed in cerebral ischemia model in rats. Morroniside (**8**) at the concentration range from 30 to 270 mg/kg body weight significantly reduced infarct volume, alleviated neurological impairment caused by ischemia/reperfusion-induced brain injury as well as enhanced progenitor cell proliferation (Wang et al., 2010; Sun et al., 2014a,b). Some special reports indicate the improvement of neurological functions in rats after feeding with iridoid glycosides from *C. officinalis* (Yao et al., 2009). Additionally, in the experimental autoimmune encephalomyelitis in animal models the administration of cornel iridoid glycoside significantly attenuated the symptoms of multiple sclerosis via an increased expression of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) as well as inhibition of JAK/STAT1 and JAK/STAT3 phosphorylation and reduction of proinflammatory mediators, such as TNF-α, IL-1β, IFN-γ, IL-17 (Yin et al., 2014; Qu et al., 2016). Iridoids of *C. officinalis* also protect glutamate-injured hippocampal cells (Jeong et al., 2012). A treatment of rats with a polyphenolic-rich dried fruits of *C. mas* resulted in increased activity of catalase in brain tissue, while in plasma it caused the opposite effect. On the other hand, the increased activity of paraoxonase-1 (PON1) was observed both in rats brain and plasma what potentiate a protective effect of PON1 with regard to the LDL and prevention of oxidative stress. Polyphenols and ascorbic acid of *C. mas* fruits were linked with this effect. Therefore, both neuroprotective and cardioprotective effects were assumed (Francik et al., 2014).

On the other hand, cornuside (**6**) as a representative for secoiridoid glycosides turned out to be an inhibitor of β-site amyloid precursor protein cleaving enzyme 1 (BACE1; IC_{50} = 55.8 μg/ml) but was a weak inhibitor of acetylocholinesterase (IC_{50} > 100 μg/ml) and butyrylocholinesterase (IC_{50} > 500 μg/ml). Other polyphenolic compounds isolated from fruits of *C. officinalis* such as tellimagrandin I (**126**), tellimagrandin II (**127**) and isoterchebin (**139**) were significant inhibitors of all enzymes engaged in the progression of neurodegeneration in Alzheimer's disease (Bhakta et al., 2017). Morroniside (**8**) was found to inhibit tau hyperphosphorylation, another Alzheimer's disease marker, via protein phosphatase 2A activation in human neuroblastoma SK-N-SH cells (Yang et al., 2016). The downregulation of mRNA expression of STIM1 and the inhibition of extracellular Ca^{2+} influx in PC12 cell line by an aqueous extract from fruits of *C. officinalis* were proposed as the potential inducing mechanisms of neurite generation and differentiation (Wang et al., 2015).

Cardioprotective Activity

In close relation to neuroprotection linked with antioxidant properties, antihyperlipidemic and cardioprotective activity of both herbal preparations were demonstrated by *in vitro* as well as *in vivo* experiments. As mentioned before these protective effects seem to be based on the same chemical constituents. In animal studies for both plant materials evidence was shown for antihypertensive effects, decreasing blood pressure and

reduction of circulating cholesterol concentration (Park et al., 2009; Rafieian-Kopaei et al., 2011; Asgary et al., 2014; Sozanski et al., 2014, 2016; Hosseinpour et al., 2017). The ethanolic extract of *C. officinalis* fruits has significantly inhibited adipogenesis (87.1%) in 3T3-L1 preadipocytes (Roh and Jung, 2012; Kang et al., 2014). In addition, it is believed that the metabolic diseases potentially correlate with endocrine hormones due to considerable role of cortisol in cholesterol metabolism (Fraser et al., 1999). One report demonstrated reduction of cortisol concentration in plasma of hamsters after supplementation of *C. mas* fruits (Lofti et al., 2014). In relation to lipid metabolism, the ability of morroniside (**8**) and 7-*O*-galloyl-D-sedoheptulose (**120**) to decrease the expression of proteins associated with lipid homeostasis SREBP-1 and SREBP-2 (sterol regulatory element binding proteins) in kidneys of mice (*db*/*db*) and rats has been shown (Yokozawa et al., 2009; Park et al., 2010). The direct cardioprotective role of cornuside present in both species has been established. The decrease of infarct volume after myocardial ischemia/reperfusion injury in rats treated with cornuside (**6**) has been assigned to the reduction of polymorphonuclear leukocytes infiltration, myeloperoxidase (MPO) activity and malondialdehyde (MDA) production in myocardial tissue as well as lower serum-levels of pro-inflammatory mediators (Jiang et al., 2011). Furthermore, this compound dilated vascular smooth muscles through endothelium-dependent NO/cGMP signaling pathway (Kang et al., 2007). Unfortunately, no convincing human studies addressing these biological activities have been published so far. Although there is one published clinical study of a fruit extract from *C. mas* demonstrating its antidiabetic effect associated with the decreasing risk factors of cardiovascular diseases (Soltani et al., 2015). These results revealed a trend in another clinical trial toward amelioration of lipid profile and vascular inflammation following addition of *C. mas* extracts to the daily diet of dyslipidemic children and adolescents but these findings need to be verified by larger scale trials (Asgary et al., 2013). However, human studies with extracts of *C. officinalis* fruits have not been published so far. Discussing these effects with respect to the ethnopharmacological reports regarding the traditional use of both species in the past some evidence for beneficial effects on heart and circulation system may exist. This concerns especially the traditional use of *C. mas* to treat disorders or complaints related to aging processes like diabetes, ischemia or cardiovascular diseases (Dinda et al., 2016; Hosseinpour-Jaghdani et al., 2017). Due to the use of *C. officinalis* in TCM as a component of complex drug mixtures (Chen et al., 2008; Liu et al., 2013; Dai et al., 2016), a clear assignment to effects on heart and circulation system is rather difficult. However, in preparations of TCM used for treatment of diseases often related to aging processes the fruits of *C. officinalis* are abundant constituents (Chinese Pharmacopoeia, 2015).

Antitumor Activity

Despite the reports of antitumor activity of *C. mas* and *C. officinalis* fruits, the data do not provide any evidence that the fruits of both species show any real antitumor activity. All results from the literature refer to cytotoxic activities in different tumor cell lines (A549, MCF-7, SKOV3, PC-3, HepG2, U937) but not to animal experiments or human studies (Chang et al., 2004; Yousefi et al., 2015). Even though a cytotoxic effect on tumor cells is observed, the antitumor activity is not fully addressed and cannot be interpreted. This kind of over-interpretation of *in vitro* studies occurs very often but has no pharmacological relevance. All polyphenolic compounds, including the most abundant constituents of dogwood fruit extracts, bind to cells in culture leading to the induction of the biological effect. Consequently, the cytotoxicity or inhibition of proliferation associated with changes in signaling pathways occur. Only a clear selectivity in cytotoxicity between tumor and non-tumor cells corresponding with appropriate animal *in vivo* experiments opens up the possibility for the statement of antitumor activity. The reported antioxidant activity for both species (Dinda et al., 2016; Hosseinpour-Jaghdani et al., 2017; Huang et al., 2018) might be a hint for protective effects against tumor-inducing free radicals but for both species only *in vitro* models have been used to demonstrate such effects (Chang et al., 2004; Chu et al., 2009; Turker et al., 2012; Yousefi et al., 2015). In particular, the flavonoids are regarded as safe and easily obtainable. For this reason, they are considered ideal candidates for cancer chemoprevention or associated agents in clinical treatment (Sak, 2014).

UNIQUE BIOLOGICAL AND PHARMACOLOGICAL ACTIVITIES OF *C. MAS* AND *C. OFFICINALIS*

General

The unique anticoagulant and ophthalmic effects of dried fruits and extracts or anthocyanin-iridoid fractions from fruits of *C. mas* were assessed in the available reports. On the other hand, the antiosteoporotic, immunomodulatory and insecticidal properties of *C. officinalis* fruits, their extracts or constituents, such as morroniside (**8**), polysaccharides or 5-hydroxymethylfurfural (**163**), and dimethyl malate (**173**) were only found. Moreover, there is no available report on the antihyperlipidemic activity of *C. officinalis*. The circumstances of needs in the traditional eastern or western medicine requiring scientific confirmation are a pivotal factor determining scientific research. For this reason, these differences between *C. mas* and *C. officinalis* might arise from the lack of dedicated studies or even from different cultural needs resulting from the geographical distribution of both species.

Cornus mas L.

Anticoagulant Effect

Historical reports about an anticoagulant effect of fruits from *C. mas* do not exist. According to the interpretation of diseases of the circulation system, their etiology was rather assumed in disturbances of the digestion tract based on the theory of the four humors. The knowledge of functions and pathological significance of platelets or the blood coagulation system is the result of research in the twentieth century. For this reason, single reports about pharmacological effects of traditionally used herbal remedies on blood coagulation

or platelet function are rather remarkable. Thus, it is worth to note that the dried fruit powder and hydromethanolic extracts of the fruits of *C. mas* showed an anticoagulant effect in animal experiments. In hypercholesterolemic rabbits the cornelian cherry powder reduced the plasma fibrinogen level after feeding over 60 days. The results indicated that consumption of this dried plant material is likely to be beneficial in atherosclerotic patients due to its reducing effects on fibrinogen (Asgary et al., 2010; Rafieian-Kopaei et al., 2011). A significant decrease in the platelet distribution width (PDW) was found in male rats treated with 50, 200, and 400 mg/kg body weight of a hydromethanolic extract over 3 weeks. The changes in the platelet-related parameters suggested platelet activity inhibition without bone marrow suppression in the treated groups in comparison with the control groups. Regarding to the secondary products present in the drug, it is evidence that antioxidants reduce the platelet activity through stimulation of prostacyclin synthesis and scavenging the synthesis-inhibiting peroxides. Recent studies have shown that polyphenols such as quercetin (**66**) and catechin (**88**), stop NADPH oxidase enzyme (present in platelets). Therefore, inhibiting the production of superoxide anion ($\cdot O_2^-$) and increasing the biological power of NO consequently regulate glycoprotein pine receptors (GPIIb-IIIa) on platelet membrane surface, that in turn inhibits platelets' activation and their adherence during inflammation and thrombosis processes. Decreasing effect of the fruit extract on platelet activity might classify it as an alternative for antiplatelet therapy in cardiovascular diseases (Abdollahi et al., 2014). Unfortunately, clinical studies investigating this aspect of activity have not been published to date. Despite some similarities in the phytochemical composition regarding the phenolic compounds between fruits from *C. mas* and *C. officinalis* anticoagulant effects of *C. officinalis* preparations are not reported in the traditional Chinese medicine. According to the theoretical fundamentals of Chinese medicine the blood coagulation system did not play any role in TCM. Nevertheless, a biochemical study also proposed an antiplatelet activity of this drug based on malic acid (**171**), citric acid (**176**), and succinic acid (**177**) identified in the fruits of *C. officinalis* (Zhang Q. -A. et al., 2013).

Ophthalmic Activity

It is believed that anthocyanins as well as other antioxidants may improve the visual functions and the visual field of patients with normal-tension glaucoma via neuroprotective effect, inhibition of lipid peroxidation and improvement of vascular blood flow into the optic nerve. The progressive optic nerve damage accompanied by morphological changes, loss of the field of vision and even increased intraocular pressure are the symptoms of so called glaucoma. An interesting study on the effect of an anthocyanin-iridoid fraction derived from fruits of *C. mas* on intraocular pressure was conducted in rabbits. Intraocular pressure was decreased by 19 and 25% after 2–3 h intraconjunctival administration of the fraction or loganic acid (**3**) solution (0.7%), respectively (Szumny et al., 2015). This activity of cornelian cherries polyphenols is likely to provide beneficial effects on vascular flow in diabetic and hypertensive retinopathy.

Cornus officinalis Sieb. et Zucc.

Antiosteoporotic Activity

Osteoporosis as a chronic disease is closely related with aging processes in the elderly. Chinese herbal medicines have been used to treat this disease for a long time and among the preparations listed in Chinese Pharmacopoeia fruits of *C. officinalis* are also included (Huang et al., 2018). Based on the modern findings in pathogenesis and treatment of osteoporosis a variety of results from biochemical and cell biological experiments are published which support the idea that natural products present in the plant preparation play a beneficial role.

Extract of *C. officinalis* significantly inhibits RANKL-mediated osteoclast differentiation in a dose-dependent manner and RANKL-induced phosphorylation of p38 and c-JUN N-terminal kinase. In the same way, the protein expression of c-Fos and NFATc1 is also suppressed as well as the RANKL-induced degradation of IκB kinase (Kim J. Y. et al., 2012). Morroniside (**8**), one the main constituent of extracts from *C. officinalis* fruits, has been studied on osteoarthritis in human chondrocytes. This compound increased the levels of type II collagen, and aggrecan as well as improved the level of proteoglycans in cartilage matrix. In this manner, it supported the survival of chondrocytes with articular cartilage thickness (Cheng et al., 2015). Summarizing, the extracts of *C. officinalis* or individual compounds inhibit osteoclast differentiation not only in *in vitro* cellular models but also in osteoporosis animal models (Xia et al., 2014). Detailed animal studies or clinical trials are not published.

Immunomodulatory Activity

The pivotal characteristics of immunomodulators in the maintenance of the human well-being and health cannot be overlooked. The sources of these medicinal remedies are part of long traditions in different regions of the world, such as traditional Chinese medicine, which have been developed through empirical experience. Traditional medicine employs a holistic approach to the prevention of disease and traditional herbal medicines are a source of components with therapeutic value (Tiwari et al., 2018). Many studies have been conducted also on preparations of *C. officinalis* and have revealed constituents that influence the innate immune system through the stimulation of macrophages and lymphocytes, and modulation of the cytokine profile, which leads to a reduction of the incidence of infections or allergic diseases.

In addition, allergic asthma is a chronic inflammatory disease of the airways, demonstrated by symptoms such as wheezing, coughing, breathlessness, and airways inflammation. Investigations in a mouse model of allergic asthma suggest that the therapeutic effects of *Corni fructus* are mediated by reduced production of Th2 cytokines (IL-5), eotaxin, and specific IgE as well as a reduced eosinophil infiltration (Kim S. H. et al., 2012). These findings are supported by animal experiments with polysaccharides from crude and processed *C. officinalis* fruits which have an enhanced effect on non-specific immunity, specific humoral immunity and specific cellular immunity in immuno-depressed mice (Du et al., 2008).

Along with the anti-inflammatory effects mentioned before, these results provide some evidence that the fruits of *C. officinalis*

show immune-protective activity. Nevertheless, the evidence by human studies is still missing.

Insecticidal Activity

An interesting biological activity is the insecticidal effect of 5-hydroxymethylfurfural (**163**) and dimethyl malate (**173**) determined in a bioassay-guided fractionation of methanolic extract from the fruits of *C. officinalis* against larvae of *D. melanogaster*. Acute toxicity against adults of *D. melanogaster* was determined showing LD50 value of 34.0 and 21.5 μg/adult for 5-hydroxymethylfurfural (**168**) and dimethyl malate (**173**), respectively (Miyazawa et al., 2003). Such investigations were reported rather infrequently. Nevertheless, they are important with respect to protect health and food quality in the past and presently. The use of such experiences could help to develop ecological strategies for modern food storage.

CONCLUSION

The utilization of plant resources for nutrition and medicinal use in the past belongs to the process of civilization. The investigation of such remarkable fruits from both species is therefore not surprising. According to the different ideas regarding the origin of diseases and methods for therapeutic treatment, the different use of fruits from *C. mas* und *C. officinalis* in the West and the East could be explained. Despite all these differences it is rather surprising that such a level of consistency is evident. The chemical composition of *C. mas* and *C. officinalis* seems to be well-established. However, the availability of missing information on some groups of compounds, such as fatty acids, carotenoids and ascorbic acid in the fruits of *C. officinalis*, is still needed. Nevertheless, fruits of both species are rich in iridoids, anthocyanins, and flavonoids. The main difference in phytochemicals concern tannins, which were found only in *C. officinalis* and may potentially determine the biological activity in a significant manner. However, the role of tannins, except for 7-*O*-galloyl-D-sedoheptulose, was not widely considered in the biological activity of *C. officinalis* fruits. Taking into consideration the close phylogenetic relationship of both species the question about tannins in fruits of *C. mas* is open. On the other hand, tannins do not rather occur in the iridoid-rich plant materials but this hypothesis requires detailed elucidation in this case. Nevertheless, the transfer of medicinal experiences from the West to the East or *vice versa* cannot be excluded but is not documented in the literature for this two species. The explanation for this consistency seems to be rather the result of testing and empirical observation. Analyzing the ethnopharmacological data available under modern scientific aspects and correlating the phytochemical composition of both species, the results show that nearly the same ingredients are also attributed for the same activity or presumed therapeutic use. However, some data concerning the phytochemical composition as well as pharmacological activity of, particularly *C. officinalis*, are still missed or limited due to their availability in Chinese. Thus, only dedicated comparative investigations of *C. mas* and *C. officinalis* fruit preparations could really provide evidence of similarities or differences in the field of their phytochemical composition and biological or pharmacological properties. Last but not least is the assignation of extracts constituents potentially responsible for the specific activity. It means that the accordance between ethnopharmacological use of both plant materials should be based on the well-established chemical composition as well as simultaneous *in vitro* and *in vivo* studies, which may facilitate the identification of active constituents. Only these studies could validate careful observations of the users or physicians in the past.

AUTHOR CONTRIBUTIONS

MC carried out a literature review in the field of phytochemistry and biological activity. MM designed the draft of the manuscript and carried out a literature review in the field of pharmacological activity. Both authors wrote the manuscript, revised and approved the final version of the manuscript.

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Anti-inflammatory and immunomodulatory effects of *Paeonia lactiflora* Pall., a traditional Chinese herbal medicine

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In China, Korea, and Japan, a decoction of the dried root without bark of *Paeonia lactiflora* Pall. has been used in the treatment of rheumatoid arthritis, systemic lupus erythematosus, hepatitis, dysmenorrhea, muscle cramping and spasms, and fever for more than 1200 years. A water/ethanol extract of the root is now known as total glucosides of peony (TGP), which contains more than 15 components. Paeoniflorin is the most abundant ingredient and accounts for the pharmacological effects observed with TGP in both *in vitro* and *in vivo* studies. The analgesic effect of TGP was confirmed in various animal models of pain, which may be mediated partly by adenosine A1 receptor. The direct anti-inflammatory effects of TGP were observed in animal models of both acute and subacute inflammation, by inhibiting the production of prostaglandin E2, leukotriene B4, and nitric oxide, and by suppressing the increase of intracellular calcium ion concentration. TGP was also reported to have protective effects of cells against oxidative stress. *In vitro*, dual effects of TGP were noted on the proliferation of lymphocytes, differentiation of Th/Ts lymphocytes, and the production of proinflammatory cytokines and antibodies. *In vivo*, TGP inhibited the delayed-type hypersensitivity in immuno-activated mice, and enhanced the delayed-type hypersensitivity in immuno-suppressed mice. In adjuvant arthritis rats, paeoniflorin exerted immunosuppressive effects. The beneficial effects of TGP in treating rheumatoid arthritis were verified by randomized controlled trials. The adverse events of TGP were mainly gastrointestinal tract disturbances, mostly mild diarrhea.

Keywords: *Paeoniae Radix*, anti-inflammation, immunomodulation, Chinese herbal medicine

Paeoniae Radix is one of the most well-known herbs in China, Korea, and Japan for more than 1200 years. Paeonies are divided into two groups: the tree Peony (also named Paeonia Moutan) and the herbaceous kinds. *Paeonia lactiflora* Pall. (also named Chinese Peony) is a herbaceous perennial flowering plant in the family Paeoniaceae with fleshy roots and annual stems. It is about 60–100 cm tall with large compound leaves 20–40 cm long. The flower buds are large and round, opening into large flowers 8–16 cm diameter, with 5–10 white, pink, or crimson petals and yellow stamens (**Figure 1**). It is native to east Asia, and is grown on dry open stony slopes, riverbanks and sparse woodland edges.

The dried root without bark of *P. lactiflora* Pall., namely *Radix Paeoniae Alba*, has been used as a medicinal herb in traditional Chinese medicine for centuries, where it is called *Bái Sháo* (literally: "White Peony"). The root is dug in summer or autumn from cultivated plants that are 4–5 years old, and cleaned with water. After removal of the bark and rootlets, it is boiled in water for a short while, dried in the sun, and then sliced. A decoction of the root has been used in the treatment of rheumatoid arthritis, systemic lupus erythematosus, hepatitis, dysmenorrhea, muscle cramping and spasms, and fever with a long history.

A water/ethanol extract of *Radix Paeoniae Alba* is known as total glucosides of peony (TGP), which contains more than 15 components, including paeoniflorin, albiflorin, oxypaeoniflorin, benzoylpaeoniflorin, oxybenzoyl-paeoniflorin, paeoniflorigenone, lactiflorin, galloylpaeoniflorin, paeonin, paeonolide, and paeonol (Zhang et al., 2001; Tan et al., 2010). Most of them are monoterpene glucosides, and their structures are shown in **Figure 2**. Among them, paeoniflorin ($C_{23}H_{28}O_{11}$, with a molecular weight of 480.45), a water-soluble compound, is the most abundant (>90%) and accounts for the pharmacological effects observed with TGP in both *in vitro* and *in vivo* studies. So the content of paeoniflorin is used for the standardization of the dosage of TGP. A preparation of TGP was approved by State Food and Drug Administration of China to enter market as a disease-modifying drug for rheumatoid arthritis in 1998. In recent years, a lot of studies describing the chemistry and the pharmacology of TGP have been published, but often in Chinese. Here, we briefly reviewed the immunomodulatory and anti-inflammatory effects of TGP.

ANALGESIC EFFECT OF TGP

Although the root of *P. lactiflora* Pall. was believed to be effective in relieving various pain in traditional Chinese medicine, the direct evidence was not obtained in modern medicine until 1988 (Wang et al., 1988). TGP suppressed writhing induced by intraperitoneal injection of acetic acid, squeak induced by footpad stimulation with electricity, and hot-plate reaction in mice and/or rats in a dose-dependent manner. Naloxone failed to abolish these effects of TGP. These data verified the analgesic effect of TGP in various animal models of pain, and suggest the effect is not mediated by the opiate receptors. The analgesic effect of TGP was further confirmed by subsequent studies (Gao et al., 2002; Lee et al., 2008;

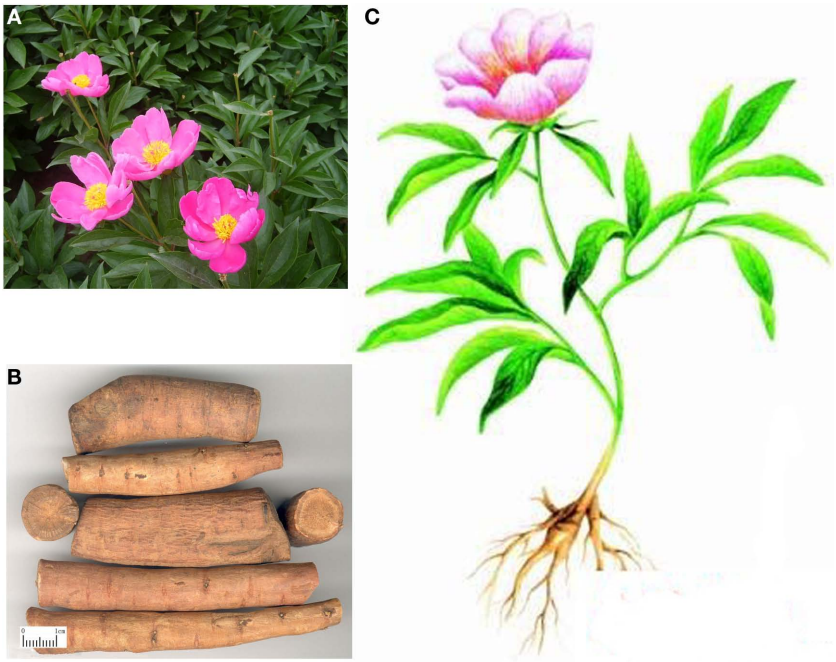

FIGURE 1 | *Paeonia lactiflora* Pall. (A) overground part of the plant; **(B)** root; **(C)** scheme of an intact plant.

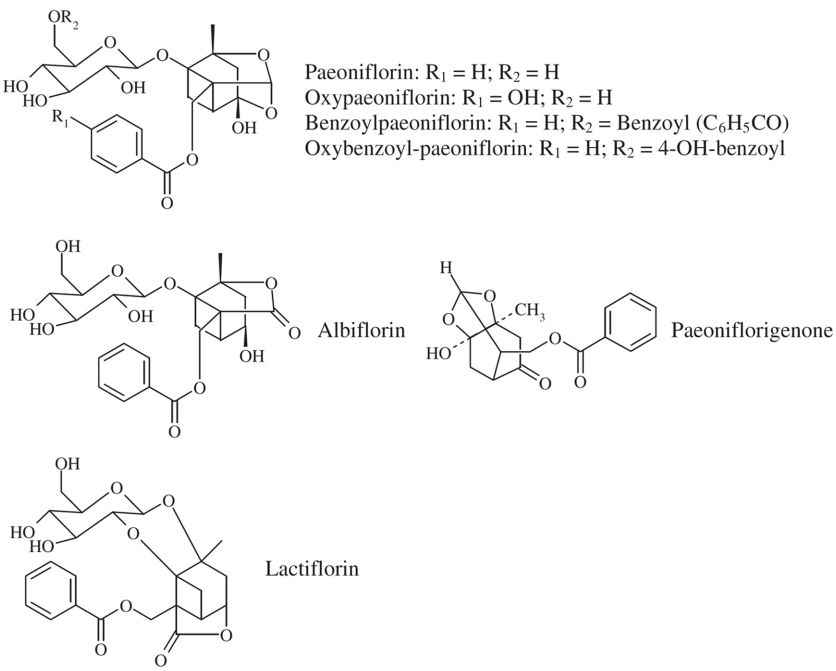

FIGURE 2 | Chemical structures of main components in total glucosides of peony.

Ou-Yang, 2008). Recently, it was demonstrated that paeoniflorin, a chief active ingredient in the root of *P. lactiflora* Pall., is effective in relieving colorectal distention (CRD)-induced visceral pain in rats with visceral hyperalgesia induced by neonatal maternal separation. Furthermore, analgesic effect of paeoniflorin is mediated, at least in part, by adenosine A1 receptor (Zhang et al., 2009).

ANTI-INFLAMMATORY EFFECT OF TGP

The direct anti-inflammatory effects of TGP were observed in animal models of both acute and subacute inflammation, such as dimethylbenzene-induced ear swelling, carrageenan-induced paw edema, subcutaneous tampon-induced granuloma, complete Freund's adjuvant-induced arthritis, and collagen-induced

arthritis (Gao et al., 2002; Zheng and Wei, 2005; Zhu et al., 2005; Xu et al., 2007; Ou-Yang, 2008). TGP also decreased the permeability of peritoneal capillary which was enhanced by acetic acid (Ou-Yang, 2008). Prostaglandin E2 (PGE2) is synthesized in substantial amounts at sites of inflammation where it acts as a potent vasodilator and causes an increase in vascular permeability and edema synergistically with other mediators. Leukotriene B4 (LTB4) is chemotactic for neutrophils and is important in the characteristic infiltration of these cells in the acute inflammatory response. PGE2 enhances the chemotactic activity of LTB4. TGP (0.001 ~ 100 mg/L) inhibited the production of LTB4 from peritoneal macrophages and the production of PGE2 from calcimycin A23187-stimulated macrophages in a dose-dependent manner (Li et al., 1992, 1994). The A23187-induced increase of intracellular calcium ion concentration [$(Ca^{2+})_i$] in macrophages was also inhibited by TGP (Li et al., 1994). Inflammation induces oxidative stress by producing oxidants like reactive oxygen species and nitric oxide (NO). NO is also a potent mediator of inflammation. TGP markedly suppressed lipopolysaccharide (LPS)-induced NO production and inducible nitric oxide synthase (iNOS) expression in rat peritoneal macrophages (Chen et al., 2008). In addition, the production of reactive oxygen species from LPS-stimulated macrophages was inhibited by higher concentrations (11.25 ~ 125 mg/L), but enhanced by lower concentrations (0.09 ~ 11.25 mg/L) of TGP in a dose-dependent manner (Liang et al., 1990). *In vivo* studies also showed that TGP inhibited the production of PGE2 by macrophages in rats with adjuvant arthritis (Li et al., 1994). Recently, paeoniflorin, the principle component of TGP, was also demonstrated to be effective in inhibiting the production of NO and PGE2 from LPS-stimulated RAW 264.7 macrophages (Kim and Ha, 2009). An *in vivo* study showed that both TGP and paeoniflorin potently inhibited passive cutaneous anaphylaxis reaction induced by IgE-antigen complex and scratching behaviors induced by compound 48/80 in mice (Lee et al., 2008). Pretreatment with paeoniflorin decreased mortality, reduced lung and kidney injury, decreased serum creatinine level, and improve systolic function of heart in mice challenged with LPS. Further experiments showed that paeoniflorin inhibited LPS-stimulated tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β release and promoted LPS-induced IL-10 production (Cao et al., 2011).

All together these data indicate that TGP exerts anti-inflammatory effects by inhibiting the production of inflammatory mediators, such as PGE2, LTB4, NO, reactive oxygen species, and proinflammatory cytokines.

IMMUNOMODULATORY EFFECTS OF TGP

DUAL EFFECTS ON PROLIFERATION AND APOPTOSIS OF LYMPHOCYTES

In vitro, dual effects of TGP on concanavalin A (ConA)-induced proliferation of mouse splenocytes were noted (Wang et al., 1991). TGP enhanced the proliferation at lower concentrations (0.05 ~ 0.4 mg/L) but inhibited the proliferation at higher concentrations (0.4 ~ 1.6 mg/L) in a dose-dependent manner. The dual effects of TGP on the proliferation of lymphocytes were confirmed by other studies (Li et al., 1990; Wang et al., 1992). In addition, TGP upregulated the differentiation of both helper T cells (Th) and suppressor T cells (Ts), and reversed the inhibitory effect of cyclosporine A on Th differentiation and the inhibitory effect of levamisole on Ts differentiation. TGP increased the ratio of Th/Ts at a lower concentration (0.2 mg/L), but decreased the ratio of Th/Ts at a higher concentration (6.0 mg/L; Wang et al., 1992). *In vivo* studies also confirmed above findings. TGP inhibited the delayed-type hypersensitivity (allergic contact dermatitis induced by 2,4-dinitrofluorobenzene) in immuno-activated mice by downregulation of Th/Ts ratio and serum level of TNF-α, and enhanced the delayed-type hypersensitivity in immuno-suppressed mice by upregulation of Th/Ts ratio (Wang et al., 1990; Chen et al., 2010). In adjuvant arthritis rats, paeoniflorin significantly inhibited the proliferation of mesenteric lymph node lymphocytes (Wu et al., 2007). In addition, paeoniflorin induces apoptosis in murine T lymphocytes and Jurkat human T cell leukemia cells through a redox-linked mechanism (Tsuboi et al., 2004). These data suggest TGP may suppress over-activated immuno-responses by inhibiting the proliferation of lymphocytes, balancing the differentiation of Th and Ts cells, and inducing the apoptosis of lymphocytes.

DUAL EFFECTS ON PRODUCTION OF PROINFLAMMATORY CYTOKINES

In vitro, dual effects of TGP on IL-1 and IL-2 production were also noted (Wei et al., 1989; Li et al., 1990). TGP enhanced IL-1 production by LPS-stimulated mouse splenocytes at lower concentrations (0.5 ~ 12.5 mg/L) but inhibited the production at higher concentrations (12.5 ~ 312.5 mg/L) in a dose-dependent manner (Li et al., 1990). TGP enhanced IL-1 production by LPS-stimulated macrophages, and IL-2 production by ConA-stimulated mouse splenocytes at lower concentrations (0.5 ~ 12.5 mg/L). However, it inhibited the production at higher concentrations (12.5 ~ 62.5 mg/L; Wei et al., 1989; Liang et al., 1990). In rats with experimental colitis, serum levels of IL-6, IL-17, and IL-23 were lowered, and the colonic expression levels of transforming growth factor-β1 (TGF-β1) and Foxp3 were elevated by treatment with TGP (Wang et al., 2010). In adjuvant arthritis rats, paeoniflorin significantly downregulated the level of proinflammatory cytokine IL-2, and upregulated the levels of IL-4 and TGF-β1 (Wu et al., 2007). These data suggest that paeoniflorin exerts the anti-inflammatory and immunomodulatory effects by balancing the function of Th1 cells and Th2 cells.

DUAL EFFECTS ON PRODUCTION OF ANTIBODIES

In vitro, dual effects of TGP on IgM-antibody production by mouse splenocytes immunized with red blood cells of sheep were found (Wang et al., 1991, 1992). TGP enhanced the production of IgM-antibodies at lower concentrations (0.1 ~ 0.4 mg/L) but inhibited the production at higher concentrations (0.4 ~ 3.2 mg/L). However, the concentration of antibodies to type II collagen in rats with collagen-induced arthritis was not changed by TGP treatment, in spite of the improvements in the hind paw swelling and the arthritis scores (Wang et al., 2011).

ANTIOXIDANT EFFECT OF TGP

TGP was also reported to have protective effects of cells against oxidative stress. Kim et al. (2009) found that TGP significantly protected primary cultures of rat cortical cells exposed to oxidative stress induced by H_2O_2. Paeoniflorin was reported to exert a neuroprotective effect on glutamate-induced neurotoxicity in PC12 cells, via inhibiting oxidative stress and Ca^{2+} overload (Mao et al., 2010). The antioxidative effect of paeoniflorin on the LPS-induced

liver inflammatory reactions and on diabetes-associated renal damage were also demonstrated by *in vivo* studies (Kim and Ha, 2010; Su et al., 2010).

THERAPEUTIC USAGE OF TGP

The anti-inflammatory and immunomodulatory effects of TGP provide the therapeutic basis in the treatment of autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus. There have been numerous clinical reports on the efficacy and safety of TGP in various diseases. Unfortunately, few of them have been substantiated by randomized controlled trials with large sample size. The first prospective, double-blind trial of TGP was conducted in 1993, and 450 patients with rheumatoid arthritis were randomized to receive TGP (1.8 g/day) or methotrexate (10 mg/week) for 12 weeks. Therapeutic response was achieved in 71.7% of TGP-treated patients and 81.7% of methotrexate-treated patients, respectively. The adverse events of TGP occurred in 13.3% patients, and most of them were gastrointestinal tract disturbances, mostly mild diarrhea. Most of the events were self-resolved in 1–2 weeks. No adverse events on hepatic, renal or hematological tests were found. The similar results were confirmed in a phase III clinical trial of 1016 patients with rheumatoid arthritis (Zhou and Li, 2003). Some pilot studies also showed therapeutic benefits of TGP in systemic lupus erythematosus (Shuai et al., 2003), Sjögren syndrome (Lu et al., 2006), ankylosing spondylitis (Wang et al., 2007), and chronic urticaria (Long et al., 2010).

CONCLUDING REMARKS

A water/ethanol extract of the dried root without bark of *P. lactiflora* Pall. is now known as TGP, and paeoniflorin is the most abundant component (>90%) of TGP. TGP is effective to relieve the pain in various animal models. TGP also inhibits both acute and subacute inflammation by suppressing the increase of intracellular calcium ion concentration, and inhibiting the production of inflammatory mediators, such as PGE2, LTB4, and NO. There are dual effects of TGP on the proliferation of lymphocytes, differentiation of Th/Ts lymphocytes, and production of proinflammatory cytokines and IgM-antibodies, which depend on the activity of immuno-responses. The underlying mechanisms of TGP responsible for the unique immunomodulatory effects need to be further studied.

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The Therapeutic Potentials of Ayahuasca: Possible Effects against Various Diseases of Civilization

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Ayahuasca is an Amazonian psychoactive brew of two main components. Its active agents are β-carboline and tryptamine derivatives. As a sacrament, ayahuasca is still a central element of many healing ceremonies in the Amazon Basin and its ritual consumption has become common among the mestizo populations of South America. Ayahuasca use amongst the indigenous people of the Amazon is a form of traditional medicine and cultural psychiatry. During the last two decades, the substance has become increasingly known among both scientists and laymen, and currently its use is spreading all over in the Western world. In the present paper we describe the chief characteristics of ayahuasca, discuss important questions raised about its use, and provide an overview of the scientific research supporting its potential therapeutic benefits. A growing number of studies indicate that the psychotherapeutic potential of ayahuasca is based mostly on the strong serotonergic effects, whereas the sigma-1 receptor (Sig-1R) agonist effect of its active ingredient dimethyltryptamine raises the possibility that the ethnomedical observations on the diversity of treated conditions can be scientifically verified. Moreover, in the right therapeutic or ritual setting with proper preparation and mindset of the user, followed by subsequent integration of the experience, ayahuasca has proven effective in the treatment of substance dependence. This article has two important take-home messages: (1) the therapeutic effects of ayahuasca are best understood from a bio-psycho-socio-spiritual model, and (2) on the biological level ayahuasca may act against chronic low grade inflammation and oxidative stress via the Sig-1R which can explain its widespread therapeutic indications.

Keywords: addiction medicine, ayahuasca, diseases of civilization, dimethyltryptamine, oxidative stress

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INTRODUCTION

Ayahuasca, a psychoactive Amazonian sacrament, has raised increased scientific and lay interest during the last two decades. Traditionally ayahuasca has been used in Ecuador, Columbia, Peru, and Brazil, where it is also known as *natema*, *hoasca*, *daime*, *yagé*, or *yajé*. The decoction is prepared by simultaneously boiling two admixture plants, the *Banisteriopsis caapi*

Abbreviations: DMT, dimethyltryptamine; ER, endoplasmic reticulum; LGI, low-grade inflammation; MAM, mitochondrion-associated membrane; MDP, mesolimbic dopamine pathways; PTSD, post-traumatic stress disorder; Sig-1R, sigma-1 receptor; UPR, unfolded-protein response.

(Malpighiaceae) containing β-carboline type alkaloids such as harmine and tetrahydroharmine; and most commonly *Psychotria viridis (Rubiaceae)*, which provides the psychoactive alkaloid DMT (McKenna, 2004; Szára, 2007). Sometimes *Psychotria viridis* is substituted by other DMT containing plants such as *Diplopterys cabrerana (formerly B. rusbyana)* of the family *Malpighiaceae*. The name ayahuasca is a compound word in Quechua language, where *aya* means soul, ancestors or dead persons and *wasca* (*huasca*) means vine or rope (Luna, 2011). Therefore, the most prevalent translation of the word is "vine of the soul". Skeptics may prefer the other linguistic alternative: "rope of death", but this paper will provide arguments favoring the former interpretation above the latter one.

Ayahuasca has been used as a central element of religious, magical, curative, initiation, and other tribal rituals for millennia (Naranjo, 1986), originally by the indigenous groups and later by the mestizo populations of the region, who respect the brew as a sacrament and value it as a powerful medicine. The indigenous and mestizo communities regularly use ayahuasca to treat physical ailments, mental problems and frequently handle their social issues, spiritual crises with the help of the brew. A Peruvian tradition called *vegetalismo* regards ayahuasca as one of the teacher plants that convey knowledge to humans (Luna, 1986), and considers the experience induced by its ingestion *trabajo* (work). In addition to its traditional and mestizo uses, ayahuasca also forms a central component of the rituals of three Brazilian syncretic churches: the *Santo Daime*, the *União do Vegetal* and the *Barquinha*. The history of these churches dates back to the first half of the 20th century, and by now they are present in 23 countries (de Rios and Rumrrill, 2008; Liester and Prickett, 2012). Obviously there is a striking discrepancy between the indigenous South American and official Western view[1] on ayahuasca use, which calls for scientific explanation and a healthy resolution.

Due to the growing popularity of the sacrament, masses of people from all parts of the world travel to the Amazon to participate in ayahuasca rituals. This unique phenomenon characterized by some as "drug tourism" (de Rios, 1994) is not as frivolous pursuit as it sounds (Grunwell, 1998), since a significant number of travelers searches for spiritual and therapeutic opportunities. The principal motivations can be characterized as: seeking improved insight, personal growth; emotional healing; and contact with a sacred nature, deities, spirits and natural energies produced by the ayahuasca (Winkelman, 2005). The trend of popularization—known as the "globalization of ayahuasca"—flows both ways, as this Amazonian tradition spreads beyond its native habitat and gets adopted into non-indigenous circles of the Western world (Tupper, 2008) either within or outside of the context of syncretic churches.

During the last couple of years several publications have been written with the goal to summarize our knowledge about ayahuasca from various perspectives (see in Labate and Cavnar, 2014). The primary aim of this article is to give an overview about the facts and hypotheses related to the possible therapeutic mechanisms of the brew in light of recent advances of the field; with the secondary aim of addressing its known adverse effects. By adhering to a biopsychosociospiritual model (Bishop, 2009) the authors will explore every level in the following order: starting with biochemistry, neuropharmacology, physiology, brain imaging, then moving to the psychological effects, social ramifications, and finally addressing spiritual implications. Efforts are taken to keep a balance among the biomedical, psycho-social and spiritual aspects of healing since "*Madre Ayahuasca, la sagrada medicina* (Mother Ayahuasca, the sacred medicine)" is best understood within this quadrilateral framework.

THE NEUROBIOLOGICAL BACKGROUND OF AYAHUASCA

From a pharmacological perspective the main ingredients of ayahuasca are DMT and the β-carboline derivative alkaloid harmine, harmaline, and tetrahydroharmine (Callaway et al., 1996). The harmine, tetrahydroharmine, and harmaline work as reversible inhibitors of the A-type isoenzyme of the monoamine oxidase (MAO), while tetrahydroharmine also exerts selective serotonin reuptake inhibitor (SSRI) effects (dos Santos, 2010). The hallucinogenic component DMT is abundant in the plant kingdom (Khan et al., 2012) and it is also present in mammalian organism; studies have detected it in human blood, brain, cerebrospinal fluid (Wallach, 2009), and the pineal gland of rats (Barker et al., 2013). While DMT is classified as an endogenous hallucinogen, together with bufotenin and 5-methoxy-DMT (Christian et al., 1977; Hollister, 1977), its exact function is yet unclear (Barker et al., 2012).

More than 50 years of research has proven to be insufficient to provide a proper neurobiological description of the role of endogenous hallucinogens. This is in part due to a paradigm problem in which these natural substances with many biological functions have been primarily studied in terms of being "hallucinogens," producing false perceptions. It is obvious that these substances play a role in producing alterations of consciousness such as dreaming, psychosis, and near death experience (Strassman, 2001). These effects presumably reflect action on serotonin (5-HT) receptors (5-HT1A, -2A and -2C) as well as the trace amine associated receptors (supposedly TAAR6) (Wallach, 2009). While the scientific knowledge about trace amine associated receptors is rapidly increasing, it is still deficient. However, the Sig-1R action of DMT may turn out to be more revealing about its physiological function (see below).

[1]The purpose of traditional ayahuasca use has been to heal both physical and spiritual ailments. *Mestizo curanderos* consider ayahuasca as a plant teacher Luna (1984). DMT has been scheduled worldwide as an illicit drug with no medical use. Due to their DMT and/or β-carboline content ayahuasca constituent plants are included as controlled substances in several countries (i.e., Australia, Bulgaria, Canada, France, Lithuania, Poland, Switzerland, and Ukraine). Ayahuasca's classification in France as *stupéfiant* illustrates well the discrepancy with *Mestizo* views.

Dimethyltryptamine exerts anxiolytic effects through 5-HT1A receptor agonism (Jacob and Presti, 2005), and its psychedelic effect is connected to its 5-HT2A receptor-activating capacity (Nichols, 2004). However, simple 5-HT receptor mediated actions are not sufficient to explain drug-induced hallucinations since 5-HT itself, and some 5-HT2A agonists (i.e., lisuride) are not hallucinogenic. Over the past two decades, it became clear that different agonists having similar binding affinities for the same sites, could elicit distinct signaling pathways within the cell. These observations are interpreted on the basis of receptor–receptor and ligand–receptor interactions such as "receptor oligomerization," "receptor trafficking," or "biased agonism" (Moreno et al., 2011; Borroto-Escuela et al., 2014) which activate different G proteins resulting in divergent intracellular cascades. **Figure 1** schematically illustrates the mechanism of receptor dimerization wherein metabotropic glutamate (mGlu2) receptors belonging to an entirely separate receptor family form a complex with the 5-HT receptor and trigger an intracellular pathway for hallucinogenic action. This may explain why lisuride which has a similar receptor binding profile to the chemically similar ergoloid lysergic acid diethylamide (LSD), lacks the psychedelic effects of its sister compound (Rogawski and Aghajanian, 1979). In case of DMT, a recent study (Carbonaro et al., 2015) concluded that while 5-HT2A receptors play a major role in mediating its effects, mGluR2 receptors likely modulate the action. Unlike the related tryptamine derivative psilocybin, DMT does not precipitate tolerance upon repeated use (Strassman et al., 1996); this produces further complications for simple receptor-based interpretations.

The latest identified target for DMT's action is the Sig-1R. Sigma receptors were originally misclassified as opioid receptors but later turned out to be non-opioid receptors of their own type. The Sig-1R subclass has been demonstrated to consist of chaperone molecules concentrated in normal cells of the brain, retina, liver, lung, heart, immune system, but also in many tumor lines (Maurice and Su, 2009). Chaperones are proteins that assist the correct folding of other protein clients. The Sig-1R chaperon has many unique features with an amino acid sequence distant from mammalian proteins and homologous to fungal sterol isomerases (Moebius et al., 1997). Sig-1R sites are concentrated in the human brain with the highest densities in the cerebellum, nucleus accumbens, and cerebral cortex (Weissman et al., 1988). Inside the cell Sig-1R is located mainly at the ER–mitochondrion interface—referred to as the MAM—and regulates cellular bioenergetics, particularly under stressful conditions (Su et al., 2010; Mori et al., 2013; Hayashi, 2015). There is another mode of Sig-1R action at the plasma membrane where it translocates under stimulation by agonists.

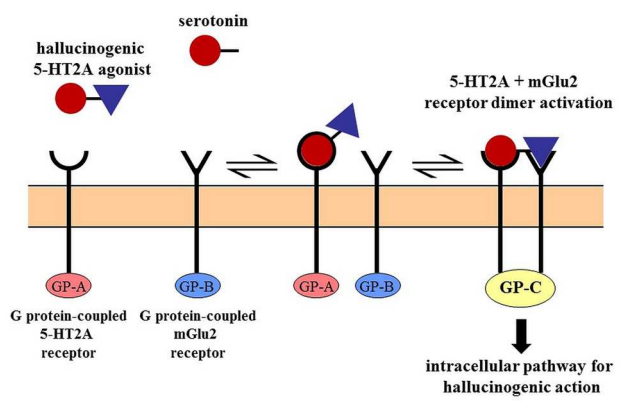

FIGURE 1 | Cross-talk between receptors by dimerization. The 5-HT2A receptor mediated hallucinogen-specific intracellular pathway requires the dimerization of the 5-HT2A receptor with the mGlu2 receptor. This unique (G protein-coupled) pathway is associated only with the dimer and not activated by either receptor alone. Serotonin lacks the mGlu2 receptor binding feature and the psychotropic effects of hallucinogens are abolished by the elimination of the mGlu2 receptor. The 5-HT2A-mGlu2 dimer is the prime target of some serotonergic hallucinogens (Moreno et al., 2011).

As an intracellular receptor localized at the MAM, Sig-1R integrates many signaling pathways and serves as a "tunnel" for lipid transport and Ca^{2+}-signaling between the ER and mitochondria (Hayashi and Su, 2007). Its involvement is critically in ion channel activities and neuronal differentiation. The wide scope and effect of ligand binding to Sig-1R indicate that Sig-1Rs are intracellular signal transduction amplifiers (Su and Hayashi, 2003). The ER-mitochondrion interface at the MAM serves as an important subcellular entity in the regulation of cellular survival via Sig-1R by enhancing the stress–response signaling (Mori et al., 2013). Sig-1R also protects the cells against reactive oxygen species and activates the antioxidant response elements (Pal et al., 2012), therefore Sig-1R agonists such as DMT may function as indirect antioxidants. More interestingly the induction of Sig-1R can repress cell death signaling: up-regulation of Sig-1R suppresses the apoptosis caused by ER stress (Omi et al., 2014). Tryptaminergic trace amines as well as neurosteroids (e.g., dehydroepiandrosterone, pregnenolon) are endogenous ligands that activate the Sig-1R (Fontanilla et al., 2009).

The Sig-1R ER chaperone function is essential for the metabotropic receptor signaling and for the survival against cellular, particularly ER stress. Dysfunctional chaperones are responsible for numerous diseases (Tsai et al., 2009). Altogether, no other receptor has ever been associated with so many different diseases as the Sig-1R. It has so far been implicated in illnesses like Alzheimer's disease, Parkinson's disease, cancer, cardiomyopathy, retinal dysfunction, perinatal and traumatic brain injury, frontal motor neuron degeneration, amyotrophic lateral sclerosis, HIV-related dementia, major depression, and psychostimulant addiction (Su, 2015). How those two modes of actions of the Sig-1R may relate to this plethora of diseases remain to be clarified but its protective influence has been verified on various aspects of cellular processes, such as calcium signaling, mitochondrial functions, ER stress, survival and apoptotic pathways (to be discussed later), and tumor cell proliferation (Tsai et al., 2014). As Sig-1Rs are expressed in the immune system, immunomodulatory functions have also been reported in the literature (Szabo et al., 2014; Dong et al., 2015).

THE POSSIBLE ROLE OF DMT IN TISSUE PROTECTION AND NEUROREGENERATION

Dimethyltryptamine is considered as a natural ligand, an endogenous agonist of the Sig-1R (Fontanilla et al., 2009). It is assumed that the Sig-1R might be involved in the DMT-induced psychedelic effects (Su et al., 2009); however, this is somewhat counterintuitive since many drugs—including non-hallucinogens—bind promiscuously to the Sig-1R with higher affinity than DMT. The results of a recent surge in Sig-1R research are pointing toward a different horizon by outlining a physiological role of DMT instead of the long-held pathological view. If the Sig-1R promotes cellular (neuronal) survival against oxidative stress (Pal et al., 2012) and regulates immune processes (Jarrott and Williams, 2015), one may attribute the same physiological role to its endogenous ligands, like DMT (Frecska et al., 2013). Since the Sig-1R is also known to regulate morphogenesis of neuronal cells, such as neurite outgrowth, synaptogenesis, and myelination (Ruscher and Wieloch, 2015); neurorestorative effects are reasonably expected from DMT. In a previous paper (Frecska et al., 2013) we concluded that the function of DMT may extend central nervous activity and involve a more universal role in cellular protective mechanisms. We provided converging evidence that while DMT is a substance which produces powerful psychedelic experiences, it is better understood not as a hallucinogenic drug of abuse, but rather an agent of significant adaptive mechanisms like neuroprotection, neuroregeneration, and immunity.

Nevertheless, immunoregulation by DMT is a bidirectional process. Sig-1Rs are expressed together with 5-HT receptors in immune cells conveying both inflammatory and anti-inflammatory signals (Szabo, 2015). These receptors are essential in the "fine-tuning" of innate and adaptive immune responses. Human clinical studies showed that ayahuasca can alter the number and distribution of blood immune cells in a way that can increase the antiviral and anti-tumor immunity of the consumer (reviewed in Frecska et al., 2013). Ayahuasca also influences the distribution of lymphocyte subpopulation: CD4 lymphocytes decrease and the number of natural killer cells increase significantly with time (dos Santos et al., 2012). The possible anti-cancer activity of the decoction makes it a promising candidate for further researches in novel pharmacotherapies (Schenberg, 2013). Furthermore, DMT may also be an adaptogen increasing the survival rate of neurons or other cell types during acute hypoxia or under chronic oxidative stress.

MECHANISMS PROPOSED AS A BASIS FOR AYAHUASCA's EFFECTS ON SYSTEMIC AND DEGENERATIVE ILLNESSES

Chronic LGI is becoming widely accepted as a common basis for many diseases of civilization (Ruiz-Núñez et al., 2013; **Table 1**). Repeated psychological stress, constant environmental pollution, and smoking behavior are associated with chronic LGI, which is one of the main causes of insulin resistance that is the pathological foundation of metabolic diseases (Aseervatham et al., 2013). Moreover, chronic LGI is involved in all stages of the atherosclerotic process and is being increasingly recognized as a universal mechanism in various chronic degenerative diseases, such as autoimmune diseases, certain cancers, neuropsychiatric diseases (e.g., Alzheimer's disease, Parkinson's disease, major depression), and osteoporosis. Dysbiosis of the gut microbiota with increased intestinal permeability ("leaky gut") are all possible root causes of LGI (Carrera-Bastos et al., 2011).[2]

Chronic LGI is closely related to oxidative stress and ER stress, and together they form a molecular web, a network interwoven with loops exacerbating each other (Chaudhari et al., 2014). Regulation of protein folding homeostasis (proteostasis) is essential for the execution of fundamental cellular functions. ER is the cellular organelle responsible for this role. Disturbance in protein folding is central to a large diversity of illnesses and growing evidences suggest ER stress as being a cardinal component in the development of a pathological condition (Díaz-Villanueva et al., 2015; Srivastava and Kumar, 2015). The cause of diseases may be various, but ER stress resulting from chronic LGI or oxidative stress may contribute to the severity and the poor prognosis of the diseased state. ER function can be altered and made dysfunctional by hypoxia, hyperglycemia, hyperlipidemia, viral infections, disturbances in cellular calcium levels, redox regulation, or by endogenous reactive oxygen species production (Chaudhari et al., 2014). These so-called stress signals exhaust the ER machinery and result in accumulation of unfolded proteins. An adaptive process called UPR is triggered with an aim to restore ER homeostasis. However, if the stress signal is severe and/or prolonged cell death pathways are elicited in form of apoptotic and pro-inflammatory reactions (Hiramatsu et al., 2015).

ER stress with UPR is thought to play a key role in neuropsychiatric illnesses such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, bipolar disorder, and in other illnesses of civilization such as atherosclerosis, diabetes, cancer, autoimmune, and cardiovas cular disorders (Chaudhari et al., 2014). All of these disorders may have common mechanism: failure of protein homeostasis. Deficits in ER-proteostasis lead to the formation of misfolded proteins characteristic of neurodegenerative diseases (Hetz and Mollereau, 2014; Plácido et al., 2014). Originally UPR has a cell protective effect: it prevents overload of ER lumen with newly synthesized proteins and activates degradation of misfolded proteins. However, misfolded proteins directly enter from ER into mitochondria and after prolonged UPR activation they

[2]Based on more and more converging evidence, it is tempting to speculate that the "Grand Unification Theory" (GUT) of medicine can be based on the gut. According to this unifying concept, a "leaky gut" lets some gut contents (e.g., bacterial endotoxins) to enter circulation and shifts the anti-inflammatory ↔ pro-inflammatory cytokine balance to pro-inflammatory direction. A long-standing pro-inflammatory condition with the simultaneous oxidative, mitochondrion, and ER stress (see in the text) can change the permeability of other membranes in the body (e.g., blood-brain barrier, synovial stratum, endothel, bronchial mucosa, and/or alveolar wall). This way the permeability problem of the gut extends to other barriers, and a universal membrane deficiency may serve as a common ground of many illnesses of civilization.

TABLE 1 | Common diseases of civilization.

• Metabolic syndrome	• Neurodegenerative diseases
• Cardiovascular diseases	• Alzheimer's dementia
• Cancer	• Autism
• Osteoarthrosis, osteoporosis	• Depression
• Autoimmune disorders	• Schizophrenia
• Chronic obstructive pulmonary disease	• Attention deficit hyperactivity disorder
• Macular degeneration	• Chronic pain syndrome

The table lists those illnesses which—due to different diet, lifestyle, and environmental factors—are more prevalent in advanced agricultural and industrial societies as compared to hunter-gatherers, traditional pastoralists and horticultural communities, or have poorer outcome in the urban environment (after; Carrera-Bastos et al., 2011; Aseervatham et al., 2013; Ruiz-Núñez et al., 2013; Srivastava and Kumar, 2015).

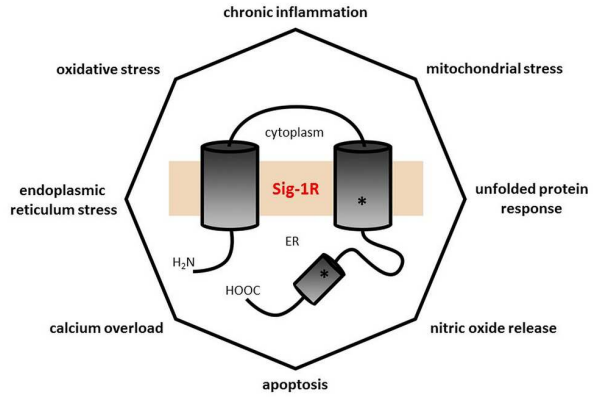

FIGURE 2 | The polygon of self-destructive forces. Every angle of this octagon represents a pathophysiological process closely related to almost all of the others, and each pathological process is known to be involved in several illnesses of civilization (see **Table 1**) with extensive overlap (e.g., Alzheimer's disease has chronic low grade inflammation, increased nitric oxide signaling, calcium dyshomeostasis, apoptosis, mitochondrial dysfunction, oxidative, and endoplasmic reticulum stress in its etiopathology). The central position of the Sig-1R illustrates its significant influence in mitigating these pathological processes. The number of angles is rather arbitrary: one may add others like insulin resistance, glutamate release, plasma membrane deficiency, etc.

cause dysfunction in energy production (Schon and Area-Gomez, 2013; Volgyi et al., 2015). Targeting Sig-1R by agonists may regulate ER stress and UPR, manage ER perturbations, regulate the formation of toxic misfolded proteins, and prevent the cell-killing apoptotic pathways (Rivas et al., 2015). Similar effects are expected from the endogenous Sig-1R ligand DMT.

Tracing Alzheimer's disease, Parkinson's disease, or major depression to inflammatory processes (chronic LGI), "leaky gut", ER stress, protein folding deficit, glutamate excitotoxicity, mitochondrial dysfunction, calcium overload, death receptor pathways, and Sig-1R involvement means following the loops of the same web of interactions since all are different aspects of the same core phenomenon. The Sig-1R located at the MAM is an excellent candidate for interfering with the conversion of environmental stress in general and psychological stress in particular into cellular stress response by its regulatory effect on signaling between the ER and mitochondrion (Hayashi, 2015). This is the point where DMT and ayahuasca take their place in this puzzle via Sig-1R action. It seems to us that the ingenuity of South American people discovered a broad-spectrum remedy, which hits the dead center of the discussed vicious circle of the malfunctioning molecular web involved in oxidative stress (**Figure 2**).

There is more to ayahuasca's therapeutic potentials besides its DMT content and above the neurobiological level. The psychological aspects will be discussed later. Here we address the other important active agents of ayahuasca, the β-carboline alkaloids, which act as selective, reversible MAO-A inhibitors (McKenna and Towers, 1984; dos Santos, 2010) with almost no effect on MAO-B (Herraiz et al., 2010). MAO inhibition is crucial as without the β-carbolines the DMT content of orally ingested ayahuasca would be broken down before crossing the blood-brain barrier. Moreover, the fact that MAO is located inside cells bound to the outer membrane of mitochondria in proximity of the Sig-1R raises the possibility that the synergy between the active compounds of ayahuasca happens not only at the periphery, but also inside neurons and glial cells. Without this intraneuronal MAO inhibition less DMT would reach the Sig-1R at the MAM. Furthermore, there are indications that the β-carboline alkaloids themselves have medicinal properties, such as anthelmintic (Hassan, 1967), antimicrobial (Ahmad et al., 1992), and vasorelaxant (Shi et al., 2001) effects, in addition to ethnopsychiatric (Shepard, 1998), sociopsychotherapeutic (Andritzky, 1989), and rehabilitative functions (Mabit et al., 1995). Harmala alkaloids have demonstrated strong psychoactive properties (Naranjo, 1967), and they act as stimulants on the central nervous system (Venault and Chapouthier, 2007). Osório's team (Osório et al., 2011) attributed an observed antidepressant effect of ayahuasca (Osório et al., 2015) to these alkaloids, which is in line with ethnographic observations suggesting that many native users of ayahuasca ascribe sacramental respect to the *B. caapi* and not the DMT containing plant constituents.

From a biological standpoint the extent to which DMT and harmine play a role in ayahuasca effects is difficult to judge since the brew contains a significant amount of bioactive substances in addition to the indole and β-carboline alkaloids. An important example of such compounds is the group of antioxidant polyphenols, which can also be linked to the observed immunomodulatory effects (Szabo et al., 2014). Antioxidants are known for their capacity of reducing inflammatory processes or even stopping them (Grimble, 1994; Geronikaki and Gavalas, 2006). Malignant transformation is also inhibited by polynucleotides through providing protection against oxidative stress for other cellular compounds (Marquardt, 1984). In addition to the immunomodulatory effects, ayahuasca may also exhibit neuroprotective and neurorestorative qualities. Hence, it has been suggested that ayahuasca can be applied therapeutically in Parkinson's and other neurodegenerative diseases (Samoylenko et al., 2010). Ayahuasca's high content of bioactive materials points toward a combined mechanism of the various effect and calls for further clinical research to reveal the detailed pharmacology of the constituents.

VEGETATIVE AND ADVERSE EFFECTS OF AYAHUASCA

Serotonin stimulation is known to affect the whole organism not just the brain. It causes vegetative changes such as increase in systolic and diastolic blood pressure, pulse rate, provokes nausea, vomiting, and pupil dilation (Boyer and Shannon, 2005). Ayahuasca was found to significantly raise the systolic and diastolic blood pressure by 35 Hgmm and the pulse rate by 26 bpm in a 2 min time interval while the rate of increase declines upon repeated intake (Riba et al., 2001). Besides actions on the vegetative nervous system ayahuasca also induces endocrine response. Elevation in prolactin, cortisone, and growth hormone levels has been reported (dos Santos et al., 2011; dos Santos et al., 2012).

While the intravenously injected DMT can cause considerable cardiac stress, it is less burdensome for humans if taken orally. Based on animal research Gable (Gable, 2007) developed a model calculation that determined the median lethal dose of DMT in 8 mg/kg for oral ingestion in human subjects. The average ceremonial dose of DMT in ayahuasca preparations is about 27 mg; therefore, the safety margin for ayahuasca is approximately 20 (Gable, 2007). Scientific sources mention only one fatal case of ayahuasca consumption (Sklerov et al., 2005). The toxicological judgment based on uncontrolled street abuse is largely influenced by cases when extra ingredients other than the two basic components (e.g., datura or tobacco) are mixed into the decoction since the intoxicating effects of these extra ingredients can in turn be attributed to ayahuasca by the toxicological reports.

The MAO-A inhibition induced by the β-carboline alkaloids presumably results in an increased level of serotonin in the neural pathways, which theoretically can lead to serotonin syndrome in extreme cases (Callaway and Grob, 1998). However, the competitive, reversible nature of the inhibition may explain the lack of well documented serotonin syndrome cases despite the globalization of ayahuasca and probable inclusion of a large number of session participants taking SSRIs. What makes the issue more complicated is that at the time of onset, ayahuasca's vegetative effects represent some sort of mild or moderate serotonin syndrome. Nevertheless, materials which may negatively interact with ayahuasca and capable to induce hyperserotonemia include ginseng (*Panax ginseng*), St John's-wort (*Hypericum perforatum*), dextromethorphan, 3,4-methylenedioxy-*N*-methylamphetamine, SSRIs or MAO inhibitors (Callaway and Grob, 1998). Furthermore, cardiovascular or endocrine problems, abnormal lipid metabolism, glaucoma, fever, and pregnancy are contraindications for ayahuasca consumption (Gable, 2007).

A tendency for psychosis or family history of mental illness predispose ayahuasca for triggering a psychotic episode or long-term depersonalization syndrome. dos Santos and Strassman (2011) reported a case where a young male individual with prior experience in the use of psychedelic substances and having previous positive experience with ayahuasca fell repeatedly into psychosis after two *Santo Daime* ceremonies. Even without proper screening the statistical probability of such cases seems to be low. The majority of the encountered problems originate from the unpreparedness of the participants, the inadequate setting (the parameters of the circumstances in which the ingestion of ayahuasca takes place), the lack of socio-cultural-cosmological embeddedness of the experiences or the lack of their integration afterward.

There is not yet any scientific evidence—or even personal reports—indicating that ayahuasca use elicits substance dependence. The ritual use of ayahuasca shows considerable differences to the traditional psychosocial harms of drug consumption (Fábregas et al., 2010). As with other psychedelic experiences an elevated emotional state may remain for a few days or weeks after ayahuasca consumption. In such cases life seems more beautiful and joyful, filled with a deeper meaning than before the experience. Therefore a feeling of emptiness or grayness can arise after the experience fades away but this is not generally the case.

CENTRAL NERVOUS SYSTEM EFFECTS OF AYAHUASCA

Riba's group (Riba et al., 2006) conducted single-photon emission computed tomography to reveal the brain areas affected by ayahuasca ingestion. Increased activity was recorded bilaterally in the anterior insula, in the fronto-medial cortical anterior cingulate of the right hemisphere and the left amygdala. The latter two play a role in the regulation of emotional arousal and the information processing of emotions. Moreover the anterior cingulate is involved in somatic attention and the experience of subjective feeling states (Bouso and Riba, 2011). By analyzing the binocular rivalry under the effect of the brew, conclusions were drawn that ayahuasca influences hemispheric dominance (Frecska et al., 2003) and gamma oscillations (Frecska et al., 2004).

Using functional magnetic resonance imaging technique de Araujo et al. (2012) found that ayahuasca induces a robust activation of occipital, temporal, and frontal areas during a closed-eyes imagery task. The consumption of the brew activated an extended network in the brain (that has previously been correlated to visual perception, memory and intention) in which the Brodmann areas BA10, BA17, BA30, and BA37 play a central role. The ayahuasca-produced stimulation of the primary visual cortex was comparable to the effect of natural images with the eyes open. Moreover, this effect correlated significantly with the occurrence of perceptual changes measured by rating scales. These authors concluded that by boosting the intensity of imagination to the same level of sensory perception ayahuasca lends a status of reality to inner experiences.

Several studies have employed electroencephalographic methods to record brain oscillatory activity after ayahuasca intake. Results indicated significant power increase in the slow gamma (36–44 Hz) band at left occipito-temporo-parietal electrodes, with tendencies to power decreases in theta and delta bands (Don et al., 1998). Another study revealed alpha power decrease at left temporal and centro-parietal sites peaking 90 min after ingestion, while decreases were found for delta

and theta waves in the parietal areas (Riba et al., 2002). The analysis of Alonso's group (Alonso et al., 2015) showed that ayahuasca preparation significantly changed the coupling of brain oscillations between anterior and posterior recording sites in the following pattern. Frontal structures decreased their influence over posterior (central, parietal, and occipital) sites which correlated with the intensity of subjective effects. On the other hand, posterior areas increased their influence over signals measured at anterior locations in parallel with the degree of incapacitation experienced (Alonso et al., 2015). These effects reflect the general action of psychedelics hypothesized by Winkelman (2007) in the proposed concept of psychointegrator, where the normal domination of cognition by frontal brain activity is replaced by intense discharges from the lower areas of the brain that are imposed on the frontal cortex.

Another recent project addressing the time course of ayahuasca effects on the electroencephalogram revealed a biphasic feature (Schenberg et al., 2015). After 50 min from ingestion of the brew there was observed a reduced alpha band activation over the left parieto-occipital cortex, followed by increased slow- and fast-gamma power (30–50 and 50–100 Hz, respectively) between 75 and 125 min. The slow-gamma power increase was located at the left centro-parieto-occipital, left fronto-temporal, and right frontal cortices while fast-gamma increases were significant at left centro-parieto-occipital, left fronto-temporal, right frontal, and right parieto-occipital areas. These effects correlated significantly with the circulating levels of ayahuasca's main ingredients, such as DMT, harmine, tetrahydroharmine, and some of their metabolites. The significance of gamma power in the broader context of consciousness involves its role in binding of information across diverse regions of the brain and in driving theta wave responses.

Based on the conceptual framework of integrated information theory Gallimore (Gallimore, 2015) supposed that the promotion of gamma oscillations is responsible for the perceptual effects of psychedelic drugs and surmised that the psychedelic state might be characterized by an increase in integration compared to a normal waking state. Gallimore referenced a small study which employed quantitative electroencephalography to measure changes in gamma oscillatory power and coherence following ayahuasca ingestion and reported a highly integrated brain state (Stuckey et al., 2005). This finding is in accordance with an earlier study showing an increase of the gamma band power in ayahuasca users (Don et al., 1998). However, contrary to these results, other studies found generalized decreases in power across all frequency bands (Riba et al., 2002, 2004). While the neuropsychological correlates and clinical relevance of these electroencephalogram patterns remain to be elucidated, Gallimore's work underscores the necessity of a comprehensive approach to ayahuasca's pharmacology and the mechanisms of its cognitive, affective, and emotional effects.

The central nervous system effects of ayahuasca are distinct from our normal resting mental states sustained by the Default Mode Network as defined by specific brain areas which are activated when the person is at rest and/or not engaged in specific mental tasks. This Default Mode Network is usually active in meta-cognition, day-dreaming, reflecting on memories, but is apparently disabled by psychedelics (Carhart-Harris et al., 2014). Psychedelics alter this relaxed brain function by reducing cerebral blood flow and the oscillatory power in brain areas of the Default Mode Network that are typically synchronized and functionally connected. Ayahuasca decreases the functional connectivity within the prefrontal cortex and in connections with other areas of the brain that are involved in a wide range of ordinary cognitive processes (Palhano-Fontes et al., 2015).

This decoupling phenomenon results in increased flexibility of high-level networks involving a more open communication among them. It permits a freer operation of the medial temporal lobe structures, which are associated with the release of cognitive states closely related to emotions and fears. The outcome is a complex mental condition characterized by increased somatic awareness and subjective feelings, but lacking the metacognitive ability for self-reflection on personal behavior and one's mentalization provided by the frontal cortex. This so-called primary cognition produces a state of heightened suggestibility because of the suspension of the frontal networks that are typically used to maintain control over mental processes and perceptions of the outside environment.

Bouso and Riba (2014) suggested that these varied effects of ayahuasca may also result from its ability to increase activity in various areas of the right hemisphere (anterior insula, anterior cingulate/fronto-medial cortex). These areas are implicated in somatic awareness, emotional arousal, feelings, and processing of emotional information. Ayahuasca also appears to increase activity in the left hemisphere's amygdala/parahippocampal gyrus structures that play a role in emotional arousal and memory, enabling ayahuasca to make repressed memories conscious and to re-experience emotions associated with them. Such apperception enables one to reprocess these memories in more constructive ways and with a potential for processing traumatic pasts in novel ways.

NEUROCHEMICAL AND PSYCHOPHYSIOLOGICAL MECHANISMS PROPOSED AS A BASIS FOR AYAHUASCA'S EFFECTS ON ADDICTION

There are a variety of biochemical and physiological mechanisms through which ayahuasca can effectively address addictions (Prickett and Liester, 2014). The inclusion of two plant species in ayahuasca provides a variety of mechanisms for direct and indirect actions on both dopaminergic and serotonergic systems. Since the effects of DMT appear to reflect the general effects of tryptamines (e.g., DMT, LSD, bufotenin, psilocin, and psilocybin) some of therapeutic mechanisms would also be shared with these related substances. The effects of the harmine alkaloids, however, would be unique to ayahuasca. Separate studies with each of these chemical classes will be necessary to distinguish their different contributions.

Liester and Prickett (2012) proposed that "ayahuasca exerts anti-addictive properties via its direct and indirect actions

on dopaminergic and serotonergic neurons in the mesolimbic pathway. Ayahuasca raises global 5-HT levels attenuating withdrawal effects and mitigating against potential dopaminergic excess when utilizing dopamine (DA) agonists. Ayahuasca balances DA in the MDP between the low levels associated with withdrawal and the elevated levels associated with initiation and reinforcement of addictive behavior" (Prickett and Liester, 2014). Therefore, the resolution of addiction through ayahuasca's therapeutic potentials may act on four levels: (1) reducing brain DA level in the MDP through effects on the 5-HT receptors, which in turn (2) interferes with the synaptic plasticity. This neurochemical mechanism is supported by (3) psychological insights and processing of repressed traumas, enhancing decision making capabilities, which allowing the person to (4) examine first person transcendental experiences.

Additional neurophysiologic mechanisms for ayahuasca's therapeutic effects involve neuroplasticity, the ability of neurons to alter their synaptic connections. Constituents of ayahuasca may affect brain derived neurotropic factor release through effects on the GABAergic and glutamatergic systems. These are involved in producing neuroplastic changes through triggering changes in gene expression which affect the architecture and communication between neurons. These exert effects on the existing neural circuits which mediate maladaptive addictive habits in stimulating the production of new circuits supporting new behaviors, with ayahuasca facilitating a neurological rewiring of the brain's reward pathways. This model is supported by animal experiments (Oliveira-Lima et al., 2015) which demonstrated that ayahuasca not only inhibits early behaviors associated with the initiation and development of alcohol addiction, but also has long-term effects in preventing the reinstatement of ethanol-induced behavioral sensitization.

PSYCHOLOGICAL AND PSYCHOSOCIAL EFFECTS OF AYAHUASCA

After an approximately 35 to 40-min latency period, the consumption of ayahuasca induces an intense modified state of consciousness that lasts approximately 4 h (McKenna, 2004). Perception, spatiotemporal orientation, beliefs about reality and the self, cognitive and emotional processes can all alter significantly during the experience. Visions of beautiful visual scenery are commonly reported together with some typical elements of the "ayahuasca world": ayahuasca beings, power animals, spirit guides, tropical motifs, vibrant, and varying geometric patterns known from the literature of the cultural anthropology of shamanism. The neurological background of the strong visual effects has been revealed by functional magnetic resonance imaging studies as it was discussed above (de Araujo et al., 2012).

Considering the psychological therapeutic benefits, the emphasis is on the induction of an introspective dream-like experience characterized by visions, autobiographic and emotional memories which increase mindfulness capacities (Soler et al., 2015), as well as on the intellectual and spiritual insights gained during the encounters with ayahuasca. Ayahuasca experiences are a constant flow of mental contents, during which knowledge is gained by intuition rather than logic. They also show a high level of overall coherence. The level of self-reflection, reminiscence, ethical sensation, prosocial behavior (Frecska, 2008), creative thinking (Frecska et al., 2012), and redemption (de Rios et al., 2002) can be increased or elicited during the experiences. Various psychological blockages and denials may enter awareness and become illuminated from multiple perspectives allowing the participants to gain insight into their maladaptive behavioral, emotional and/or cognitive patterns. These psychodynamic contents are often accompanied by an enhanced internal moral attitude that forces the participants to face their deep thoughts and emotions that confront them with earlier wrongdoings, self-deceptions, and lies (Frecska, 2011). Repressed memories can surface causing emotional catharsis and opening the way to abreaction, relief, and remission. The strong serotonergic effect of ayahuasca and the internal phenomenon of being held or guided by an intelligent power can be considered responsible for the observation found in many personal reports that indicate that the retraumatization by the experience is avoided by reaching a certain flip-point, a "slew-round" to a previously inaccessible, corrective positive aspect of the emotional pattern. In such cases post traumatic growth becomes possible.

The emotional charge of ayahuasca experiences often follows a sine wave. Kjellgren et al. (2009) described an initial "contractile frightening state" during which participants frequently confront their innermost fears: fear of insanity, fear of death, paranoid thoughts or the despair of cosmic loneliness and outcast. Distressful somatic symptoms such as dizziness, diarrhea, nausea, and vomiting may also arise and become essential part of the process, carrying the subjective experience of purge and relief. If the subject is able to surrender, this unpleasant initial phase is usually followed by a sudden transformation of the experience into transcendental experiences, reflections, changed worldview and/or new orientation to life.

It is frequently reported—especially in what Metzner (Metzner, 1998) called hybrid shamanic and psychotherapeutic ritual setting—that participants arrive at ayahuasca ceremonies with a predefined personal intention such as asking for healing, guidance, teaching or a solution to a personal problem. These intentions seem to support the experience by two means. They seem to provide a basic structure to the unconscious materials that arise during the experience as well as a narrative frame for the interpretation and integration of the experience.

Scientific interest toward ayahuasca has grown rapidly over the last decades and so the most pregnant directions of its possible therapeutic use have begun to be outlined. However, the illicit legal status of the brew that ensues from its DMT content imposes heavy impediments to its scientific understanding. Many findings therefore come from investigations carried out among the members of the Brazilian syncretic churches, where the legal use of ayahuasca primarily serves religious aims instead of therapeutic ones.

The Hoasca Project was first to provide scientific findings regarding the psychopharmacological and psychological effects of ayahuasca (Grob et al., 1996). The study involved 15 members

of the *União do Vegetal* and suggested that the long-term use of ayahuasca resulted in positive behavioral and lifestyle changes in the participants' lives. An unexpected finding was the possible anti-addictive effect of the brew as revealed from the reports of several participants of the study who had struggled with alcohol and substances abuse before having joined the *União do Vegetal*. Two decades later a similar study involving 32 members of *Santo Daime* arrived at the same conclusions (Halpern et al., 2008).

Barbosa's team (Barbosa et al., 2005) concluded that the use of ayahuasca increased assertiveness, joy of life and liveliness among the members of the Brazilian syncretic churches, while da Silveira and others (da Silveira et al., 2005) found that adolescents who regularly consume ayahuasca show less signs of anxiety, are more optimistic, self-confident, insistent, and emotionally mature than their peers. Similarly, results of psychometric tests applied by dos Santos' group (dos Santos et al., 2007) revealed that after ayahuasca use, individuals respond with less anxiety to states that involve hopelessness and resemble panic, while measures of state- or trait-anxiety remain unaffected. Recently Barbosa's team (Barbosa et al., 2012) performed a meta-analysis on publications listed on PubMed with the aim to summarize current knowledge about the effect of ayahuasca on health. It concludes that the consumption of ayahuasca is safe and under certain conditions may even be beneficial. Results of a longitudinal prospective study on a large population of regular users showed no signs of cognitive impairment and the decoction had no negative effect on coping strategies or the general mental health (Bouso et al., 2012). While there are occasional reports of ayahuasca users dying during the episode, they typically reflect underlying health conditions or prolonged neglect of participants during rituals.

The prolonged social contact among participants that is typical of ayahuasca based treatments provides the opportunity for developing the social support that is crucial to recovery from many mental illnesses including addiction. The ceremonial context enhances bonding among participants that can facilitate therapeutic processes, especially through the provision of social support and the enforcement of social norms that encourage an abstinent lifestyle. The participants in ayahuasca ceremonies of the churches provides social support for managing stress and gives a sense of belonging that motivates lasting behavioral changes.

Since DMT is known to be a very potent 5-HT agonist, it can decrease impulsive behavior and facilitate social interactions (Frecska, 2008). A rapid 5-HT receptor action can explain the traditional indication of ayahuasca use in crisis prevention and occasioning redemption (de Rios et al., 2002). The prosocial, cohesive action effect of ayahuasca is reflected in the quality of the elicited subjective experience, which commonly involves ethical lessons (Shanon, 2002). Ayahuasca is highly revered by mestizo curanderos as a stern moral teacher (Luna, 1986).

PSYCHOTHERAPEUTIC AND SPIRITUAL EFFECTS OF AYAHUASCA

Ayahuasca experiences often reflect psychodynamic effects that contribute important therapeutic outcomes through providing a connection with significant aspects of the person's past, elevating repressed memories into consciousness where they can play a role in psychological healing through restructuring. A frequent theme mentioned by victims of abuse and recovered addicts is that the ayahuasca-induced visions helped them to recover long-forgotten memories of traumatic events that they were then able to work through, providing a basis for restructuring their personal life (Loizaga-Velder and Verres, 2014). Ayahuasca-induced insights facilitate self-reflection, producing changes in self perspectives that can trigger psychodynamics insights which provide solutions to personal problems that underlie maladaptive lifestyles. Ayahuasca helps resolve personal conflicts by providing conscious insights into patterns of psychological functioning that underlie pathological behaviors such as substance abuse and dependence. Participants of ayahuasca rituals often report insights that enable acceptance of previously denied problems and dysfunctional patterns. The visionary state of consciousness produced by ayahuasca can also provoke reflections on personal relationships which provided the motivation for making the changes necessary to resolve interpersonal problems.

Hence, ayahuasca's effects appear to evoke psychodynamic mechanisms and psycholytic effects that can augment access to pre-conscious and unconscious memories. This release of repressed emotions can catalyze healing processes by contributing to the resolution of traumas by releasing the person from dysfunctional habits that underlie the dynamics of addiction and many other behavioral problems. Psycholytic processes engendered by ayahuasca also promote an awareness of the likely future outcomes and personal consequences of maladaptive behaviors, providing a motivation for change. Personal accounts of addicts reveal that the ayahuasca experiences led many of them to perceive that their drug use was leading them down a path of self-destruction that would lead to their death. Ayahuasca might produce death experiences, sometime a sense that one was dying, or a vision of oneself as dead as a consequence of drug use. These experiences led to realizations that helped them to make radical changes in their behavior by providing additional motivation to make necessary changes in personal behavior and lifestyle (Loizaga-Velder and Verres, 2014). A basic effect of ayahuasca on psychological process involves a confrontation with oneself, forcing a greater personal awareness that facilitates a reconstruction or restructuring of the nature of oneself (Fernández and Fábregas, 2014). A reassessment of the past provides the basis for an experience of cleansing from the past events and the basis for new perspectives into one's patterns of behavior.

Speculating on the psychotherapeutic effects of ayahuasca, Naranjo (1979) suggested that its effects are similar to that of an intense psychotherapy. He attributed the prime therapeutic effect of ayahuasca to its harmaline content instead of DMT. Trichter (2010) claimed that the therapeutic effect reported in personal anecdotes results from the psychological work being carried out at a much deeper level than in the case of traditional psychotherapeutic methods. Mabit (2007) listed eleven factors that contribute to the brew's therapeutic effect, one of which is its ability to lower defenses and to reveal ego defense mechanisms.

This in turn allows repressed unconscious materials to enter consciousness and extinguish the emotional charge of past traumatic experiences, helping the participants to temporarily experience thus far unknown emotional states and cognitive patterns; and through them they better understand the means and directions of adjustments needed in their lives.

Maté (2014) proposed that ayahuasca is capable of treating many conditions because both physical and psychological conditions can be based in unconscious psychological conditions. Psychedelics assist by bringing these dynamics into consciousness, initiating a process of liberating the person from these influences. Maté (2014) also suggested that while deep psychological dynamics may emerge into awareness during ayahuasca ceremonies, their therapeutic potential depends on trained guidance to bring these potentials to fruition. Successful treatment with ayahuasca requires an experienced person to provide structure and guidance to effectively orient to the visions, the therapeutic purpose, and the development of the experience across sessions. Lacking qualified assistance in achieving their full integration, important experiences may not produce benefits. Nonetheless, he emphasizes that in the right supportive circumstances, ayahuasca can help provide the insights and personal meanings that can help resolve the underlying dynamics of addiction by triggering visions of the emotional states and traumatic imprints.

Frecska (2011) supposes that the experience consists of repeated sequences of deconditioning and reintegration. This can be conceived as a "secure" form of regression, which makes the correction of maladaptive cognitive and emotional structures (personal network of concepts, maladaptive patterns, etc.) possible. Echenhofer (2012) endeavored to draw parallels with experiences from other spiritual traditions and divides the process of deconditioning and reintegration into three subphases: (1) form dismantling and healing, (2) form creation, and (3) form expression.

Ayahuasca also produces transcendent and mystical experiences, the "peak experiences" that led to the "psychedelic" paradigm of LSD treatment that was based in recognition that these substances provide an effective treatment for alcoholism by changing the individual's personal awareness, self-perceptions and worldview. A significant dimension of the spiritual experience was a transformation of personal consciousness in ways that eliminated the craving for drugs. Mystical or spiritual experiences reported during the ayahuasca sessions are frequently said to have a life changing effect on those bearing them, sometimes setting them off on a path of spiritual mission (Krippner and Sulla, 2000).[3] Although subjective accounts have limitations as they are vulnerable to memory distortion and self-defense mechanisms, the high rate of such reports is remarkable. Furthermore, these early observations are in line with the experimental findings of Griffiths' team at the Johns Hopkins University (Griffiths et al., 2011) using psilocybin. By analyzing the reports of many 100s of ayahuasca experiences Shanon (Shanon, 2002) came to the conclusion that the experiences can sometimes have such a deep effect that the individuals may feel they are no longer the same person.

The psychotherapeutic effect, however, does not only depend on the experience and its phenomenal content or depth. In addition, it depends on how much the insights gained during the experiences become integrated into the everyday life of the participants afterward. Without adequate integration any experience loses its therapeutic potential in time. House (2007) warned that psychedelic experiences can carry the feeling that the desired psychological change happened during the experience itself. Such feelings are, however, illusionary and by diverting the participants from real integration they may cause more harm than benefit.

Another possible pitfall emphasized by Trichter (2010) is the phenomenon of spiritual bypassing, which occurs when individuals escape into repeated spiritual practice in order to avoid their psychological blockages. As a result an unhealthy relationship may be developed with the given spiritual tradition and techniques, which disguises the real psychological problems. The integration of the experiences can best be supported by psychotherapy carried out by an expert. Given the uncertain legal status of ayahuasca in many countries, however, this is only possible afterward, separate from the ritual (Jungaberle et al., 2008; Oak et al., 2015). Such professional assistance for the integration is, however, not at all straight forward since the number of therapists who are familiar with handling such unusual experiences is generally very low. Considering that the number of people seeking out ayahuasca ceremonies keeps growing every year, the rate of related scientific research and professional education has become crucial and requires attention.

USE OF AYAHUASCA IN THE TREATMENT OF ADDICTIONS

The potentials of ayahuasca administration in substance use disorders illustrate the necessity of an integrative view spanning from the biological to spiritual levels. In recent years there has been a growing scientific interest in the possible therapeutic use of ayahuasca in the treatment of addictions. Various forms of clinical research have attempted to reveal the mechanisms that lie under the commonly reported anti-addictive effects of ayahuasca (see Winkelman, 2014 for review). Loizaga-Velder and Pazzi (2014) revealed the bases of ayahuasca therapies with an empirical study of therapists who used ayahuasca professionally in addictions treatment and addicts who participated in ayahuasca treatment programs based on indigenous shamanistic approaches. These authors noted that most patients reported therapeutic effects involving: "augmenting body awareness, reducing drug-craving, triggering different types of emotional processes (catharsis, perception of previously suppressed emotions, generating inner resources for coping with emotions or urges to use), supporting introspection (self-analysis, eliciting awareness of addiction, and its adverse effects on oneself and others), and enhancing self-efficacy (becoming aware of positive aspects of oneself, thus improving

[3] This was exactly the way every Brazilian syncretic church *União do Vegetal*, *Santo Daime*, and *Barquina* got started!

self-esteem and confidence to stay sober)" (Loizaga-Velder and Pazzi, 2014).

Successful treatment of addictions involves a range of physical effects of ayahuasca, including the purgative and emetic effects, bodily sensations, the evoked visions and the recall of significant past memories. The physical experiences produced are interpreted within the ritual context, one's personal circumstances and with respect to the recovery processes. Ayahuasca activates a body-oriented dynamics that provides relief from stress, as well as a detoxification experience derived from the emetic effects which may produce an attenuation of craving. Within the shamanistic traditions, the vomiting or purge is seen as a part of the process of detoxification of the body, not only the elimination of toxins, but also the expulsion of morbid emotional and mental conditions. Loizaga-Velder and Pazzi (2014) noted that many report that the emetic effect experienced through vomiting is seen as providing an elimination of troublesome emotions as well as provoking diverse emotional dynamics that trigger psychological and spiritual reactions.

One important institution where ayahuasca is applied therapeutically is the Takiwasi Center for Rehabilitation of Drug Addicts and for Research on Traditional Medicines in Tarapoto, Peru (Mabit, 2007). The program integrates traditional ayahuasca rituals with physical, psychological, and spiritual activities into treatments that address a range of factors contributory to addiction. Founded in 1992 by Jaques Mabit, a French medical doctor, the Takiwasi uses a mixed approach of Western psychotherapy and traditional Amazonian medicine based on local herbs. The success is attributed not to the ayahuasca alone, but the use of other plants, the ritual setting, the community life and the interactions with therapists. Within the Takiwasi program the traditional medicines and rituals are combined with transpersonal psychology and modern social techniques to guide the personal transformation of addicts, using the ayahuasca ritual to produce profound alterations of mind that change addicts' outlook on life, on their spiritual strength and faith.

The Institute of Applied Amazonian Ethnopsychology (see Fernández and Fábregas, 2014) is another group which has applied shamanistic perspectives to the treatment of addictions with ayahuasca. The approach is based on a minimalist model, without extensive focus on ritual healing processes, but nonetheless integrates influences from the Brazilian *Santo Daime* religion, shamanism, Eastern meditative disciplines and transpersonal psychology. The approach also includes other healing techniques outside of the ayahuasca sessions such as: "individual psychotherapy; workshops on emotions, breathing techniques, psychodrama, and bibliotherapy; and family constellation therapy [and] ... other practices such as massage, colonic irrigation, shiatsu, and naturopathy" (Fernández and Fábregas, 2014). While the community context is used to counter the extreme self-centeredness of the addict, the treatment also involved solitary periods during ayahuasca sessions when the participants would retreat to their own cabins for periods of introspection, self-reflection and engaging their own healing processes based on self-regeneration from within the person.

Some centers have developed structured studies for monitoring their therapeutic outcome. In an observational study Thomas' group (Thomas et al., 2013) followed 12 members of a Canadian First Nations community, a subpopulation especially prone to addictions due to a mixture of social and psychological factors including transgenerational historic traumas and cultural dislocation. These authors found that participation in ayahuasca retreats correlated with enhanced mindfulness, personal empowerment, hopefulness, improvements in quality of life, and increased subjective feelings of connection with self, others, spirit and nature as rated with the Difficulty in Emotion Regulation Scale, Hope Scale, Philadelphia Mindfulness Scale, and the McGill Quality of Life questionnaires. In addition, the four Week Substance Use Survey also suggested that this form of ayahuasca-assisted group therapy might be associated with reductions in substance use, particularly reductions in problematic cocaine use.

Cross-sectional and longitudinal case-control studies showed that ritual and religious ayahuasca users present fewer alcohol-related problems than control groups and that illicit drug use diminished after joining ayahuasca churches (Grob et al., 1996; Doering-Silveira et al., 2005; Halpern et al., 2008; Fábregas et al., 2010). It is an open question whether ayahuasca has anti-addictive properties *per se* or if the social factors (e.g., religious social reinforcement) are the primary factor in producing these results. However, known neurochemical and psychophysiological actions of ayahuasca constituents provide evidence that pharmacological factors are a significant feature of ayahuasca's anti-addiction effects.

DISCUSSION

Evaluating the health benefits and risks of a remedy of plant origin is more difficult than that of assessing synthetized compound. In the case of ayahuasca such an evaluation is also complicated by the admixture nature of the brew, the strong psychoactive effects, the setting of its typical administration, and by the legal and financial impediments. As far as the plant components of the brew are concerned, significant differences in health potentials may stem not only from the particular species of the DMT plant being used (*Psychotria viridis* or *Diplopterys cabrerana*), but from the variety of the *B. caapi* (e.g., *cielo*, *trueno*, black, or red, etc.) in the mixture. A range of interactions may emerge between the plant alkaloids. Among those the most important occurs between the β-carbolines MAO inhibitor effect and the DMT content. MAO activity may vary considerably between individuals and across sessions (for stress-related and endogenous inhibitor mediated changes; see Obata, 2007). Since the enzyme may not be fully saturated by the competitive inhibitor, or it can be overloaded with other monoamines, the same DMT content may result in different plasma levels. Moreover, the same plasma DMT level can lead to different intraneuronal concentrations depending on the efficacy of the active DMT-uptake processes into the brain, neurons, and vesicles (reviewed in Frecska et al., 2013). The ayahuasca's effect on suggestibility interferes with the conditions of set

and setting (Barbosa et al., 2005) and favors stronger placebo response. To make this issue more difficult: ayahuasca cannot be camouflaged easily for double-blind administration. While in most of the countries ayahuasca is not scheduled as rigorously as its DMT constituent, this doesn't mean that its legal status is unambiguous and it can be studied on par with other plant remedies (St. John's wort, for example). Plant medicines do not enjoy the financial support of pharmaceutics introduced and promoted by the industry. Patenting ayahuasca would violate indigenous people's right over their intellectual heritage and such use could constitute biopiracy; these circumstances further undercut potential financial interest and industrial support.

The wide spectrum of ayahuasca's effects—as was outlined in the text—from biological to psychosocial (even spiritual) may also complicate the issue, but on the other hand it makes this plant remedy more interesting and promising for future explorations. The Sig-1R mediated action places ayahuasca research in the new stream of medical investigations, namely, it fits nicely into the emerging unifying concept of systemic and degenerative illnesses. It would be intriguing to see how a shamanic potion can be useful against wide array of civilization diseases. Ayahuasca-induced psychological effects like increased insight, reframing of cognitive structures, increased imaginary, and cathartic emotions promise potentials for ayahuasca use in psychedelic assisted psychotherapy by means of facilitating interventions based on insight oriented, cognitive, guided affective imagery, and cathartic techniques (Loizaga-Velder, 2014). The anti-impulsive, prosocial, cohesive action of ayahuasca should be tested in detention centers with young delinquents, and there is another important field of interest: combat-related post-traumatic stress.

Social isolation, lack of trust, violent outbursts, emotional numbing, and vivid recollection of traumatic experiences are often present in PTSD, a condition difficult to treat. Combat-related PTSD patients have been significantly less responsive to conventional therapies than other PTSD populations (e.g., victims of rape, survivors of natural disasters, participants of life-threatening accidents, or members of rescue-teams facing mass casualties). Veterans with PTSD may require treatment tailored to the unique nature of combat, military culture, and their individual circumstances (Yehuda, 2008). Generally, combat-related traumatic experiences can be complicated by aggravating factors: combat veterans are typically multi-traumatized over an extended period of extremely distressful time, frequently have survival guilt, guilt over killing enemy combatants, causing collateral damage, being responsible for friendly fire, and they usually witnessed the dismemberment and death of their comrades (Frecska, 2011). Our hypothesis is that—similar to methylenedioxy-methamphetamine (MDMA) which has already been tested (Mithoefer et al., 2013)—ayahuasca can also improve treatment of PTSD through enhancing trust and social feelings. In addition to these beneficial core effects, in proper settings it may also elicit "moral lessons" with subsequent relief and redemption. Additionally, the induced visions can be integrated into a cognitive re-exposure therapy with desensitization and reprocessing. The latter is the most effective cognitive treatment recognized by the National Center for PTSD. Ayahuasca facilitated cognitive exposure therapy could possibly cut down the long desensitization period necessary in the traditional psychotherapeutic approach.

The ideal scientific assessment of ayahuasca therapies are admittedly lacking, with the double-blind clinical studies a practical challenge. From the perspective of medical and research ethics, the lack of such studies should not be seen as reasons for absolutely prohibiting treatments with ayahuasca, however. In order to make our point, we would like to refer to the statements of Sackett et al. (1996), who warned for an intricate approach toward evidenced based medicine: "Evidence based medicine is the conscientious, explicit, and judicious use of current best evidence in making decisions about the care of individual patients. The practice of evidence-based medicine means integrating individual clinical expertise with the best available clinical evidence from systematic research" (Sackett et al., 1996).

In short, evidence-based medicine is the integration of experience collected in clinical practice with evidence gained from trial based research (evidenced based medicine = trial based medicine + clinical practice). Indeed, Sackett and his coworkers (Sackett et al., 1996) go on: "Evidence based medicine is not restricted to randomized trials and meta-analyses. It involves tracking down the best external evidence with which to answer our clinical questions." and "...some questions about therapy do not require randomized trials (successful interventions for otherwise fatal conditions) or cannot wait for the trials to be conducted. And if no randomized trial has been carried out for our patient's predicament, we must follow the trail to the next best external evidence and work from there." Not only the ayahuasca treatment programs for drug addicts have shown considerable expertise on the side of clinical practice, but formal assessments cited above support therapeutic ayahuasca use under certain conditions. Therefore, while randomized controlled trials are needed, these should not be seen as an absolute necessity to justify further use. In our opinion, the studies that are available do justify the application of ayahuasca at least in the treatment of addictions.

Randomized clinical trials typically use double blind controls to assess the role of placebo or expectancy effects. The use of double blind controls is particularly problematic for ayahuasca. Ideal double-blind clinical trials may be forever beyond the methodological possibility given the ritual elements. Double-blind clinical studies will be particularly challenging in terms of appropriate pharmacological and ritual control conditions. What would constitute a placebo substitute for ayahuasca might be found, but the ritual aspect that is implicated in treatment success also requires some control. But such studies are to separate drug effects from set and setting—such as ritual. It is, however, these combined physiological and ritual elements of ayahuasca that appear to have the effects on treatment outcomes. The shamanistic approaches attribute therapeutic effects to a variety of factors, including pharmacological as well as psychological, but most significantly the interactions of the biological and personal levels with the spiritual. For example, the evidence for ayahuasca as an effective treatment for addictions is from practices that espouse a view that emphasizes the interaction of a sacrament

with physiological effects with the interpersonal dynamics of ritual. Consequently, the more one agrees to the importance of addressing the spiritual aspects of human experiences as part of therapy by extending the Engelian paradigm (Engel, 1977) to the biopsychosociospiritual model (Bishop, 2009), the more one should adhere to the clinical practice side of the Sackett's equation, since targeting spiritual levels is beyond current scientific approaches.

Nevertheless, studies on ayahuasca as a therapy can attempt to isolate the physiological effects from ritual by controls. Double blind studies will also be necessary to determine the effects of the separate components (DMT vs. harmine) as well as their interactions. These should have to incorporate additional controlled trials to determine long term risks and benefits, and relative effectiveness in comparisons with other procedures or agents used to treat addictions.

To date we lack controlled ayahuasca and DMT studies largely due to administrative prohibitions. The necessary phases of evaluation for the psychedelic medicines have to rely on an integration of various forms of validation through a "triangulation" that combines data derived from: animal research; epidemiological research on general risks and adverse reactions; medical case studies and personal accounts of those who have received these substances as treatments; and process oriented research that assess pre and post-treatment conditions with a variety of standardized tools—as Alper and Lotsof (2007) pointed out in their assessment of ibogaine. While administrative regulations considerably impede research, they have not entirely obstructed it, but in terms of clinical applications they have been absolutely prohibitive. We would argue, however, that although treatment with these substances is ruled out by their Schedule I classification, their use as remedies is justified in that they reduce the patient's severe suffering without causing disproportionate harm to others (i.e., other people, or to the State's interest).

We are not going to see major pharmaceutical companies address the need for evaluation of ayahuasca or other plants as medicines. There are no financial incentives. So how can physicians and patients make use of such treatments? In addition to patients' predicaments their choices and preferences are other factors which should not totally be ignored in good clinical care. There are multiple levels of society at which we need to act to change the current political climate that regulates these substances. Public pressure on federal regulatory agencies is central to advancing experimental and treatment use of these substances. This influence involves general education, education of the media, and activities in public health and policy organizations, as well as private funding of research and corporate developments. The arguments from the cumulative scientific, clinical, ethnographic and cross-cultural evidence regarding the immense potentials of these substances are the basis for a public education approach to facilitate professional development, media coverage and popular pressure upon the government to change federal regulations and procedures. In summary, education, public policy development, and collective political action, rather than just more science, is necessary for changing opportunities for the use of ayahuasca in treatment of some of the most ravaging social diseases of our times.

AUTHOR CONTRIBUTIONS

All authors made significant contributions to the preparation of the manuscript and approved it before submission. EF developed the paper design, wrote the biological section, edited the References, and finalized the manuscript. PB wrote the first draft of the manuscript and elaborated on the psychological components. MW worked on the addictological aspects and revised the style of the manuscript.

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REVIEW
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Gymnadenia conopsea (L.) R. Br.: A Systemic Review of the Ethnobotany, Phytochemistry, and Pharmacology of an Important Asian Folk Medicine

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Gymnadenia conopsea (L.) R. Br. (*Orchidaceae*) is a perennial herbaceous orchid plant that grows widely throughout Europe and in temperate and subtropical zones of Asia. In China, its tuber has been used in traditional Chinese medicines, Tibetan medicines, Mongolian medicines and other ethnic medicines, and taken to treat numerous health conditions. The present paper provides a review of the traditional uses, phytochemistry, biological activities, and toxicology to highlight the future prospects of the plant. More than 120 chemical compounds have been isolated, and the primary components are glucosides, dihydrostilbenes, phenanthrenes, aromatic compounds, and other compounds. *G. conopsea* and its active constituents possess broad pharmacological properties, such as the tonifying effect, anti-oxidative activity, anti-viral activity, immunoregulatory, antianaphylaxis, antigastric ulcer, sedative, and hypnotic activities, etc. However, overexploitation combined with the habitat destruction has resulted in the rapid decrease of the resources of this plant, and the sustainable use of *G. conopsea* is necessary to study. Meanwhile, the toxicity of this plant had not been comprehensively studied, and the active constituents and the mechanisms of action of the tuber were still unclear. Further, studies on *G. conopsea* should lead to the development of scientific quality control and new drugs and therapies for various diseases; thus, its use and development require additional investigation.

Keywords: ***Gymnadenia conopsea*, traditional medicine, glucosides, tonifying activity, anti-viral activity**

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Abbreviations: BSA, Bovine Serum Albumin; CAT, Catalase; DNA, Deoxyribonucleic Acid; DPPH, 1,1-diphenyl-2-picrylhydrazyl; DTH, Delayed type hypersensitivity; HBsAg, Hepatitis B Surface Antigens; HBV, Hepatitis B virus; HDL-C, High Density Lipoprotein-Cholesterol; HE, Hemoglobin Electrophoresis; HIV-1, Human Immunodeficiency Virus-1; HPLC, High-performance Liquid Chromatography; HPLC-DAD-MS^n, High-performance Liquid Chromatography-diode Array Detection-tandem Mass Spectrometry; HPSEC-MALLS/RID, High Performance Size Exclusion Chromatography Coupled with Multi-angle Laser Light Scattering/refractive Index Detector; IC_{50}, 50% inhibition concentration; LD_{50}, Median Lethal Dose; LDL-C, Low Density Lipoprotein-Cholesterol; M_w, Weight-average Molecular Weight; MDA, Malondialdehyde; MeOH, Methyl Alcohol; PACE, Polysaccharide Analysis by Carbohydrate Gel Electrophoresis; PCR, Polymerase Chain Reaction; SOD, superoxide dismutase; TC, Total Cholesterol; TCM, Traditional Chinese Medicine; TG, Triglyceride; WHO, World Health Organization.

INTRODUCTION

Gymnadenia conopsea (L.) R. Br. (*Orchidaceae*) is a perennial herbaceous flowering plant that is distributed from 200 to 4700 m altitude throughout northern Europe, including England, Ireland, Russia, etc., and temperate and subtropical zones in Asian countries, including Nepal, China, Japan, and the Korean peninsula (Commission of Flora Reipublicae Popularis Sinicae, 2004; http://frps.eflora.cn/frps). For thousands of years, due to the prominent effects on invigorating the spleen, nourishing the lungs and blood, regenerating body fluid, and controlling bleeding with astringents, the tubers of *G. conopsea* was ascribed as a reinforcing agent of traditional medicines in China. It has been primarily used to treat kidney asthenia, cough, and dyspnea induced by lung asthenia, consumption diseases, neurasthenia, chronic diarrhea, morbid leucorrhea, chronic hepatitis, and other diseases in some Asian countries (Chinese Materia Editorial Committee, State Chinese Medicine Administration Bureau, 2002). Because the contour of the tuber is similar to the palm of the human hand, the tuber was given the Chinese name *Shou Zhang Shen*, meaning "ginseng likes palm hands" (**Figure 1**). In 1977, the tuber of *G. conops*ea was listed in the Pharmacopeia of the People's Republic of China (Committee for the Pharmacopoeia of P.R. China, 1977). Now, it is widely used as a folk medicine and traditional health food by Tibetans, Mongolians, the Han people, and other ethnic groups in China.

Because of the marked therapeutic effects and nutritional actions, researchers have widely investigated the properties of the tuber of *G. conopsea* Modern pharmacological studies have shown that it possesses broad pharmacological properties and can be used in the following treatments: tonifying effect, antioxidative, anti-viral, gastric ulcer prevention, anti-aging, immunoregulatory, antianaphylaxis, sedative, hypnotic, etc. Most of these actions have closely matched traditional uses. The chemical compounds from this plant have also been extensively studied, and glucosides, toluylenes, dihydrostilbenes, phenanthrenes, aromatic compounds, and other compounds have been isolated and identified.

In this review, advances in the ethnobotanical, phytochemical, biological and pharmacological activities, and toxicology of *G. conopsea* are presented and critical assessment. And the data supports its use and exploitation in new drugs.

BOTANICAL DESCRIPTION

Commission of Flora Reipublicae Popularis Sinica According to the description by Meekers et al. (2012) and the Commission of Flora Reipublicae Popularis Sinicae (2004), *Gymnadenia conopsea* (L.) R. Br. is an apolycarpic, perennial, terrestrial, and fragrant orchid herb that belongs to the *Gymnadenia* genus of *Orchidaceae* family, and distributs at forests, grasslands, and waterlogged meadows from 200 to 4700 m altitude. It has about 69 synonyms of this species, but only *Gymnadenia conopsea* (L.) R. Br. is an accepted and approved name in the World. The stem is 20–60 cm, erect, slim, terete, or angled above and leafy with 2–3 brown membranous sheaths at the base. The leaves are green with dimensions of 5.5–15 × 1–2.5 cm, and the lower leaves are erect to slightly spreading, more, or less narrowly oblong-lanceolate or linear-lanceolate, obtuse to subacute, and slightly hooded at the apex, entire, keeled, and folded and have 1–2 or more veins on each side of the midrib; the upper 2–3 leaves are smaller, lanceolate or bract-like, and taper to a fine point. The bracts are green and usually tend toward violet at the edges, and they are lanceolate and taper to a fine point at the apex. The raceme is 5.5–15 cm long and ranges in color from pale pink to lilac (rarely white or bright magenta), and it is strongly scented with a flowering season from July to August. Inflorescence 11–26 cm, slender; peduncle with one to a few scattered, lanceolate bracts 1.5–6 cm; rachis 4–12 cm, densely many flowered; floral bracts lanceolate, often longer than ovary and flower, apex long acuminate-caudate. And flowers fragrant, pink, rarely pinkish white; ovary 5–8 mm including pedicel. Dorsal sepal broadly elliptic to broadly ovate-elliptic; lateral sepals reflexed, obliquely ovate, 4–5.5 × 3–4 mm, 3-veined, margin revolute, apex acute. Petals obliquely ovate-triangular, 3-veined, apex acute; lip spreading, broadly cuneate-obovate. There are two tubers with dimensions of 14–30 × 7.5–24 mm; the tubers are palmate lobed with thick segments that are tapering and obtuse, pressed together, and split halfway to the base into 3–6 lobes. The short and thick roots are sparse and grow horizontally or even toward the soil surface. The fruit has dimensions of 8.6–9.3 × 2.6–2.7 mm, and they are erect with six ribs. The seeds have dimensions of 0.3 × 0.1 mm and are produced in large numbers (http://www.theplantlist.org[1]; www.efloras.org[2]; **Figure 1**).

TRADITIONAL USES

G. conopsea is widely distributed in northern Europe and certain Asian countries. Like some TCM, *G. conopsea* is not used in folk medicine, and just has been considered a fragrant orchid plant in some European countries. So, studies on the ethnopharmacology and clinical uses of the plant have been mainly focused on Asian countries, such as China, Nepal, and Japan.

In China, *G. conopsea* is primarily distributed in Heilongjiang, Jilin, Liaoning, Hebei, Shanxi, Shangxi, Gansu, Sichuan, and Yunnan Provinces and Inner Mongolia and Xizang Autonomous Regions at altitudes of 265–4700 m (Commission of Flora Reipublicae Popularis Sinicae, 2004). The tuber has been employed as a reinforcing agent of folk medicines and widely used as traditional Chinese medicine (Han national medicine), Tibetan medicine, Mongolian medicine, Baiyao (the Bai national medicine), Chaoyao (the Korean national medicine), and Naxiyao (the Naxi national medicine) to treat various diseases in China. The tuber has also been employed as a health care product with other medicines or food to improve the body and prevent illness, and could be made to tincture and galenical to treat impotence and the bronchial asthma in China and Russia, respectively (Mamedov and Craker, 2001; Matsuda et al., 2004; Gutierrez, 2010). But at the same time, because of the rare resource of this plant, the tuber of *Gymnadenia crassinervis*, *Coeloglossum viride* var. *bracteatum*, and *Spiranthea lancea* have

[1]The Plant List, version 1 (Accessed January 01, 2010).
[2]The Flora of China.

FIGURE 1 | Photographs of *Gymnadenia conopsea* (L.) R. Br. and the tuber. We also thanks for the provider of **Figure 1**, Zhou Yao from http://www.plantphoto.cn.

been used as substitutes for *G. conopsea* in certain regions, such as Tibet region (Xie et al., 2005; Zi, 2008; Xue et al., 2009).

As a traditional Tibetan medicine, the *G. conopsea* tuber has been widely used in the Tibetan region to treat lung disease and weakening by invigorating the kidney and moisturizing the lungs. The plant is known as "Wangla," and it has been recorded in the "Sibuyidian," the classical book of Tibetan medicine, since the eighth century. In Tibetan medicine, it could be used as a single medicine or as one composition mixed with other medicines to treat diseases. For example, after grinding to powder, it (30 g) concoction with bee honey (40 g), *Rhizome Gastrodiae* (30 g), *Radix Phlomii* (30 g), *Herb Drosera peltat* (30 g), and *Rhododendron parvifolium* (30 g) could be used to treat impotence, spermatorrhea, anemia, and insomnia for 3 g twice per day (Chinese Materia Editorial Committee, State Chinese Medicine Administration Bureau, 2002). And according to the database of Tibetan prescriptions, out of 4500 traditional prescriptions in Tibet Autonomous Region, the *G. conopsea* tuber was used 104 times (rate of 2.3%), and 33 prescriptions were used to invigorate the body, strengthen the Yang, and lengthen human life; 26 prescriptions were used to treat kidney diseases; 12 prescriptions were used to treat gout and arthromyodynia diseases; 11 prescriptions were applied to treat lung disease; 7 prescriptions were used to treat eye diseases; and other prescriptions were used to treat parasitic diseases and additional diseases (Ji et al., 2009; Xue et al., 2009). Currently, the tuber of *G. conopsea* is combined with other medicines in various preparations to treat a number of diseases. Five preparations have been listed in the Chinese Pharmacopeia and approved by the State Food and Drug Administration of China of the People's Republic of China. Medicines such as "Shi Wei Shou Shen Powder" and "Fu Fang Shou Shen Wan" have been widely used to treat kidney asthenia, impotence and spermatorrhea, among other disorders (http://www.sfda.gov.cn, 2014; **Table 1**). Except the above effects on the clinic, the tuber of *G. conopsea* also could be used to treat hepatitis B by folk doctors only in the Tibetan region (Chinese Materia Editorial Committee, State Chinese Medicine Administration Bureau, 2002). At the same time, it has also been used as a common food item by local people in the Tibetan region, where it is usually cooked with vegetables and rice. For example, the tuber is used as an important ingredient in the famous dish "Shiguo Ji" (chicken cooked in a stone hotpot).

In the traditional Mongolian medicine, the tuber of *G. conopsea* is named "Erihaoteng" and has been historically recorded by many classical folk medicine books of the Inner Mongolia Autonomous Region. In additional, it has been widely used to treat kidney asthenia, lumbago and leg pain, light scurvy, spermatorrhea, and impotence (Gege et al., 2013). The tuber of *G. conopsea* and other traditional medicines have been combined into different preparations to treat various diseases. For example, the tuber and an additional 36 medicines are known as Shouzhangsheng-37 pills, which are used to treat kidney cold and asthenia, edema, tinnitus, spermatorrhea, impotence, stomach diseases, dyspepsia, and other diseases, and this preparation has been approved by the State Administration of Traditional Chinese Medicine of the People's Republic of China (http://www.sfda.gov.cn, 2014; (Si and Liu, 2013); **Table 1**). Meanwhile, Wu et al. (2014) reported that in clinical practice of Mongolian medicine, after administrating orally two Shenzhujin pills (the tuber is the primary component) for three times per day for 21 days, the kidney deficiency of one patient (65 years) have been cured.

TABLE 1 | Preparations in which *Gymnadenia conopsea* (L.) R. Br. was the primary component as listed in the Chinese Pharmacopeia and approved by the government*.

Preparation name	Main compositions	Usage
Shi Wei Shou Shen San	Tuber Gymnadenia, Cinnamomi Cortex, Myristicae Semen, Piperis Longi Fructus, Asparagi Radix, Granati Pericarpium, Canavaliae Semen, Carthami Flos, Moschus, Bear Gall.	Invigorating the kidney and treating spermatorrhea.
Fu Fang Shou Shen Wan	Tuber Gymnadenia, Polygonati Rhizoma, Cynomorii Herba, Chebulae Fructus, Rhizoma Mirabilis Himalaica, Asparagi Radix, Cordyceps, Tribuli Fructus, Rhizoma Przewalskia Tangutica, Herba Pleurospermum.	Warming the kidney and activating yang. Treating insufficiency of kidney-YANG, damage of essence, impotence, and spermatorrhea, etc.
Shou Shen Shen Bao Jiao Nang	Tuber Gymnadenia, Polygonati Rhizoma, Asparagi Radix, Folium Rhododendron Anthopogonoides, Cordyceps.	Warming the kidney and invigorating yin. Treating giddy dazzled, tinnitus, lumbar genu aching, and limp is faint induced by kidney asthenia.
Shou Zhang Shen 37 Wan	Tuber Gymnadenia, Calcite, Alpiniae Oxyphyliae Fructus, Granati Pericarpium, Zingiberis Rhizoma, Feces Trogopterori, Myristicae Semen, Piperis Longi Fructus, Gecko, Chebulae Fructus, Sal Ammoniac, Folium Rhododendron Anthopogonoides, etc.	Invigorating the kidney and strengthening yang. Treating kidney asthenia, edema, tinnitus, impotence, and spermatorrhea, etc.
Fu Fang Shou Shen Yi Zhi Jiao Nang	Tuber Gymnadenia, Polygoni Multiflori Radix Praeparata, Acanthopanacis Senticosi Radix, Polygonati Rhizoma, Angelicae Sinensis Radix, Asteragali Radix, Lycii Fructus, Schisandrae Chinensis Fructus, Corni Fructus, Rhizoma Polygala, Acori Tatarinowii Rhizoma, Paeoniae Radix Rubra.	Invigorating the kidney and liver, and treating the deficiency of liver and kidney, amnesia, palpitation, and insomnia induced by Qi and blood hemophthisis.

**Cited from "Chinese Pharmacopeia" and the Website: http://www.sfda.gov.cn.*

Furthermore, the tuber of *G. conopsea* is also used in Korean national medicine, Bai national medicine, and Naxi national medicine, where it is known as Yinyang Cao, Foshousheng, and Kaishelabei, respectively. However, it has been used as a reinforcing agent within all of these traditional medicines and used to treat similar diseases as previously indicated, such as invigorating the body, strengthening the Yang, etc. (Jia and Li, 2005). But up to now, except the experience-based uses of this plant, the relevant evidence-based clinical uses and the safety data scientifically are very rare and should be investigated further.

PHYTOCHEMISTRY

Approximately 129 compounds have been isolated and identified from *G. conopsea* to date, and most of compounds isolated from the tuber. Forty-nine glucosides compounds (one of the most important components) were identified, which could be divided into benzylester glucosides and other glucosides. Meanwhile, dihydrostilbenes, phenanthrenes, aromatic compounds, polysaccharides, and other compounds were also isolated and reported (**Table 2**; **Figure 2**). The different chemical compositions of *G. conopsea* provide the foundation for its different pharmacology activities.

Glucosides

As one of the most important components, glucosides are widely studied and separated from the tuber of *G. conopsea*. According to the structural conformation of the glucosides, they have been divided into benzylester glucosides and other glucosides.

Benzylester Glucosides

Benzylester glucosides are produced by combining 2-isobutyl tartaric acid or 2-isobutyl hydroxysuccinic acid with 4-glycosylbenzyl alcohol. According to the differences in their organic acids, they are classified as (2R, 3S)-2-isobutyl tartaric acid derivates and (2R)-2-isobutyl hydroxysuccinic acid derivatives. Approximately 29 compounds have been isolated and identified. In 2006, Morikawa et al. (2006a,b) isolated the following compounds: gymnoside I (**1**), gymnoside II (**2**), gymnoside III (**3**), gymnoside IV (**4**), gymnoside V (**5**), gymnoside VI (**6**), gymnoside VII (**7**), gymnoside VIII (**8**), gymnoside IX (**9**), gymnoside X (**10**), loroglossin (**11**), dactylorhin A (**12**), dactylorhin **B** (**13**)and militarine (**14**). Meanwhile, (−)-4-[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyloxy] benzyl alcohol (**15**), (+)-4-[α-D-glucopyranosyl-(1→4)-β-D-glucopyranosyloxy] benzyl alcohol (**16**), (−)-4-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyloxy] benzyl alcohol (**17**), (−)-4-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyloxy] benzyl ethyl ether (**18**), (−)-(2R,3S)-1-(4-β-D-glucopyranosyloxybenzyl)-2-O-β-D-glucopyranosy]-4-{4-[α-D-glucopyranosyl-(1→4)-β-D-glucopyranosyloxy]benzyl}-2-isobutyltartrate (**19**), (−)-(2R,3S)-1-(4-β-D-glucopyranosyloxybenzyl)-2-O-β-D-glucopyranosyl-4-{4-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyloxy] benzyl}-2-isobutyltartrate (**20**), (−)-(2R,3S)-1-{4-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyloxy]benzyl}-2-O-β-D-glucopyranosyl-4-(4-β-D-glucopyranosyloxybenzyl)-2-isobutyl-tartrate (**21**), (−)-(2R,3S)-1-(4-β-D-glucopyranosyloxybenzyl)-4-{4-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyloxy]benzyl}-2-isobutyl tartrate (**22**), (−)-(2R,3S)-1-(4-β-D-glucopyranosyloxybenzyl)-4-methyl-2-isobutyltartrate (**23**) and (−)-(2R)-2-O-β-D-glucopyranosyl-4-(4-β-D-glucopyranosylbenzyl)-2-isobutyltartrate (**24**) were isolated from the tuber in 2008. And the further studies showed that at 10^{-5} M, the inhibition rates of the above compounds foracetylcholine esterase were <10%, and the positive drug donepezil gave an inhibition rate of 77.2%. Then, the results presented that at 10^{-5} M, the inhibition rates of the

TABLE 2 | Compounds isolated from *Gymnadenia conopsea* (L.) R. Br. (the structure of the primary compounds are illustrated in Figure 2).

No.	Names	Parts	References
1	Gymnoside I	Tuber	Morikawa et al., 2006a
2	Gymnoside II	Tuber	Morikawa et al., 2006a
3	Gymnoside III	Tuber	Morikawa et al., 2006a
4	Gymnoside IV	Tuber	Morikawa et al., 2006a
5	Gymnoside V	Tuber	Morikawa et al., 2006a
6	Gymnoside VI	Tuber	Morikawa et al., 2006a
7	Gymnoside VII	Tuber	Morikawa et al., 2006a
8	Gymnoside VIII	Tuber	Morikawa et al., 2006b
9	Gymnoside IX	Tuber	Morikawa et al., 2006b
10	Gymnoside X	Tuber	Morikawa et al., 2006b
11	Loroglossin	Tuber	Morikawa et al., 2006b
12	Dactylorhin A	Tuber	Morikawa et al., 2006b
13	Dactylorhin B	Tuber	Morikawa et al., 2006b
14	Militarine	Tuber	Morikawa et al., 2006b
15	(−)-4-[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyloxy] benzyl alcohol	Tuber	Zi, 2008; Zi et al., 2008
16	(+)-4-[α-D-glucopyranosyl-(1→4)-β-D-glucopyranosyloxy] benzyl alcohol	Tuber	Zi, 2008; Zi et al., 2008
17	(−)-4-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyloxy] benzyl alcohol	Tuber	Zi, 2008; Zi et al., 2008
18	(−)-4-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyloxy] benzyl ethyl ether	Tuber	Zi, 2008; Zi et al., 2008
19	(−)-(2R,3S)-1-(4-β-D-glucopyranosyloxybenzyl)-2-O-β-D-glucopyranosy]-4-{4-[α-D-glucopyranosyl-(1→4)-β-D-glucopyranosyloxy]benzyl}-2-isobutyltartrate	Tuber	Zi, 2008; Zi et al., 2008
20	(−)-(2R,3S)-1-(4-β-D-glucopyranosyloxybenzyl)-2-O-β-D-glucopyranosyl-4-{4-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyloxy]benzyl}-2-isobutyltartrate	Tuber	Zi, 2008; Zi et al., 2008
21	(−)-(2R,3S)-1-{4-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyloxy] benzyl}-2-O-β-D-glucopyranosyl-4-(4-β-D-glucopyranosyloxybenzyl)-2-isobutyltartrate	Tuber	Zi, 2008; Zi et al., 2008
22	(−)-(2R,3S)-1-(4-β-D-glucopyranosyloxybenzyl)-4-{4-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyloxy]benzyl}-2-isobutyltartrate	Tuber	Zi, 2008; Zi et al., 2008
23	(−)-(2R,3S)-1-(4-β-D-glucopyranosyloxybenzyl)-4-methyl-2-isobutyltartrate	Tuber	Zi, 2008; Zi et al., 2008
24	(−)-(2R)-2-O-β-D-glucopyranosyl-4-(4-β-D-glucopyranosylbenzyl)-2-isobutyltartrate	Tuber	Zi, 2008; Zi et al., 2008
25	Dactylorhin E	Tuber	Zi, 2008
26	Coelovirins A	Tuber	Zi, 2008
27	Coelovirins B	Tuber	Zi, 2008
28	Coelovirins D	Tuber	Zi, 2008
29	Coelovirins E	Tuber	Zi, 2008
30	4-Hydroxybenzyl-β-D-glucopyranoside	Tuber	Yang, 2009
31	4-Methylphenyl-β-D-glucopyranoside	Tuber	Yang, 2009
32	4-Hydroxyphenyl-β-D-glucopyranoside	Tuber	Yang, 2009
33	4-Methoxymethylbenzyl-β-D-glucoside	Tuber	Morikawa et al., 2006b
34	bis (4-hydroxybenzyl)-ethermono-β-D-glucopyranoside	Tuber	Morikawa et al., 2006b
35	4-(β-D-glucopyranosyloxy) benzoic aldehyde	Tuber	Zi, 2008
36	4-(β-D-glucopyranosyloxy) benzyl ethyl ether	Tuber	Zi, 2008
37	Phenyl-β-D-glucopyranoside	Tuber	Morikawa et al., 2006b
38	4-Formylphenyl-β-D-glucopyranoside	Tuber	Morikawa et al., 2006b
39	Benzyl-β-D-glucopyranoside	Tuber	Morikawa et al., 2006b
40	trans-ferulic acid-β-D-glucoside	Tuber	Zi, 2008
41	cis-ferulic acid-β-D-glucoside	Tuber	Zi, 2008
42	N^6-(4-hydroxybenzyl) adenine riboside	Tuber	Zi, 2008
43	Daucosterol	Tuber	Li et al., 2007
44	Dioscin	Tuber	Li et al., 2001
45	Dactylose A	Tuber	Morikawa et al., 2006b
46	Dactylose B	Tuber	Morikawa et al., 2006b
47	n-Butyl-β-D-fructopyranose	Tuber	Li et al., 2001

(Continued)

TABLE 2 | Continued

No.	Names	Parts	References
48	Thymidine	Tuber	Morikawa et al., 2006b
49	Batatasin III	Tuber	Matsuda et al., 2004
50	3′-O-methylbatatasin III	Tuber	Matsuda et al., 2004
51	3′,5-Dihydroxy-2-(4-hydroxybenzyl)-3-methoxybibenzyl	Tuber	Matsuda et al., 2004
52	3,3′-Dihydroxy-2-(4-hydroxybenzyl)-5-methoxybibenzyl	Tuber	Matsuda et al., 2004
53	Gymconopin D	Tuber	Matsuda et al., 2004
54	3,3′-Dihydroxy-2,6-bis(4-hydroxybenzyl)-5-methoxybibenzyl	Tuber	Matsuda et al., 2004
55	5-O-methylbatatacin III	Tuber	Yoshikawa et al., 2005
56	2-(4-Hydroxybenzyl)-3′-O-methylbatatacin III	Tuber	Yoshikawa et al., 2005
57	Arundinin	Tuber	Yoshikawa et al., 2005
58	Arundin	Tuber	Yoshikawa et al., 2005
59	Bulbocodin C	Tuber	Yoshikawa et al., 2005
60	Bulbocodin D	Tuber	Yoshikawa et al., 2005
61	Gymconopin A	Tuber	Matsuda et al., 2004
62	Gymconopin B	Tuber	Yoshikawa et al., 2005
63	Gymconopin C	Tuber	Yoshikawa et al., 2005
64	1-(4-Hydroxybenzyl)-4-methoxy-9,10-dihydrophenanthrene-2,7-diol	Tuber	Matsuda et al., 2004
65	1-(4-Hydroxybenzyl)-4-methoxyphenanthrene-2,7-diol	Tuber	Matsuda et al., 2004
66	Dihydrophenanthrene-4,5-diol	Tuber	Matsuda et al., 2004
67	2-Methoxy-9,10-4-methoxy-9,10-dihydrophenanthrene-2,7-diol	Tuber	Matsuda et al., 2004
68	Blestriarene A	Tuber	Matsuda et al., 2004
69	Blestriarene A	Tuber	Matsuda et al., 2004
70	Blestriarene A	Tuber	Matsuda et al., 2004
71	9, 10-Dihydro blestriarene B	Tuber	Matsuda et al., 2004
72	9,10–9′,10′-Dihydro blestriarene C	Tuber	Matsuda et al., 2004
73	4-Hydroxybenzyl alcohol	Tuber	Cai et al., 2006
74	4-Hydroxybenzyl aldehyde	Tuber	Cai et al., 2006
75	Pinoresinol	Tuber	Morikawa et al., 2006b
76	4-Hydroxybenzoic acid	Tuber	Yue et al., 2010
77	Vanillic acid	Tuber	Yue et al., 2010
78	Trans-p-coumaric acid	Tuber	Yue et al., 2010
79	Cis-p-coumaric acid	Tuber	Yue et al., 2010
80	4-Benzaldehyde	Tuber	Morikawa et al., 2006b
81	4-Hydroxybenzyl methyl ether	Tuber	Zi, 2008
82	3,5-Methoxy benzaldehyde	Tuber	Morikawa et al., 2006b
83	4-[(4-Hydroxyphenyl)methoxy]-benzenemethanol	Tuber	Morikawa et al., 2006b
84	4,4′-Dihydroxydiphenyl methane	Tuber	Morikawa et al., 2006b
85	Phenol	Tuber	Morikawa et al., 2006b
86	5-Hydroxymethyl furfural	Tuber	Morikawa et al., 2006b
87	1,2-Dihydroxy benzene	Tuber	Morikawa et al., 2006b
88	2,6-Dimethoxy phenol	Tuber	Morikawa et al., 2006b
89	Eugenol	Tuber	Morikawa et al., 2006b
90	4-Hydroxybenzene	Tuber	Morikawa et al., 2006b
91	4-Methoxy phenylpropanol	Tuber	Morikawa et al., 2006b
92	4-Ethoxy phenylpropanol	Tuber	Morikawa et al., 2006b
93	Contra-hydroxybenzyl dithioether	Tuber	Morikawa et al., 2006b
94	Syringol	Tuber	Morikawa et al., 2006b
95	Syringaldehyde	Tuber	Morikawa et al., 2006b
96	Gastrodin	Tuber	Yue et al., 2010
97	4-Hydroxybenzoic aldehydes	Tuber	Zi, 2008
98	4-Hydroxybenzoic acid	Tuber	Yue et al., 2010

(Continued)

TABLE 2 | Continued

No.	Names	Parts	References
99	3-Hydroxybenzoic acid	Tuber	Yue et al., 2010
100	4-Hydroxyisophthalic acid	Tuber	Yue et al., 2010
101	3-Methoxy-4-hydroxybenzoic acid	Tuber	Yue et al., 2010
102	Arctigenin	Tuber	Yue et al., 2010
103	erythro-buddlenol E	Tuber	Zi, 2008
104	Lappaol A	Tuber	Yue et al., 2010
105	Lappaol F	Tuber	Yue et al., 2010
106	2-Hydroxy-2-(4-hydroxyphenylmethyl)-4-methylcyclopent-4-en-1,3-dione	Tuber	Zi, 2008
107	2-Hydroxy-3-(4-hydroxyphenyl)-4-hydroxymethylcyclopent-2-enone	Tuber	Zi, 2008
108	3,3′-Dihydroxy-4-(4-hydroxybenzyl)-5-methoxybibenzyl	Tuber	Morikawa et al., 2006b
109	2-C-(4-hydroxybenzyl)-α-L-xylo-3-ketohexulofuranosono-1,4-lactone	Tuber	Morikawa et al., 2006b
110	Arabinose	Tuber	Sun et al., 2010
111	Xylose	Tuber	Sun et al., 2010
112	Mannitose	Tuber	Sun et al., 2010
113	Galactose	Tuber	Sun et al., 2010
114	Glucose	Tuber	Sun et al., 2010
115	Secoxyloganin	Tuber	Zi, 2008
116	Quercitin-3, 7-di-O-β-D-glucopyranoside	Tuber	Zi, 2008
117	Kaempferol-3-β-glycosido-7-β-glycoside	Flower	Schonsiegel and Ingomar, 1969
118	Quercetin-3-β-glycosido-7-glycoside	Flower	Schonsiegel and Ingomar, 1969
119	Astragalin	Flower	Schonsiegel and Ingomar, 1969
120	Isoquercetin	Flower	Schonsiegel and Ingomar, 1969
121	Cyclo(L-Leu-L-Tyr)	Tuber	Zi, 2008
122	Cyclo(L-Leu-L-Pro)	Tuber	Zi, 2008
123	Cyclo(L-Val-L-Tyr)	Tuber	Zi, 2008
124	Cyclo(L-Ala-L-Phe)	Tuber	Zi, 2008
125	Thymidine	Tuber	Yue et al., 2010
126	β-sitoterol	Tuber	Yue et al., 2010
127	Octadecylselyl acid	Tuber	Yue et al., 2010
128	Di-(p-hydroxybenzyl) disulfide	Tuber	Li et al., 2007
129	4.4′-Dihydroxybenzyl sulfoxide	Tuber	Li et al., 2007

above compounds formonoamine oxidase-B were <15.2%, and the positive drug pargyline had an inhibition rate of 94.5% (Zi et al., 2008). Meanwhile, dactylorhin E (**25**), coelovirins A (**26**), coelovirins B (**27**), coelovirins D (**28**), and coelovirins E (**29**) also were isolated (Zi, 2008).

Other Glucosides

Other glucosides have also been isolated from the tuber of *G. conopsea*. In 2009, Yang identified 4-hydroxybenzyl-β-D-glucopyranoside (**30**), 4-methylphenyl-β-D-glucopyranoside (**31**), and 4-hydroxyphenyl-β-D-glucopyranoside (**32**). The following compounds have also been isolated (Li et al., 2001; Morikawa et al., 2006b; Zi, 2008): 4-methoxymethylbenzyl-β-D-glucoside (**33**), *bis* (4-hydroxybenzyl)-ether mono-β-D-glucopyranoside (**34**), 4-(β-D-glucopyranosyloxy) benzoic aldehyde (**35**), 4-(β-D-glucopyranosyloxy)benzyl ethyl ether (**36**), phenyl-β-D-glucopyranoside (**37**), 4-formylphenyl-β-D-glucopyranoside (**38**), benzyl-β-D-glucopyranoside (**39**), *trans*-ferulic acid-β-D-glucoside (**40**), *cis*-ferulic acid-β-D-glucoside (**41**), N^6-(4-hydroxybenzyl)adenine riboside (**42**), daucosterol (**43**), dioscin (**44**), dactylose A (**45**), dactylose B (**46**), n-butyl-β-D-fructopyranose (**47**), and thymidine (**48**).

Dihydrostilbenes

Dihydrostilbenes are other important components of *G. conopsea*. The basic parental nucleus of these compounds is dihydrogen alkene. The C-2, -3, -4, -5, -6 and -3′ positions of the basic nucleus areusually substituted. In 2004 and 2005, batatacin III (**49**), 3′-O-methylbatatacin III (**50**), 3′,5-dihydroxy-2-(4-hydroxybenzyl)-3-methoxybibenzyl (**51**), 3,3′-dihydroxy-2-(4-hydroxybenzyl)-5-methoxybibenzyl (**52**), gymconopin D (**53**), 3,3′-dihydroxy-2,6-bis(4-hydroxybenzyl)-5-methoxybibenzyl (**54**), 5-O-methylbatatacin III (**55**), 2-(4-hydroxybenzyl)-3′-O-methylbatatacin III (**56**), arundinin (**57**), arundin (**58**), bulbocodin C (**59**), bulbocodin D (**60**) were isolated and identified from the tuber, respectively (Matsuda et al., 2004; Yoshikawa et al., 2005).

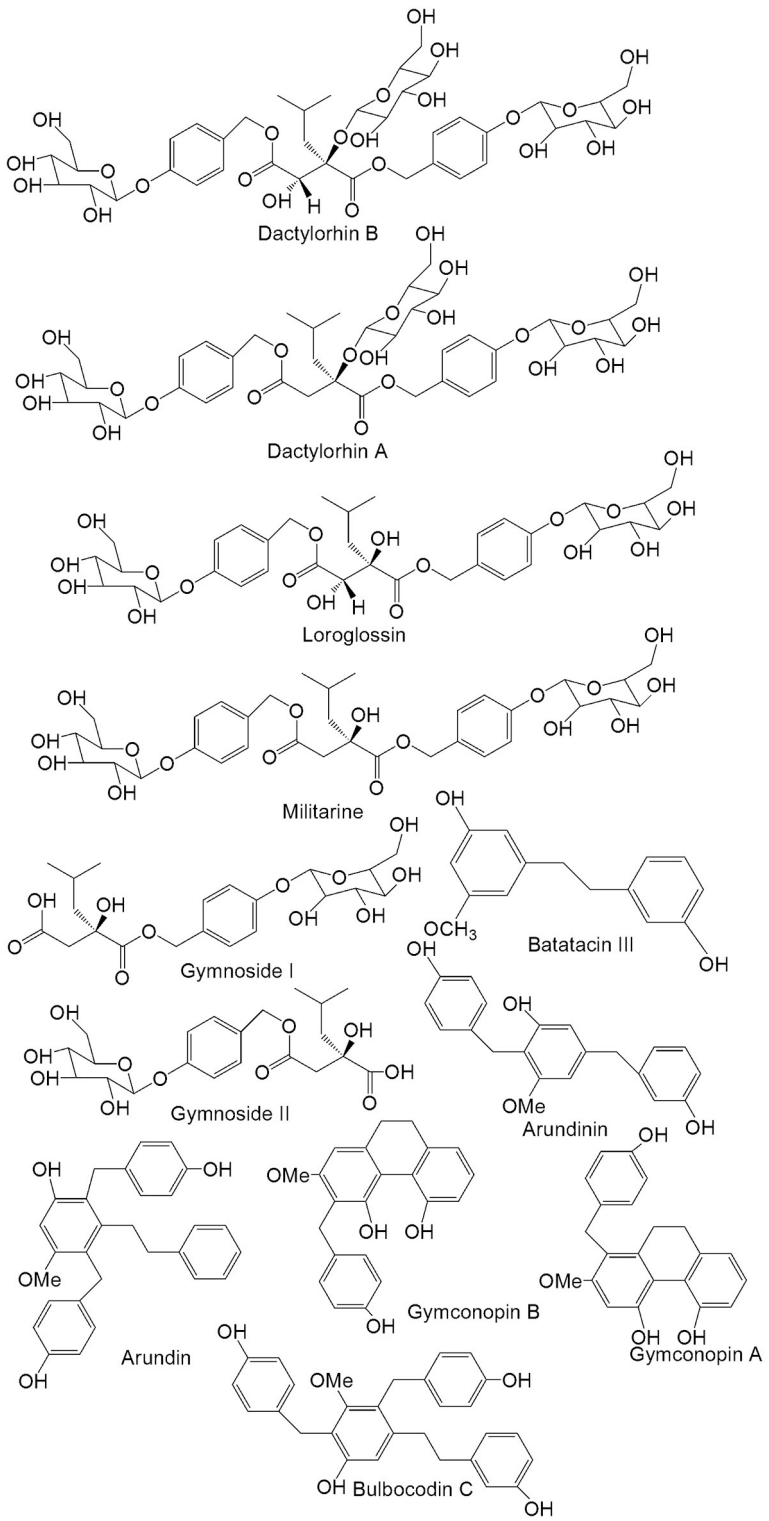

FIGURE 2 | Chemical structures of the primary compounds from the tuber of *Gymnadenia conopsea* (L.) R. Br.

Phenanthrenes

A group of phenanthrene compounds was isolated from the tuber of *G. conopsea* and studied in recent decades, including gymconopin A (**61**), gymconopin B (**62**), gymconopin C (**63**), 1-(4-hydroxybenzyl)-4-methoxy-9,10-dihydrophenanthrene-2,7-diol (**64**), 1-(4-hydroxybenzyl)-4-methoxyphenanthrene-2,7-diol

(**65**), 2-methoxy-9,10-dihydrophenanthrene-4,5-diol (**66**), 4-methoxy-9,10-dihydrophenanthrene-2,7-diol (**67**), blestriarene A (**68**), blestriarene A (**69**), blestriarene A (**70**), 9, 10-dihydro blestriarene B (**71**), and 9,10–9′,10′-dihydro blestriarene C (**72**) (Matsuda et al., 2004; Yoshikawa et al., 2005).

Aromatic Compounds

Approximately 20 aromatic compounds from *G. conopsea* have been studied, and most of them are phenol compounds. In 2006, Cai et al. (2006) isolated 4-hydroxybenzyl alcohol (**73**) and 4-hydroxybenzyl aldehyde (**74**) from the tuber. Then, pinoresinol (**75**), 4-hydroxybenzoic acid (**76**), vanillic acid (**77**), *trans*-p-coumaric acid (**78**), *cis*-p-coumaric acid (**79**), 4-benzaldehyde (**80**), 4-hydroxybenzyl methyl ether (**81**), 3,5-methoxy benzaldehyde (**82**), 4-[(4-hydroxyphenyl)methoxy]-benzenemethanol (**83**), 4,4′-dihydroxydiphenyl methane (**84**), phenol (**85**), 5-hydroxymethyl furfural (**86**), 1,2-dihydroxybenzene (**87**), 2,6-dimethoxy phenol (**88**), eugenol (**89**), 4-hydroxybenzene (**90**), 4-methoxy phenylpropanol (**91**), 4-ethoxy phenylpropanol (**92**), contra-hydroxybenzyl dithioether (**93**), syringol (**94**), syringaldehyde (**95**), gastrodin (**96**), 4-hydroxybenzoic aldehydes (**97**), 4-hydroxybenzoic acid (**98**), 3-hydroxybenzoic acid (**99**), 4-hydroxyisophthalic acid (**100**), 3-methoxy-4-hydroxybenzoic acid (**101**), arctigenin (**102**), erythro-buddlenol E (**103**), lappaol A (**104**), lappaol F (**105**), 2-hydroxy-2-(4-hydroxyphenylmethyl)-4-methylcyclopent-4-en-1,3-dione (**106**), 2-hydroxy-3-(4-hydroxyphenyl)-4-hydroxymethylcyclopent-2-enone (**107**), 3,3′-dihydroxy-4-(4-hydroxybenzyl)-5-methoxybibenzyl (**108**), and 2-c-(4-hydroxybenzyl)-α-L-xylo-3-ketohexulofuranosono-1,4-lactone (**109**) were isolated and identified from the tuber of *G. conopsea* (Morikawa et al., 2006b; Zi, 2008; Yue et al., 2010).

Polysaccharides

In 2009, Yang (2009) studied the four extraction and purification methods for the polysaccharides of *G. conopsea*. The results showed that the optimum parameters for solvent extraction were 80°C for 2.5 h, a ratio of material to water of 1:30 with three repetitions, which yielded a polysaccharide extraction rate of up to 11.83%. The microwave extraction method were 250 W for 20 min, a ratio of material to water of 1:30 with one repetition, and the extraction rate was up to 13.56%. The ultrasound extraction method was 240 W, 60°C for 10 min, and three repetitions, and the extraction rate was 15.24%. Finally, the parameters for cellulose extraction were to add cellulose at 1% of the tuber concentration for 4 h at 50°C and Ph 4.8, and the extraction rate was 16.58%. Thus, the cellulose method is the best among the four methods. Meanwhile, vapor phase chromatography was adopted to analyze the monosaccharides from the polysaccharides, and the results showed that the polysaccharides are composed of arabinose (**110**), xylose (**111**), mannitose (**112**), galactose (**113**), and glucose (**114**) (Sun et al., 2010). And in 2014, the water soluble polysaccharides from the tuber of *G. conopsea* collected at seven regions in China were investigated and compared using high performance size exclusion chromatography coupled with multi-angle laser light scattering/refractive index detector (HPSEC-MALLS/RID) and saccharide mapping based on polysaccharide analysis by carbohydrate gel electrophoresis (PACE), respectively. The results showed that the weight-average molecular weight (M_w) and the radius of gyration ($<S^2>z^{1/2}$) of polysaccharides were ranging from 4.46 × 105 to 7.41 × 105 Da and 73.3–94.2 nm, respectively. And the exponent (v) values of $<S^2>z^{1/2} = kM_w^v$ were ranging from 0.36 to 0.42, which indicated that α-1,4- and β-1,3(4)-glucosidic, α-1,5-arabinosidic, β-1,4-mannosidic and α-1,4-D-galactosiduronic linkages existed in globular polysaccharides. The further studies results that the nitric oxide released from RAW 264.7 cells induced by polysaccharides were significantly affected by their α-1,5-arabinosidic and β-1,3(4)-glucosidic, especially α-1,4-D-galactosiduronic and β-1,4-mannosidic linkages (Lin et al., 2015).

Other Compounds

From *G. conopsea*, six flavones were isolated from the flower, including secoxyloganin (**115**), quercitin-3, 7-di-O-β-D-glucopyranoside (**116**), kaempferol-3-β-glycosido-7-β-glycoside (**117**), and quercetin-3-β-glycoside-7-glycoside (**118**), astragalin (**119**) and isoquercetin (**120**) (Schonsiegel and Ingomar, 1969; Zi, 2008). Then, cyclo(L-Leu-L-Tyr) (**121**), cyclo(L-Leu-L-Pro) (**122**), cyclo(L-Val-L-Tyr) (**123**), cyclo(L-Ala-L-Phe) (**124**), thymidine (**125**), β-sitoterol (**126**), and octadecylselyl acid (**127**) were isolated (Yue et al., 2010; Zi et al., 2010). And di-(p-hydroxybenzyl) disulfide (**128**) and 4.4′-dihydroxybenzyl sulfoxide (**129**) also were identified (Li et al., 2007). Lan (2005) determined the trace element contents of the tuber of *Gymnadenia conopsea* (L.) R. Br., and the results showed that the Cu, Mn, Fe, Zn, Ni, Se, Cr, K, Ca, Mg, Cd, and Pb contents in μg/g were 7.6, 31.6, 60.3, 20.1, 2.1, 6.0, 2.0, 19,000, 4100, 1800, 0.16, and 3.31, respectively.

PHARMACOLOGY

Tonifying Effect and Anti-Fatigue Activity

In China, the *G. conopsea* tuber has been used as a reinforcing agent for traditional medicines, and it has historically been used to treat kidney asthenia, lung asthenia, consumption diseases, neurasthenia, impotence, spermatorrhea, and other diseases. According to the theory of traditional Chinese medicine, kidney asthenia is related to weakness in the body. Thus, the tonifying activity (kidney-yang-tonifying activity) was the primary subject of study in China. The results showed that after administering orally 2 and 1 g/kg the tuber for 10 days, the medicine relieved the symptoms in mice with yang deficiencies that had been induced by hydrocortisone (25 mg/kg, i.g.), increased the body weight of kidney-yang-deficient mice (7.82 and 5.56 g, $P > 0.05$), prolonged the retention time on the rotating bar (19.57 ± 10.21 min, $P < 0.01$, and 5.90 ± 2.47 min, $P < 0.05$) and increased the kidney indexes of treated mice compared with those of the model group. And the increased weight and time on the rotating bar for the untreated control groups were 8.71 g ($P > 0.05$) and 24.20 ± 13.95 min ($P < 0.01$), respectively. Further, study showed that the tubers could also increase DNA synthesis in the spleen (48.89 ± 18.68 and 46.63 ± 13.80 μg/g, $P < 0.01$), kidneys (14.26 ± 5.34 and 5.94 ± 4.88 μg/g, $P > 0.05$), and liver (42.94 ± 13.95

μg/g and 42.02 ± 17.69, $P < 0.05$) of mice with yang deficiencies that were induced by hydrocortisone compared with the mice in the model group (27.25 ± 13.18 μg/g, $P < 0.01$, 4.90 ± 4.82 μg/g, $P > 0.05$, and 19.02 ± 15.49 μg/g). These results suggested that the tuber has a significant tonifying effect on the kidney and strengthens the bodies of mice with kidney deficiencies that were induced by hydrocortisone (Lin, 2009).

In 2011, Zhao and Liu (2011) studied the anti-fatigue activity of the tuber of *G. conopsea* in mice. After administering orally (i.g.) aqueous extracts to mice (40, 20, and 10 g/kg) for 6 days, the swimming times were markedly increased (1441.6, 1357.0, and 1249.9 s, $P < 0.01$) during the weight-bearing swimming tests in a dose-dependent manner. The swimming times of the control and positive groups (*Radix ginseng*, 20 g/kg) were 491.1 s and 1685.9 s ($P < 0.01$). According to the processing method of traditional Mongolia medicine in China, the tuber should be decocted in goat or cow milk before clinical use. After processing with a different method, Jin and Wang (2009) studied and compared the strengthening effects of the tuber of *G. conopsea* tonics. The results showed that compared with the control group (saline water, 6.38 ± 4.22 min), a goat milk decoction (2 g/kg. i.g.) could significantly increase the swimming time of mice (19.44 ± 12.6 min, $P < 0.01$); the unprocessed group (2 g/kg) and cow's milk decoction group (2 g/kg) had swimming times of 9.09 ± 9.81 min and 6.43 ± 7.21 min ($P > 0.05$), respectively, but they did not show significant difference. All of the decoctions could increase the SOD activity and MDA content compared with that of the control group. Thus, the researchers indicated that after processing with goat milk, the tuber have a better effect with the strengthening tonics than unprocessed medicine (**Table 4**).

Anti-Oxidant Activity

The anti-oxidant activity of the tuber of *G. conopsea* was first comprehensive studied in 2006. The results showed that after isolating by Diaion HP-20 column chromatography (reverse-phase silica gel columnchromatography, Chromatorex ODSDM1020T, 100–200 mesh, Fuji Silysia Chemical, Ltd.), the MeOH-eluted fraction showed the radical scavenging activities for DPPH radical (SC_{50} = 59.1 μg/ml) and.O_2^- (IC_{50} = 13.3 μg/ml), but without the inhibitory activity on xanthine oxidase (IC_{50} > 100 μg/ml). Meanwhile, the radical scavenging activities for DPPH radical and·O_2^- exhibiting inhibitory activity on formozan formation and xanthine oxidase of the acetone-eluted fraction were 55.4, 33.2, and 29.7 μg/ml, respectively. But H_2O-eluted fraction didn't present any activities. Then, the anti-oxidant 13 compounds were studied further, some compounds showed the marked radical scavenging activities for DPPH radical and·O_2^- exhibiting inhibitory activity on formozan formation and xanthine oxidase of 13 compounds (Morikawa et al., 2006b; **Table 3**). Meanwhile, the study simultaneously showed that at a concentration of 10^{-6} mol/L, arctigenin, lappaol A, and lappaol F have anti-oxidative activities that inhibit Fe^{2+}-cystine-induced lipid peroxidation in rat liver microsomes with inhibitory rates of 53, 59, and 52%, respectively, relative to that of vitamin E at 35% (Zi et al., 2008).

In 2010, Li et al. (2010) screened and evaluated the aqueous extract, acidic aqueous extract (pH = 3), 60% ethanol extract, 95% ethanol extract, n-butanol extract, n-butanol saturated by aqueous extract and ethyl acetate extracts of the tuber of *G. conopsea* for their radical scavenging capacity. The results showed that the radical scavenging capacity of the aqueous and acidic aqueous (pH = 3) extracts were weak. The radical scavenging activities (IC_{50}) of the other extracts were 0.1266, 0.4537, 0.3151, and 0.1305 mg /ml. Thus, the investigators indicated that the 95% ethanol extract was the most effective among the six extracts (**Table 4**).

Anti-Viral Activity

In 2008, Zi (2008) studied the anti-HIV activity of certain compounds from the tuber of *G. conopsea* at a concentration of 10^{-5} mol/L in relation to the drugs zidovudine (10^{-7} mol/L, 85.6%) and lamivudine (10^{-8} mol/L, 47.4%), and the inhibition rates of these compounds against the VSVC/HIV-luc model in 293 cell lines were 0.6–13.3% (**Table 3**).

In Tibet, *G. conopsea* has primarily been used by local people to treat chronic hepatitis B. In 2002, Lu et al. (2002) studied the anti-HBV activity. The results showed that after treating the patient's serum that contained hepatitis B for 4 h, the medicine (0.03, 0.06, and 0.12 mg/L) could inhibit eight hemagglutin units of HBsAg. Thus, they indicated that the tuber has medium anti-HBV activity. In 2003, Kimura et al. (2003) studied the inhibitory effect of Tibetan medicinal plants on viral polymerases. The results showed that at 100 μg/ml, 28 species of 76 medicines presented the anti-RTase activity with the more than 70% inhibition rates. But after adding BSA to these drugs, except the fruit of *Terminalia chebula* and *Areca catechu,* other species didn't show the anti-RTase activity, including *G. conopsea* (Kimura et al., 2003; **Table 4**).

Sedative and Hypnotic Activities

In 2009, Lin (2009) studied the sedative and hypnotic effects of the tuber of *Gymnadeniae* tubers at different dosages. In the sedative test, administering the tuber (2 and 1 g/kg i.g.) for 30 min, it could reduce the spontaneous activity of mice within 5 min (6.63 ± 3.18 and 5.36 ± 3.44) at inhibitory rates of 37.44% ($P < 0.05$) and 45.60% ($P < 0.05$), and the control group (normal saline) and positive group (diazepam, 0.004 g/kg) exhibited inhibition of 10.60 ± 5.77 and 3.66 ± 1.47 (an inhibition of 64.21%, $P < 0.01$), respectively. The tubers could also decrease the frequency of upward-raising motion in mice forelimbs within 5 min, especially for the high dose group at 15.42 ± 11.77 times ($P < 0.05$). The upward-raising frequency of the control and positive groups were 22.11 ± 6.92 and 9.37 ± 3.65 ($P < 0.01$), respectively.

In the mesmerism test, the medicine could prolong sleeping times at doses of 2 and 1 g/kg i.g. (32.94 ± 14.84 min, $P < 0.01$; and 28.85 ± 11.28 min, $P < 0.05$, respectively) in mice and decrease the latency time (3.00 ± 0.82 min, and 3.34 ± 0.52 min, $P > 0.05$, respectively) induced by pentobarbital sodium over the threshold dose (50 mg/kg) compared with mice in the control group (13.96 ± 8.45 and 4.80 ± 1.85 min); the positive groups were 45.31 ± 12.31 min ($P < 0.01$) and 1.05 ± 0.31 min ($P < 0.05$). This treatment also increased the number of sleeping mice (18 and 18) and latency time (3.11 ±

TABLE 3 | Activities of specific compounds from *Gymnadenia conopsea* (L.) R. Br.

Compounds	Effects	*In vitro* test	References
Gymconopin A GymconopinB 2-Methoxy-9,10-dihydrophenanthrene-4,5-diol 4-Methoxy-9,10-dihydrophenanthrene-2,7-diol 1-(4-Hydroxybenzyl)-4-methoxy-9,10-dihydrophenanthrene-2,7-diol 1-(4-Hydroxybenzyl)-4-methoxyphenanthrene-2,7-diol Blestriarene A BatatacinIII 3′,5-Dihydroxy-2-(4-hydroxybenzyl)-3-methoxybibenzyl3,3′-Dihydroxy-2-(4-hydroxybenzyl)-5-methoxybibenzyl 3,3′-Dihydroxy-2,6-bis-(4-hydroxybenzyl)-5-methoxybibenzyl α-Tocopherol (+)-Catechin	Anti-oxidant activity	Radical scavenging activities for DPPH radical and.O_2^- exhibiting inhibitory activity on formozan formation and xanthine oxidase at 29.2, 45.8, and >100 μM; 33.4, 21.5, and >100 μM; 31.2 μM, >100 μM and null; 12.7, 0.95, and 44.0 μM; 8.2, 0.19, and 30.5 μM; 15.7, 9.4, and >100 μM; 5.8, 0.27, and 4.5 μM; >40, 82.8, and >100 μM; >40, 9.3, and 72.9 μM; >40, 13.4, and 45.1 μM; >40, 13.4, and 65.2 μM; 11.0, null and null; 6.0, 1.5, and >10 μM, respectively.	Morikawa et al., 2006b
Arctigenin Lappaol A Lappaol F	Anti-oxidant activity	Anti-oxidative activity inhibiting Fe^{2+}-cystine activity induced in rat liver microsomal lipid peroxidation with inhibitory rates of 53, 59, and 52%, compared with that of vitamin E at 35%.	Zi et al., 2008
(−)-4-[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyloxy]benzyl alcohol(−)-4-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyloxy] benzyl ethyl ether(−)-(2R,3S)-1-[4-β-D-glucopyranosyloxybenzyl]-4-methyl-2-isobutyltartrate Cyclo[gly-L-S-(4-hydroxybenzyl)]cys 2-Hydroxy-2-(4-hydroxyphenylmethyl)-4-methylcyclopent-4-en-1,3-dione 2-Hydroxy-3-(4-hydroxyphenyl)-4-hydroxymethylcyclopent-2-enone Coelovirins E Dactylorhin E Dactylorhin B Militarine Gastrodin	Anti-HIV activity	At a concentration of 10^{-5} mol/L, the inhibition rates of these compounds against VSVC/HIV-luc model in 293 cell lines were 9.0, 5.0, 6.2, 11.9, 11.3, 0.6, 13.3, 5.1, 10.0, 2.4, and 0.6%, respectively. Comparisons with the positive drugs zidovudine (10^{-7} mol/L, 85.6%) and lamivudine (10^{-8} mol/L, 47.4%) were performed.	Zi, 2008

1.00 min, $P < 0.01$; and 2.56 ± 0.49 min, $P < 0.05$), and it prolonged their sleeping times (28.22 ± 10.50 min, $P < 0.01$; and 20.81 ± 9.22 min, $P < 0.05$) relative to that of the control group (7, 0.92 ± 1.58 and 8.56 ± 15.53 min), which was induced by pentobarbital sodium at less than the threshold dose (45 mg/kg), and the diazepam group (20, 6.35 ± 0.82 and 38.22 ± 3.84; $P < 0.01$). These results indicated that the tuber of *G. conopsea* has marked sedative and hypnotic dose-dependent effects, and its mechanism of action requires further research (**Table 4**).

Preventing and Treating Gastric Ulcers

With normal saline as the control, the inhibitory effect of the tubers on gastric ulcers was induced by hydrochloride-ethanol solution (7.5 ml/kg for 3 days, i.g.). The macroscopic and pathological results showed that at doses of 2 and 1 g/kg, the treatment could relieve the pathology index of gastric ulcers induced by hydrochloride-ethanol solution and decrease the gastric ulcer index (0.05 ± 0.13 cm and 0.22 ± 0.38 cm) at inhibitions of 88.86% ($P < 0.01$) and 48.88%, respectively. The biochemical test showed that after treatment with *Gymnadeniae* tubers, the serum (9.30 ± 4.18 nmol/ml, $P < 0.01$; and 13.71 ± 3.89 nmol/ml) and gastric (1.10 ± 1.19 nmol/ml, $P < 0.05$; and 0.96 ± 0.69 nmol/ml) MDA contents could be decreased in rats. The gastric ulcer index, MDA content in the serum and gastric ulcer index of the model group were 0.44 ± 0.18 cm, 14.17 ± 4.88 and 3.81 ± 5.35 nmol/ml, respectively. The above results suggest that the tubers exert a degree of inhibition on gastric ulcers that were induced by the hydrochloride-ethanol mixture (Lin, 2009). Jiang et al. (2009) also studied the beneficial effect of the tuber of *G. conopsea* on acute gastric ulcers in rats that were induced by hydrochloride-ethanol solution (7.5 ml/kg for 3 days). After drug administration orally (2 and 1 g/kg) for 30 min, the gastric ulcer areas were decreased with inhibitions of 88.86 ($P < 0.01$) and 48.88 ($P < 0.01$); the inhibition by a commonly prescribed drug (ranitidine, 1.95 g/kg) was 91.12 ($P < 0.01$). The

TABLE 4 | The biological activities of *Gymnadenia conopsea* (L.) R. Br.

Pharmacological activities	Plant parts	Processing method/extract	Test system	Animal or test organism	Positive drug	References
Tonifying activity	Tuber	Powder, no extract	*In vivo*	Mice induced by hydrocortisone (25 mg/kg, i.g.)	–	Lin, 2009
Anti-fatigue activity	Tuber	Aqueous extract	*In vivo*	Mice	Radix ginseng, 20 g/kg	Zhao and Liu, 2011
	Tuber	Goat and cow milk decoction	*In vivo*	Mice	–	Jin and Wang, 2009
Anti-oxidant activity	Tuber	Methanol, aqueous and acetone-eluted fraction	*In vitro*	Radical scavenging assay	–	Morikawa et al., 2006b
	Tuber	Aqueous, acidic aqueous, 60% ethanol t, 95% ethanol, n-butanol, and n-butanol extracts	*In vitro*	Radical scavenging assay	–	Li et al., 2010
Anti-viral activity	Tuber	Aqueous extract	*In vitro*	The patient's serum with hepatitis B	–	Lu et al., 2002
Sedative and hypnotic activities	Tuber	Powder, no extract	*In vivo*	Mice	Diazepam	Lin, 2009
Preventing and treating gastric ulcers	Tuber	Powder, no extract	*In vivo*	Mice induced by a hydrochloride-ethanol solution	–	Lin, 2009
	Tuber	Powder, no extract	*In vivo*	Mice induced by a hydrochloride-ethanol solution	–	Jiang et al., 2009
Immunoregulatory Activity	Tuber	The polysaccharide	*In vivo*	Mice	–	Shang et al., 2014
Anti-aging activity	Tuber	Shouzhangshen-37 pill (the tuber of *G. conopsea* is the primary component of this pill)	*In vivo*	Mice induced by injecting D-galactose	Vitamin E	Si and Liu, 2013
Anti-hyperlipidemia activity	Tuber	Ethanol extracts	*In vivo*	Hyperlipidemia rats	Lovastatin	Zhang et al., 2013
Antianaphylaxis activity	Tuber	Methanolic extract	*In vivo*	Mice	–	Matsuda et al., 2004
Anti-silicosis activity	Tuber	60% ethanol extract	*In vivo*	Rat lung interstitial fibrosis	–	Wang et al., 2007, 2008
	Tuber	Ethanol extract	*In vivo*	Rat lung interstitial fibrosis	–	Zeng et al., 2007
	Tuber	Ethanol extract	*In vivo*	Rat lung interstitial fibrosis	–	Chen, 2008

pathology of the gastric ulcers was markedly improved by these drugs (**Table 4**).

Immunoregulatory Activity

As a traditional folk medicine, the tuber of *G. conopsea* has been widely indicated to have good tonifying and immunoregulatory activities (Chinese Materia Editorial Committee, State Chinese Medicine Administration Bureau, 2002; Li et al., 2006). In 2014, Shang et al. (2014) first evaluated the immunoregulatory functions of the polysaccharide of the tuber of *G. conopsea* in mice. After administering orally the polysaccharide (100, 50, and 10 μg/g) and distilled water for 28 days, the serum lysozyme content (104.8 ± 7.8, 102.5 ± 2.8, and 100.1 ± 7.3, all $P < 0.01$), thymus index (0.42 ± 0.03, $P < 0.01$; 0.35 ± 0.01, $P < 0.01$; and 0.25 ± 0.02) and spleen index (0.92 ± 0.07, $P < 0.01$; 0.85 ± 0.04, $P < 0.01$; and 0.79 ± 0.10, $P < 0.05$) were significantly improved relative to that of the control group (86.7 ± 2.8 mg/L, 0.22 ± 0.02, and 0.72 ± 0.05 mg/g). In the delayed type hypersensitivity (DTH) test, the drugs could promote the genesis of DTH and induce ear swelling, especially for the high dose group (30.0 ± 0.4 mg, $P < 0.01$) compared with the control group (15.6 ± 0.5 mg). The polysaccharides could significantly improve the phagocytic function of mouse macrophages, and the phagocytosis rates were 69.6 ± 1.3 ($P < 0.01$), 58.6 ± 0.5 ($P < 0.05$) and 49.2 ± 1.0 ($P < 0.05$) compared with that of the control group at 40.8 ± 0.7. These results suggest that the polysaccharide has a marked immunoregulatory function (**Table 4**).

Anti-Aging Activity

In 2013, Si and Liu (2013) studied the anti-aging effects and mechanisms of the Shouzhangshen-37 pill on subacute aging in mice (the tuber of *G. conopsea* is the primary component of this pill). The subacute aging model mice were induced by injecting D-galactose (120 mg/kg) once per day for 7 weeks. High (2.4 g/kg), middle (1.2 g/kg), and low (0.6 g/kg) doses of Shouzhangshen-37 pills and a common drug (VE, 38.9 mg/kg) were also administered i.g. for 7 weeks. After the treatment, the anti-aging effects and mechanisms were evaluated by observing learning and memory ability with step-through tests, the indexes of the brain, thymus, and spleen were calculated by measuring the SOD, CAT, and MDA contents of brain tissues and observing the pathomorphism changes in mouse cerebral tissue by HE coloration. The results showed that compared with the model group, the drugs could enhance the memory ability and decrease

the number of mistakes within 300 s (1.54 ± 0.53, $P < 0.05$; 1.26 ± 0.42, $P < 0.01$; and 1.71 ± 0.82, $P < 0.05$) compared with that of the model group (2.11 ± 0.81) and VE group (1.26 ± 0.42, $P < 0.01$). In addition, the brain tissue index (12.97 ± 1.89 mg/g, $P < 0.05$; 13.39 ± 1.39 mg/g, $P < 0.01$; and 13.05 ± 2.35 mg/g, $P < 0.05$), spleen (4.53 ± 0.66 mg/g, $P < 0.05$; 4.76 ± 0.82 mg/g, $P < 0.01$; and 4.58 ± 0.65 mg/g, $P < 0.05$), and thymus (1.11 ± 0.12 mg/g, $P < 0.05$; 1.21 ± 0.20 mg/g, $P < 0.01$; and 1.14 ± 0.25 mg/g, $P < 0.05$;) were improved compared with the model group (10.43 ± 1.66, 3.63 ± 0.55, and 0.91 ± 0.18 mg/g). The results of the biochemical test showed that the SOD activity (170.8 ± 20.3 U/mg, $P < 0.05$; 180.6 ± 14.7 U/mg, $P < 0.01$; and 177.3 ± 20.1 U/mg, $P < 0.05$) and CAT activity (6.51 ± 0.22, 7.14 ± 0.72, and 6.32 ± 0.23 U/mg, all $P < 0.05$) were increased and MDA content (3.21 ± 0.98 nmol/mg, $P < 0.05$; 3.17 ± 0.73 nmol/mg, $P < 0.01$; and 3.36 ± 0.81 nmol/mg, $P < 0.05$) was decreased compared with that of the model group (156.9 ± 14.5, 5.24 ± 0.51 U/mg, and 4.31 ± 0.47 nmol/mg). These results suggest that the Shouzhangshen-37 pill has an anti-aging effect in subacute aging mice. The mechanism may enhance the antioxidant activity in brain tissue, decrease the MDA content in brain tissue, protect the brain nerve cells, and improve memory ability in mice (**Table 4**).

Anti-Hyperlipidemia Activity

In 2013, Zhang et al. (2013) studied the effects of the tuber of *G. conopsea* ethanol extracts on the blood lipids and liver function of experimental hyperlipidemia rats. The results showed that after treating the hyperlipidemia model rats that had been induced by high-fat diets with ethanol extracts (at 5, 2.5, and 1.25 g/kg, i.g.), the TC and LDL-C serum contents of the model rats were not improved; however, the TG and HDL-C contents were decreased at 1.35, 0.89, 0.97 mmol/L ($P < 0.01$), and 0.49 ($P < 0.05$), 0.53 and 0.49 mmol/L ($P < 0.05$) compared with the model group (2.43 and 0.64 mmol/L), and the commonly prescribed drug lovastatin also showed markedly decreased TG and HDL-C content ($P < 0.01$). In addition, the ALT and AST activities in the serum were also inhibited at10.71, 10.82, 8.21 U/L ($P < 0.05$), 6.97 ($P < 0.01$), 13.60 ($P < 0.05$), and 18.02 U/L compared with that of the model group, and the commonly prescribed drug results were 8.53 ($P < 0.05$) and 10.38 ($P < 0.01$). These results suggest that the tuber could reduce blood lipids and protect the liver function in experimental hyperlipidemia rats (**Table 4**).

Antianaphylaxis Activity

In 2004, Matsuda et al. (2004) studied the effects of the methanolic extract of the *G. conopsea* tuber. The results showed an anti-allergic effect on passive cutaneous anaphylaxis reactions in the ears of mice. The inhibitory effects of the principal constituents on β-hexosaminidase release, which acts as a marker of degranulation in RBL-2H3 cells, were examined, and five phenanthrenes (gymconopin B, 4-methoxy-9,10-dihydrophenanthrene-2,7-diol, 1-(4-hydroxybenzyl)-4-methoxyphenanthrene-2,7-diol, 1-(4-hydroxybenzyl)-4-methoxy-9,10-dihydrophenanthrene-2,7-diol, blestriarene A) and six dihydrostilbenes (gymconopin D, batatasin III, 3′-*O*-methylbatatasin III, 3,3′-dihydroxy-2-(4-hydroxybenzyl)-5-methoxybibenzyl, 3′,5-dihydroxy-2-(4-hydroxybenzyl)-3-methoxybibenzyl, and 3,3′-dihydroxy-2,6-bis(4-hydroxybenzyl)-5-methoxybibenzyl) were found to inhibit antigen-induced degranulation by 65.5–99.4% at 100 μM in RBL-2H3 cells (**Table 4**).

Anti-Silicosis Activity

Silicosis is an important occupational disease caused by the inhalation of silica dust, and it is characterized by lung interstitial fibrosis. In 2007 and 2008, Wang et al. (2007, 2008) studied the effects of silica exposure on collagen synthesis in rat lungs and mechanisms of anti-oxidative stress by using a 60% ethanol extract of the tuber of *G. conopsea*. Silicotic animal models were established by surgically directing the tracheal instillation of silica into rat lungs. After administering the ethanol extract orally (8 g/kg, per day), the rats were sacrificed and the samples were collected to assay the relative index at 7, 14, 21, 28, and 60 days. The results showed that the extract could reduce the lung/body weight ratio of rats (8.6, 6.99, $P < 0.01$; 7.25, $P < 0.05$; 7.97, $P < 0.05$; and 9.75 mg/g, $P < 0.05$) relative to the model group (11.04, 9.28, 9.11, 11.98, and 13.91 mg/g), and the extract could also improve pathological changes in the lung. This treatment could also ameliorate silica-induced pulmonary fibrosis by decreasing the type I and type III collagen-positive area percentage in the lungs of rats exposed to silica at different time points. These studies also indicated that the ethanol extract could decrease the MDA content (4.78, $P < 0.01$; 5.39, $P < 0.05$; 5.48, $P < 0.05$; 5.29, $P < 0.05$; and 4.35 mmol/L) and increase the SOD (302.67, 243.95, $P < 0.01$; 293.38, $P < 0.05$; 277.74, $P < 0.05$; and 243.36 KU/L) and GPx (2199.58, 2359.34, $P < 0.01$; 2538.66, $P < 0.01$; 2422.41, $P < 0.05$; and 2298.04 U/L) activities relative to that of the model group (6.43, 6.88, 6.98, 6.12, and 5.82 mmol/L, 314.84, 183.62, 219.41, 218.44, and 226.50 KU/L, and 2489.50, 1015.34, 1227.83, 1814.90, and 1867.38 U/L). In 2007, Zeng et al. (2007) found that the ethanol extract could reduce the TNF-α integral optical density (86.86, 105.1, 122.09, 108, and 94.88, all $P < 0.01$) of rat lungs compared with that of the model group (116.98, 140.1, 220.19, 140.9, and 110.9) at 7, 14, 21, 28, and 60 days, respectively. Before and after treating with a 60% ethanol extract, Chen (2008) studied the differential gene expression profiles of rat lung tissue in the early stage exposure to silica. After treating with the extract (8 g/kg, per day, i.g.), there were 308 and 231 up-regulated and down-regulated genes among a total of 539 available genes compared with that of the model group. Up-regulated pathways might be associated with cell adhesion molecules, notch signaling pathways, and leukocyte transendothelial migration and cell communication. Down-regulated pathways might be related to genes in the linkage of complementary and coagulation cascades, hematopoietic cell lineages, urea cycle and Alzheimer's disease. The chip results were verified by using real-time PCR for SOD and HMOX genes with the same trends. Thus, the investigators indicated that the alcohol extract may inhibit silicosis progress during early exposure periods and attenuate airs acculitis and fibrosis of the lung. The results also showed that the ethanol extract (8 g/kg, per day, i.g.) has the reverse

effect on silicosis, and this process involved the cathepsin D precursor SEC14-like protein 3 and peroxiredoxin-1 (Chen, 2009; **Table 4**).

Other Activities

In 2008, Zi (2008) studied the anti-cancer activity of certain compounds from the tuber of *G. conopsea*. The results showed that the IC_{50}-value of (−)-4-[β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyloxy] benzyl alcohol against human hepatoma cancer cells (Bel7402) was 3.818 μM compared with the commonly prescribed drug camptothecine at 12.5 μM. At a concentration of 10^{-6} mol/L, gastrodin was active against serum deprivation-induced SH-SY5Y apoptosis.

At a dose of 5 mg/kg (i.p.), dactylorhin B, coelovirin E and (2R,3S)-1-(4-hydroxybenzyl)-2-hydroxy-4-(4-hydroxybenzyl)-2-isobutyltartrate could improve mouse memory deficits induced by scopolamine with a neuroprotective effect. Coelovirin E and (2R,3S)-1-(4-hydroxybenzyl)-2-hydroxy-4-(4-hydroxybenzyl)-2-isobutyltartrate could extend the latent time for the step-down test in mice with improvements of 65 and 106%, respectively (Zi, 2008; **Table 4**).

PREPARATIONS AND QUALITATIVE AND QUANTITATIVE ANALYSIS

G. conopsea has been widely used as a folk medicine in the treatment or prevention of diseases for thousands of years in China, but it has not been listed in the Chinese pharmacopeia because there is a lack of scientific quality standards to control the quality of the tuber since 1977 (Committee for the Pharmacopoeia of P.R. China, 1977, 2000; Chinese Materia Editorial Committee, State Chinese Medicine Administration Bureau, 2002). Recently, experts have attempted to formulate the proper quality standard by analyzing the active compounds and determining the contents of plants from different habitats with different chromatographic equipment.

Now, as a strategy to control the quality of folk medicines, chemical fingerprint analyses have been accepted by the WHO (1991), State Food and Drug Administration of China (2000), and other authorities, which has been recognized as a rapid and reliable method of identifying and qualifying herbal medicines. Cai et al. (2006) first developed a high-performance liquid chromatography-diode array detection-tandem mass spectrometry (HPLC-DAD-MS^n) method for the chemical fingerprint analysis and rapid identification of major compounds in *G. conopsea* tubers. An HPLC separation was performed by using a linear gradient at room temperature (20°C) and a flow rate of 0.7 ml/min with an Inertsil C_{18} ODS-3 column. The gradient elution started with a methanol: water mixture (20:80, v/v), and the methanol content was increased to 100% within 60 min; the detection wavelength for fingerprint analysis was 270 nm. Adenosine, 4-hydroxybenzyl alcohol, 4-hydroxybenzyl aldehyde, dactylorhin B, loroglossin, dactylorhin A, and militarine were well-separated and identified from the tuber of 10 samples, which were collected from Sichuan, Qinghai, and Hebei Provinces and Tibet Autonomous Region of China and Nepal. Xue et al. (2013) also studied the fingerprint of *G. conopsea* by using HPLC. Their samples were separated on a Kromasil C_{18} (4.6 × 250 mm, 5 μm) column and gradient-eluted with a mixture of methanol and water (0.04% phosphoric acid) at a flow rate 0.7 ml/min; the detection wavelength was set at 222 nm, and the column temperature was 30°C. The results showed that 13 common peaks were selected, and the method validation met the technical requirements of a fingerprint. The similarity of 12 samples from the different regions of the Xizang Autonomous Region and Qinghai Province was 0.904–0.989. The above two methods are simple, practical and reliable. The combined use of the two fingerprints confirmed the identification and quality assessment of *G. conopsea*. In 2009, after investigating the optimal extraction method, Li et al. (2009) firstly developed a HPLC method for the simultaneous quantification of five glucosyloxybenzyl 2-isobutylmalates in the tubers of *G. conopsea*, which was collected at five regions in China. The optimal extraction conditions was the direct reflux with 70% ethanol for 1 h, and the compounds are separated on an Agilent Hydrosphere C18 (150 × 4.6 mm i.d., 5 μm) column using a mobile phase of acetonitrile-water including 0.3% acetic acid (adjusted with 36% acetic acid) with gradient elution at a flow rate of 1.0 mL/min. Detection is set at a UV wavelength of 221.5 nm. The recovery of the method is 97.7–101.0%, and linearity ($r > 0.9998$) is obtained for all the analytes. The results showed that the contents of dactylorhin B, dactylorhin E, loroglossin, dactylorhin A, and militarine were 1638.28, 100.19, 279.91, 717.22, and 101.78 mg/g (Lijiang City, Yunan Province), 665.82, 92.12, 248.15, 507.65, and 138.57 mg/g (Weixian City, Hebei Province), 707.84, 85.64, 322.63, 705.70, and 137.30 mg/g (Kangding City, Sichuan Province), 2207.30, 204.46, 604.81, 2213.39, and 228.04 mg/g (Xizang Autonomous Region), and 4073.89, 156.81, 3060.63, 2230.67, and 563.56 mg/g (Xining City, Qinghai Province). Yang et al. (2009) and Yang (2010) determined the contents of four marker compounds from the tubers of *Gymnadenia conopsea* (L.) R. Br. by HPLC, and the tubers were collected from 24 regions, including the Sichuan Province, Xizang Autonomous Region, Jilin Province, Heilongjiang Province, Qinhai Province, and other regions in China. At a detection wavelength of 222 nm, the mobile phases of methanol (A) and water (B) were used in a gradient elution at 0.7 ml/min. The initial condition was 15–20% A in 0–10 min, which increased to 45% A in 20 min and 70% A in 35 min. The results showed that the highest contents of dactylorhin A, dactylorhin B, loroglossin, and militarine from the rhizomes of24 different regions were 1.428 and 1.638 mg/g from Lulang County in the Xizang region and 1.148 and 0.521 mg/g from Jilin Province. In 2011, the gastrodin contents were assayed by HPLC, and the results showed that the highest content was 9.113 mg/g in Tongde County of Qinghai Province, China, followed by 7.785 mg/g (Beinagou region, Qinghai Province), 4.881 mg/g (Huangnan Zeku region, Qinghai Province), 4.309 mg/g (Guoluo region, Qinghai Province), 4.285 mg/g (Batang Grassland, Qinghai Province), 3.895 mg/g (Nangqian County, Qinghai Province), 3.719 mg/g (Maixiu Forest Farm, Qinghai Province), 3.242 mg/g (Jinyingtan, Qinghai Province), 3.024 mg/g (Linzhi County, Xizang Autonomous

Region), 3.103 mg/g (Chengduo, Qinghai Province), 2.734 mg/g (Bomi County, Xizang Autonomous Region), and 2.268 mg/g (Beishan Forest Farm, Huzhu County, Qinghai Province; Xue et al., 2011).

Meanwhile, the content of four marker constituents of the tubers with the climatic factors were determined and analyzed by HPLC in 2014. The results showed that the samples collected from Milin County in the Xizang Autonomous Region had the highest gastrodin content at 2.725 mg/g, and the Shennongjia region of Hubei Province had lowest content at 0.374 mg/g. The samples collected from Kangding County of Sichuan Province, Aba region of Sichuan Province and Naqu County of the Xizang Autonomous Region had the highest contents of adenosine, 4-hydroxybenzyl alcohol and 4-hydroxybenzaldehyde at 1.485, 1.505, and 1.048 mg/g, respectively. These findings suggest that the average temperature and annual average variations in monthly temperature change along with seasonal variations, and the highest temperature, variations in the scope of the annual temperature, average temperature of the most humid season, average temperature of the hottest season, annual rainfall, rainfall during the driest season, rainfall during the coldest season, and latitude and altitude of the habitat can have an effect on the active ingredient content and quality of the tuber of *G. conopsea* (Zhang et al., 2014).

The study results also indicated that the samples collected from Huzhu County of Qinghai Province had the highest contents of gastrodin (4.9242 mg/g), dactylorhin A (8.2274 mg/g), and militarine (7.4645 mg/g; Yao and Lin, 2014).

TOXICITY

In 2007, Bai and Zheng (2007) evaluated the toxicity of the tuber of *G. conopsea*. In acute toxicity test, after administering the tuber (1.00, 2.15, 4.64, and 10 g/kg, i.g.) to mice and rats, the behavior, and growth of the animals did not change after 2 weeks compared to the control group ($P > 0.05$), and the LD_{50} was more than 10 g/kg. The genotoxicity of the medicine was then studied, and the results showed that the rates of bone marrow and sperm abnormalities were not changed compared with that of the control group, but the sperm abnormality rates of the group administered a common drug (cyclophosphamide, 30 mg/kg) changed markedly ($P < 0.01$). And after administering the tuber (1.67, 3.33, and 6.67 g/kg, i.g) to rats for 30 days, the parameters of blood routine, biochemical indexes, organ coefficient, and organ pathology of rats haven't marked changed compared the control group ($P > 0.05$). So they thought that the tuber didn't present the apparent toxicity.

CONCLUSIONS AND FUTURE PERSPECTIVES

As an important traditional medicine in China, the tuber of *G. conopsea* has been historically used in a number of clinical applications. However, lack of scientific quality standard to control the quality of the tuber has been hampered to the development of this plant. Up to now although investigators have begun studying and developing the quality of the tuber and its preparations by analyzing the marker compounds and determining the contents of plants from different habitats, a scientific method to control the quality of these products has not yet been developed and approved by government. Thus, a characteristic chemical and biological index should be established to monitor and evaluate quality of samples and maintain their clinical and pharmaceutical stability, and the method of accurately controlling the quality of the tuber in traditional medicine and preparations should be studied further.

With the excavation and abundant use of *G. conopsea*, as well as the over-grazing and disorder tourism resulted in the habitat destruction, the resources of this plant were rapid decreased. At the same time, because of the low reproductive capacity of this plant, once the resources and habitat have been destroyed, the species does not recover easily. In 2000, *G. conopsea* has been listed in the grade Π section of endangered species (Gesang and Gesang, 2010). Recently, investigators have begun studying and developing methods of cultivating this plant, such as tissue culture and artificial breeding, but this technology is still not applied in industry (Bao et al., 2008). Therefore, the sustainable use of *G. conopsea* is necessary to investigate. Of course, along with the decrease of resources, the studies on the aerial part should be paid more attention, especially for finding new active compounds and bioactivities.

In a word, phytochemical and pharmacological studies of *G. conopsea* have received great interest, and an increasing number of extracts and active compounds have been isolated that have demonstrated tonifying activity, anti-viral activity and immunoregulatory activity, among others. However, validating the correlations of the ethnomedicinal uses and pharmacological effects should be carried out further, and the toxicity of this plant also should be studied systematically. Meanwhile, the poor quality control, decreased resources, the increasing gap between more experience-based traditional uses and less evidence-based clinical trials for the tuber of *G. conopsea* has created challenges. Therefore, this plant should be studied and developed further, especially in the resource conservation.

AUTHOR CONTRIBUTIONS

XS and JZ conceived the review; XS, XG, XM wrote the manuscript; HP collected the literatures; and YL edited the manuscript. All the authors read and approved the final version of the manuscript.

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REVIEW
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Moringa Genus: A Review of Phytochemistry and Pharmacology

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Moringa is a genus of medicinal plants that has been used traditionally to cure wounds and various diseases such as colds and diabetes. In addition, the genus is also consumed as a source of nutrients and widely used for purifying water. The genus consists of 13 species that have been widely cultivated throughout Asia and Africa for their multiple uses. The purpose of this review is to provide updated and categorized information on the traditional uses, phytochemistry, biological activities, and toxicological research of *Moringa* species in order to explore their therapeutic potential and evaluate future research opportunities. The literature reviewed for this paper was obtained from PubMed, ScienceDirect, and Google Scholar journal papers published from 1983 to March 2017. *Moringa* species are well-known for their antioxidant, anti-inflammatory, anticancer, and antihyperglycemic activities. Most of their biological activity is caused by their high content of flavonoids, glucosides, and glucosinolates. By documenting the traditional uses and biological activities of *Moringa* species, we hope to support new research on these plants, especially on those species whose biological properties have not been studied to date.

Keywords: ***Moringaceae*, *Moringa*, glucosinolates, pharmacological activity, phytochemistry**

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INTRODUCTION

The genus *Moringa* is one of the genera found in the Moringaceae family along with *Anoma* and *Hyperanthera*. It is well-known as the "drumstick" or "horseradish" family. The *Moringa* genus comprises 13 species distributed through southwest Asia, southwest Africa, northeast Africa, and Madagascar. The species and their distributions are listed in **Table 1**.

Among the 13 species, current research is limited to *Moringa oleifera, Moringa stenopetala*, *Moringa concanensis,* and *Moringa peregrina*. As the other species are endemic to Madagascar and Northeast Africa, they are being evaluated less as there is less exploration for naturally occurring bioactive substances in these locations. In contrast, *M. oleifera*, which is native to India, is being studied widely. As a result, the species has been cultivated throughout the world, specifically in Asia, Latin America, Florida, the Caribbean, and the Pacific Islands (Fahey, 2005).

The species in this genus can be categorized into three groups depending on their type of trunk (Olson and Rosell, 2006). *M. stenopetala, Moringa drouhardii, Moringa ovalifolia*, and *Moringa hildebrandtii* have bloated water-storing trunks and are known as bottle trees. Meanwhile, *M. peregrina, M. concanensis*, and *M. oleifera* have slender trunks. The remaining species are tuberous shrubs endemic to Northeast Africa. *Moringa* species are also resistant to drought, and can grow fast without needing much care.

The *Moringa* genus has traditionally been widely used to improve health. Kings and queens used *Moringa* to improve their alertness and to maintain healthy skin. Indian warriors were fed

TABLE 1 | List of *Moringa* species throughout the world.

Species	Country	Trivial name
M. arborea Verdcourt	Kenya, Somalia	–
M. borziana Mattei	Kenya, Somalia	–
M. concanensis Nimmo	India	–
M. drouhardii Jumelle	Southern Madagascar	–
M. hildebrandtii Engler	Southwest Madagascar	Hildebrandt's Moringa
M. longituba Engler	Kenya, Southeast Ethiopia, Somalia	*Moringa tubiflora*
M. oleifera Lam.	India	Horseradish, Ben-oil Drumstick, Kelor
M. ovalifolia Dinter ex Berger	Namibia, Southwest Angola	Phantom Tree, Ghost Tree, African Moringo
M. peregrina Forssk. Ex Fiori	Red Sea, Arabia, Northeast Africa	Ben tree, wispy-needled Yasar tree, Wild drumstick tree, Yusor, Al Yassar, Al Ban
M. pygmaea Verdcourt	North Somalia	
M. rivae Chiovenda	Kenya, Ethiopia	Swanjehro
M. ruspoliana Engler	Kenya, Ethiopia, Somalia	
M. stenopetala (Baker f.) Cufodontis	Kenya, Southwest Ethiopia, Somalia	Cabbage tree, Haleko, Shelagda, Shiferaw

All listed species were validated taxonomically from The Plant List (www.theplantlist.org, V1.1, 2013), Bihrmann's Caudiciforms (www.bihrmann.com).

M. oleifera leaves to enhance their energy and help to relieve their pain and stress during war (Mahmood et al., 2010). Other traditional uses of the genus are in healing skin infections, anxiety, asthma, wounds, fever, diarrhea, and sore throats.

The genus is well-known for its multiple uses. The seeds are used for purifying water, the leaves as nutrition supplements, the oil as a biofuel, the trunks as gum, the flowers as honey, and all of the plant parts can also be used for medicinal purposes (Fahey, 2005). *M. oleifera*, which is also known as the "Miracle Tree" and "Mother's Best Friend," has been named the most nutrient-rich plant. Other than having a high concentration of vitamin A, vitamin C, potassium, and calcium, the plant contains all the essential amino acids (Mahmood et al., 2010).

Various research has been conducted on this genus to study its biological properties, especially on *M. oleifera* that has been under study since the 1970s (Mahmood et al., 2010). Currently, it is well-known that the plant has anti-inflammatory, antioxidant, anticancer, and antidiabetic activities. Recently, more research has been conducted on other species such as *M. concanensis, M. stenopetala*, and *M. peregrina*. However, no profound research on other species has been found. This review will primarily compile all the traditional uses, phytochemical contents, and biological activities of the *Moringa* genus, aiming to encourage new research on other species.

TRADITIONAL USES

All the different parts of *Moringa* plants have been reported to have medicinal values. The traditional medicinal uses of *Moringa* species are tabulated in **Table 2**. Other than the species listed in **Table 2**, *Moringa arborea, Moringa longituba, Moringa borziana, Moringa pygmaea*, and *M. hildebrandtii* have also been reported to have medicinal value but have not been mentioned in connection with any specific activity. *Moringa* species are highly nutritious which benefits people in terms of providing daily nutritional supplements and boosting their immune systems (Popoola and Obembe, 2013). Mahmood et al. (2010) reported that *Moringa* leaves contain vitamin C, vitamin A, and high concentrations of essential amino acids. In addition, because the species are resistant to drought, *Moringa* species become valuable during the dry season when other vegetables are not available. In fact, *M. stenopetala* can be found in all households in the Gamo Gofa zone (Seifu, 2014). Mathur (2005) stated in his book that *Moringa* leaves are highly nutritious, with two times more protein than yogurt, four times more vitamin A than carrots, three times more potassium than bananas, seven times more vitamin C than oranges, and four times more calcium than milk. Zaghloul et al. (2012) reported that *M. peregrina* was also used as fodder to increase animal weight while *M. oleifera* contains high amount of zeatin that has been used as a natural plant growth enhancer and helps to increase crop yields (Leone et al., 2015b).

As well as their medicinal uses, *M. drouhardii, M. longituba, M. peregrina, M. stenopetala,* and *M. oleifera* have been used as coagulants to help clarifying water in addition to having antimicrobial activity (Bazrafshan et al., 2012; Dalvand et al., 2016). The high oleic acid content and high stability of *M. ovalifolia, M. stenopetala*, and *M. oleifera* seed oil makes it suitable to use as edible oil, cosmetic oil, biodiesel, and lubrication oil for machinery and watches (Rashid et al., 2008). Other than that, *M. stenopetala* is also used to expel snakes (Seifu, 2014), while the unique stature of *M. hildebrandtii* with its large trunk, and leaves and its scented flowers, is used to mark special occasions. It is also planted around the graves of the Mahabo tribe (Olson and Razafimmandimbison, 2000).

PHYTOCHEMISTRY

Moringa species contain various phytoconstituents such as alkaloids, saponins, tannins, steroids, phenolic acids, glucosinolates, flavonoids, and terpenes. The diversity of these phytochemicals in the genus contributes to its numerous pharmacological uses. About 110 compounds were identified from the genus and are tabulated in **Figure 1** and **Table 3**. Some of these compounds showed positive results when tested for various biological activities. In addition to these 110 compounds, the genus contains more compounds as detected by GC-MS. Regardless of the high phytochemical contents of the genus, the constituents of only specific species had been explored, namely *M. concanensis, M. peregrina, M. stenopetala*, and *M. oleifera*, and most of the studies focused on the leaves of the plants.

Flavonoids

The *Moringa* genus has high antioxidant activity mainly due to its high content of flavonoids. Most of the flavonoids present in the genus are in the flavanol and glycoside form. The most

TABLE 2 | Ethnomedicine of *Moringa* species.

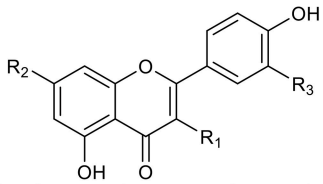
Species	Part	Traditional uses	References
M. concanensis	Bark	Reduce pain, abortifacient	Patil and Patil, 2005
	Leaves	External tumors	Chitravadivu et al., 2009
	Resin	Fire burn wounds	
M. drouhardii	Bark	Colds and coughs	Olson, 1999
M. peregrina	Leaves	Skin rashes, paralysis	Odee et al., 2002
	Bark	Disinfectant to speed up wound healing	Marwah et al., 2007
	Pods	Infantile paralysis or convulsions	Miller et al., 1988
	Leaves, roots	Malaria, hypertension, stomach disorder, expel retained placenta, asthma, diabetes	Mekonnen et al., 1999
M. rivae	Leaves	Weakness of thigh and calf muscles	Forest Department, 2016
	Gum	Arthritis	
M. ruspoliana	–	Eye and throat infections, tsetse fly bites, livestock diseases, abdominal pains, sexually transmitted diseases	Odee et al., 2002
M. stenopetala	Leaves	Flu	Teklehaymanot and Giday, 2010
	Leaves	Diabetes and disorders associated	Habtemariam, 2016
	Root	Malaria, stomach pain, diabetes	Mekonnen, 2002
	Leaves	Malaria, hypertension, expel retained placenta, stomach pain, visceral leishmanial, diabetes, wound healing, common cold	
	Bark	Cough	Teklehaymanot and Giday, 2010
	Root	Epilepsy, help during labor	
M. oleifera	Leaf	Diarrheal, dysentery, colitis, sores, skin infection, anemia, cuts, scrapes, rashes, sign of aging	Silver, 2017
	Gum	Fevers, dysentery, asthma, dental decay	
	Seeds	Warts	
	Leaves	Antibacterial, antimalarial	Parrotta, 1993
	Oil	Gout, acute rheumatism	
	Flowers	Tumor, inflammation, hysteria, enlargement of spleen, muscle diseases, aphrodisiac substances	Anwar et al., 2007; Yabesh et al., 2014
	Roots	Toothache, anthelmintic, ant paralytic	Anwar et al., 2007; Popoola and Obembe, 2013; Sivasankari et al., 2014
	Barks	Aiding digestion, stomach pain, poor vision, ulcer, hypertension, joint pain, anemia, diabetes	Popoola and Obembe, 2013; Yabesh et al., 2014
	Leaves	Cardiac stimulants, malaria, arthritis, diseases of the skin, hypertension, typhoid fevers, swellings, parasitic diseases, diabetes, cuts, contraceptive remedy, genio-urinary ailments, boost immune system, elicit lactation	Anwar et al., 2007; Kasolo et al., 2010; Abe and Ohtani, 2013; Popoola and Obembe, 2013; Sivasankari et al., 2014; Yabesh et al., 2014

common flavonoids of the genus are rutin (**1**), quercetin (**3**), rhamnetin (**6**), kaempferol (**8**), apigenin (**9**), and myricetin (**14**). Optimization research has been conducted to discover the best way to extract flavonoids from *M. oleifera* with highest yield. As a result, subcritical ethanol extraction yielded 26.7% more flavonoid than a reflux method (Wang et al., 2017).

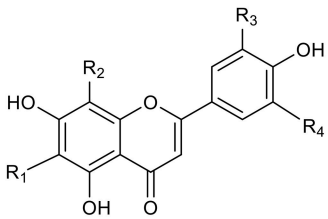
Glucosinolate

Moringa species contain abundant glucosinolates. The most abundant glucosinolate present in the species is 4-*O*-(*α*-*L*-rhamnopyranosyloxy)-benzyl glucosinolate (**30**), also known as glucomoringin (GMG). Three isomers of 4-*O*-(*α*-*L*-acetylrhamnopyrosyloxy)-benzyl glucosinolate (**31–33**) were also detected in *M. oleifera* leaves, depending on the maturity and physiological properties of the leaves (Leone et al., 2015b).

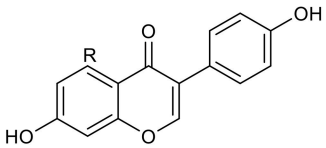
Disruption of plant tissues usually from cutting or chewing caused the release of myrosinase which, when in contact with glucosinolates produces isothiocyanates. The most abundant isothiocyanate found in the genus, 4-[(*α*-*L*-rhamnosyloxy) benzyl] isothiocyanate (GMG-ITC) (**34**), is derived from 4-*O*-(*α*-*L*-rhamnopyranosyloxy)-benzyl glucosinolate (**30**). Recently, isothiocyanates have become a major research interest of *Moringa* for their various biological activities such as their anticancer, antidiabetic, antimicrobial, and anti-inflammatory effect (Park et al., 2011; Padla et al., 2012; Waterman et al., 2014, 2015). The alkylation of isothiocyanates with proteins and DNA contribute to their biological activity (Nibret and Wink, 2010).



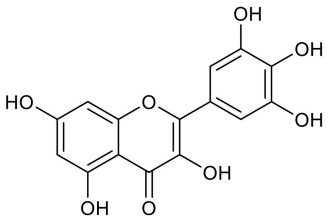
	R_1	R_2	R_3
1	O-GlcRha	OH	OH
2	H	O-rhamnosyl	OCH_3
3	OH	OH	OH
4	O-Glc	OH	OH
5	O-Glc	OH	H
6	OH	OCH_3	OH
7	OH	OH	OCH_3
8	OH	OH	H



	R_1	R_2	R_3	R_4
9	H	H	H	H
10	OCH_3	OCH_3	OCH_3	OCH_3
11	H	H	OH	H



	R
12	OH
13	H



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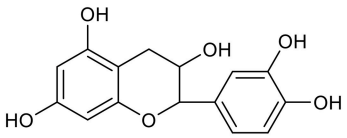

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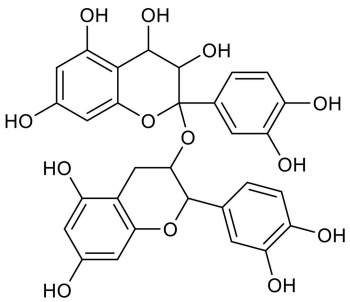

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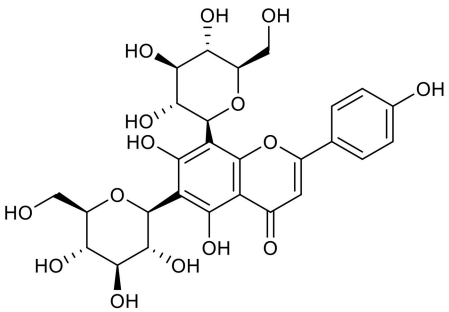

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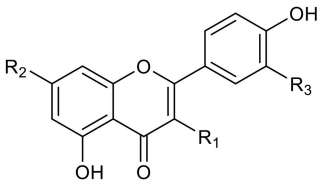
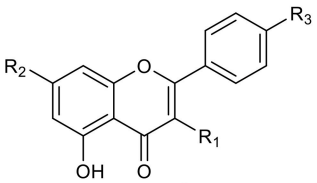
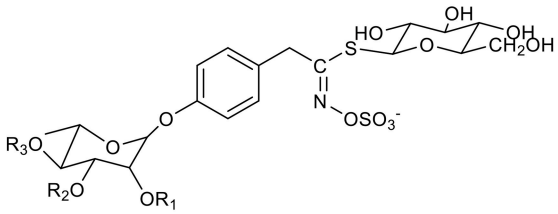


	R_1	R_2	R_3
18	O-Glc	OH	OH
19	O-GlcMal	OH	OH
20	O-rhamnopyranosyl (1-6)- glucopyranoside	OH	OH
21	O-Glc	OH	H
22	O-GlcMal	OH	H
23	O-GlcRha	OH	H
24	O-rhamnosyl	OH	H

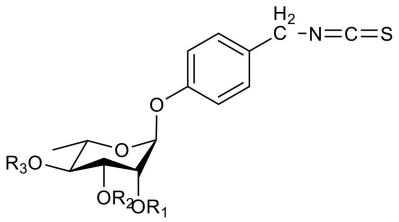


	R_1	R_2	R_3
25	O-2″,3″-Diacetylglucoside	OH	OCH_3
26	O-[Glucosyl-(1‴ → 2″)]-[rhamnosyl (1⁗ → 6″)-glucoside	O-rhamnose	OH
27	O-(2″-Galloylrhamnoside)	OH	OCH_3
28	O-(2″-Galloylrutinoside)	O-Rhamnose	OCH_3
29	O-[Rhamnosyl-(1‴ → 2″)]-[rhamnosyl (1⁗ → 4″)-glucoside	O-Rhamnose	OH

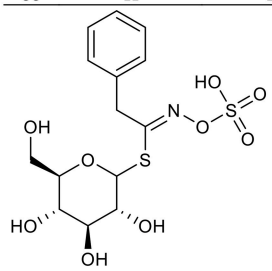
FIGURE 1 | Continued



	R_1	R_2	R_3
30	H	H	H
31	Ac	H	H
32	H	Ac	H
33	H	H	Ac



	R_1	R_2	R_3
34	H	H	H
35	Ac	H	H
36	H	Ac	H
37	H	H	Ac



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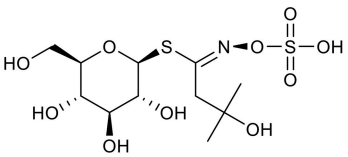

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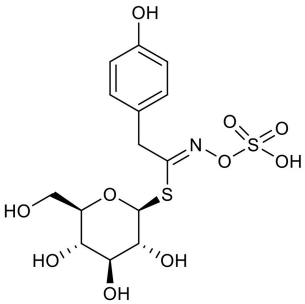

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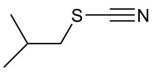

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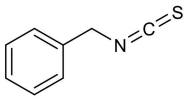

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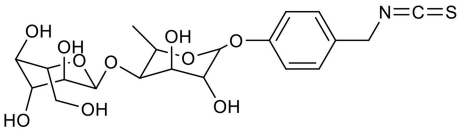

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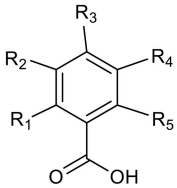
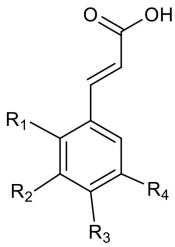
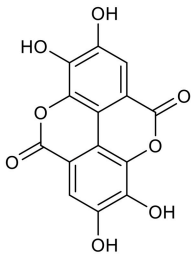


	R_1	R_2	R_3	R_4	R_5
44	H	OH	OH	OH	H
45	H	H	H	H	OH
46	OH	H	H	OH	H
47	H	OCH_3	OH	OCH_3	H



	R_1	R_2	R_3	R_4
49	H	OCH_3	OH	H
50	H	OH	OH	H
51	OH	H	H	H
52	H	H	OH	H
53	H	OCH_3	OH	OCH_3



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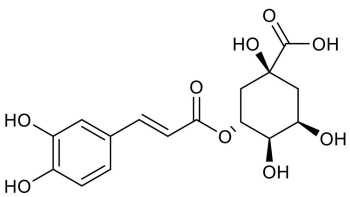

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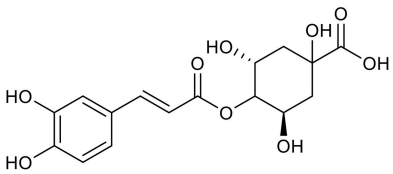

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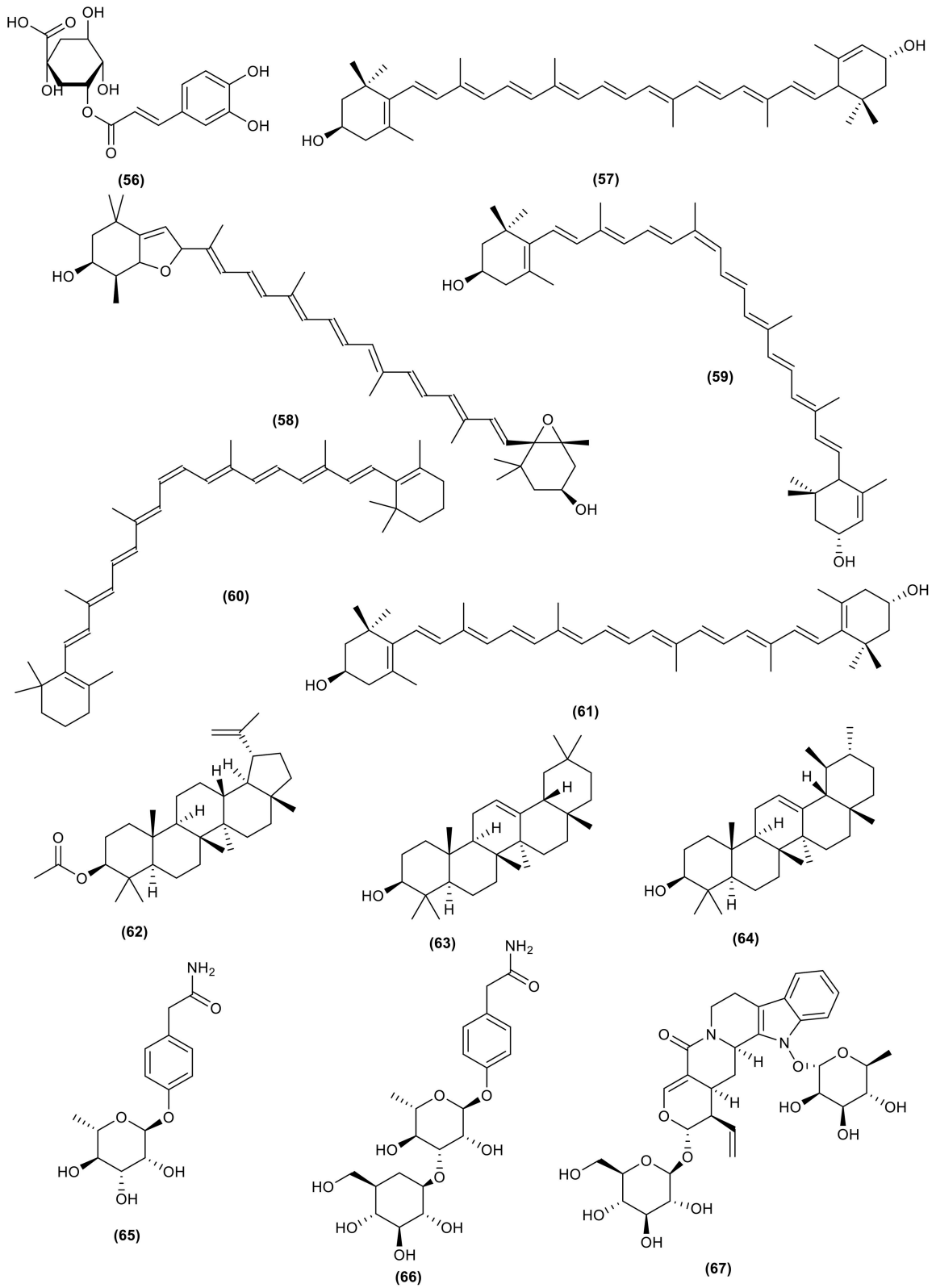
FIGURE 1 | Continued



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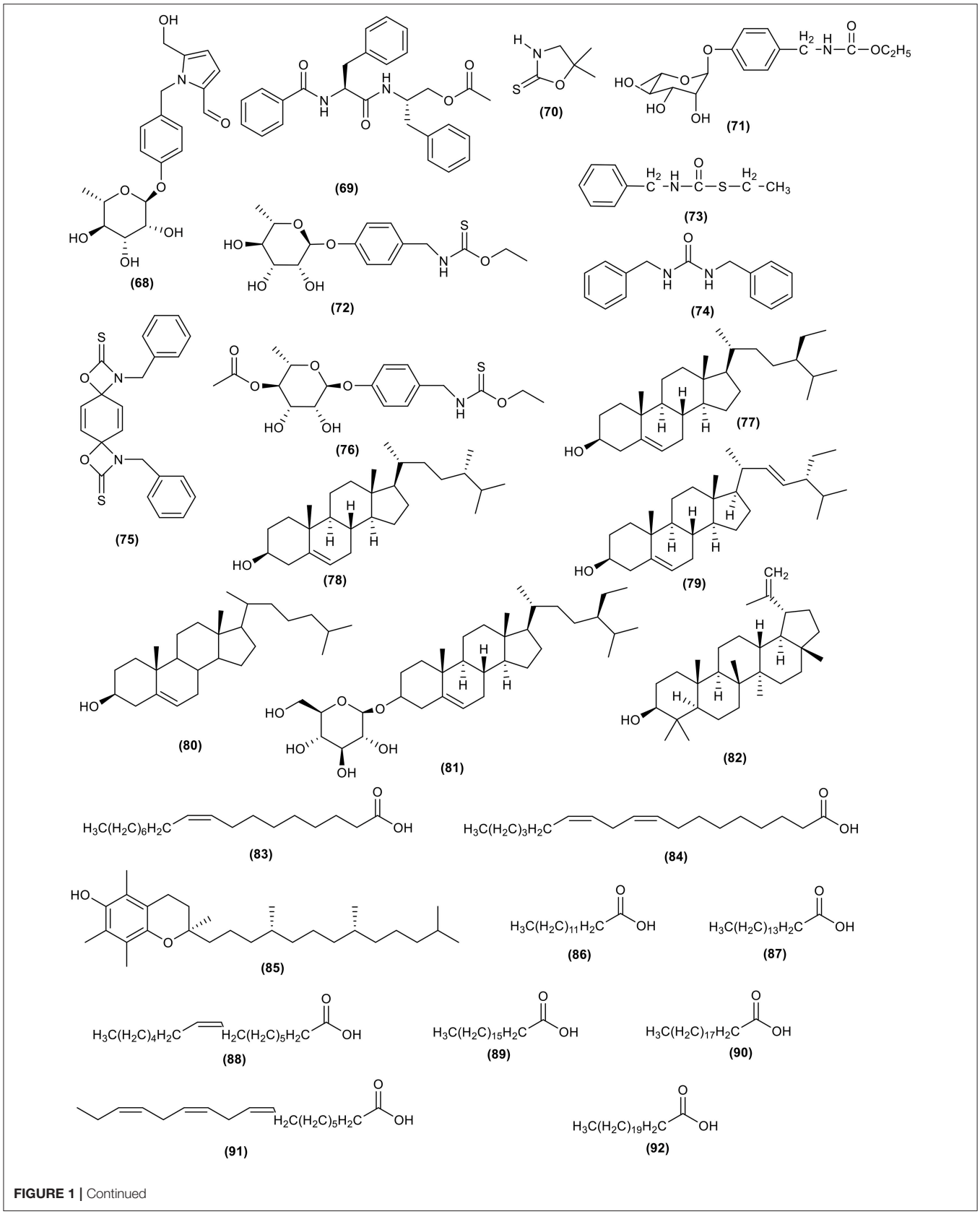

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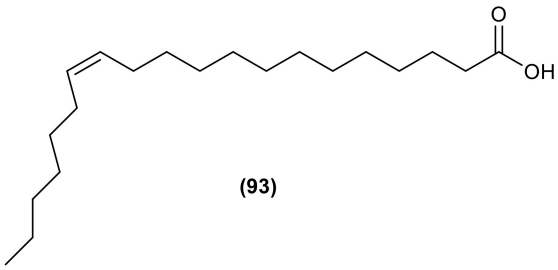
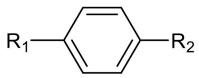
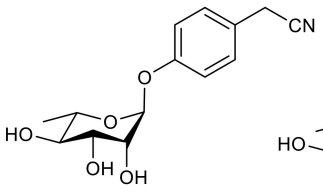
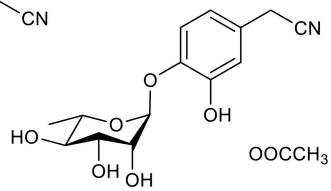
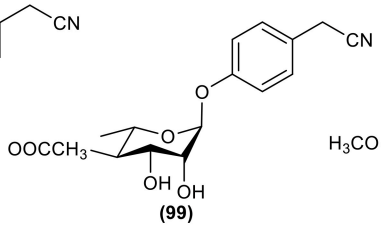


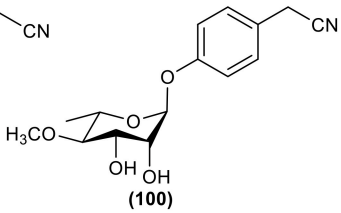


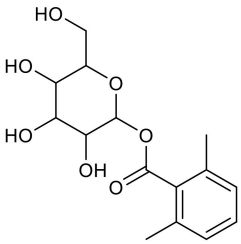
	R_1	R_2
94	CO_2H	O-Glucose
95	CO_2H	O-Rhamnosyl-(1″ → 2′)-glucose
96	CHO	O-Glucose

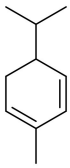


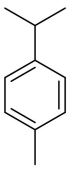


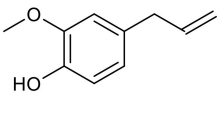


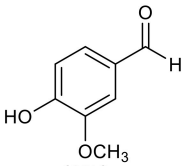


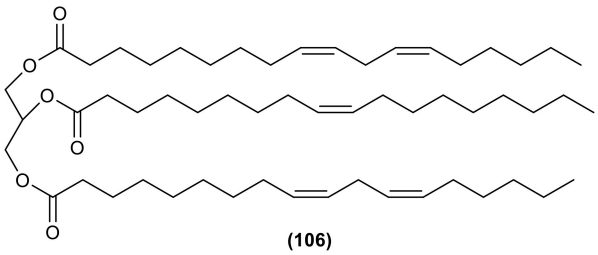


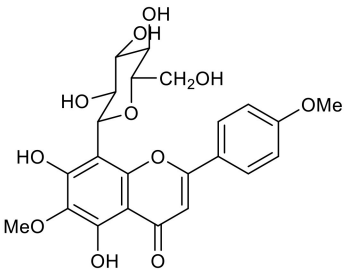


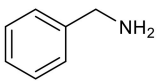


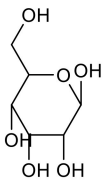












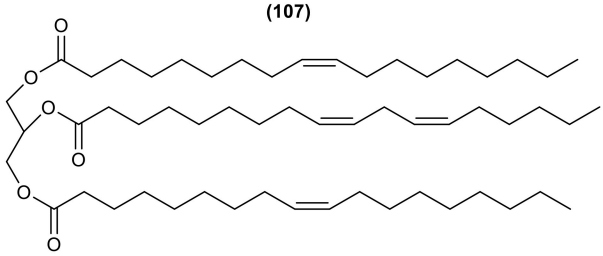


FIGURE 1 | Structures of compounds from *Moringa* species.

Dehshahri et al. (2012a) initiated an *in vitro* callus culture study of *M. peregrina* to induce the production of isothiocyanates but the study did not manage to produce any. Isothiocyanate is commonly present as a volatile oil and is not stable at room temperature. In contrast, *M. oleifera's* isothiocyanate is very stable and present as a solid at room temperature because of the additional sugar moiety on its structure (Tumer et al., 2015).

Phenolic Acid

M. oleifera leaves contain gallic acid (**44**) as their major phenolic acid. Ellagic acid (**48**), ferulic acid (**49**), caffeic acid (**50**), *o*-coumaric acid (**51**), and chlorogenic acid (**54**), are also detected in the leaves and gentisic acid (**46**), syringic acid (**47**), ρ-coumaric acid (**52**), and sinapic acid (**53**) were detected in trace amounts (Leone et al., 2015a,b). Other phenolic

TABLE 3 | Chemical constituents of *Moringa* species.

No.	Compound name	Species	Part	References
FLAVANOIDS AND FLAVANOL GLYCOSIDES				
1	Rutin	*M. stenopetala, M. peregrina, M. oleifera*	Leaves, aerial, leaves	Elbatran et al., 2005; Manguro and Lemmen, 2007; Devaraj et al., 2011; El-Alfy et al., 2011; Habtemariam and Varghese, 2015; Leone et al., 2015b
2	Chryseriol-7-*O*-rhamnoside	*M. peregrina*	Aerial	Elbatran et al., 2005; El-Alfy et al., 2011
3	Quercetin	*M. peregrina, M. oleifera*	Aerial, leaves	Elbatran et al., 2005; Devaraj et al., 2011; El-Alfy et al., 2011; Leone et al., 2015b
4	Isoquercetin	*M. oleifera*	Leaves	Vongsak et al., 2014
5	Astragalin	*M. oleifera*	Leaves	Vongsak et al., 2014
6	Rhamnetin	*M. peregrina*	Aerial	El-Alfy et al., 2011
7	Isorhamnetin	*M. oleifera*	Leaves	Leone et al., 2015b
8	Kaempferol	*M. oleifera*	Leaves	Manguro and Lemmen, 2007; Devaraj et al., 2011; Leone et al., 2015b
9	Apigenin	*M. oleifera, M. peregrina*	Leaves, aerial	Abdel-Rahman Tahany et al., 2010; El-Alfy et al., 2011; Leone et al., 2015b
10	6,8,3,5-Tetramethoxyapigenin	*M. peregrina*	Aerial	Elbatran et al., 2005
11	Luteolin	*M. oleifera*	Leaves	Leone et al., 2015a
12	Genistein	*M. oleifera*	Leaves	Leone et al., 2015a
13	Daidzein	*M. oleifera*	leaves	Leone et al., 2015a
14	Myricetin	*M. oleifera*	Leaves, seed	Lalas and Tsaknis, 2002; Leone et al., 2015a
15	Epicatechin	*M. oleifera*	Leaves	Leone et al., 2015a
16	Procyanidins	*M. oleifera*	Root, stem barks	Atawodi et al., 2010
17	Vicenin-2	*M. oleifera*	Leaves	Muhammad et al., 2016
18	Quercetin-3-*O*-glucoside	*M. oleifera*	Leaves, seeds	Manguro and Lemmen, 2007; Leone et al., 2015b; Maiyo et al., 2016
19	Quercetin-3-*O*-(6″-malonyl) glucoside	*M. oleifera*	Leaves	Leone et al., 2015a
20	Quercetin 3-*O*-α-*L*-rhamnopyranosyl(1-6)-β-*D*-glucopyranoside	*M. peregrina*		Dehshahri et al., 2012b
21	Kaempferol-3-*O*-glucoside	*M. oleifera*	Leaves	Leone et al., 2015a
22	Kaempferol-3-*O*-(6″-malonyl) glucoside	*M. oleifera*	Leaves	Leone et al., 2015a
23	Kaempferol-3-rutinoside	*M. oleifera*	Leaves	Leone et al., 2015a
24	Kaempherol-3-*O*-α-rhamnoside	*M. oleifera*	Leaves	Manguro and Lemmen, 2007
25	Kaempferide 3-*O*-(2″,3″-diacetylglucoside)	*M. oleifera*	Leaves	Manguro and Lemmen, 2007
26	Kaempferol-3-*O*-[β-glucosyl-(1→ 2)]-[α-rhamnosyl-(1→ 6)]-β-glucoside-7-*O*-α-rhamnoside	*M. oleifera*	Leaves	Manguro and Lemmen, 2007
27	Kaempferide-3-*O*-(2″-*O*-galloylrhamnoside)	*M. oleifera*	Leaves	Manguro and Lemmen, 2007
28	Kaempferide-3-*O*-(2″-*O*-galloylrutinoside)-7-*O*-α-rhamnoside	*M. oleifera*	Leaves	Manguro and Lemmen, 2007
29	Kaempferol-3-*O*-[α-rhamnosyl-(1→2)]-[α-rhamnosyl-(1→4)]β-glucoside-7-*O*-α-rhamnoside	*M. oleifera*	Leaves	Manguro and Lemmen, 2007
GLUCOSINOLATE AND ISOTHIOCYANATE				
30	4-(α-*L*-rhamnopyranosyloxy) benzyl glucosinolate (glucomoringin)	*M. oleifera, M. stenopetala*	Leaves, seed	Mekonnen and Drager, 2003; Leone et al., 2015b; Tumer et al., 2015
31	4-[(2′-*O*-acetyl-α-*L*-rhamnosyloxy) benzyl] Glucosinolate	*M. oleifera*	Leaves	Leone et al., 2015b; Tumer et al., 2015
32	4-[(3′-*O*-acetyl-α-*L*-rhamnosyloxy) benzyl] Glucosinolate	*M. oleifera*	Leaves	Leone et al., 2015b; Tumer et al., 2015

(Continued)

TABLE 3 | Continued

No.	Compound name	Species	Part	References
33	4-[(4′-*O*-Acetyl-α-*L*-rhamnosyloxy)benzyl] Glucosinolate	*M. oleifera*	Leaves	Leone et al., 2015b; Tumer et al., 2015
34	4-[(α-*L*-rhamnosyloxy) benzyl] Isothiocyanate	*M. oleifera, M. peregrina*	Leaves	Tumer et al., 2015
35	4-[(2′-*O*-acetyl-α-*L*-rhamnosyloxy) benzyl] Isothiocyanate	*M. oleifera*	Leaves	Tumer et al., 2015
36	4-[(3′-*O*-acetyl-α-*L*-rhamnosyloxy) benzyl] Isothiocyanate	*M. oleifera*	Leaves	Tumer et al., 2015
37	4-[(4′-*O*-acetyl-α-*L*-rhamnosyloxy) benzyl] Isothiocyanate	*M. oleifera, M. stenopetala*	Leaves	Mekonen and Gebreyesus, 2000; Tumer et al., 2015
38	Benzyl glucosinolate (glucotropaeolin)	*M. oleifera*	Seed	Saini et al., 2016
39	Glucoconringiin	*M. stenopetala*	Seed, leaves, roots	Mekonnen and Drager, 2003
40	Sinalbin	*M. oleifera*	Leaves	Leone et al., 2015b
41	Isobutylthiocyanate/Isothiocyanate	*M. stenopetala, M. peregrina*	Seed oil, stem, seed	Nibret and Wink, 2010; Dehshahri et al., 2012a
42	Benzyl isothiocyanate	*M. stenopetala*	Seed oil	Nibret and Wink, 2010
43	4-[(β-*D*-glucopyranosyl-1->4-α-*L*-rhamnopyranosyloxy) benzyl] Isothiocyanate	*M. oleifera*	Leaves, seeds	Maiyo et al., 2016
PHENOLIC ACID				
44	Gallic acid	*M. oleifera*	Leaves	Verma et al., 2009
45	Salicylic acid	*M. oleifera*	Leaves	Leone et al., 2015a
46	Gentisic acid	*M. oleifera*	Leaves	Leone et al., 2015a
47	Syringic acid	*M. oleifera*	Leaves	Leone et al., 2015a
48	Ellagic acid	*M. oleifera*	Leaves	Verma et al., 2009; Leone et al., 2015a
49	Ferulic acid	*M. oleifera*	Leaves	Verma et al., 2009; Leone et al., 2015a
50	Caffeic acid	*M. oleifera*	Leaves	Leone et al., 2015a
51	*o*-Coumaric acid	*M. oleifera*	Leaves	Leone et al., 2015a
52	*p*-Coumaric acid	*M. oleifera*	Leaves	Leone et al., 2015a
53	Sinapic acid	*M. oleifera*	Leaves	Leone et al., 2015a
54	Chlorogenic acid	*M. oleifera,*	Leaves	Verma et al., 2009; Leone et al., 2015b
55	Cryptochlorogenic acid	*M. oleifera*	Leaves	Vongsak et al., 2014
56	Neochlorogenic acid	*M. peregrina*	Aerial	El-Alfy et al., 2011
Terpene				
57	All-*E*-lutein	*M. oleifera*	Pods	Teixera et al., 2014; Saini et al., 2016
58	All-*E*-luteoxanthin	*M. oleifera*	Pods	Saini et al., 2016
59	13-*z*-Lutein	*M. oleifera*	Pods	Saini et al., 2016
60	15-*z*-β-Carotene	*M. oleifera*	Pods	Saini et al., 2016
61	All-*E*-Zeaxanthin	*M. oleifera*	Pods	Saini et al., 2016
63	β-Amyrin	*M. peregrina*	Aerial	El-Alfy et al., 2011
64	α-Amyrin	*M. peregrina*	Aerial	El-Alfy et al., 2011
Alkaloid and Sterol				
65	4′-hydroxyphenylethanamide-α-*L*-rhamnopyranoside (marumoside A)	*M. oleifera*	Leaves	Sahakitpichan et al., 2011
66	3″-*O*-β-*D*-glucopyranosyl derivatives (marumoside B)	*M. oleifera*	Leaves	Sahakitpichan et al., 2011
67	*N*, α-*L*-Rhamnopyranosyl vincosamide	*M. oleifera*	Leaves	Panda et al., 2013

(Continued)

TABLE 3 | Continued

No.	Compound name	Species	Part	References
68	Pyrrolemarumine-4″-*O*-α-*L*-rhamnopyranoside	*M. oleifera*	Leaves	Sahakitpichan et al., 2011
69	Aurantiamide acetate	*M. oleifera*	Roots	Sashidara et al., 2009
70	5,5-Dimethyloxazolidine-2-thione	*M. stenopetala*	Seeds	Mekonnen and Drager, 2003
71	*O*-Ethyl-4-[(α-*L*-rhamnosyloxy)-benzyl] carbamate	*M. oleifera*	Seeds	Guevara et al., 1999
72	Niazimicin	*M. oleifera*	Leaves, seeds	Guevara et al., 1999; Jung, 2014
73	*N*-benzyl, S-ethylthioformate	*M. oleifera*	Root bark	Nikkon et al., 2003
74	1, 3-Dibenzyl urea	*M. oleifera*	Roots	Sashidara et al., 2009
75	Pterygospermin	*M. oleifera*	Seeds	Das et al., 1957
76	Niaziminin	*M. oleifera*	Leaves	Murakami et al., 1998
77	β-sitosterol	*M. peregrina, M. oleifera*	Oil, aerial, leaves, seed	Abdel-Rahman Tahany et al., 2010; El-Alfy et al., 2011; Abd El Baky and El-Baroty, 2013; Maiyo et al., 2016
78	Campesterol	*M. peregrina,*	Oil	Abd El Baky and El-Baroty, 2013
79	Stigmasterol	*M. peregrina,*	Oil	Abd El Baky and El-Baroty, 2013
80	Cholest-5-en-3-ol	*M. stenopetala*	Root	Tesemma et al., 2013
81	β-Sitosterol-3-*O*-β-*D*-galactopyranoside	*M. oleifera, M. peregrina*	Stem bark	Abdel-Rahman Tahany et al., 2010; Bargah and Das, 2014
82	Lupeol	*M. peregrina*	Leaves	Safaeian et al., 2015
OTHERS				
83	Oleic acid	*M. oleifera, M. drouhardii, M. ovalifolia, M. peregrina, M. stenopetala, M. concanensis, M. hildebrandtii*	Oil, oil, oil	Kleiman et al., 2008; Nibret and Wink, 2010; Gaikwad et al., 2011; Abd El Baky and El-Baroty, 2013
84	Linoleic acid	*M. oleifera, M. drouhardii, M. ovalifolia, M. peregrina, M. stenopetala, M. concanensis, M. hildebrandtii*	Oil	Kleiman et al., 2008; Abd El Baky and El-Baroty, 2013
85	Tocopherols	*M. concanensis*	Leaves	Vijayakumar and Sumathi, 2016
86	Myristic acid	*M. oleifera, M. drouhardii, M. ovalifolia, M. peregrina, M. stenopetala, M. hildebrandtii*	Oil	Kleiman et al., 2008; Nibret and Wink, 2010; Abd El Baky and El-Baroty, 2013
87	Palmitic acid	*M. oleifera, M. drouhardii, M. ovalifolia, M. peregrina, M. stenopetala, M. concanensis, M. hildebrandtii*	Oil, roots	Kleiman et al., 2008; Nibret and Wink, 2010; Abd El Baky and El-Baroty, 2013; Faizi et al., 2014
88	Palmitoleic acid	*M. oleifera, M. drouhardii, M. ovalifolia, M. peregrina, M. stenopetala, M. concanensis*	Oil	Abd El Baky and El-Baroty, 2013

(Continued)

TABLE 3 | Continued

No.	Compound name	Species	Part	References
89	Stearic acid	*M. oleifera, M. drouhardii, M. ovalifolia, M. peregrina, M. stenopetala, M. concanensis, M. hildebrandtii*	Oil	Kleiman et al., 2008; Abd El Baky and El-Baroty, 2013
90	Arachidic acid	*M. oleifera, M. drouhardii, M. ovalifolia, M. peregrina, M. stenopetala, M. concanensis, M. hildebrandtii*	Roots, oil	Kleiman et al., 2008; Abd El Baky and El-Baroty, 2013; Faizi et al., 2014
91	Linolenic acid	*M. oleifera, M. drouhardii, M. ovalifolia, M. peregrina, M. stenopetala*	Oil	Abd El Baky and El-Baroty, 2013
92	Behenic acid	*M. oleifera, M. drouhardii, M. ovalifolia, M. peregrina, M. stenopetala, M. concanensis, M. hildebrandtii*	Oil	Kleiman et al., 2008; Abd El Baky and El-Baroty, 2013
93	Paullinic acid	*M. oleifera, M. drouhardii, M. ovalifolia, M. peregrina, M. stenopetala, M. concanensis, M. hildebrandtii*	Oil	Kleiman et al., 2008; Abd El Baky and El-Baroty, 2013
94	Benzoic acid 4-*O*-β-glucoside	M. oleifera	Leaves	Manguro and Lemmen, 2007
95	Benzoic acid 4-*O*-α-rhamnosyl-(1→2)-β-glucoside	*M. oleifera*	Leaves	Manguro and Lemmen, 2007
96	Benzaldehyde 4-*O*-β-glucoside	*M. oleifera*	Leaves	Manguro and Lemmen, 2007
97	Niazirin	*M. peregrina, M. oleifera*	Seeds, leaves	Faizi et al., 1994; Shanker et al., 2007; Sahakitpichan et al., 2011
98	Niaziridin	*M. oleifera*	Pods, leaves	Shanker et al., 2007
99	Niazirinin	*M. oleifera*	Leaves	Faizi et al., 1994
100	4-(4′-*O*-methyl-α-*L*-rhamnosyloxy) benzyl nitrile	*M. peregrina*	Seeds	El-Haddad et al., 2002
101	*M*oringyne	*M. oleifera*	Seeds	Memon et al., 1985
102	α-Phellandrene	*M. oleifera*	Oil	Ogunbino et al., 2009
103	*p*-Cymene	*M. peregrina, M. oleifera*	Seed, oil	Ogunbino et al., 2009; Dehshahri et al., 2012a
104	Eugenol	*M. oleifera*	Bark	Al-Asmari et al., 2015
105	Vanillin	*M. oleifera*	Leaves, fruits, seed	Singh et al., 2009
106	1,3-Dilinoleoyl-2-olein	*M. stenopetala*	Roots	Bekele et al., 2013
107	6-Methoxy-acacetin-8-C-β-glucoside	*M. peregrina*	Aerial	El-Alfy et al., 2011
108	Benzylamine	*M. oleifera*		Iffiu-Soltesz et al., 2010
109	*D*-allose	*M. oleifera*	Leaves	Al-Asmari et al., 2015
110	1,3-Dioleoyl-2-linolein	*M. stenopetala*	Roots	Bekele et al., 2013

acid constituents in the species are tabulated in **Figure 1** and **Table 3**.

Terpenes

Teixera et al. (2014) and Saini et al. (2014) reported that the major carotenoid detected in *M. oleifera* leaves is lutein (**57**). Saini et al. (2014) reported that *M. oleifera* did not contain α-carotene which can usually be found in green leafy plants. The author assumed that all of the α-carotene had been fully converted into lutein. Other carotenoids that can be found in the plant are all-*E*-luteoxanthin (**58**), 13-*Z*-lutein (**59**), 15-*Z*-β-carotene (**60**), and all-*E*-zeaxanthin (**61**) (Saini et al., 2014). Lupeol acetate (**62**), β-amyrin (**63**), and α-amyrin (**64**) were isolated from an *n*-hexane fraction of an ethanol extract of the aerial part of *M. peregrina* (El-Alfy et al., 2011).

Alkaloids

Two new pyrrole alkaloid glycosides were isolated from *M. oleifera* leaves, marumoside A (**65**) and marumoside B (**66**) together with pyrrolemarumine-4″-*O*-α-*L*-rhamnopyranoside (**68**) (Sahakitpichan et al., 2011).

Sterols

A sterol glycoside, namely β-sitosterol-3-*O*-β-*D*-galactopyranoside (**81**), was isolated from a chloroform extract of *M. oleifera* stem bark (Bargah and Das, 2014). The main steroidal components in *M. peregrina* oil were β-sitosterol (**77**) (56.76%), campesterol (**78**) (23.24%), and stigmasterol (**79**) (8.11%) (Abd El Baky and El-Baroty, 2013). β-sitosterol (**77**) was isolated from the leaves and seeds of *M. oleifera* (Maiyo et al., 2016) and an acetone extract of *M. stenopetala* root wood has been reported to contain cholest-5-en-3-ol (**80**) (Tesemma et al., 2013).

Others

Shanker et al. (2007) reported that two nitrile glycosides, niazirin (**97**) and niaziridin (**98**), were observed by reverse phase HPLC. From the three parts of *M. oleifera* tested, only the leaves and pods showed peaks for these nitrile glycosides while no corresponding peaks were detected from the bark. The content of niazirin (**97**) was higher in the leaves while the pods contained a higher concentration of niaziridin (**98**). 6-methoxy-acacetin-8-C-β-glucoside (**107**) was first isolated from an ethanolic extract of the aerial part of *M. peregrina* (El-Alfy et al., 2011).

The major fatty acids of *M. peregrina* are oleic acid (18:1) (**83**) 65.36% and linoleic acid (18:3) (**84**) 15.32%. The oil also contains a high content of tocopherols (**85**) and phenols (Abd El Baky and El-Baroty, 2013). Oleic acid (**83**) (18:1), linoleic acid (**84**) (18:2), myristic acid (**86**) (14:0), palmitic acid (**87**) (16:0), palmitoleic acid (**88**) (16:1), stearic acid (**89**) (18:0), arachidic acid (**90**) (20:0), linolenic acid (**91**) (18:3), behenic acid (**92**) (22:0), and paullinic acid (**93**) (20:1) are present in *M. oleifera, M. drouhardii, M. ovalifolia, M. peregrina,* and *M. stenopetala. M. concanensis* contains all the fatty acids listed above except myristic acid (**86**) and linolenic acid (**91**). *M. hildebrandtii* also contains all the fatty acids listed except for palmitoleic acid (**88**) and linolenic acid (**91**) (Kleiman et al., 2008). The species contain high oleic acid (**83**) levels from 68 to 79%. However, the fatty acid content is dependent on the location where the oil is obtained. *M. oleifera* seed oil has a nutty flavor and has light yellow color (Nadeem and Imran, 2016). The oil contains a high concentration of oleic acid (**83**) which constitutes 75–77% of the fatty acid composition of the seeds.

BIOLOGICAL ACTIVITIES

Antioxidant

The high phenolic content of *Moringa* species contributes to their high antioxidant activity. Phenolic compounds stabilize radicals produced in cells by donating or accepting electrons, hence acting as antioxidants. A water extract of *M. stenopetala* leaves had higher DPPH (1,1-di-phenyl-2-picrylhydrazyl) inhibition (IC_{50}: 40 μg/mL) than a similar extract of *M. oleifera* leaves (IC_{50}: 215 μg/mL). Rutin (**1**) also possessed high antioxidant activity (IC_{50}: 5 μg/mL) in a DPPH assay. An HPLC analysis showed a higher content of rutin (**1**) in *M. stenopetala*, making it a stronger antioxidant than *M. oleifera* (Habtemariam and Varghese, 2015). A methanol fraction of *M. peregrina* leaves showed DPPH inhibition (IC_{50}: 17.07 μg/mL) which was comparable to ascorbic acid's DPPH inhibition (IC_{50}: 13.68 μg/mL) (Al-Owaisi et al., 2014). Based on HPLC results, *M. peregrina* leaves' hexane fraction did not contain phenolic compounds, but did possess radical scavenging activity. Abd El Baky and El-Baroty (2013) reported that *M. peregrina* seed oil also had significant antioxidant activity compared to the common antioxidants BHA, α-tocopherol, and BHT. *M. ovalifolia*, specifically its bark, contained quercetin (**3**), kaempferol (**8**), and myricetin (**14**) that showed antioxidant activity by increasing ferric reducing activity and inhibiting DPPH activity (Ananias, 2015). A study reported that pre-treatment of *M. peregrina* leaves could prevent the plasma hydrogen peroxide concentration from rising at doses of 200 and 400 mg/kg (Safaeian et al., 2015). It also reduced the elevated hydrogen peroxide concentration in plasma and increased the ferric reducing antioxidant at doses of 400 mg/kg. Santhi and Sengottuvel (2016) reported that a methanol extract of *M. concanensis* leaves inhibited DPPH activity, hydroxyl radicals, reducing power, and superoxide anion radicals. The hydroxyl radical inhibition of the extract (IC_{50}: 45.3 μg/mL) was stronger than that of ascorbic acid (IC_{50}: 58.2 μg/mL). Ndhlala et al. (2014) studied the phytochemical properties, and the antioxidant and antimicrobial activities of *M. oleifera* from 13 different cultivars around the world. It was found that *M. oleifera* from different cultivars had different antioxidant, phytochemical, and antimicrobial profiles. *M. oleifera* from one of Thailand's cultivars was five times stronger in inhibiting DPPH radical scavenging activity than ascorbic acid was.

Verma et al. (2009) reported that the most active fraction of a *M. oleifera* leaf hydromethanolic extract was the ethyl acetate fraction. The fraction inhibited DPPH by IC_{50} at 0.04 mg/mL, which was comparable with quercetin (**3**) activity that had inhibited DPPH with an IC_{50}-value of 0.02 mg/mL. As well as *in-vitro* tests, the ethyl acetate fraction of *M. oleifera* leaves has been tested on CCl_4 intoxicated rats (Verma et al., 2009). The extract increased superoxide dismutase (SOD), catalase (CAT),

and reduced glutathione (GSH) levels. Pre-treatment of the *M. oleifera* leaf hydroethanolic extract also counteracted hepatoxicity induced by paracetamol in Sprague-Dawley rats by reducing lipid peroxidation levels and normalizing antioxidant enzyme levels (Uma et al., 2010). A study observed that *M. oleifera* leaf extract reduced DNA breakage in KB cells in addition to increasing their antioxidant enzymes and inhibiting lipid peroxidation (Sreelatha and Padma, 2011). The antioxidant activity of leaf, oil, and seed ethanolic extracts of *M. oleifera* have been reported to display renal protective and hepatoprotective activity against gamma radiation, $HgCl_2$, acetaminophen, and arsenic (Gupta et al., 2007; Fakurazi et al., 2008; Sinha et al., 2011; Abarikwu et al., 2017). Agrawal et al. (2015) observed that *M. oleifera* hydro alcoholic root extract acted synergistically with curcumin and with piperine in inhibiting oxidative stress induced by beryllium in rats. By controlling GSH level, *M. oleifera* ethanol extract reduced glucose-induced cataractogenesis of isolated goat eye lenses (Kurmi et al., 2014). This activity was also observed in the flavonoid fraction of the *M. oleifera* leaves that reduced selenite-induced cataractogenesis in rat pups (Sasikala et al., 2010).

A study stated that myricetin (**14**) from *M. oleifera* seeds had stronger antioxidant activity than α-tocopherol and BHT (Lalas and Tsaknis, 2002). A leaf extract of *M. oleifera* contained isoquercetin (**4**), astragalin (**5**), and crypto-chlorogenic acid (**54**). The leaf extract of the plant, together with the compounds, reduced reactive oxygen species in HEK-293 cells that were induced by H_2O_2 (Vongsak et al., 2015). The compound that had the highest antioxidant activity was determined to be isoquercetin (**4**) as it increased the mRNA expression levels of CAT, heme oxygenase 1, and SOD. Maiyo et al. (2016) isolated two compounds from *M. oleifera* seeds and leaves that showed antioxidant activity: quercetin-3-*O*-glucoside (**18**) displayed significant antioxidant activity while 4-(β-*D*-glucopyranosyl-1->4-α-*L*-rhamnopyranosyloxy) benzylisothiocyanate (**43**)'s activity was moderate.

Ngamukote et al. (2016) conducted a study on the effects of *M. oleifera* extract on the fasting plasma glucose (FPG) concentrations of healthy volunteers, in addition to its antioxidant activity. The extract reduced plasma malondialdehyde (MDA) levels, increased Trolox equivalent antioxidant activity, increased the ferric reducing ability of the plasma, and did not change the FPG concentration compared with that of healthy volunteers that were fed only warm water.

Anti-convulsant

Experiments to discern the effects of *M. concanensis* leaf ethanol extract on the maximal electroshock seizure test and the pentylene tetrazole-induced convulsion test were conducted on Swiss albino mice (Joy et al., 2013). For both of the tests, *M. concanensis* inhibited mortality compared to control group in which deaths resulted. The study reported that the extract might block either calcium channels, sodium channels, or NMDA receptors, or has GABA agonist activity.

Anticancer

The main pathway for the anticancer activity of *Moringa* species is by inhibiting proliferation through apoptosis. **Table 4** summarizes the cancerous cell lines that have been inhibited by *Moringa* species. Methanol crude extracts of *M. concanensis* root bark inhibited the proliferation of hepatocellular carcinoma (Hep-G2) cells through intrinsic pathways by regulating caspase 9 and caspase 3 while reducing the mitochondrial membrane potential of the cells (Vijayarajan and Pandian, 2016). (4′-*O*-acetyl-α-*L*-rhamnopyranosyloxy)benzyl isothiocyanate (**37**) and niazimicin (**70**) were responsible for the regulation of caspase 9 activity (Tiloke et al., 2013). *M. oleifera* leaf extract decreased the proliferation of B16F10 melanoma cells in addition to causing roughly 22% cancerous cell death (Gismondi et al., 2013). It caused apoptosis at the sub G1-area and induced cell arrest at the G2/M phase. The extract increased the $p27^{Kip1}$, p^{53}, and $p21^{WAF1/Cip1}$ levels of the cells. Moringin (**34**) inhibited malignant astrocytoma cells by oxidative stress-mediated apoptosis through Bax and p53 activation pathways (Rajan et al., 2016). Nibret and Wink (2010) reported that seed oil from *M. stenopetala* inhibited the proliferation of HL-60 cells with IC_{50}: 11.63 μg/mL. The bioactive compound reported was benzyl isothiocyanate (**42**), that had high cytotoxic activity against HL-60 cell with IC_{50}: 4.62 μg/mL.

An ethanol extract of *M. stenopetala* leaves and seeds reduced Hep-G2 activity and increased LDH leakage in a dose and time-dependent manner (Mekonnen et al., 2005). 4-(β-*D*-glucopyranosyl-1->4-α-rhamnopyranosyloxy)-benzylisothiocyanate (**43**) had stronger anticancer activity than quercetin-3-*O*-glucoside (**18**) against Caco-2 and Hep-G2 cells (Maiyo et al., 2016). The presence of eugenol (**101**) in *M. oleifera* bark inhibited the activity of E2F1/survivin and the presence of *D*-allose (**109**) in *M. oleifera* leaves inhibited cancer cells in the G1 phase of MDA-MB-231 and HCT-8 cells (Al-Asmari et al., 2015).

A water extract of *M. oleifera* pods exhibited suppressive effects on dextran sodium sulfate- and azoxymethane-induced mouse colon carcinogenesis (Budda et al., 2011). The extract reduced COX-2 proteins and iNOS expression in addition to reducing the PCNA index of the mice. The extract also reduced the multiplicity and incidence of the tumors. The study reported that the high content of omega-9 oleic fatty acid (**83**) in the extract, that possesses anti-inflammatory activity, could modulate this cell proliferation. Alternately, glucomoringin (**30**) might also be responsible for this antitumor activity.

A hydro alcoholic extract of *M. oleifera* also exhibited antitumorigenic activity by balancing xenobiotic metabolism between Phase I and Phase II (Bharali et al., 2003). The extract increased Cyt P450 and Cyt b5 activity in Phase I while increasing glutathione S-transferase, glutathione reductase, and glutathione peroxidase, and reducing the GSH levels that are responsible for Phase II. The study also reported that the extract might act as a "blocking agent" in reducing xenobiotic substrates for Phase II. In addition, the extract increased CAT concentration while reducing mouse skin papilloma genesis and lipid peroxidation.

Antimicrobial

Various research has been conducted on *Moringa* species for their antimicrobial activity. **Table 5** summarizes the antimicrobial

TABLE 4 | Cell line studied for anticancer activity of *Moringa* species.

Species	Cancerous cell line inhibited	References
M. concanensis	Hep G2	Vijayarajan and Pandian, 2016
M. oleifera	A549, Hep-G2, Panc-1, p34, COLO 357, MDA-MB-231, HCT-8, MCF-7, HeLa, CACO-2, L929, HCT-16, PC3, K562, THP-1, T47D, HL-60, Colo-205	Monera et al., 2008; Waiyaput et al., 2012; Berkovich et al., 2013; Tiloke et al., 2013; Al-Asmari et al., 2015; Diab et al., 2015; Elsayed et al., 2015; Jung et al., 2015; Madi et al., 2016
M. peregrina	MCF-7, Hep G2, HCT 116	El-Alfy et al., 2011; Abd El Baky and El-Baroty, 2013
M. stenopetala	HL-60, Hep-G2	Mekonnen et al., 2005; Nibret and Wink, 2010

activity of each species. *Moringa* species have been widely used as water purifiers and antiseptics for water treatment because of their high antimicrobial activity. Hexane and methanol seed extracts of both *M. oleifera* and *M. stenopetala* showed inhibition against waterborne pathogens, particularly against *Salmonella typhii, Vibrio cholera*, and *Escherichia coli* (Walter et al., 2011). Most of the extracts showed better inhibition in lower concentrations.

Ethyl acetate, acetone, and ethanol extracts of *M. oleifera* seeds, roots, leaves, and a mixture, were assessed for their dental antibacterial and antifungal activity (Elgamily et al., 2016). All of the extracts showed inhibition of *Streptococcus aureus* and *Streptococcus mutans* with the ethanol extract and leaf extract showing the highest inhibition. In contrast, none of the extracts showed inhibition against *Candida albicans*. Another study reported that higher concentrations of *M. oleifera* seeds were needed to inhibit the growth of *C. albicans* (Saadabi and Abu Zaid, 2011). The ethanolic leaf extract of *M. oleifera* was formulated into mouthwash and toothpaste (Elgamily et al., 2016). As a result, the toothpaste showed inhibition of *Streptococcus aureus*, *S. mutans*, and *C. albicans,* but the mouthwash only showed antimicrobial activity. The ethanol extracts of the seeds and leaves of *M. oleifera* also exhibited inhibition against the dermatophytes *Trichophyton mentagrophytes, Microsporum canis, Trichophyton rubrum*, and *Epidermophyton floccosum* (Chuang et al., 2007).

Hexane, ethyl acetate, methanol, and chloroform extracts of *M. oleifera* leaves were also tested on different diarrhea associated bacteria: *Serratia marcescens, Shigella dysenteriae, Enterobacter* sp., *E. coli, Klebsiella pneumoniae*, and *Salmonella* sp. (Rahman et al., 2010). All of the extracts exhibited antibacterial activity against the bacteria with minimum inhibitory concentrations ranging from 62.5 to 1,000 μg/mL and zones of inhibition of 8–23.2 mm. Peixoto et al. (2011) reported that aqueous and ethanolic extracts of *M. oleifera* leaves showed inhibition against *Staphylococcus aureus, Vibrio parahaemolyticus, Enterococcus faecalis*, and *Aeromonas caviae*. In contrast, the extracts showed negative result on *E. coli, Salmonella enteritidis*, and *Pseudomonas aeruginosa*. Throughout the study it was observed that the extract showed stronger inhibition against gram-positive species than gram-negative species.

The bioactive compounds isolated from *M. stenopetala* were cholest-5-en-3-ol (**80**), oleic acid (**83**), and palmitic acid (**87**) (Tesemma et al., 2013). From the compounds listed, cholest-5-en-3-ol (**80**) possessed the strongest antibacterial activity against *E. coli*. Abdel-Rahman Tahany et al. (2010) isolated lupeol acetate (**62**), β-amyrin (**63**), α-amyrin (**64**), β-sitosterol (**77**), and β-sitosterol-3-*O*-β-*D*-glucoside (**81**) from *M. peregrina* and these compounds showed higher inhibition toward bacteria than toward fungi. β-Sitosterol (**77**) and β-sitosterol-3-*O*-β-*D*-glucoside (**81**), particularly, only possessed antibacterial activity. It was also reported that *M. concanensis* showed a synergistic effect with *Hugonia mystax* and *Curcuma neilgherrensis* with better activity against *E. coli*, *P. aeruginosa*, and *K. pneumoniae* than when tested alone against the bacteria (Karmegam and Nagaraj, 2017).

Nikkon et al. (2003) isolated aglycon of deoxy-niazimicine (*N*-benzyl, *S*-ethyl thioformate) (**73**) from a chloroform extract of *M. oleifera* root bark and this compound showed stronger inhibition toward *Staphylococcus aureus, S. dysenteriae, Shigella boydii, S. typhii, P. aeruginosa, C. albicans*, and *Aspergillus flavus* growth than a crude extract. 4-[(α-*L*-rhamnosyloxy)-benzyl] isothiocyanate (**34**) from *M. oleifera* and *M. stenopetala* seeds had minimal bactericidal concentrations of 56 μmol/l for *Bacillus subtilis* and 40 μmol/l for *Mycobacterium phlei* (Eilert et al., 2007). This isothiocyanate, together with 4-[(4′-*O*-acetyl-α-*L*-rhamnosyloxy)-benzyl] isothiocyanate (**37**) from *M. oleifera* seeds, exhibited antimicrobial activity against *Staphylococcus aureus, Staphylococcus epidermis, B. subtilis, E. floccosum*, and *T. rubrum* (Padla et al., 2012). Zaffer et al. (2014) reported that an ethyl acetate extract of *M. oleifera* bark showed higher inhibition toward *Pseudomonas fluorescens, Staphylococcus aureus, Bacillus megaterium*, and *Citrobacter freundii* than methanol, chloroform, and aqueous extracts of the same part of the plant.

Torondel et al. (2014) reported the effectiveness of dried and wet *M. oleifera* leaf powder as a hand-washing product in healthy volunteers. The results showed that, only the highest dose of *M. oleifera*, 4 g, showed levels of inhibition of *E. coli* comparable to a non-medicated liquid soap. The study reported that this activity was not related to the mechanical friction produced while washing hands. As aqueous preparations of *M. oleifera* leaf powder exhibited stronger microbial inhibition than dried preparations, the study reported that this activity might be due to saponin presence in the extract that had surfactant properties.

Antitrypanosomal

In the *Moringa* genus, *M. stenopetala* showed significant antitrypanosomal activity. *M. stenopetala* leaf acetone extract and root ethanol extract inhibited the infective stages of *Trypanosoma brucei* (Mekonnen, 2002). A methanol extract of *M. stenopetala* maintained 100% survival against *Trypanosoma congolense* (Kifleyohannes et al., 2014). It also minimized PCV which is a method of controlling anemia. Dichloromethane and methane extracts of the seeds had low trypanocidal activity while the essential oils that contain mainly benzyl isothiocyanate (**42**) and isobutyl isothiocyanate (**41**) showed potent trypanocidal

TABLE 5 | Microorganisms studied for antimicrobial activity of *Moringa* species.

Species	Microorganism inhibited	References
M. concanensis	*Proteus vulgaris, Lactobacillus brevis, Pseudomonas* sp. *Staphylococcus* sp., *Micrococcus luteus, Bacillus* sp., *Lactobacillus bulgaricus, Aspergillus sojae, Aspergillus niger, Aspergillus oryzae, Aspergillus flavus, Candida albicans, Vibrio cholera, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*	Balamurugan and Balakrishnan, 2013; Karmegam and Nagaraj, 2017
M. oleifera	*Aspergillus flavin, Trichoderma* sp., *Staphylococcus aureus, Shigella dysenteriae, Shigella boydii, Bacillus megaterium, Escherichia coli, Enterobacter aerogenes, Providencia stuartii, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus subtilis, Mycobacterium phlei, Bacillus cereus, Sarcina lutea, Basidiobolus ranarum, Basidiobolus haptosporus, Propionibacterium acnes, Staph epidermis, Strep pyogenes, Trichophyton mentagrophytes, Microsporum canis, Trichophyton rubrum, Epidermophyton floccosum, Pseudomonas fluorescens, Citrobacter freundii, Serratia marcescens, Enterobacter* sp., *Salmonella* sp., *Proteus vulgaris, Streptococcus mutans, Vibrio parahaemolyticus, Enterococcus faecalis, Aeromonas cavieae, Streptococcus aureus, Streptococcus mutans, Penicillium expansum, Penicillium digitatum, Penicillium aurantiogriseum, Penicillium citrinum, Aspergillus niger* spp., *Microcytis aeruginosa, Salmonella typhii, Vibrio cholera, Candida albicans*	Nwosu and Okafor, 1995; Chuang et al., 2007; Lurling and Beekman, 2010; Rahman et al., 2010; Peixoto et al., 2011; Saadabi and Abu Zaid, 2011; Walter et al., 2011; Padla et al., 2012; Rattanasena, 2012; Galuppo et al., 2013; Marrufo et al., 2013; Patel et al., 2014; Zaffer et al., 2014; Eyarefe et al., 2015; Dzotam et al., 2016; Elgamily et al., 2016
M. ovalifolia	*Escherichia coli, Bacillus cereus, Enterococcus faecalis*	Shailemo et al., 2016
M. peregrina	*Escherichia coli, Staphylococcus aureus, Candida albicans, Pseudomonas aeruginosa, Staphylococcus epidermis, Candida glabrata, Klebsiella pneumoniae, Enterobacter cloacae, Candida tropicalis, Enterococcus* sp., *Aeromonas hydrophila*	Abdel-Rahman Tahany et al., 2010; Lalas et al., 2012; El-Awady et al., 2015
M. stenopetala	*Salmonella typhii, Vibrio cholera, Escherichia coli, Shigella* spp., *Staphylococcus aureus, Candida albicans, Pseudomonad aeruginosa*	Walter et al., 2011; Tesemma et al., 2013; Seifu, 2014

activity (Nibret and Wink, 2010). Petroleum ether, chloroform, methanol, and aqueous fractions of *M. oleifera* leaves, stem bark, and roots inhibited *Trypanosoma brucei brucei* (Ibrahim et al., 2014). Ayyari et al. (2013) reported that 4-[(α-*L*-rhamnosyloxy) benzyl] isothiocyanate (**34**) from *M. peregrina* aerial parts inhibited *Trypanosoma brucei rhodesiense* with an IC_{50} of 0.1 μM. With a dose 10 mg/kg for four consecutive days, the compound reduced parasitemia by 95% but the parasitemia increased again after 10 days of infection. The compound was reported to have the potential to become a high quality antitrypanosomal drug as it inhibited the parasite at specific enzymes and irreversibly inhibited trypanothione reductase. An acetone extract of *M. stenopetala* leaves and an ethanol extract of *M. stenopetala* root wood inhibited *Trypanosoma brucei* trypomastigotes with ED_{50}-values of 10 and 9.2 μg/mL, respectively (Mekonnen et al., 1999).

Antileishmanial

Mekonnen and Gessesse (1998) reported that high concentrations of *M. stenopetala* extract, 600 to 1,500 μg/mL, changed *Leishmania donovani* promastigotes' shape and resulted in the loss to their flagella in 40–95% of them. A root extract of the plant inhibited *Leishmanial aethiopica* in mice (Bekele et al., 2013). 1,3-Dilinoleoyl-2-olein (**106**) and 1,3-dioleoyl-2-linolein (**110**) from the roots inhibited the promastigotes and amastigotes stages of the parasite. The activity of 1,3-Dilinoleoyl-2-olein (**106**) in inhibiting the promastigotes stage was comparable to that of the positive control, miltefosine.

Kaur et al. (2014) reported that a 70% ethanolic extract of *M. oleifera* roots and a methanolic extract of *M. oleifera* leaves displayed antileishmanial activity against *L. donovani* promastigotes. The ethyl acetate fraction of a methanolic extract inhibited leishmaniasis with an IC_{50} of 27.5 μg/mL. Niazinin, isolated from the ethyl acetate fraction, showed the most antileishmanial activity with an IC_{50} of 27.5 μg/mL. Singh et al. (2015) observed antileishmanial activity from different parts of *M. oleifera*, the bark, leaf, stem, flower, and root. The flower, especially from the ethyl acetate fraction, showed the most potent activity against *L. donovani* promastigotes in infected macrophages by inhibiting parasite viability in a dose- and time-dependent manner. The extract also reduced parasite activity in both the spleen and the liver of Balb/c mice.

Antiviral

M. oleifera extract possessed antiviral activity against the herpes simplex virus type 1 (HSV-1) by inhibiting more than 50% of plaque formation at a 100 μg/mL dose (Lipipun et al., 2003). The extract inhibited phosphonoacetate-resistant HSV-1 and kinase-deficient HSV-1 strains in mice. At a dose of 750 mg/kg, the extract reduced the mortality of the infected mice by prolonging their mean survival time and delaying the development of skin lesions. An aqueous extract of *M. oleifera* leaves activated cellular immunity in mice that were infected with HSV-1 by reducing the virus concentration and limiting herpetic skin lesion development (Kurokawa et al., 2016). *M. peregrina* seed oil was also reported to have antiviral effects against HSV (Soltan and Zaki, 2009). Murakami et al. (1998) reported that 4-[(4′-*O*-acetyl-alpha-*L*-rhamnosyloxy) benzyl] isothiocyanate (**37**) and niaziminin (**76**) inhibited Epstein-Barr virus activation. *M. oleifera* also showed inhibition against the foot and mouth disease virus at concentrations of 1–50 μg/mL (Younus et al., 2016).

A buffer extract of *M. oleifera* fruits exhibited anti-HBV activity and a hydroalcoholic extract of the plant's leaves reduced the ccDNA level of HBV in HepG2 cells (Waiyaput et al., 2012). According to a survey, *M. oleifera* was being used as a supplement toward antiretroviral therapy for HIV infection, but no further research has been conducted on the efficiency of the plant as an antiviral substance (Monera and Maponga, 2010).

Antihyperglycemic, Antihyperlipidemic, and Hypocholesterolemic

Ethanol and aqueous extracts of the aerial parts of *M. peregrina* exhibited antihyperglycemic activity against streptozotocin diabetic rats by lowering their blood glucose levels (El-Alfy et al., 2011). The hexane fraction from an ethanol extract of the plant reduced blood glucose levels by 64–77.44% for 3 h after 30 min of administration. The study suggested that this activity might be because the fraction contained the antihyperglycemic compounds lupeol acetate (**62**) and β-sitosterol (**77**). *M. stenopetala* leaf hydroalcoholic extract was reported to inhibit certain enzymes that related to hyperglycemia and hyperlipidia, such as maltase, sucrase, pancreatic cholesterol esterase, pancreatic lipase, and pancreatic α-amylase (Toma et al., 2014). The leaf extract also reduced cholesterol, triglycerides, and the glucose lipid profile in fructose-induced rats (Geleta et al., 2016b).

Sangkitikomol et al. (2014) evaluated the effects of *M. oleifera* leaf hydroethanolic extract on advanced glycation end products. Throughout the study, the extract reduced the mRNA expression of PPARα1, PPAR-γ, and HMG-CoAR that are responsible for maintaining lipid homeostasis. The extract inhibited the formation of AGE in HepG2 cells at doses of 2.5–10 mg/mL. *M. oleifera* aqueous leaf extract also inhibited formation of both non-fluorescent and fluorescent advanced glycation end products by reducing monosaccharides, in addition to reducing the oxidation of thiols and protein carbonyl content (Nunthanawanich et al., 2016).

A study reported that an aqueous extract of *M. oleifera* leaves increased insulin levels and decreased insulin resistance, helping to combat hyperglycemia in diabetic rats (Tuorkey, 2016). The extract reduced creatinine and urea levels from damaged kidneys and increased the immune tolerance of the diabetic rats by increasing the activity of CD69, INF-γ, and CD44. Insulin-like protein was observed in the seed coat of *M. oleifera* (Paula et al., 2016). This protein had antigenic epitopes similar to insulin and displayed hypoglycemic activity on oral administration. The same activity was observed by a protein from *M. oleifera* leaves (Paula et al., 2017). The protein reduced blood glucose levels after single and repeated doses of the extract. It displayed antioxidant activity by increasing CAT levels and reducing MDA levels. It also cross-reacted with anti-insulin antibodies which proved that it might have antigenic epitopes similar to insulin. *M. oleifera* leaf extract reduced FPG level, post-prandial levels, blood glycated hemoglobin, total cholesterol, non-HDL-C, HDL-C, VLDL-C, and LDL-C in Type 2 diabetic patients (Kumari, 2010; Nambiar et al., 2010; Ghiridhari et al., 2011). Leaf powder capsules (4 g) significantly increased the secretion of insulin in healthy subjects (Anthanont et al., 2016).

M. oleifera seed extract reduced lipid peroxidation and increased antioxidant enzyme activity in streptozotocin-induced mice (Al-Malki and El Rabey, 2015). It also reduced the immunoglobin IgG and IgA activity of the mice which correlated to the reduction of IL-6 that is responsible for glucose homeostasis and pancreatic beta cell activity. The seed extract normalized the activity of both damaged kidneys and pancreases without changing the pathology of the mice. It was reported that the bioactive compounds involved were quercetin (**3**), kaempferol (**8**), glucomoringin (**30**), and chlorogenic acid (**54**) and that they had various biological activities such as their anticancer, antioxidant, hypotensive, anti-inflammatory, hypoglycemic, and antidyslipidemic effects. Other than these compounds, isothiocyanates (**34–37**) had also been reported to show antidiabetic activity (Waterman et al., 2015). A study reported that benzylamine (**108**) isolated from *M. oleifera* reduced the plasma cholesterol, body weight gain, hyperglycemic responses, and fasting blood glucose levels of high fat diet-induced mice (Iffiu-Soltesz et al., 2010).

Barbagallo et al. (2016) reported that *M. oleifera* reduced IL6 expression and had protective effect toward adipocytes by inducing the expression of heme-oxygenase-1. The extract increased IRS-1 gene expression which is responsible for insulin resistance and a shortage of which causes type 2 diabetes. In addition, the extract also induced thermogenesis during the differentiation of adipose tissue by upregulating the activity of mediators of thermogenesis, specifically the uncoupling protein, PPARα, sirtuin 1, and coactivator 1 α. *M. oleifera* extract (100 μg/mL) showed the same behavior as 0.4 μg/mL pravastatin in inhibiting HMG-CoA reductase and decreasing cholesterol biosynthesis (Duangjai et al., 2011). *M. oleifera* also displayed synergistic effects with sitagliptin showing antihyperglycemic activity and delaying lenticular opacity in diabetic rats (Olurishe et al., 2016).

A different reaction of HDL level was observed between normal rabbits and hypercholesterolemic rabbits fed with *M. oleifera*. *M. oleifera* decreased HDL levels in normal rabbits but increased HDL levels in hypercholesterolemic rabbits (Mehta et al., 2003; Nunthanawanich et al., 2016). The lipid profiles of the aorta, heart, and liver were reduced in the hypercholesterolemic rabbits.

Antifertility

Mekonnen (2002) reported that an ethanolic extract of *M. stenopetala* leaves reduced fertility by 73.3%. The extract exhibited oxytocic activity on guinea pig and mouse uteri. The extract also increased the smooth muscle of the mice uteri that might lead to contraction, thus rejecting implantation. *M. concanensis* stem bark also inhibited implantation by 46% using 400 mg/kg of the extract (Ravichandiran et al., 2007). The reaction was solvent-dependent. The ethyl acetate fraction showed the lowest anti-implantation activity in contrast to chloroform, petroleum ether, and an ethanol extract.

It was reported that *M. oleifera* leaf extract aborted 100% of implantation in seven rats (Sethi et al., 1988). The extract was administered after 5–10 days after mating. The aqueous root extract of the plant did not promote a favorable condition of the uterus for implantation of the fertilized eggs (Prakash et al., 1987). A high dose of the root extract, 600 mg/kg, had anti-progestational activity, preventing the formation of the deciduoma in rats (Shukla et al., 1988). It also reduced the protein concentration for the formation of the uterus (Prakash et al., 1988).

Anti-inflammation

A study reported that an ethanolic extract of the *M. concanensis* flower and fruit inhibited inflammation by 78.4 and 44.08%, respectively (Rao et al., 2008; Jayabharathi and Chitra, 2011). An extract of the aerial part of *M. peregrina* decreased the effect of peritorial inflammation and reduced the permeability of small blood vessels (Elbatran et al., 2005). Ethanolic and aqueous extracts of *M. peregrina* seeds inhibited fresh egg albumin-induced acute inflammation in rats at doses of 100–300 mg/kg p.o (Koheil et al., 2011).

The major anti-inflammation mechanism reported for *M. oleifera* was the inhibition of the NF-κB pathway. Four fractions of *M. oleifera* leaf (hexane, chloroform, ethyl acetate, and butanol) reduced IL-1β, IL-6, PGE2, TNF-α, and nitric oxide production in LPS macrophages (Arulselvan et al., 2016). Among the fractions, ethyl acetate possessed the strongest inhibition effects and was therefore further analyzed. The extract blocked the nuclear translocation of NF-κB and increased inhibitor κB expression, which was also observed in a fruit extract of *M. oleifera*. Higher concentrations (500 and 1,000 μg/mL), especially from chloroform fraction, were reported to be cytotoxic. Kooltheat et al. (2014) reported that an ethyl acetate extract of *M. oleifera* leaves suppressed the expression of RelA. A hydro ethanolic flower extract also reduced the activity of inflammatory mediators and proinflammatory cytokines such as PGE2, IL-6, IL-1β, TNF-α, NF-κB, iNOS, NO, and COX2 in LPS-induced RAW264.7 macrophages (Tan et al., 2015). In addition, the extract increased the activity of the anti-inflammatory cytokines IL-10 and 1κB-α. Among the different parts of *M. oleifera*, the fruit showed the highest activity in reducing NO release induced by LPS in RAW264.7 cells (Lee et al., 2013).

Sashidara et al. (2009) isolated aurantiamide acetate (**69**) and 1,3-dibenzyl urea (**74**) from *M. oleifera* roots that exhibited inhibition toward IL-2 activity. Aurantiamide acetate (**69**) also inhibited TNF-α activity. 4-[(α-*L*-rhamnosyloxy)benzyl]isothiocyanate (**34**) and 4-[(4′-*O*-acetyl-α-L-rhamnosyloxy)benzyl]isothiocyanate (**37**) from *M. oleifera* leaves displayed anti-inflammatory activity by regulating IL-1β and iNOS expression in addition to reducing the production and expression of inflammatory markers in RAW macrophages (Waterman et al., 2014). 4-[(α-*L*-rhamnosyloxy)benzyl]isothiocyanate (**34**) exhibited anti-inflammatory and antioxidant activity against cerebral tissue damage induced by cerebral ischemia reperfusion in rats (Galuppo et al., 2015b). The compound reduced iNOS, phospho-ERK p42/44, TNF-α, MMP-9, p-selectin, and NFκBp65 nuclear translocation/IκB-alpha cytosolic degradation. A study also noted that 4-[(2′-*O*-acetyl-α-*L*-rhamnosyloxy) benzyl]isothiocyanate (**35**) suppressed COX-2 and iNOS activity in addition to inhibiting the expression of NO in RAW264.7 mouse macrophage cells induced by LPS (Park et al., 2011). The compound inhibited NF-κB activation, the phosphorylation of ERK1/2, IκBα the phosphorylation of IKKα/β, and increase degradation of IκBα. The isothiocyanate showed stronger activity than other isothiocyanates such as benzyl isothiocyanate and sulforaphane. 4-[(α-*L*-rhamnosyloxy)benzyl]isothiocyanate (**34**), 4-[(2′-*O*-acetyl-α-*L*-rhamnosyloxy)benzyl]isothiocyanate (**35**), 4-[(3′-*O*-acetyl-α-*L*-rhamnosyloxy)benzyl] isothiocyanate (**36**), and 4-[(4′-*O*-acetyl-α-*L*-rhamnosyloxy)benzyl] isothiocyanate (**37**) inhibited the production of NO (Cheenpracha et al., 2010).

Galuppo et al. (2014) reported that 4-[(α-*L*-rhamnosyloxy) benzyl] isothiocyanate (**34**) exhibited anti-inflammatory activity toward multiple sclerosis cascades. The compound specifically reduced the imbalance of Bax/BCl-2 and inhibited TNF-α activity on myelin oligodendrocyte glycoprotein 35–55-induced C57B1/G male mice. The compound also inhibited GSK3β levels and normalized the Wnt-β-catenin pathway in experimental autoimmune encephalomyelitis mice (Giacoppo et al., 2016). The compound attenuated apoptosis in addition to suppressing PPARγ activation. 4-[(α-*L*-rhamnosyloxy) benzyl]isothiocyanate (**34**), together with α-cyclodextrin, inhibited the phosphorylation of p38 and Akt in LPS-induced inflammation in RAW 264.7 macrophage cells (Giacoppo et al., 2017). Another study reported that quercetin (**3**) and kaempferol (**8**) inhibited transcription 1(STAT-1) (Tan et al., 2015).

A study reported that a methanol extract of *M. oleifera* leaves reduced the edematogenic effect of carrageenan-induced and histamine-induced paw edema (Adedapo et al., 2015). The extract reduced the number of writhes of mice induced by acetic acid. The analgesic activity of the extract was recorded to be higher than that of the reference drug, indomethacin, at doses of 100 and 200 mg/kg. The inflammatory response after administration of an *M. oleifera* ethanolic leaf extract was observed on atopic dermatitis mice and human keratinocytes (Choi et al., 2016). The extract reduced mannose receptor mRNA, retinoic acid-related orphan receptor γT, and thymic stromal lymphopoietin expression in ear tissue. From an *in vitro* assay, it was observed that the extract reduced mitogen-activated protein kinases, CCL17, IL-6 pro-inflammatory cytokine-related mRNA, TNF-α, and IL-1β expression. *M. oleifera* pod extract also inhibited the elevation of protein levels and mRNA of cyclooxygenease-2, TNF-α, IL-6, and iNOS by inhibiting the phosphorylation of mitogen-activated protein kinases and κB proteins (Muangnoi et al., 2012).

M. oleifera hydroethanolic and methanolic leaf extracts improved the cellular and humoral immunity of normal and immunosuppressed mice in a dose-dependent manner (Gupta et al., 2010; Nfambi et al., 2015). The extract increased the phagocytic index, weight of the thymus and spleen, antibody titer, and white blood cell and neutrophil concentration. An ethanolic extract of *M. oleifera* seeds inhibited the reaction of delayed-type hypersensitivity by reducing mean foot pad thickness on mice (Mahajan and Mehta, 2010). The immunosuppressive activity of the extract was observed by its ability to down-regulate phagocytosis by macrophages. The ethanolic extract of the seeds also reduced white blood cell, and leukocyte concentration which usually leads to an immunity reaction. It increased paw edema which typically resulted in type IV hypersensitivity occurring. Methanolic extracts of *M. oleifera* leaves exhibited analgesic effects by reducing mechanical allodynia and thermal hyperalgesia in Freund's adjuvant arthritis-induced rats (Manaheji et al., 2011). In contrast, *M. oleifera* methanolic root extracts only reduced thermal hyperalgesia in the

rats. The activity of the root and leaf extracts were comparable with indomethacin activity. In addition, a combination of root and leaf extracts showed higher reduction of thermal hyperalgesia in lower doses. An ethanol extract of *M. oleifera* leaves and its major compounds, quercetin-3-*O*-glucoside (**18**), kaempferol-3-*O*-glucoside (**21**), and crypto chlorogenic acid (**55**), showed anti-inflammatory activity, inhibiting the migration and chemotactic oxidation of polymorphonuclear leukocytes (Vongsak et al., 2013).

Moringa species also inhibited ulcers induced by non-steroidal anti-inflammatory drugs. *M. oleifera* extract reduced gastric lesions from acetylsalicylic acid, serotonin, and indomethacin (Pal et al., 1995), while an extract of the aerial part of *M. peregrina* reduced gastric lesions from indomethacin (Elbatran et al., 2005). A hydroalcoholic extract of *M. oleifera* seeds reduced the lesion severity and size of acute colitis induced by acetic-acid in rats (Minaiyan et al., 2014). The extract also reduced the activity of TNF-α, IL-4, IL-6, and myeloperoxide which are usually responsible for inflammatory bowel diseases. Debnath and Guha (2007) reported that an *M. oleifera* aqueous leaf extract reduced the mean ulcer index in addition to increasing 5-HT concentration and enterochromaffin cell concentration. The extract protected against ulcer formation in aspirin-induced rats by stimulating enterochromaffin cells in the gastrointestinal tract via 5-HT_3 receptors (Debnath et al., 2011).

The ethyl acetate fraction of *M. oleifera* leaves obtained from a hydroethanolic extract of the plant increased normal human dermal fibroblast migration and cell proliferation (Gothai et al., 2016). It was observed that a higher dose, more than 125 μg/mL, reduced cell proliferation. High concentrations of phenols in the extract might induced caspase and apoptosis. The migration observed also displayed a 9% better activity than the positive control, allantoin. The leaf ethyl acetate extract contained 4-[(2′-*O*-acetyl-α-*L*-rhamnosyloxy)benzyl]isothiocyanate (**35**) and 4-[(3′-*O*-acetyl-α-*L*-rhamnosyloxy)benzyl]isothiocyanate (**36**), both of which have anti-inflammatory activity. Muhammad et al. (2016) reported that a leaf extract of *M. oleifera* proliferated tissue cells, hence reducing the wound size of diabetic foot ulcers. It downregulated the activity of the inflammatory mediators TNF-α, IL-1β, IL-6, iNOS, and COX-2. The extract also increased angiogenesis activity by reducing the time needed for the wound healing phase, in which vascular endothelial growth factor activity occurs. The study reported that the bioactive compound for this activity was vicenin-2 (**17**). An aqueous fraction of *M. oleifera* leaves reduced scar areas and increased the closure rates of wounds in addition to increasing the granuloma and skin breaking strength, granuloma dry weight, and hydroxyproline content of albino rats (Rathi et al., 2006). *M. oleifera* protease activity from leaf and root aqueous extracts exhibited proteolytic, fibrinolytic, and fibrinogenolytic activity on blood clotting (Satish et al., 2012). The protease showed similar activity to plasmin and thrombin. The protease also attenuated apoptosis in addition to suppressing PPAR gamma activation. Bhatnagar et al. (2013) reported that an *M. oleifera* seed extract, together with *Acacia arabica* biopolymers, had potential as a wound dressing material. In addition to being good antimicrobial substances, the biopolymers were biodegradable and could absorb water from 415 to 935%. They also shortened the time taken to activate partial prothrombin and thromboplastin.

Antihypertension

Safaeian et al. (2015) showed that an *M. peregrina* leaf extract, administered before and during the administration of dexamethasone to rats, prevented their systolic blood pressure from increased but did not reduce already increased systolic blood pressure. The bioactive dose, 400 mg/kg, showed low but significant antihypertensive activity. The compounds that were reported responsible for this activity were quercetin (**3**), apigenin (**9**), and lupeol (**82**) which had been previously reported to possess antihypertension activity. Safaeian et al. (2015) mentioned that the plants used were obtained during summer and this might affect the concentration of bioactive compounds in the plant. This correlated to a study that observed that the total phenolics in *M. oleifera* harvested in winter was higher than that in plants harvested in summer (Shih et al., 2011).

The vasodilatory activity of an *M. stenopetala* leaf crude extract and fraction were reported by Geleta et al. (2016a). The activity was observed in the thoracic aorta of guinea pigs that had been induced by various vascular contraction agents. Aqueous and 70% ethanol crude extracts of the leaves reduced the vascular contraction induced by potassium chloride, methylene blue, epinephrine, and glibenclamide, in addition to inhibiting the increment of fructose-induced blood pressure in rats in a dose-dependent manner. The extract possessed alkaloids and glycosides that might have cause stronger activity than observed with the fractions. In the study, the extract had the greatest activity toward vascular contraction induced by potassium chloride either when the thoracic aorta was attached to the endothelium, or not. Geleta et al. (2016a) suggested that the extract blocked Ca^{2+} channels during this activity. The endothelium might have stimulated relaxing factors that increased the extract's activity.

A study reported that an ethanol extract of *M. oleifera* leaves reduced pulmonary arterial blood pressure immediately after administration of monocrotaline to rats (Chen et al., 2012). As pulmonary hypertension is usually related to increased reactive oxygen species in the system, the antioxidant activity of the extract that increased SOD activity might be the largest contribution toward its antihypertension activity on pulmonary hypertension. The bioactive compounds reported in this study were niazirin (**97**) and niaziridin (**98**). *M. oleifera* seeds also exhibited cardioprotective activity in spontaneous hypertensive rats (Randriamboavonjy et al., 2016). The extract increased cardiac diastolic function and reduced nocturnal heart rate without modifying the blood pressure of the rats. It also reduced fibrosis and the thickness of the left ventricular relative, interseptal, and anterior walls, in addition to reducing cardiac triglyceride levels. Plasmatic prostacyclin and PPAR-α and γ, activity was also increased by the extract. The study concluded that the extract exhibited antifibrotic and antihypertrophic activity that helped protect cardiac function in hypertension rats.

In addition, it was reported that the antioxidant activity of an *M. oleifera* leaf butanolic extract helped to reduce cardiac necrosis and oxidative stress in isoproterenol-induced rats (Panda, 2015).

The extract reduced cardiac lipid peroxidation and increased cardiac antioxidants. The free radicals in the cardiac region were inhibited by the extract with IC_{50}-values of 19.92 ± 1.19 μg/mL which was comparable with quercetin (**3**) activity (IC_{50}-value of 19.95 ± 1.17 μg/mL). The extract also reduced inflammation and necrosis to almost normal myofibrillar structure. *N*,*α*-*L*-rhamnopyranosyl vincosamide (**67**) exhibited cardioprotective activity by reducing the level of serum cardiac markers and myocardial necrosis in addition to normalizing the cardiac histology of isoproterenol-induced rats (Panda et al., 2013).

Antispasmodic

Hydro alcoholic extracts of *M. peregrina* leaves and seeds were investigated for their antispasmodic activity on ileum spasms (Sadraei et al., 2015). The leaves inhibited ileum spasms induced by potassium chlorine (IC_{50}: 439 ± 108 μg/mL), electrical field stimulation (IC_{50}: 314 ± 92 μg/mL), and acetylcholine (IC_{50}: 365 ± 61 μg/mL). It was observed that the seed extract displayed better inhibition than the leaves against ileum spasms induced by potassium chloride (IC_{50}: 87 ± 18 μg/mL), electrical field stimulation (IC_{50}: 230 ± 51 μg/mL), and acetylcholine (IC_{50}: 118 ± 18 μg/mL). The aqueous extract of *M. oleifera* seeds exhibited higher inhibition activity against acetylcholine-induced contractions (ED_{50} of 65.6 mg/mL) (Caceres et al., 1992).

Others

A study reported that *M. oleifera* exhibited antidepressant activity (Kaur et al., 2015). The crude extract showed positive results in the forced swim test, tail suspension test, and locomotor activity test. The activity was enhanced with co-administration of the SSRI depression drug, fluoxetine. In addition, high doses of the extract, particularly 2,000 mg/kg, did not exert toxicity on the tested mice. Akanni et al. (2014) reported that an ethanol extract of *M. oleifera* leaves exhibited antileukemic activity against benzene-induced leukemic Wistar rats. The extract normalized the leukemic condition, increased GSH, and reduced MDA levels in the rats. Galuppo et al. (2015a) reported that isothiocyanate, specifically 4-[(*α*-*L*-rhamnosyloxy)-benzyl] isothiocyanate (**34**), isolated from *M. oleifera* delayed ALS development. The compound reduced PARP-1 activity in addition to promoting Nrf-2 activity. The study suggested that the isothiocyanate interfered with motor neuron degeneration and ALS development in the SOD1 rats. Upon administration of 125 mg/kg *M. oleifera* leaf extract, rats' antibody against the Salmonella typhimurium "O" antigen increased to 50%. The extract also increased the concentration of serum immunoglobulins, white blood cells, and neutrophils, hence increasing the humoral immune response of the rats (Jayanthi et al., 2015).

TOXICITY

Awodele et al. (2012) reported that an aqueous extract of *M. oleifera* leaves did not produce any mortality in Wistar albino mice at orally administered doses of up to 6,400 mg/kg. Higher doses (3,200 and 6,400 mg/kg) did triggered dullness and reduced locomotion in the rats. There was no significant difference observed on the rats' sperm quality, or on their hematological, histological, and biochemical parameters. The LD_{50}-value was determined to be 1,585 mg/kg. In another study, it was reported that high doses of *M. oleifera* leaves (3,000 mg/kg) caused the presence of micro nucleated polychromatic erythrocytes in the femur bone marrow of Sprague-Dawley rats (Asare et al., 2012). The study reported that doses of more than 3,000 mg/kg caused acute toxicity and increased the urea levels of the rats. The study reported that this was caused by high concentrations of nitrogenous compounds in the *M. oleifera*, potentially from proteins.

CONCLUSION

Various research has been conducted to evaluate the traditional uses of *Moringa* species and all of the research supported the traditional claims. However, there are still an abundance of traditional uses that have not been evaluated, especially in species other than *M. oleifera* and *M. stenopetala*. Hence, further research is needed to exploit the many uses of *Moringa* species.

AUTHOR CONTRIBUTIONS

NA obtained the literatures and wrote the manuscript while KH and EK gave ideas and edited the manuscript.

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REVIEW
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Lamiaceae: An Insight on Their Anti-Allergic Potential and Its Mechanisms of Action

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The prevalence of allergic diseases such as asthma, allergic rhinitis, food allergy and atopic dermatitis has increased dramatically in recent decades. Conventional therapies for allergy can induce undesirable effects and hence patients tend to seek alternative therapies like natural compounds. Considering the fact above, there is an urgency to discover potential medicinal plants as future candidates in the development of novel anti-allergic therapeutic agents. The Lamiaceae family, or mint family, is a diverse plant family which encompasses more than 7,000 species and with a cosmopolitan distribution. A number of species from this family has been widely employed as ethnomedicine against allergic inflammatory skin diseases and allergic asthma in traditional practices. Phytochemical analysis of the Lamiaceae family has reported the presence of flavonoids, flavones, flavanones, flavonoid glycosides, monoterpenes, diterpenes, triterpenoids, essential oil and fatty acids. Numerous investigations have highlighted the anti-allergic activities of Lamiaceae species with their active principles and crude extracts. Henceforth, this review has the ultimate aim of compiling the up-to-date (2018) findings of published scientific information about the anti-allergic activities of Lamiaceae species. In addition, the botanical features, medicinal uses, chemical constituents and toxicological studies of Lamiaceae species were also documented. The method employed for data collection in this review was mainly the exploration of the PubMed, Ovid and Scopus databases. Additional research studies were obtained from the reference lists of retrieved articles. This comprehensive summarization serves as a useful resource for a better understanding of Lamiaceae species. The anti-allergic mechanisms related to Lamiaceae species are also reviewed extensively which aids in future exploration of the anti-allergic potential of Lamiaceae species.

Keywords: Lamiaceae, anti-allergic, allergy, hypersensitivity, mast cell, β-hexosaminidase, eosinophil, histamine

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INTRODUCTION

Allergy is one of the manifestations of an abnormal regulation of the immune system. It can present as a mild to severe disorder, such as allergic rhinitis, food allergies, asthma, conjunctivitis, angioedema, urticaria, eczema, insect allergies and life-threatening anaphylaxis (Galli et al., 2008). Nowadays, allergy has become a global health concern. The cases of allergic disorders are increasingly rising and have reached an alarming rate. This statement is supported by some of the statistical figures provided by Pawankar (2014). According to Pawankar (2014), there are roughly 300 million

individuals experiencing asthma and approximately 200 to 250 million individuals experiencing food allergies around the world. Additionally, 1/10 of the population experienced medication hypersensitivities and around 400 million people suffered from allergic rhinitis. Allergy can be very irritating to an extent that can greatly affect the quality of life, lead to an economic burden and can even jeopardize one's life (Pawankar, 2014). Seeing the staggering pattern of morbidity and mortality caused by allergic disorders, this health issue must not be neglected and must be taken seriously with the active involvement of patients and healthcare professionals.

Currently, there are many treatment options for allergic disorders. Some of the widely used therapeutics are anti-histamine drugs, corticosteroids, leukotriene inhibitors and mast cell stabilizers to treat and control allergic conditions (Cota et al., 2012). All these medications are found to be efficactive in alleviating allergic symptoms. However, the drugs do not actually cure the allergy conditions. Instead, long term consumption of such drugs has been associated with undesirable side effects and sometimes may worsen the conditions (Han et al., 2017). Some examples of common side effects encountered by anti-histamine agents users are dry mouth, drowsiness, gastrointestinal disturbances, headache, agitation, confusion, etc. (Simon and Simons, 2008). As for corticosteroids, they work effectively in relieving allergic disorders, like allergic asthma, eczema, allergic rhinitis, etc. However, they often brings about undesirable side effects to patients in a long-term therapy. For instance, patients who are using inhaled corticosteroids for asthma control are likely to encounter undesirable effects like oral candidiasis (Fukushima et al., 2003) and adrenal suppression (Robert Webb, 1981). Patients with eczema who usually use topical steroidal treatment can develop Cushing's syndrome (Tiwari et al., 2013) and skin thinning (Atherton, 2003) as well as easy bruising (Coondoo et al., 2014). Due to the limitations of modern medicines, there is an increasing interest in using complementary and alternative medicine, particularly herbal medicine for allergy conditions management (Engler et al., 2009).

Undeniably, medicinal plants have been widely utilized as healing modalities for both preventive and curative purposes. They play an extremely crucial role in human health. In recent years, there has been a growing trend in the world population with as many as 80% of people globally relying on the use of herbal medicinal products and supplements for their primary healthcare needs (Schuster, 2001; Ekor, 2013). This increasing demand and interest in the use of herbal medicinal products has encouraged new drug discoveries and developments (Ekor, 2013). In fact, many active ingredients of new drugs are derived from medicinal plants proven to be remarkably important in aiding drug discovery and development (Katiyar et al., 2012). Hence, studies need to be actively conducted on plants in order to identify possible candidates as safer and effective anti-allergic agents in future.

The Lamiaceae family is one of the biggest plant families among flowering plants, consisting of 236 genera with a coverage of more than 7,000 species (Khoury et al., 2016). It is also an important herbal family which comprises a wide array of plants with biological and medical applications (Uritu et al., 2018). Lamiaceae species often have four-angled or quadrangular stems with the presence of glandular hairs (Harley et al., 2004). Their roots are usually made of branched tap root. Their flowers are typically hypogynous and bilaterally symmetric with five united petals and sepals (Kokkini et al., 2003; Ramasubramania Raja, 2012; Carović-Stanko et al., 2016). The leaves are simple and arranged oppositely, each pair at a right angle to the previous one or whorled (Harley et al., 2004). Fruits are made of four dry one-seeded nutlets (Kokkini et al., 2003). Seeds are non-endospermic (Ramasubramania Raja, 2012). The environment adaptation of Lamiaceae is highly varied. The species predominantly distribute in the summer rainfall areas but also occur in winter rainfall areas. The species usually can be found in habitats which are dry, rocky, woodland or grassland, along forest margins and in fynbos (Will and Claßen-Bockhoff, 2014). The diversity of Lamiaceae species is mainly concentrated in Mediterranean regions and a small portion of them inhabit Australia, Southwest Asia and South America (Kokkini et al., 2003).

Khoury et al. (2016) reported that the high content of volatile compounds has contributed to many medicinal properties in Lamiaceae species. Historically, Lamiaceae plants have been reported to be effective in alleviating a range of conditions like exhaustion, weakness, depression, memory enhancement, circulation improvement, strengthening of fragile blood vessels, skin allergies and asthma (Wang et al., 2004; Naghibi et al., 2010; Ramasubramania Raja, 2012). In the Eastern Himalayan region of India, several Lamiaceae species have been utilized traditionally to treat certain conditions. For instance, the leaves of *Clerodendrum serratum* have been used as a traditional remedy for eye disorders. Moreover, the leaves of *Elsholzia blanda* is used to relieve itching conditions. The seed of *Perilla frutescens* is also claimed to be effective against fever and headache (Kala, 2005). Meanwhile, in China, the Chinese tea brewed using the leaves of *Salvia officinalis* is used as a traditional remedy to treat tonsillitis and hypertension (Li et al., 2013). Another Lamiaceae species, *Scutellaria baicalensis* has been extensively used as traditional Chinese medicine (TCM) for thousands of years. It is known as Huang Qin in Chinese. The decoction prepared from dried roots is used as a traditional remedy for diarrhea, dysentery, hypertension, hemorrhaging, insomnia, inflammation and respiratory infections (Zhao et al., 2016). In Mediterranean regions, like Lebanon, *Mentha spicata* is formulated into infusions to ease digestive disorders, arthritis, gastritis. The infusion is also used as an antiemetic and antimicrobial agent (Khoury et al., 2016). The medicinal uses of commonly used Lamiaceae species are summarized in **Table 1**.

This review is particularly focused on the summarization of the anti-allergic activities of the Lamiaceae family linked to the phytochemistry and ethnopharmacology reported in research studies. In addition to anti-allergic activities, toxicological investigations of Lamiaceae species are also highlighted in this review.

ANTI-ALLERGIC ACTIVITY

The abundance of species within the Lamiaceae family has led to a variety of medicinal uses, making the family pharmacologically

TABLE 1 | Medicinal uses of commonly used Lamiaceae species.

Plant name	Country/region	Local name/ common name	Medicinal use	Plant part used	Mode of preparation	References
Clerodendrum petasites (Lour.) S.Moore	Thailand	Thao yaai mom	Asthma	Aerial part	The aerial part is prepared as tea or alcoholic extract.	(Hazekamp et al., 2001)
Clerodendrum serratum (Linn.) Moon	Arunachal Pradesh, India	No information	Eye disorders	Leaves	No information.	(Kala, 2005)
Elsholtzia blanda (Benth.) Benth.	Arunachal Pradesh, India	No information	Itching conditions	Leaves	No information.	(Kala, 2005)
Epimeredi indica (L.) Rothm	China	Guang Fan Feng	Rheumatoid arthritis, bones and muscles ache, skin ulcer, hemorrhoids, eczema	Whole plant	The whole plant is used to prepare as medicinal bath.	(Li et al., 2006)
Mentha arvensis Linn.	Western Himalayas	Pudina	Stomach problems, allergy, liver and spleen disease, asthma, indigestion, rheumatic pains, arthritis	Leaves	Leaves are made as salad and formulated into infusion respectively.	(Khan and Khatoon, 2007)
	Korea	Bak-ha	Analgesic, local vasodilator, skin irritant, antispasmodic agent, acute mastitis, allergic dermatitis and skin itching	Aerial part	Sometimes combine with other herbs as traditional remedy.	(Shin, 2003)
Mentha longifolia (L.) L.	West Bengal, India	Junglipudina	Menstrual disorders, pulmonary infection, congestion, asthma, urinary tract infections, indigestion, back pain, headache and to fasten wound healing process	Leaves	The leaves are formulated into extract.	(Sinhababu and Arpita, 2013)
	India	No information	Carminative, stimulant, antiseptic and febrifuge	Leaves and flower tops	No information.	(Sinhababu and Arpita, 2013)
Mentha spicata Linn.	Thessaloniki, Greece	No information	Common cold and cough	Aerial part	No information.	(Karousou et al., 2007)
	Lebanon	No information	Digestive disorders, arthritis, gastritis, antiemetic and antimicrobial agents	No information	Formulated as infusions.	(Khoury et al., 2016)
Perilla frutescens (Linn.) Britton	India	No information	Arthritis	Seed oil	The oil is extracted from the plant seed and massaged onto the arthritis part.	(Singh, 1997)
	Arunachal Pradesh, India	No information	Fever and headache	Seed	No information.	(Kala, 2005)
Prunella vulgaris Linn.	Iberian Peninsula	No information	External antiseptic	Aerial part	No information.	(Rigat et al., 2015)
Salvia miltiorrhiza Bunge	China	Danshen	Promoting cardiovascular health by improving blood circulation to remove blood stasis, clearing heart heat to relieve restlessness and cooling blood to remove carbuncle	Root	The root is air-dried and made into decoctions and pills. Nowadays, the root is widely formulated into various preparations, such as tablets, capsules, granules, injections, oral liquids, sprays and tea.	(Su et al., 2015)
Salvia officinalis Linn.	China	No information	Tonsillitis and hypertension	Leaves	The leaves are brewed as tea.	(Li et al., 2013)
Salvia plebeia R. Brown	Korea	Baem-Cha-Zu-Ki	Skin inflammatory disease and asthma	No information	No information	(Choi et al., 2014; Shin and Kim, 2002)
Scutellaria *baicalensis* Georgi	China	Huang Qin	Diarrhea, dysentery, hypertension, hemorrhaging, insomnia, inflammation and respiratory infections	Root	The dried root is used to prepare decoctions.	(Zhao et al., 2016)

TABLE 1 | Continued

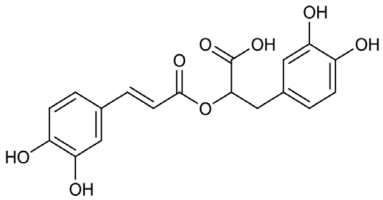
Plant name	Country/region	Local name/ common name	Medicinal use	Plant part used	Mode of preparation	References
Thymus serpyllum Linn.	Uttar Pradesh, India	No information	Headache, dysentery and vomiting	No information	Prepared as decoction.	(Singh, 1997)
Thymus vulgaris Linn.	Indonesia	No information	Asthma and other respiratory disorders	Leaves	No information	(Ikawati et al., 2001)
Vitex negundo Linn.	Uttar Pradesh, India	No information	Pain, swelling and eye inflammation	Leaves	The leaves are prepared as paste and applied onto the sprains to relieve pain. The leaf juice is used as drops to reduce eye inflammation.	(Singh, 1997)
Vitex trifolia Linn.	Indonesia	No information	Asthma and other respiratory disorders	Leaves	No information	(Ikawati et al., 2001; Alam et al., 2002)

important. The diversity is believed to be due to the wide variety of biologically active constituents in this plant family. Each species comprises a mixture of phytochemicals which attributes to the bioactivity of the plant (Carović-Stanko et al., 2016). Phytochemical investigations of the Lamiaceae family have demonstrated the presence of various bioactive compounds such as flavonoids (da Silva et al., 2015; Mamadalieva et al., 2017; Aghakhani and Kharazian, 2018), alkaloids (Malik et al., 2003; Asghari et al., 2017), phenolics (Berdowska et al., 2013; Zielińska and Matkowski, 2014; Skendi et al., 2017), lignans (Hong et al., 2009; Brandão et al., 2017), terpenoids (Ye et al., 2018), saponins (Ramasubramania Raja, 2012; Shah et al., 2014), etc. All these chemical constituents contribute to multidirectional pharmacological activities. Some of the remarkable bioactivities reported within this plant family are anti-allergic (Malik et al., 2003; Makino et al., 2003; Kim et al., 2009), anti-inflammatory (Borges et al., 2018), antimicrobial (Khoury et al., 2016; Cocan et al., 2018), free radical scavenging (Khaled-Khodja et al., 2014; Politeo et al., 2018), antinociceptive (Hwang et al., 2018; Uritu et al., 2018), anti-cancer activities (Nguyen et al., 2018; Sajjadi et al., 2018), etc. Many pharmacological activities of the Lamiaceae family have been widely studied and investigated. However, this study is mainly focused on the potential biologically active candidates with promising anti-allergic activity from Lamiaceae species in order to provide a direction in the discovery of potential novel, safe and efficacious natural anti-allergic agents in future. In the past, numerous *in vitro*, *in vivo* and *ex vivo* studies have been conducted and evaluated on the plant parts of Lamiaceae species to investigate the anti-allergic potential of Lamiaceae plants. **Figure 1** and **Table 2** show a summarization of the remarkable anti-allergic activities of the Lamiaceae family. The mechanisms of anti-allergic activities of Lamiaceae species are extensively discussed in this review.

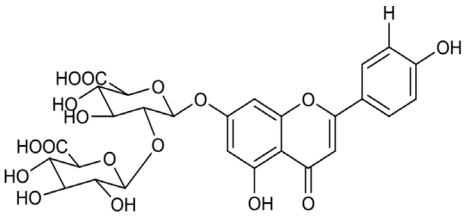
INHIBITION OF ALLERGEN-SPECIFIC IGE

Immunoglobulin E (IgE) is of central importance in the regulation of immune responses against parasitic infestations and most importantly, it is also recognized as a main mediator for immediate-type allergies or type I hypersensitivity reactions, such as allergic asthma, rhinitis, atopic diseases, anaphylaxis, etc. (Al-Mughales, 2016). It is a potent mast cell activator able to trigger mast cell degranulation and downstream responses with a minute amount (Actor, 2014). It exists in trace amounts in plasma but the amount can be substantially elevated in allergic reactions (Gould and Beavil, 1998; van der Burg et al., 2014). It possesses an additional constant region, CH_4, which particularly restricts it to bind to high affinity IgE receptors on mast cells and basophils (Flaherty, 2012). Upon the first encounter with the antigen, the plasma cells start to produce IgE molecules. The secreted IgE molecules bond to the high affinity IgE receptors (FcεRI) on the mast cells and basophils surfaces via their Fc portion, forming IgE-FcεRI complexes. In this form, the half-life of IgE can be prolonged to two to three weeks or sometimes can even be retained on the cell surface for months (Actor, 2014; van der Burg et al., 2014). Upon the re-exposure to the same antigen, the antigen will cross-link with the IgE-FcεRI complexes which then lead to mast cell degranulation (Actor, 2014). Inhibition of IgE production and IgE-mast cell cross-linking are particularly essential to prevent the progression to mast cell degranulation.

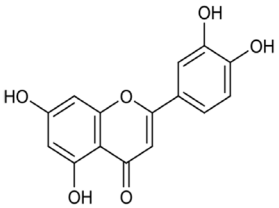
There are several studies demonstrating that Lamiaceae species have a suppressive effect on IgE levels and IgE-mast cell cross-linking. These findings could be useful to recognize potential treatment options for allergic disorders. From the study of Sharma et al. (2018), results revealed that the essential oil of *Mentha arvensis* significantly decreased ($P < 0.001$) the serum IgE level in OVA-sensitized mice at a concentration of 200 μl/kg. The study successfully identified three compounds in the essential oil, which are menthol, menthone and 1,8-cineole, with particularly large percentage contents of menthol. However, the compound which contributed to the anti-allergic activity was not known (Sharma et al., 2018). Therefore, this provides a clue for further findings on the possible anti-allergic compound in future. In the work of Lee et al. (2006), it was proposed that the aqueous extract of *Mosla dianthera* exhibited anti-allergic effects through an *in vivo* model. When the mice were sensitized with compound 48/80 and anti-DNP IgE, intraperitoneal pretreatment of 1–1,000 mg/kg of aqueous extract resulted in a dose-related reduction in passive cutaneous anaphylaxis (PCA) reaction (Lee et al., 2006). Similar activities were displayed by the aqueous extract of species *Perilla frutescens* (Shin et al., 2000), *Phlomis umbrosa*



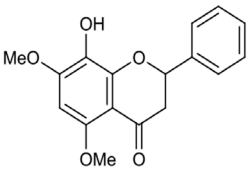
Rosmarinic acid **(1)**



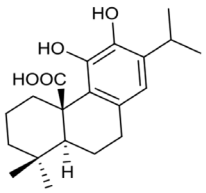
Apigenin 7-*O*-[ß-glucuronosyl (2 ➔ 1) ß-glucuronide] **(2)**



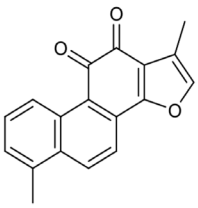
Luteolin **(3)**



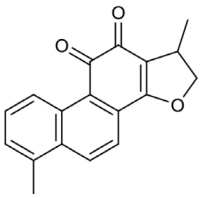
8-hydroxy-5,7-dimethoxyflavanone **(4)**



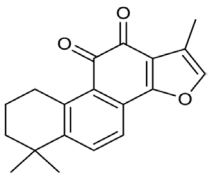
Carnosic acid **(5)**



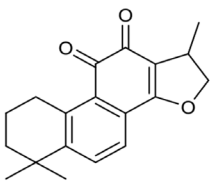
Tanshinone I **(6)**



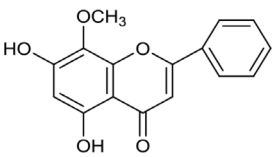
15,16-dihydrotanshinone I **(7)**



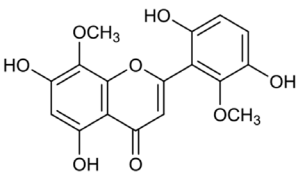
Tanshinone IIA **(8)**



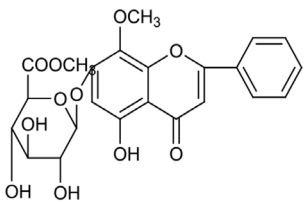
Cryptotanshinone **(9)**



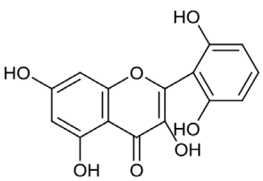
Wogonin **(10)**



Ganhuangenin **(11)**

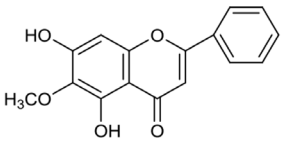


Wogonoside **(12)**

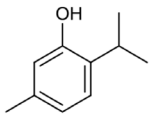


3,5,7,2',6'-pentahydroxyl flavanone **(13)**

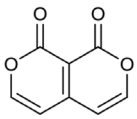
FIGURE 1 | Continued



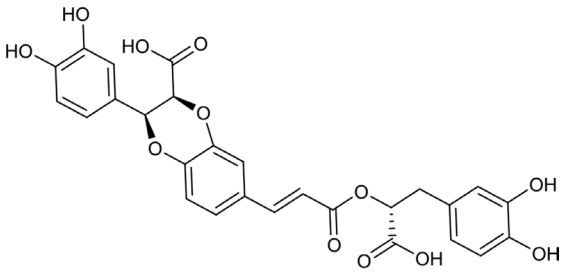
Oroxylin A **(14)**



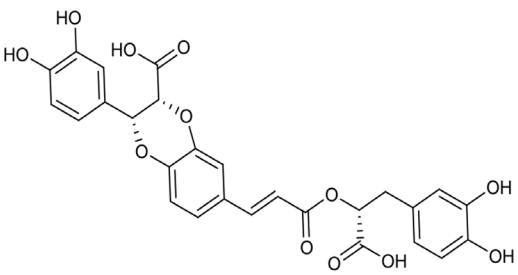
2-isopropyl-5-methylphenol **(15)**



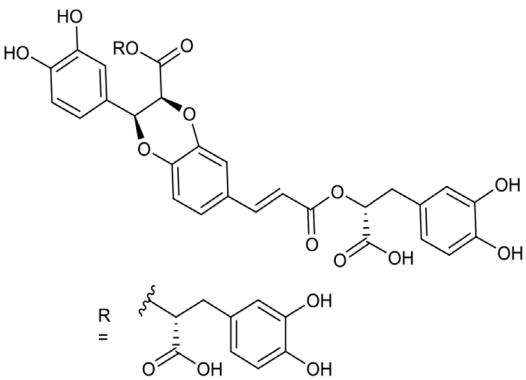
1H,8H-pyrano [3,4-c] pyran-l,8-dione **(16)**



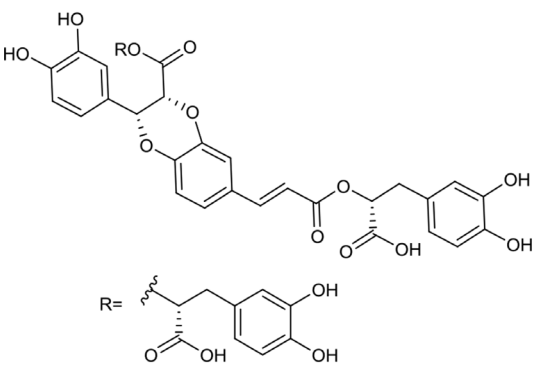
Clinopodic acid C **(17)**



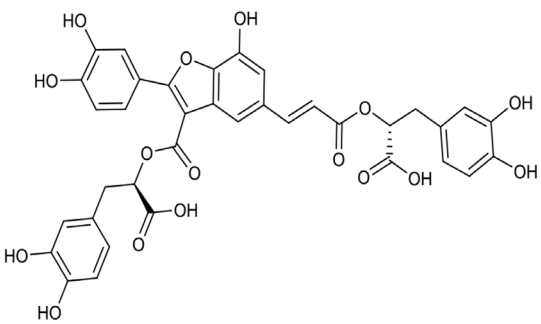
Clinopodic acid E **(18)**



Lycopic acid A **(19)**

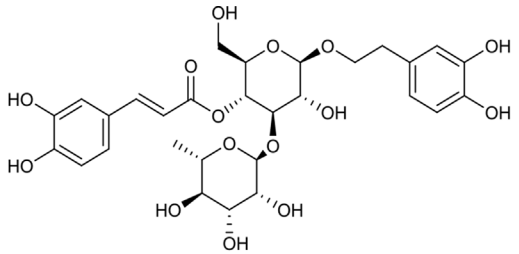


Lycopic acid B **(20)**

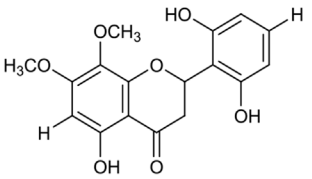


Scizotenuin A **(21)**

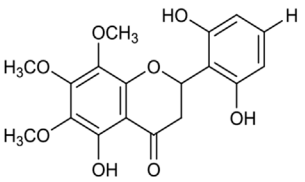
FIGURE 1 | Continued



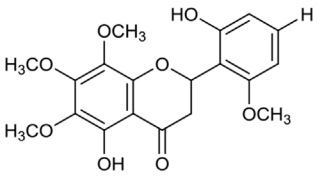
Acteoside **(22)**



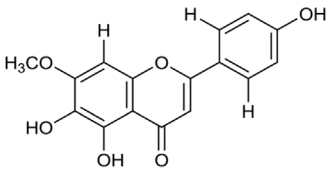
5,2',6'-trihydroxy-7,8-dimethoxyflavanone **(23)**



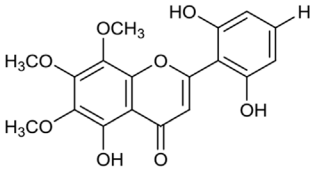
5,2',6'-trihydroxy-6,7,8-trimethoxyflavanone **(24)**



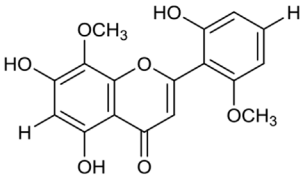
5,2'-dihydroxy-6,7,8,6'-tetramethoxyflavanone **(25)**



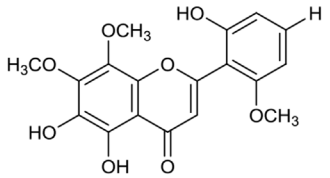
Hispidulin **(26)**



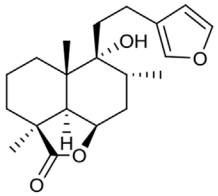
5,2',6'-trihydroxy-6,7,8-trimethoxyflavone **(27)**



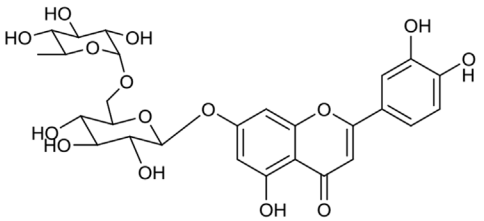
5,7,2'-trihydroxy-8,6'-dimethoxyflavone **(28)**



5,6,2'-trihydroxy-7,8,6'-trimethoxyflavone **(29)**

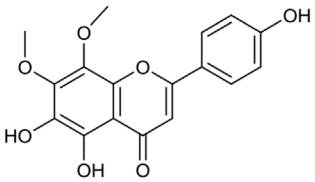


Marrubiin **(30)**

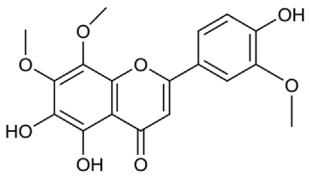


Luteolin-7-*O*-rutinoside **(31)**

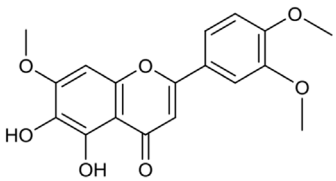
FIGURE 1 | Continued



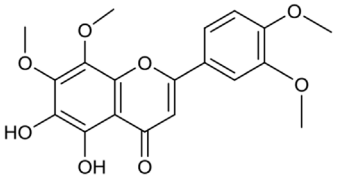
5,6,4'-trihydroxy-7,8-dimethoxyflavone **(32)**



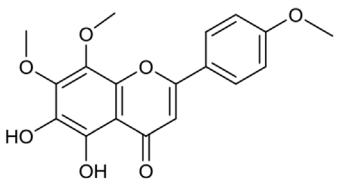
5,6,4'-trihydroxy-7,8,3'-trimethoxyflavone **(33)**



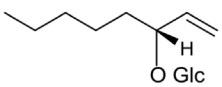
5,6-dihydroxy-7,3',4'-trimethoxyflavone **(34)**



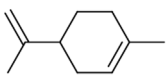
5,6-dihydroxy-7,8,3',4'-tetramethoxyflavone **(35)**



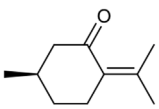
5,6-dihydroxy-7,8,4'-trimethoxyflavone **(36)**



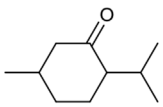
(3R)-1-octan-3-yl ß-D-glucopyranoside **(37)**



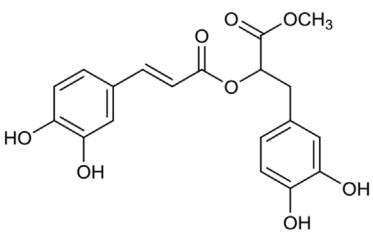
Limonene **(38)**



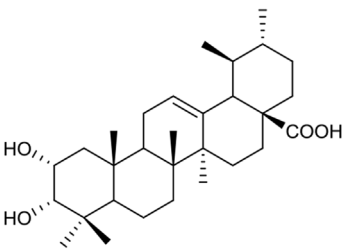
Pulegone **(39)**



Menthone **(40)**

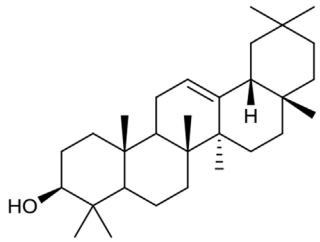


Rosmarinic acid methyl ester **(41)**

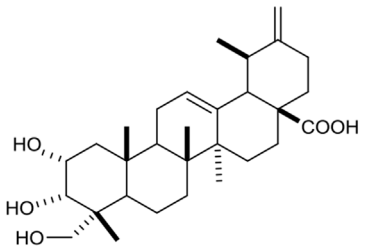


2α,3α-dihydroxyurs-12-en-28-oic acid **(42)**

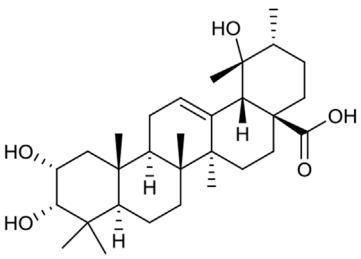
FIGURE 1 | Continued



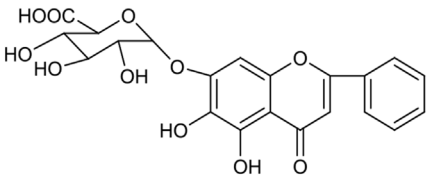
ß-amyrin **(43)**



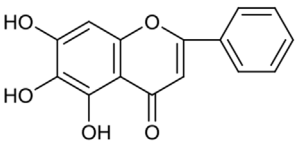
2α,3α,23-trihydroxyursa-12,20(30)-dien-28-oic acid **(44)**



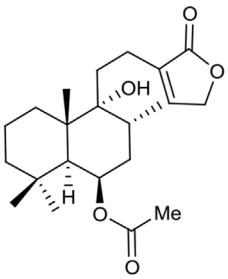
Euscaphic acid **(45)**



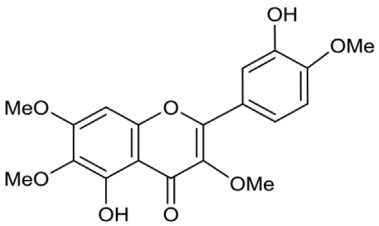
Baicalin **(46)**



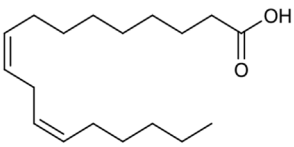
Baicalein **(47)**



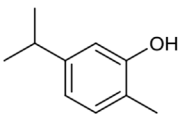
Viteosin-A **(48)**



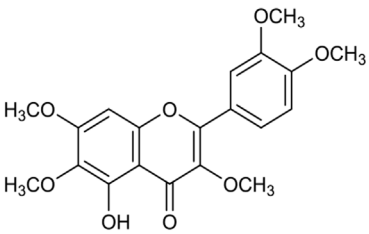
Vitexicarpin **(49)**



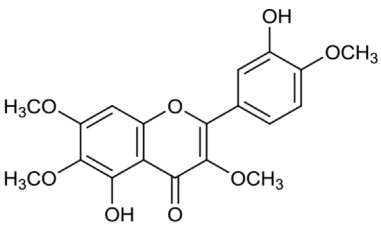
Linoleic acid **(50)**



Carvacrol **(51)**



5-hydroxy-3,6,7,3',4'-pentamethoxyflavone **(52)**



Casticin **(53)**

FIGURE 1 | Chemical structures of phytochemicals isolated from Lamiaceae species with anti-allergic activity.

TABLE 2 | Mechanism of action of extracts and isolates of Lamiaceae species with anti-allergic activity.

Plant name	Plant part used	Isolated compound/ extract used	Chemical class	Assay type	Mechanism of action/conclusion	References
Clerodendron phlomidis Linn.	Leaves	Aqueous extract	–	*In vivo*	Concentration of 100 mg/kg potently reduced blood eosinophil count, mast cell degranulation and histamine release in sensitized mice.	(Vadnere et al., 2007)
				Ex vivo	Potent antagonizing effect of histamine-induced goat tracheal contraction at doses of 4 mg/ml and 10 mg/ml.	(Vadnere et al., 2007)
Clerodendrum serratum (Linn.) Moon	Root and stem	Aqueous extract	–	*In vivo*	Root extract resulted in no significant increase of leucocyte and eosinophil count at 260 mg/kg in milk-induced leucocytosis mice and prolonged PCD at 156 mg/kg in egg albumin-sensitized guinea pigs.	(Bhangare et al., 2012)
Clerodendron trichotomum Thunb.	Leaves	Acteoside **(22)**	Phenylpropanoid glycoside	*In vivo*	At dose of 50 mg/kg significantly inhibited eosinophil infiltration, decreased histamine content and phospholipase A_2 activity in BALF while at 25 mg/kg, recruitment of leukocytes was suppressed and inhibited sRaw in both IAR and LAR in sensitized guinea pigs model.	(Lee et al., 2011b)
Clinopodium gracile (Benth.) Matsum var. multicaule	Whole plant	Aqueous extract	–	*In vivo*	Compound 48/80-induced mice were observed with concentration-dependently reduced anaphylactic death with intraperitoneally administration at concentrations ranging from 1-100 mg/kg and the same reduction manner was seen in IgE-mediated PCA reaction.	(Park et al., 2010)
				In vitro	Dose-dependent inhibition of histamine release inhibition from RPMC and HMC-1 cells respectively across 1-100 μg/ml. Allergic inflammation reduced with the attenuation of intracellular calcium, NF-κB, gene expression and secretion of TNF-α and IL-6 stimulated by PMACI in HMC-1 cells.	(Park et al., 2010)
Dracocephalum argunense Fisch.	Whole plant	Aqueous extract	–	*In vivo*	Significant inhibition of systemic anaphylaxis with intraperitoneal administration of aqueous extract in mice at concentration range of 0.01–1 g/kg. Serum histamine and PCA inhibition were reduced in a dose-dependent manner.	(Kim et al., 2006; Kim and Shin, 2006)
				In vitro	Decreased intracellular calcium and histamine release from RPMC in dose dependent manner with concentrations of 0.001–1 mg/ml. TNF-α and IL-6 gene expression in HMC-1 cells were inhibited across doses ranging from 0.01–1 mg/ml with the involvement of NF-κB attenuation.	(Kim et al., 2006; Kim and Shin, 2006)

TABLE 2 | Continued

Plant name	Plant part used	Isolated compound/ extract used	Chemical class	Assay type	Mechanism of action/conclusion	References
Elsholtzia ciliate (Thunb.) Hyland	Whole plant	Aqueous extract	–	*In vivo*	Serum histamine, systemic anaphylaxis and PCA reaction observed with dose-related inhibition at concentrations of 10–1,000 mg/kg.	(Kim et al., 2011)
				In vitro	Significantly inhibited histamine release at 10 and 100 μg/ml of aqueous extract and recorded with reduction of PMACI-stimulated intracellular calcium at 100 μg/ml of aqueous extract pretreatment in HMC-1 cells. Gene expression and production of IL-6, IL-1β and TNF-α were suppressed through inhibition of NF-κB activation and p38 MAPK pathway with concentrations ranging from 1-100 μg/ml.	(Kim et al., 2011)
Isodon japonicas Hara	Whole plant	Aqueous extract	–	*In vivo*	Challenged-mice protected from systemic allergic death and PCA with intraperitoneal administration of extract at concentration of 0.1 g/kg.	(Kim et al., 2004; Shin et al., 2004)
				In vitro	Dose-dependently decreased histamine release from RPMC stimulated by compound 48/80 or anti-DNP IgE at doses ranging from 0.001-1 mg/ml and reduced gene expression and production of TNF-α and IL-6 in PMACI-stimulated HMC-1 cells.	(Kim et al., 2004; Shin et al., 2004)
Lagochilus leiacanthus Fisch. & C.A.Mey.	Whole plant	5,2',6'-trihydroxy-7,8-dimethoxyflavanone **(23)**, 5,2',6'-trihydroxy-6,7,8-trimethoxyflavanone **(24)**, 5,2'-dihydroxy-6,7,8,6'-tetramethoxyflavanone **(25)**, Oroxylin-A **(14)**, Hispidulin **(26)**, 5,2',6'-trihydroxy-6,7,8-trimethoxyflavone **(27)**, 5,7,2'-trihydroxy-8,6'-dimethoxyflavone **(28)**, 5,6,2'-trihydroxy-7,8,6'-trimethoxyflavone **(29)**	Flavonoid	*In vitro*	Significantly inhibited the release of β-hexosaminidase from RBL-2H3 cells with IC_{50} values ranging from 13.5-48.9 μM.	(Furukawa et al., 2011)
Lycopus lucidus Turcz.	Aerial part	Rosmarinic acid **(1)**, clinopodic acid C **(17)**, lycopic acid A **(19)**, clinopodic acid E **(18)**, lycopic acid B **(20)**, scizotenuin A **(21)**	Phenylpropanoid	*In vitro*	Acted as hyaluronidase inhibitor with IC_{50} of 309, 80.1, 134, 82.8, 141 and 241 μM respectively.	(Murata et al., 2010)

TABLE 2 | Continued

Plant name	Plant part used	Isolated compound/ extract used	Chemical class	Assay type	Mechanism of action/conclusion	References
	Whole plant	Aqueous extract	–	*In vivo*	Systemic anaphylactic death and PCA reaction reduced dose-dependently across concentrations of 0.005–0.1 g/kg in sensitized mice.	(Shin et al., 2005)
				In vitro	Histamine release potently reduced in compound 48/80 or anti-DNP IgE-stimulated RPMC corresponded to decreased intracellular calcium at dose range of 0.01–1 mg/ml. Attenuation of NF-κB caused a reduction in downstream cytokines, such as TNF-α and IL-6 expression.	(Shin et al., 2005)
Marrubium vulgare Linn.	Aerial part	Marrubiin **(30)**	Furane labdane diterpene	*In vitro*	Maximal inhibition (67.6 ± 4%) of OVA-induced allergic oedema was achieved at dose of 100 mg/kg in actively sensitized mice.	(Stulzer et al., 2006)
Melissa officinalis Linn.	Leaves	Rosmarinic acid **(1)**	Phenylpropanoid	*In vitro*	Exhibited potent suppressive effect on hyaluronidase with 1.0 ± 0.3% of enzyme activity.	(Ippoushi et al., 2000)
Mentha arvensis Linn.	Leaves, roots and stem	Ethanolic and aqueous extract	–	*In vivo*	100 μg/ml ethanolic leaf and root extract showed potent histamine inhibition at 57% and 53% respectively in mice.	(Malik et al., 2003)
	Whole plant	Aqueous extract	–	*In vivo*	Anal administration of 0.05 g/kg extract protected mice from anaphylactic death. PCA reaction reduced dose-dependently with intraperitoneal, oral and intravenous administration of extract.	(Shin, 2003)
				In vitro	Significant reduction in histamine release at 0.1 and 1 mg/ml of extract in compound 48/80-induced and anti-DNP IgE-mediated model in RPMC. TNF-α production reduced significantly at concentration of 0.1 mg/ml in RPMC.	(Shin, 2003)
	–	Essential oil	–	*In vivo*	Dose-dependently reduced histamine-induced bronchoconstriction in guinea pigs at 200 and 400 μl/kg and significantly reduced eosinophil count, serum IgE level and BALF eosinophils in OVA-sensitized mice at 200 μl/kg.	(Sharma et al., 2018)
Mentha haplocalyx Briq.	Aerial part	Ethanolic extract	–	*In vivo*	Observed with inhibition of eosinophil infiltration, T_H2 cytokines (IL-4, IL-5) expression and production in OVA-sensitized mice.	(Lee et al., 2011a)

TABLE 2 | Continued

Plant name	Plant part used	Isolated compound/ extract used	Chemical class	Assay type	Mechanism of action/conclusion	References
Mentha piperita Linn.	Leaves	50% ethanolic eluate	–	*In vivo*	Decreased nasal responses in antigen-induced rats at 300 and 1,000 mg/kg.	(Inoue et al., 2001)
				In vitro	Recorded with potent inhibition of histamine release from RPMC with IC_{50} of 2.55 (1.42–3.94) μg/ml.	(Inoue et al., 2001)
	Leaves	Luteolin-7-*O*-rutinoside **(31)**	Flavonoid glycoside	*In vivo*	Reduced frequency of sneezing at 100 and 300 mg/kg while nasal rubbing was seen in antigen-induced rats at 100 mg/kg or more.	(Inoue et al., 2002)
				In vitro	Suppression of histamine release from compound 48/80-induced RPMC with IC_{50} value of 21.9 μM.	(Inoue et al., 2002)
Mentha piperita var. *citrata* (Ehrh.) Briq.	Leaves	5,6,4'-trihydroxy-7,8-dimethoxyflavone **(32)**, 5,6,4'-trihydroxy-7,8,3'-trimethoxyflavone **(33)**, 5,6-dihydroxy-7,3',4'- trimethoxyflavone **(34)**, 5,6-dihydroxy-7,8, 3', 4'-tetramethoxyflavone **(35)**, 5,6-dihydroxy-7,8, 4'-trimethoxyflavone **(36)**	Flavonoid	*In vitro*	Significantly reduction of β-hexosaminidase release from RBL-2H3 at IC_{50} range of 2.4–6.7 μM.	(Sato and Tamura, 2015)
Mentha spicata L. var. *crispa* Benth.	Leaves	5,6-dihydroxy-7,8,3',4'-tetramethoxyflavone **(35)**, 5,6,4'-trihydroxy-7,8,3'-trimethoxyflavone **(33)**, (3R)-1-octan-3-yl β-D-glucopyranoside **(37)**	Flavonoid and aliphatic glycoside	*In vivo*	Demonstrated β-hexosaminidase release suppression from rat basophils at 56, 6.4 and 560 μM respectively.	(Yamamura et al., 1998)
Minthostachys verticillata (Griseb.) Epling	Leaves and stems	Essential oil	–	*In vitro*	β-hexosaminidase release from human basophils was diminished by 32.15% to 39.72% as comparable to dexamethasone and theophylline.	(Cariddi et al., 2007)
	Leaves and stems	Limonene **(38)**, Pulegone **(39)**, Menthone **(40)**	Monoterpene	*In vivo*	**(38)** maximally suppressed PCA reaction in challenged-mice at 250 mg/kg.	(Cariddi et al., 2011)
				In vitro	The combination of the three constituents in essential oil significantly suppressed the production of IL-13 from human PBMC. Potent inhibitory effect on β-hexosaminidase release from human basophils was observed with a concentration range of 10–40 μg/ml.	(Cariddi et al., 2011)
Mosla chinensis Max.	Whole plant	Aqueous extract	–	*In vivo*	Observed with concentration dependent suppression of systemic anaphylaxis and PCA reaction with doses of ranging from 10–1,000 mg/kg in sensitized mice.	(Kim et al., 2012)
				In vitro	Decreased intracellular calcium caused a dose-dependent reduction of histamine release from RPMC and reduced NF-κB activation resulted in decreased downstream TNF-α, IL-6, IL-8 expression in PMACI-stimulated HMC-1 cells.	(Kim et al., 2012)

TABLE 2 | Continued

Plant name	Plant part used	Isolated compound/ extract used	Chemical class	Assay type	Mechanism of action/conclusion	References
Mosla dianthera Maxim.	Whole plant	Aqueous extract	–	*In vivo*	Challenged-mice recorded with zero mortality due to systemic shock with pretreatment of 1,000 mg/kg and reduced PCA reaction in a dose-dependent manner across the range of 1–1,000 mg/kg.	(Lee et al., 2006)
				In vitro	Attenuation of histamine release from RPMC at doses ranging from 0.001–1 mg/ml and decreased intracellular calcium, NF-κB activation, gene expression and secretion of TNF-α, IL-6, IL-8 in PMACI-stimulated HMC-1 cells.	(Lee et al., 2006)
Nepeta bracteata Benth.	Whole plant	Crude aqueous extract	–	*In vivo*	Medium dose (not specified dose) exhibited the most potent reduction in the number of T_H17 cells, increased number of Treg cells in OVA-sensitized mice and decreased eosinophil infiltration in BALF.	(Wang et al., 2016)
Ocimum basilicum Linn.	Leaves	Acetone and hydro-methanol extract	–	*In vitro*	Achieved histamine release suppressive effect of 35.35% and 50.76% respectively at 100 μg/ml.	(Kaur et al., 2018)
Ocimum gratissimum Linn.	Leaves	Methanolic extract	–	*In vivo*	Significantly suppressed number of eosinophils, decreased IL-4 level, reduced level of eosinophil peroxidase in BALF and lungs and decreased airway mucus hypersecretion at 100 mg/kg.	(Costa et al., 2012)
Ocimum sanctum Linn.	Leaves and seeds	Dried and fresh leaves ethanolic extract, volatile oil from fresh leaves and fixed oil from seeds	–	*In vivo*	Guinea pigs were protected from both histamine-induced and acetylcholine-induced preconvulsive dyspnoea with pretreatment of fresh leaves ethanolic extract, volatile oil and fixed oil.	(Singh and Agrawal, 1991)
	Leaves	Ethanolic extract	–	*In vivo*	Exhibited significant mast cell stabilizing potential, inhibition of IgE and delayed onset of histamine-induced bronchospasm with 64.25 ± 9.51%, 25.80 ± 4.85 ng/ml and 440 s respectively.	(Sridevi et al., 2009)
	Leaves	Ethanolic extract and isolated flavonoidal fraction	–	*In vivo*	Sensitized rats showed with significant mast cell stabilization of 67.24 ± 2.94% with extract administration and 60.48 ± 2.72% with fraction administration.	(Choudhary, 2010)
Perilla frutescens Britton	Whole plant	Aqueous extract	–	*In vivo*	Exhibited dose-dependent inhibition of compound 48/80-induced plasma histamine release at concentration range of 0.01–1 g/kg in sensitized rats. Marked suppression of PCA reaction at doses of 0.1 and 1 g/kg.	(Shin et al., 2000)
				In vitro	At concentration range of 0.001–1 mg/ml, histamine release and TNF-α production were decreased dose-dependently in stimulated RPMC. cAMP level in RPMC significantly increased at 1 mg/ml.	(Shin et al., 2000)

TABLE 2 | Continued

Plant name	Plant part used	Isolated compound/ extract used	Chemical class	Assay type	Mechanism of action/conclusion	References
	Whole plant	Rosmarinic acid **(1)**, Apigenin 7-*O*-[β-glucuronosyl(2→1) β-glucuronide] **(2)**	Flavonoid and flavonoid glycoside	*In vivo*	**(1)** and **(2)** suppressed PCA reaction in sensitized mice with percentage inhibition of 41% and 32% respectively.	(Makino et al., 2001)
	Leaves	Luteolin **(3)**	Flavonoid	*In vivo*	Sensitized mice showed with decreased oxazolone-induced ear odema with oral administration of 1 mg of **(3)**. Dose-dependent inhibition of TNF-α production occurred at dose range of 1–1,000 μg.	(Ueda et al., 2002)
	Leaves	Rosmarinic acid **(1)**	Phenylpropanoid	*In vivo*	Equivalent PCA reaction suppression was achieved by 19 mg/kg of **(1)** as compared to 150 mg/kg of tranilast, the positive control.	(Makino et al., 2003)
	Leaves	Rosmarinic acid **(1)**	Phenylpropanoid	*In vivo*	Oral administration of **(1)** by *Der f*-sensitized mice caused reduction in allergen-specific immunoglobulin, eosinophil infiltration, eosinophil counts in BALF, eotaxin, IL-4 and IL-5 expression at concentration of 1.5 mg/day.	(Sanbongi et al., 2004)
	Leaves	Ethanol extract	–	*In vivo*	T_H2 cytokines (IL-5 and IL-13), serum IgE level, eosinophil infiltration, histamine and eotaxin in BALF were suppressed in OVA-sensitized BALB/c mice.	(Chen et al., 2015)
	Leaves	Luteolin **(3)**	Flavonoid	*In vivo*	Compound 48/80- or serotonin-induced scratching behaviour and vascular permeability were reduced dose-dependently at 5, 10 and 20 mg/kg in sensitized mice.	(Jeon et al., 2014)
				In vitro	Dose-dependent reduction of compound 48/80-induced histamine release from RPMC marked at 5, 10 and 20 μM. Diminished production of TNF-α (31.9%-76.8%) and IL-1β (27.3%-81.2%) in PMACI-stimulated HMC-1 cells at a range of 5–20 μM of **(8)**.	(Jeon et al., 2014)
	Leaves	Aqueous fraction	–	*In vivo*	DNFB-sensitized mice experienced 35% reduction of ear swelling symptom at dose of 100 μg/ml.	(Heo et al., 2011)
				In vitro	At 100 μg/ml, eosinophil counts reduced by 73.7% accompanied with decreased expression of MMP-9 and IL-31 in mice ear tissues. T-bet protein expression was augmented and resulted in T_H1/T_H2 balance.	(Heo et al., 2011)
	Leaves	Methanolic extract	–	*In vitro*	Dose-dependent reduction of IL-4, IL-5, IL-13 and GM-CSF production in DP2-stimulated BEAS-2B cells at concentration range of 5-50 μg/ml with involvement of decreased phosphorylation of JNK and p38.	(Liu et al., 2013)
	Leaves	Rosmarinic acid methyl ester **(41)**	Phenolic compound	*In vitro*	Possessed potent inhibitory activity on β-hexosaminidase from RBL-2H3 cells with IC_{50} of 9.9 ± 0.8 μg/ml.	(Zhu et al., 2014)

TABLE 2 | Continued

Plant name	Plant part used	Isolated compound/ extract used	Chemical class	Assay type	Mechanism of action/conclusion	References
	Leaves	8-hydroxy-5,7-dimethoxyflavanone **(4)**	Flavanone	*In vivo*	Oral administration of **(4)** inhibited PCA reaction at dose of 5 mg and allergic nasal response at dose of 1.5 mg.	(Kamei et al., 2017)
				In vitro	Significant suppressive effect on histamine release from RBL-2H3 cells was observed with IC_{50} of 68.5 μM.	(Kamei et al., 2017)
	Leaves	Crude extract	–	*In vivo*	Decreased serum IgE level was observed in the blood of *Der f*-challenged mice.	(Komatsu et al., 2016)
				In vitro	Reduced $CD4^+/CD8^+$ ratio in splenic T lymphocytes with percentage of 1.50 ± 0.07%.	(Komatsu et al., 2016)
Phlomis umbrosa Turcz.	Roots	Aqueous extract	–	*In vivo*	At dose of 1 g/kg, plasma histamine release only recorded with 0.023 ± 0.002 μg/ml in compound 48/80- sensitized mice. Anal, oral and intraperitoneal administration of 0.01–1 g/kg extract resulted in dose-dependent reduction in PCA reaction.	(Shin et al., 2008; Shin and Lee, 2003)
				In vitro	Significant inhibition of histamine release from RPMC activated by compound 48/80 or anti-DNP IgE recorded at doses of 0.1 mg/ml and 1 mg/ml. Extract attenuated the secretion of IL-1β, IL-6 and TNF-α in PMACI-stimulated HMC-1 cells.	(Shin et al., 2008; Shin and Lee, 2003)
Pogostemon cablin (Blanco) Benth	–	Aqueous extract	–	*In vivo*	Intraperitoneal administration of extract caused a dose-related suppression of systemic anaphylaxis induced by compound 48/80 in sensitized mice across doses of 10–1,000 mg/kg. At doses ranging from 1–1,000 mg/kg, PCA reaction induced by DNP-HSA was reduced in a concentration-dependent manner in sensitized rats.	(Yoon et al., 2016)
				In vitro	Suppressed the release of histamine and β-hexosaminidase from RPMC across concentrations of 1–1,000 μg/ml. Expression and secretion of TNF-α, IL-6 and IL-8 was inhibited in HMC-1 cells at doses of 1–100 μg/ml due to the attenuation of NF-κB activation.	(Yoon et al., 2016)
	–	Patchouli oil	–	*In vivo*	Significant reduced PCA reaction in ovalbumin-challenged rats and suppressed delayed-type hypersensitivity at doses of 20, 40 and 80 mg/kg of Patchouli oil.	(He et al., 2013)
				Ex vivo	Decreased contraction responses in guinea pig ileum at concentrations of 0.01, 0.02 and 0.04 mg/ml of Patchouli oil.	(He et al., 2013)

TABLE 2 | Continued

Plant name	Plant part used	Isolated compound/ extract used	Chemical class	Assay type	Mechanism of action/conclusion	References
Prunella vulgaris var. *lilacina* Nakai	Whole plant	2α,3α-dihydroxyurs-12-en-28-oic acid **(42)**	Triterpenoid	*In vitro*	Significant suppressive effect on β-hexosaminidase from RBL-2H3 with IC_{50} value of 57 μM.	(Ryu et al., 2000)
	Whole plant	Aqueous extract	–	*In vivo*	At doses of 0.5 and 1 g/kg, sensitized rats completely protected from anaphylatic death. Oral administration of extract with doses ranging from 0.001–1 g/kg dose-dependently reduced PCA reaction.	(Kim et al., 2007; Shin et al., 2001)
				In vitro	Inhibition of intracellular calcium level caused downstream decreased release of histamine from RPMC in a concentration dependent manner with concentration range of 0.001–1 mg/ml. RPMC also showed with significant reduction of TNF-α production with the pretreatment of 0.01 mg/ml and 0.1 mg/ml.	(Kim et al., 2007; Shin et al., 2001)
	Spike	β-amyrin **(43)**, 2α,3α,23-trihydroxyursa-12,20(30)-dien-28-oic acid **(44)**, Euscaphic acid **(45)**	Triterpenoid	*In vitro*	Observed with inhibition of histamine release from HMC-1 cells with 46.7%, 57.9% and 54.2% respectively.	(Choia et al., 2016)
Rosmarinus officinalis Linn.	Leaves	Carnosic acid **(5)**	Polyphenol	*In vivo*	PCA reaction was significantly suppressed at 100 mg/kg in sensitized mice with percentage inhibition of 67.1%.	(Mizushina et al., 2014)
				In vitro	**(5)** inhibited β-hexosaminidase release from PMACI A23187-stimulated RBL-2H3 cells at 10 μM.	(Mizushina et al., 2014)
Salvia miltiorrhiza Bunge	Roots	15,16-dihydrotanshinone-I **(7)**, Cryptotanshinone **(9)**	Diterpene	*In vitro*	**(7)** and **(9)** significantly suppressed the release of β-hexosaminidase from RBL-2H3 cells with IC_{50} values of 16 ± 2.4 μM and 36 ± 3.7 μM respectively.	(Choi and Kim, 2004; Ryu et al., 1999)
	Leaves	Ethanolic extract	–	*In vivo*	Oral administration of 25–100 mg/kg extract dose-dependently inhibited PCA reaction in anti-DNP IgE-stimulated rats.	(Yang et al., 2008)
				In vitro	Dose-related inhibition of COX-1 and COX-2-dependent prostaglandin D_2 production observed with IC_{50} values of 3.96 and 21.54 μg/ml respectively in BMMC. Suppression of leukotriene C_4 generation and β-hexosaminidase release was seen in BMMC with IC value of 2.6 and 22.4 μg/ml.	(Yang et al., 2008)
	Rhizome	Tanshinone I **(6)**, 15,16-dihydrotanshinone-I **(7)**, Tanshinone IIA **(8)**, Cryptotanshinone **(9)**	Diterpene	*In vivo*	**(6)**, **(7)**, **(8)** and **(9)** significantly suppressed PCA reaction at dose of 50 mg/kg with percentage inhibition of 59%, 49%, 35% and 32% respectively in sensitized mice.	(Trinh et al., 2010a)
				In vitro	Potent inhibition of IL-4 and TNF-α expression by **(6)**, **(7)** and **(8)** at dose of 50 μM in RBL-2H3 cells.	(Trinh et al., 2010a)

TABLE 2 | Continued

Plant name	Plant part used	Isolated compound/ extract used	Chemical class	Assay type	Mechanism of action/conclusion	References
	Roots	15,16-dihydrotanshinone-I **(7)**	Diterpene	*In vitro*	**(7)** at 20 μM produced 90% suppression on degranulation and generation of prostaglandin D_2 and leukotriene C_4 in IgE/Ag-stimulated BMMC through inhibition of FcεRI-mediated Syk-dependent signal pathway.	(Li et al., 2015)
Salvia plebeia R. Brown	Whole plant	Aqueous extract	–	*In vivo*	No anaphylactic death occurred in compound 48/80-induced rats with intraperitoneal administration of 0.5 and 1 g/kg extract. At doses ranging from 0.01–1 g/kg, intraperitoneal and oral administration of extract showed with dose-dependent inhibition of PCA reaction.	(Shin and Kim, 2002)
				In vitro	Concentration-dependent reduction histamine release from RPMC activated by compound 48/80 or anti-DNP IgE at a concentration range of 0.001–1 mg/ml. TNF-α production from RPMC was significantly inhibited at concentrations of 0.01–1 mg/ml whereas cAMP level in RPMC significantly elevated compared with that of basal cells.	(Shin and Kim, 2002)
	Whole plant	Ethanol extract	–	*In vivo*	Oral administration of 100 mg/kg extract significantly suppressed serum IgE level, serum histamine, eosinophil count, pro-inflammatory cytokines (IFN-γ and TNF-α) expression, T_H1, T_H2 and T_H17 cytokines expression in *Der f*-sensitized mice.	(Choi et al., 2014)
Schizonepeta tenuifolia (Benth.) Briq.	Whole plant	Aqueous extract	–	*In vivo*	100% protection from systemic anaphylaxis was observed with doses of 0.5 and 1 g/kg in compound 48/80-challenged rats whereas a marked suppression in PCA reaction was seen in orally administered of 0.1 and 1 g/kg of anti-DNP IgE-sensitized rats.	(Shin et al., 1999)
				In vitro	Significant inhibition of compound 48/80 or IgE-mediated histamine release from RPMC was marked at concentration range of 0.01–1 mg/ml. A potent inhibition of TNF-α production observed at 1 mg/ml with a content of 0.889 ± 0.747 ng/ml.	(Shin et al., 1999)
	Whole plant	Extract with phosphate buffered saline/olive oil (P/O) in proportion of (9:1) mixture	–	*In vivo*	Skin thickening and hyperplasia of epidermis and dermis in DNCB-sensitized mice remarkably decreased by 38.15% and 42.37% respectively with treatment of 1% of extract in P/O (9:1) mixture.	(Choi et al., 2013)
				In vitro	DNCB-induced mice observed with reduced serum levels of IgE, TNF-α and IL-6, recorded with 46.26%, 41.97% and 70.42% inhibition respectively with the treatment of 1% of extract in P/O (9:1) mixture.	(Choi et al., 2013)

TABLE 2 | Continued

Plant name	Plant part used	Isolated compound/ extract used	Chemical class	Assay type	Mechanism of action/conclusion	References
	–	Aqueous extract	–	*In vitro*	Pro-inflammatory cytokines (IL-6, IFN-γ and TNF-α) and pro-allergic T_H2 cytokines (IL-4 and IL-13) in RBL-2H3 were decreased with the treatment of 100 μg/ml of aqueous extract. β-hexosaminidase release from RBL-2H3 cells reduced significantly at dose of 10 μg/ml.	(Lin et al., 2018)
Scutellaria baicalensis Georgi	Roots	Wogonin **(10)**, Ganhuangenin **(11)**, Wogonoside **(12)**, 3,5,7, 2',6'-pentahydroxyflavanone **(13)**	Flavonoid	*Ex vivo*	**(10)**, **(11)** and **(12)** significantly inhibited the production of IgE from concanavalin A-stimulated rat spleen lymphocytes at concentrations of 10.0 and 100.0 μM. Histamine and leukotriene B_4 release from rat PEC was markedly suppressed at dose of 100 μM for all flavonoids.	(Lim, 2003; Lim et al., 2003)
	Roots	Aqueous extract	–	*In vivo*	Oral administration of 50 mg/kg extract selectively inhibited the release of IL-5 in mice.	(Kim et al., 2010)
	Roots	Baicalin **(46)**	Flavone glycoside	*In vitro*	Histamine and leukotriene release from OVA-sensitized guinea pig lung mast cells were potently suppressed at doses of 10, 30 and 60 μg. The standardized extract of **(46)** exhibited a more potent outcome than pure **(46)** at 60 μg only.	(Kim et al., 2010)
	Whole plant	Ethanolic extract	–	*In vivo*	Exhibited 6.6% inhibition of PCA reaction in sensitized-rats at 280 mg/kg.	(Jung et al., 2012)
				In vitro	40% reduction of histamine content in compound 48/80-stimulated RPMC with dose of 10 μg/ml. Significant reduced production of TNF-α and IL-8 in PMACI-stimulated HMC-1 cells with inhibition of MAPK activation at concentration range of 1–100 μg/ml.	(Jung et al., 2012)
	Roots	Aqueous extract	–	*In vivo*	Topical application of 5% extract reduced DNFB-induced cutaneous reaction by 31% as compared to control group.	(Kim et al., 2013)
				In vitro	Significantly suppressed β-hexosaminidase release from RBL-2H3 at doses of 125, 250 and 500 ppm with percentage inhibition of 19%, 34% and 60% respectively.	(Kim et al., 2013)
	Whole plant	Crude ethanol extract	–	*In vivo*	OVA-sensitized mice were protected from food allergy anaphylactic death by 60% and observed with significantly suppression of OVA-specific IgE, IL-17, T_H2 cytokines (IL-4, IL-5, IL-10, IL-13) and T_H1 cytokines (IFN-γ and IL-12) with the treatment of 25 mg/kg of extract.	(Shin et al., 2014b)

TABLE 2 | Continued

Plant name	Plant part used	Isolated compound/ extract used	Chemical class	Assay type	Mechanism of action/conclusion	References
	–	Wogonin **(10)**, Baicalin **(46)**, Baicalein **(47)**	Flavonoid	*Ex vivo*	**(10)** suppressed the production of T_H2 cytokines (IL-4, IL-5, IL-10, IL-13) and IFN-γ without causing cytotoxicity in OVA-sensitized mice splenocytes at 50 μmol/ml as compared to **(46)** and **(47)**.	(Shin et al., 2014a)
				In vivo	Oral administration of 1 mg/kg of **(15)** potently decreased the production of OVA-specific IgE, IL-5, IL-10 and IL-13 in sensitized mice.	(Shin et al., 2014a)
	Roots	Aqueous extract	–	*In vivo*	Attenuation of DNCB-induced epidermal thickness, leukocytes infiltration, serum IgE, IL-4, IFN-γ and TNF-α production in BALB/c mice skin.	(Kim et al., 2016)
	Root	Ethanol extract, acetone extract and ethyl acetate extract	–	*In vivo*	The highest inhibitory activity against 4-AP-induced allergic skin pruritus, histamine-induced paw swelling, ear PCA reaction, anaphylaxis ear swelling and total serum IgE level was seen with 1.42 g/kg of ethanol extract in sensitized mice.	(Li et al., 2014)
	Rhizome	Baicalin **(46)**, Baicalein **(47)**, Oroxylin A **(14)**	Flavonoid	*In vivo*	**(46)** which was orally administered metabolized into **(47)** and **(14)**. Metabolite, **(14)** possessed a more potent anti-histamine activity, seen with significant reduced histamine-induced scratching behaviour and vascular permeability in sensitized mice at doses of 20 and 50 mg/kg.	(Trinh et al., 2010b)
				In vitro	Metabolite of **(46)**, **(14)** remarkably inhibited the contraction of guinea pig ileum with IC_{50} value of 0.28 mmol/L.	(Trinh et al., 2010b)
	–	Oroxylin A **(14)**	Flavonoid	*In vivo*	Significantly reduced eosinophils infiltration in BALF and airway hyperresponsiveness in OVA-sensitized mice. Potent attenuation of serum IgE level, T_H2 cytokines (IL-4, IL-5 and IL-13) production and NF-κB activation with oral administration of 15, 30 and 60 mg/kg.	(Zhou et al., 2016)
	Roots	Linoleic acid **(50)**	Fatty acid	*Ex vivo*	Significantly suppressed the production of IL-4, IL-5, IL-10 and IL-13 but enhanced secretion of IFN-γ and IL-12, resulted in T_H1/T_H2 balance at 50 μg/L.	(Jung et al., 2017)
	–	Baicalein **(47)**	Flavonoid	*In vivo*	**(47)** induced $CD4^+$ $FOXP_3^+$ T cell differentiation in ovalbumin-sensitized mice at concentrations of <10 μmol/L without causing cell death.	(Bae et al., 2016)

TABLE 2 | Continued

Plant name	Plant part used	Isolated compound/ extract used	Chemical class	Assay type	Mechanism of action/conclusion	References
Stachys riederi var. *japonica* Miq.	Whole plant	Aqueous extract	–	*In vivo*	Dose-dependent inhibition was resulted in the occurrence of systemic anaphylaxis at concentration range of 0.005–1 g/kg and significant reduction of PCA reaction at concentrations of 0.1 and 1 g/kg.	(Shin, 2004)
				In vitro	At doses of 0.1 and 1 mg/ml, a significant decrease release of histamine from RPMC and diminished secretion of TNF-α and IL-6 from HMC-1 cells.	(Shin, 2004)
Teucrium japonicum Houttuyn	Whole plant	Aqueous extract	–	*In vivo*	Serum histamine release was significantly reduced at 100 and 1,000 mg/kg, with 500 mg/kg as the effective dose that completely protected compound 48/80-induced mice from systemic anaphylaxis. At doses ranging from 1–1,000 mg/kg, a dose-dependent inhibition of PCA reaction was resulted in anti-DNP IgE-challenged mice.	(Kim et al., 2009)
				In vitro	Significant reduction of compound 48/80-induced intracellular calcium and downstream histamine release from RPMC was observed at 1 mg/ml. Gene expression of TNF-α was diminished dose-dependently at 0.01–1 mg/ml with the involvement of NF-κB in PMACI-stimulated HMC-1 cells.	(Kim et al., 2009)
Thymus vulgaris Linn.	Leaves	2-isopropyl-5-methylphenol **(15)**	Monoterpenoid phenolic	*In vivo*	Dose-dependently inhibited recruitment of inflammatory cells, reduced airway hyperreponsiveness, suppressed level of OVA-specific IgE, T_H2 cytokines in BALF at concentrations of 4, 8 and 16 mg/kg.	(Zhou et al., 2014)
	Leaves	n-hexane extract	–	*In vivo*	Portrayed intermediate inhibitory activity on histamine release with 46.22 ± 0.08%.	(Ikawati et al., 2001)
	–	2-isopropyl-5-methylphenol **(15)**, Carvacrol **(51)**	Monoterpenoid phenolic	*In vivo*	**(15)** and **(51)** reduced delayed-type hypersensitivity by 26% and 50% respectively in ovalbumin-sensitized mice.	(Gholijani and Amirghofran, 2016)
				Ex vivo	Both compounds led to reduction of IL-2, IFN-γ, IL-4, IL-17 level and T-bet expression but increased level of IL-10 and TGF-β in mice splenocytes cultures.	(Gholijani and Amirghofran, 2016)
Vitex negundo Linn.	Leaves	Aqueous subfraction of ethyl acetate fraction	–	*In vitro*	Mast cell stabilizing activity with 80.99 ± 0.7231% was observed in rat mesenteric mast cells at dose of 500 μg/ml.	(Patel and Deshpande, 2011)
	Leaves	5-hydroxy-3, 6, 7, 3',4'-pentamethoxyflavone **(52)**	Flavonoid	*In vivo*	200 mg/kg extract demonstrated significant reduction of eosinophil count in BALF and serum bicarbonate level in egg albumin sensitized guinea pigs.	(Patel and Deshpande, 2013)

TABLE 2 | Continued

Plant name	Plant part used	Isolated compound/ extract used	Chemical class	Assay type	Mechanism of action/conclusion	References
Vitex rotundifolia Linn.	Fruits	Aqueous extract	–	*In vivo*	Dose-dependent reduction of systemic anaphylaxis reaction in compound 48/80-induced rats across concentration range of 0.0001–1 g/kg. Significant suppression of PCA reaction marked at doses of 0.5 and 1 g/kg in sensitized rats.	(Shin et al., 2000)
				In vitro	Histamine release from RPMC was reduced in a dose-dependent manner at dose range of 0.001–1 mg/ml and marked inhibition of TNF-α production at 0.001 mg/ml.	(Shin et al., 2000)
	Fruits	1H,8H-pyrano[3,4-c]pyran-1,8-dione **(16)**	Pyran	*In vivo*	Serum IgE, eosinophil counts and IL-5 production in BALF were significantly suppressed by 43%, 82% and 34% respectively. IL-4 and IL-5 level significantly decreased in $CD4^+$ T cells in T_H2 skewed condition with treatment.	(Lee et al., 2009)
				In vitro	Eosinophil migration and eotaxin production were reduced by 48% and 70% respectively at 10 μg/ml in A549 cell media.	(Lee et al., 2009)
	Fruits	Casticin **(53)**	Flavonoid	*In vitro*	Up to 63% of eosinophil migration inhibition was observed in A549 cell media with pretreatment of 10 μg/ml. Eotaxin level was reduced from concentration range of 0.1–10 μg/ml in A549 cells.	(Koh et al., 2011)
	–	Aqueous extract	–	*In vivo*	Oral administration of 100 mg/kg extract caused 86% inhibition of eosinophilia, reduction of T_H2 cytokines (IL-4, IL-5, IL-13) and TNF-α level in BALF and decreased serum IgE level in ovalbumin-sensitized mice.	(Bae et al., 2013)
Vitex trifolia Linn.	Leaves	n-hexane and ethanolic extract	–	*In vitro*	Both n-hexane and ethanolic extract highly suppressed histamine release by 80.13 ± 3.95 and 81.58 ± 0.24% respectively in RBL-2H3 cells.	(Ikawati et al., 2001)
	Leaves	Viteosin-A **(48)**, Vitexicarpin **(49)**	Flavonoid	*Ex vivo*	**(48)** and **(49)** respectively reduced histamine-induced tracheal contraction by 27.1% and 66.2% at 0.00013 M and percentage increased to 47.9% and 97.2% respectively when raised to 0.0004 M.	(Alam et al., 2002)
Zataria multiflora Boiss.	Whole plant	Hydro-ethanolic extract	–	*In vitro*	Observed with increment of Treg cells, T_H1/T_H2 ratio, IFN-γ/IL-4 ratio, IFN-γ and $FOXP_3$ expression. Significant reduction of T_H2 and T_H17 cells and decreased expression of IL-4, IL-17 and TGF-β occurred in sensitized mice spleen cells.	(Kianmehr et al., 2017)
	Seeds	Hydro-ethanolic extract	–	*In vivo*	Increased IFN-γ and decreased IL-4 were resulted in ovalbumin-sensitized guinea pigs with the oral administration of extract.	(Boskabady et al., 2013)
				In vitro	Achieved T_H1/T_H2 balance with enhanced ratio of IFN-γ/IL-4 in PHA-stimulated hPBMC.	(Boskabady et al., 2013)

(Shin et al., 2008), *Salvia plebeia* (Shin and Kim, 2002), *Schizonepeta tenuifolia* (Shin et al., 1999) and *Teucrium japonicum* aqueous extract (Kim et al., 2009). Sridevi et al. (2009) highlighted that the ethanolic extract of *Ocimum sanctum* at 400 mg/kg effectively reduced mortality (41%) due to anaphylactic shock-induced bronchospasm in tested subjects with a significant drop ($P < 0.001$) in serum IgE level to 25.80 ± 4.85 ng/ml ($P < 0.001$), as compared to sensitized control (125.06 ± 9.66 ng/ml). These findings confirm that the anti-allergic potential of *O. sanctum* is worthwhile to be further explored.

Over the past two decades, many studies have been conducted on *P. frutescens* species to explore and determine their anti-allergic potential. For example, Makino et al. (2001) isolated rosmarinic acid **(1)** and apigenin 7-*O*-[β-glucuronosyl (2→1) β-glucuronide] (**2**) from *P. frutescens*, which demonstrated anti-allergic activity with potent suppression of PCA reaction in antigen-challenged mice with inhibition of 41% ($P < 0.01$) and 32% ($P < 0.05$) respectively. Meanwhile, Ueda et al. (2002) isolated rosmarinic acid **(1)**, caffeic acid and luteolin **(3)** from the leaves of *P. frutescens* and tested them for respective anti-allergic effects with oxazolone-induced ear edema test. Interestingly, only luteolin **(3)** showed an inhibitory effect on oxazolone-induced ear edema at 1 mg, whereas the other compounds did not show any inhibitory activity (Ueda et al., 2002). However, in a continuation of the work by Makino et al. (2003), the results suggested that the anti-allergic titer of rosmarinic acid **(1)** was 8 folds higher than the conventional anti-allergic drug tranilast, where 19 mg/kg of rosmarinic acid **(1)** was sufficient to achieve an equivalent PCA reaction suppression as 150 mg/kg of tranilast. Such a potent anti-allergic effect from rosmarinic acid **(1)** is certainly exciting and worthy to be further studied in the development of anti-allergic agents. In light of the study by Chen et al. (2015), an OVA-induced murine model of allergic asthma was employed. Results demonstrated a promising reduction in serum IgE level in the OVA-sensitized mice with 320 μg of ethanolic extract of *P. frutescens* leaves and hence amelioration of asthmatic symptoms (Chen et al., 2015). A similar reduction outcome was obtained in the study using *Der f* (*Dermatophagoides farinae*) mite-induced atopic dermatitis murine model with oral administration of *P. frutescens* leaves extract (Komatsu et al., 2016). In the latest work by Kamei et al. (2017), a new active principle, 8-hydroxy-5,7-dimethoxyflavanone (PDMF) **(4)** was isolated from the leaves of *P. frutescens* and demonstrated to have a potent suppressive effect on PCA reaction in anti-DNP IgE-stimulated-BALB/c mice. In addition, sneezing frequency, the allergic rhinitis nasal response, was also reduced with 1.5 mg of PDMF **(4)** after the BALB/c mice were challenged with Japanese cedar pollen grains (Kamei et al., 2017). Considering all these evidences together, *P. frutescens* possesses a great potential to be developed as an effective anti-allergic agent as shown in allergic asthma, atopic dermatitis and allergic rhinitis models.

According to Mizushina et al. (2014), the isolated compound carnosic acid **(5)**, from the *Rosmarinus officinalis* leaves, possesses the ability to suppress PCA reaction at a dose of 100 mg/kg in sensitized mice with percentage inhibition of 67.1%. Interestingly, the inhibition caused by carnosic acid **(5)** was greater than that of tranilast, a frequently used anti-allergic drug. In fact, tranilast at 100 mg/kg only inhibited PCA reaction by 23.9% as compared to 67.1% suppression by carnosic acid **(5)**, which was approximately 2.8 folds stronger than tranilast (Mizushina et al., 2014). Hence, it can be assumed that carnosic acid **(5)** has a strong potential to be used as anti-allergic compound. In another account, *Schizonepeta tenuifolia* demonstrated a 46.26% reduction in serum IgE level with a treatment of 1% of *S. tenuifolia* extract with phosphate buffered saline/olive oil (P/O) in proportion of (9:1) mixture in DNCB-induced BALB/c mice (Choi et al., 2013).

Salvia miltiorrhiza is a perennial herb which is well known to have tanshinones as principal bioactives. This plant is widely employed as traditional remedy, particularly in TCM (Li et al., 2015). Over the past decade, researchers have conducted studies to investigate its anti-allergic effect. Yang et al. (2008) proposed that a dose-dependent reduction of PCA reaction occurred at 25–100 mg/kg of ethanol extract of *S. miltiorrhiza* leaves ($P < 0.001$). When the IgE-stimulated rats were fed with 50 mg/kg of extract, the PCA reaction appeared to decrease by approximately 36.4% (Yang et al., 2008). A study conducted by Trinh et al. (2010a) highlighted the potency of the PCA reaction inhibition exhibited by the active principles of *S. miltiorrhiza* in the following manner: tanshinone I **(6)** (59%) > 15,16-dihydrotanshinone I **(7)** (49%) > tanshinone IIA **(8)** (35%) > cryptotanshinone **(9)** (32%). Another species from the same genus, *S. plebeia*, was reported to suppress *Der f*-induced elevated serum IgE level in BALB/c mice of atopic dermatitis model at a concentration of 100 mg/kg of ethanolic extract (Choi et al., 2014). Therefore, this finding suggests that *S. plebeia* could be a good candidate for atopic dermatitis treatment in future.

For more than 2,000 years, Chinese people have recognized the dried root of *Scutellaria baicalensis* as a very valuable medicinal herb and many people have regarded it as the golden herb due to its diverse medicinal uses. It is traditionally known as Huang-Qin and it is now listed officially in the Chinese Pharmacopoeia (Zhao et al., 2016). It is widely employed in TCM as treatment for cardiovascular diseases and bleeding disorders, such as hematemesis, hematuria and metrorrhagia (Yoon et al., 2009; Chen et al., 2013). In recent years, it has started to emerge as potentially possessing anti-allergic properties as many studies were actively carried out to investigate its anti-allergic effect. Lim et al. (2003) highlighted that *S. baicalensis* root contains active constituents that are particularly useful against allergic diseases. Four flavonoids were isolated from the root of *S. baicalensis*, wogonin (WG) **(10)**, ganhuangenin (GHG) **(11)**, wogonoside (WGS) **(12)** and 3,5,7,2',6'-pentahydroxyl flavanone (PHF) **(13)**. WG **(10)**, GHG **(11)** and WGS **(12)** were found to potently suppress the production of IgE from the concanavalin A (ConA)-stimulated spleen lymphocytes obtained from Sprague–Dawley rats, at 10 and 100 μM, except PHF **(13)**, even when tested with the highest dose (Lim et al., 2003). This outcome could be due to the structural differences with methoxy substitution and their respective positions on the polyphenolic ring (Lim, 2002). Meanwhile, Li et al. (2014) evaluated the efficacy of three different extracts (ethanol, acetone and ethyl acetate extract) of *S. baicalensis* against allergic reactions. Result revealed that ethanol extract showed the most promising outcome among the three extracts. It demonstrated the highest inhibitory activity against mice ear PCA reaction with percentage inhibition of 55.17% at a dose of 1.42 g/kg.

With the same dose, the total serum IgE level was recorded at the lowest level (3.23 ± 1.05 IU/ml) as compared to treatment with the other two extracts and was comparable to the positive control, 0.1 g/kg of sodium cromoglycate (3.19 ± 1.14 IU/ml) (Li et al., 2014). In the latest work of Zhou et al. (2016), another active constituent, oroxylin A **(14)**, isolated from *S. baicalensis*, was reported to cause a potent suppression ($P < 0.01$) in serum IgE levels in OVA-sensitized mice (Zhou et al., 2016). Other than oral administration, the efficacy of topical application on anti-allergic effect was also evaluated. In the study of Kim et al. (2013), the aqueous extract of *S. baicalensis* was topically applied onto DNFB-induced ear swelling and result suggested that the cutaneous reaction significantly reduced ($P < 0.05$) by 31% with 5% of extract (Kim et al., 2013). In a continuation of the study from the same researchers a few years later, in addition to cutaneous reactions, the serum IgE level in DNCB-induced contact dermatitis was also proven to be suppressed by topical treatment of *S. baicalensis* aqueous extract (Kim et al., 2016). Considering these results, *S. baicalensis* showed to be effective against IgE production and thus to have preventive effects towards allergy. Hence, it is suitable to be further developed as natural anti-allergic agent.

Furthermore, Zhou et al. (2014) demonstrated that 2-isopropyl-5-methylphenol (thymol) **(15)** from *Thymus vulgaris* portrayed a dose-dependent reduction trend in the production of IgE with the pretreatment of 4 mg/kg, 8 and 16 mg/kg of thymol **(15)** in OVA-challenged mice. Among the three concentrations used, 16 mg/kg of thymol **(15)** showed a comparable inhibition ($P < 0.01$) of OVA-specific IgE with positive control, dexamethasone (Zhou et al., 2014). Likewise, *Vitex rotundifolia* also showed similar inhibitory activity with 43% suppression in serum IgE level with the treatment of *V. rotundifolia*'s phytoconstituent, 1H,8H-pyrano [3,4-c] pyran-l,8-dione (PPY) **(16)** (Lee et al., 2009).

INHIBITION OF MAST CELLS AND BASOPHILS DEGRANULATION

A few species of Lamiaceae have successfully displayed compelling mast cell stabilizing activity. For instance, Vadnere et al. (2007) evaluated the mast cell stabilizing activity of *Clerodendron phlomidis* using *in vivo* murine system. The mast cell stabilization was achieved by using leaf aqueous extract of *C. phlomidis* in tested mice. Results revealed that 100 mg/kg of extract was able to confer protection as high as 73.25% from mast cell degranulation which was almost comparable to the standard drug, disodium cromoglycate, that exhibited a protection of 83.75% (Vadnere et al., 2007). Furthermore, *O. sanctum* leaves were also studied for their mast cell stabilization activity in the research of Choudhary (2010). The leaves were prepared into ethanolic extract and flavonoidal fraction isolated from ethanolic extract. The albino rats were fed orally with 100 and 200 mg/kg of leaf ethanolic extract after sensitization. The results demonstrated that 62.44 ± 3.80% and 67.24 ± 2.94% of mast cell stabilization activity was respectively recorded at 100 and 200 mg/kg of ethanolic extract. Meanwhile, significant inhibition of mast cell degranulation was also seen with 75 and 150 mg/kg of isolated flavonoidal fraction, which marked with 54.62 ± 1.76% and 60.48 ± 2.72% respectively (Choudhary, 2010). In addition to that, Patel and Deshpande (2011) employed an *in vitro* assay to evaluate the inhibitory activity of mast cell degranulation of *Vitex negundo*. The rat mesenteric mast cells were stimulated with compound 48/80 to induce mast cell degranulation. At the end of experiment, the numbers of intact and disrupted mast cells were counted and compared. Result suggested that the number of intact mast cells was more than that of disrupted mast cells after the pretreatment with 500 μg/ml of aqueous sub-fraction of *V. negundo*. The mast cell protection was significant, which marked with a percentage of 80.99 ± 0.7231% ($P < 0.001$) (Patel and Deshpande, 2011).

In a study conducted by Murata et al. (2010), it was found that *Lycopus lucidus* contains bioactive compounds that contribute to anti-allergic activity through inhibition of hyaluronidase enzyme (Murata et al., 2010). Hyaluronidase is an enzyme that cleaves hyaluronic acid in an extracellular matrix of connective tissue and is well known for being involved in allergic reactions by causing increased capillary permeability (Sakamoto et al., 1980). Inhibition of this enzyme is known to have suppressive effect on mast cell degranulation, which is a hallmark manifestation of allergy (Asada et al., 1997). Therefore, inhibition of hyaluronidase enzyme can thus become one of the targets to prevent the occurrence of allergy. Murata et al. (2010) isolated 22 compounds from dried aerial parts of *L. lucidus*. Amongst the 22 compounds isolated, only six of them were identified to possess hyaluronidase inhibitory activity. Isolated rosmarinic acid **(1)** was previously identified as a good hyaluronidase inhibitor. It was set as the positive control in this study and marked with hyaluronidase inhibition with IC_{50} value of 309 μM. By comparison, the other five constituents were considered as strong hyaluronidase inhibitors with smaller values of IC_{50} as compared to the positive control. The potencies of inhibition of the six phytoconstituents were arranged in a descending manner: clinopodic acid C **(17)** (IC_{50} value: 80.1 μM) > clinopodic acid E **(18)** (IC_{50}: 82.8 μM) > lycopic acid A **(19)** (IC_{50}: 134 μM) > lycopic acid B **(20)** (IC_{50}: 141 μM) and scizotenuin A **(21)** (IC_{50}: 241 μM) > rosmarinic acid **(1)** (IC_{50}: 309 μM) (Murata et al., 2010). This has provided scientific evidence to support future research on *L. lucidus* for its anti-allergic potential. Similarly, in the work of Ippoushi et al. (2000), it was found that the leaf methanolic extract of *Melissa officinalis*, which is also commonly known as lemon balm, possessed the highest hyaluronidase inhibition among 46 plants tested, achieving as low as only 1.0 ± 0.3% of enzyme activity (Ippoushi et al., 2000). The potency of lemon balm in suppressing hyaluronidase is worthy to be further explored, so that it can become an anti-allergic therapeutic in future. Taken together, these outcomes suggest that these species can be used as potential novel anti-allergic therapeutic agents through mast cell degranulation inhibition.

INHIBITION OF ALLERGIC MEDIATORS AND SECRETORY GRANULES

Following mast cell degranulation, various chemical mediators are released from the mast cells or basophils. One of the most

prominent mediators release from mast cells is histamine. Histamine was discovered as a potent vasoactive agent in 1911 by Dale and Laidlaw and recognized as a major contributor to allergic diseases (White, 1990; Xie and He, 2005). Histamine participates in both early and late phase allergic reactions. In the early phase of allergic reactions, the histamine released from degranulated mast cells and basophils triggers an array of acute allergic symptoms which can be seen within minutes. Some of the examples of allergic symptoms are increased vascular permeability causing redness, swelling, itchiness and pain, bronchoconstriction, anaphylaxis, etc. (Jutel et al., 2009). The onset of immediate allergic reactions is followed by the late phase responses (LAR) which contribute to more sustained inflammation. During the LARs, histamine acts as the chemoattractant to effector cells, notably T_H2 lymphocytes, eosinophils, and basophils for recruitment to the inflammatory site and hence is implicated in the pathogenesis of late phase chronic allergic inflammatory reactions (He et al., 1997; Galli et al., 2008).

In addition, β-hexosaminidase is another popular biomarker used to evaluate mast cell degranulation in many the allergy studies (Kuehn et al., 2010). Similar to histamine, it is produced and stored as secretory granules within mast cells and basophils. However, unlike histamine, it does not have any significant involvement or contribution to allergic reactions. Instead, β-hexosaminidase was reported to have the ability to confer host defense against bacterial infection (Fukuishi et al., 2014). Nevertheless, it is widely employed as a mast cell degranulation marker because it is released together with histamine during the degranulation process (Cota et al., 2012; Huang et al., 2016). As compared to histamine, the release of β-hexosaminidase is slower and persists for a longer time. This makes β-hexosaminidase a better indicator for mast cell degranulation detection than histamine (Huang et al., 2016). Apart from histamine and β-hexosaminidase, mast cells also contain other mediators like eicosanoids, such as prostaglandin D_2 and leukotrienes C_4, chemotactic factors and immunoregulatory cytokines (Xiang et al., 2001; Parikh et al., 2003). During degranulation, these substances are also released from mast cells and basophils through exocytosis. Therefore, they are also used as biomarkers of mast cell degranulation in the allergy studies.

Anti-allergic activity of plants can be evaluated through the potency of the plant in suppressing the mediators, secretory granules or any functional changes induced by the mediators as aforementioned. In a study by Vadnere et al. (2007), a significant inhibition ($P < 0.05$) in histamine-induced tracheal contraction was seen at concentrations of 4 and 10 mg/ml of leaf aqueous extract of *Clerodendron phlomidis* on isolated goat tracheal chain. At the same time, intraperitoneally administered leaf aqueous extract of *C. phlomidis* at concentrations of 100 mg/kg showed a potent reduction ($P < 0.05$) in histamine-induced vascular leakage in sensitized murine model (Vadnere et al., 2007). Lee et al. (2011) explored the possibility of *C. trichotomum* on anti-asthmatic potential employing *in vivo* guinea pig model. A phenylpropanoid glycoside, acteoside **(22)** was isolated from the ethyl acetate fraction from *C. trichotomum* leaves. At dose 25 mg/kg, specific airway resistance (sRaw) was significantly ($P < 0.05$) inhibited in immediate phase response (IAR) and LAR in ovalbumin-challenged guinea pigs by 32.14% and 55.88% respectively. The result seemed promising as it was superior to positive controls, 5 mg/kg dexamethasone (55.88%) and 10 mg/kg disodium cromoglycate (52.94%) in LAR. Meanwhile, concentration of 50 mg/kg of acteoside **(22)** was significantly ($P < 0.05$) against the histamine release and phospholipase A_2 (PLA_2) activity in BALF in which the histamine content was marked with 26.40 ± 1.96% and PLA_2 activity recorded with only 28.08 ± 2.05% (Lee et al., 2011b).

Park et al. (2010) investigated the anti-allergic potential of *Clinopodium gracile* using both *in vivo* and *in vitro* studies. In the *in vivo* study, the mice were treated with compound 48/80 to induce systemic anaphylaxis after the pretreatment with intraperitoneal administration of aqueous extract of *C. gracile* with concentrations ranging from 1–100 mg/kg. The mortality of mice due to anaphylactic shock was assessed. Results revealed that the systemic anaphylactic death event was reduced concentration-dependently, in which the doses of 50 mg/kg and above were identified as the effective doses that prevented the mice from fatal anaphylactic shock. The reduction of systemic anaphylaxis corresponded to the reduced serum histamine. On the other hand, aqueous extract of *C. gracile* was also shown to significantly ($P < 0.05$) suppress the release of histamine in *in vitro* RPMC and HMC-1 cells assays. The suppressive effect occurred in a dose-dependent manner with the concentrations ranging from 1–100 μg/ml. As aforementioned, calcium influx is pivotal in the releasing of secretory granules and mediators from mast cell degranulation. Therefore, a reduction of intracellular calcium level can result in the inhibition of chemical mediators such as histamine and β-hexosaminidase release (Oka et al., 2005). In the same study, intracellular calcium level was also evaluated in HMC-1 cell line. Results showed that pretreatment with aqueous extract of *C. gracile* caused a suppression in intracellular calcium level induced by PMACI. Hence, this finding is supported by the theory which suggests the involvement of intracellular calcium in the inhibition of histamine release from mast cells. In addition, mast cell-mediated hypersensitivity also occurs with the involvement of NF-κB and inflammatory cytokines. NF-κB activation is essential to regulate downstream pro-inflammatory cytokine expression, such as TNF-α and IL-6 which play a critical role in initiating and sustaining the allergic inflammatory responses (Blackwell and Christman, 1997). Therefore, the attenuation of NF-κB activation causes a reduction in downstream inflammatory cytokines gene expression and hence produces suppressive effects in allergic inflammation. NF-κB-dependent transcriptional activity was evaluated through luciferase activity assay. Results revealed that treatment with aqueous extract significantly ($P < 0.05$) inhibited the activation of NF-κB and its downstream cytokine expression (TNF-α and IL-6) at doses of 1 and 10 μg/ml. These findings provide evidence that *C. gracile* is has the potential to be developed as an anti-allergic agent in the future, given its potential in reducing allergic inflammation (Park et al., 2010). Similarly, aqueous extracts of *Dracocephalum argunense* (Kim and Shin, 2006; Kim et al., 2006), *Elsholtzia ciliata* (Kim et al., 2011), *Isodon japonicus* (Kim et al., 2004;

Shin et al., 2004), *Lycopus lucidus* (Shin et al., 2005), *Mentha arvensis* (Shin, 2003), *Mosla chinensis* (Kim et al., 2012), *M. dianthera* (Lee et al., 2006), *Perilla frutescens* (Shin et al., 2000), *Phlomis umbrosa* (Shin et al., 2008), *Pogostemon cablin* (Yoon et al., 2016), *Prunella vulgaris* (Shin et al., 2001), *Salvia plebeia* (Shin and Kim, 2002), *Schizonepeta tenuifolia* (Shin et al., 1999), *Stachys riederi* (Shin, 2004), *Teucrium japonicum* (Kim et al., 2009) and *Vitex rotundifolia* (Shin et al., 2000) also exhibited similar outcomes as portrayed in the experiment conducted using *C. gracile*.

In another account, Inoue et al. (2001) prepared four different extracts and separated fractions (50% ethanolic extract, water eluate, 50% ethanolic eluate and ethanolic eluate) from *M. piperita* leaves to investigate their anti-allergic potential on allergic rhinitis. It was found that 50% ethanolic eluate exhibited the most potent inhibition to histamine release, with an IC_{50} value of 2.55 μg/ml and exerted its antagonizing effect on nasal responses at doses of 300 and 1,000 mg/kg in antigen-challenged rats (Inoue et al., 2001). In the continuation of their previous work, Inoue et al. (2002) focused on the 50% ethanolic eluate to isolate the active compounds responsible for the anti-allergic effect. Following the isolation of the compounds, a total of eight chemical constituents were considered. However, among the eight compounds, luteolin-7-*O*-rutinoside **(31)** was found to be the most effective in suppressing histamine release from compound 48/80-induced RPMC with IC_{50} value of 21.9 μM. The nasal responses were reduced at doses of 100 and 300 mg/kg of luteolin-7-*O*-rutinoside **(31)** (Inoue et al., 2002). Recently, Sato and Tamura (2015) evaluated anti-allergic activity of *M. piperita* leaves using β-hexosaminidase assay. Five major flavonoids components, 5,6,4'-trihydroxy-7,8-dimethoxyflavone **(32)**, 5,6,4'-trihydroxy-7,8,3'-trimethoxyflavone **(33)**, 5,6-dihydroxy-7,3',4'-trimethoxyflavone **(34)**, 5,6-dihydroxy-7,8,3',4'-tetramethoxy flavone **(35)** and 5,6-dihydroxy-7,8,4'-trimethoxyflavone **(36)** isolated from leaves dichloromethane extract were all shown to possess potent anti-allergic activity. However, 5,6,4'-trihydroxy-7,8,3'-trimethoxyflavone **(33)** and 5,6-dihydroxy-7,8,3',4'-tetramethoxyflavone **(35)** proved to have the strongest inhibitory activity on β-hexosaminidase release from RBL-2H3 cells, which were recorded with IC_{50} values of 2.4 and 3.0 μM respectively. Their safety profiles also seemed promising as they had relatively lower cytotoxicity than another typical natural anti-allergic substance, luteolin **(3)** (Sato and Tamura, 2015). Therefore, this might indicate that 5,6,4'-trihydroxy-7,8,3'-trimethoxyflavone **(33)** and 5,6-dihydroxy-7,8,3',4'-tetramethoxyflavone **(35)** can be potentially developed into safe and effective anti-allergic agents. Another species from the same genus, *M. spicata*, was shown to have similar activity with its flavones, 5,6,4'-trihydroxy-7,8,3'-trimethoxyflavone **(33)** and 5,6-dihydroxy-7,8,3',4'-tetramethoxyflavone **(35)** and aliphatic glycoside, (3R)-1-octan-3-yl β-D-glucopyranoside **(37)**. The potency of inhibitory activities was arranged in a descending manner: 5,6,4'-trihydroxy-7,8,3'-trimethoxyflavone **(33)** with IC_{50} of 6.4 μM > 5,6-dihydroxy-7,8,3',4'-tetramethoxyflavone **(35)** with IC_{50} of 56 μM > (3R)-1-octan-3-yl β-D-glucopyranoside **(37)** with IC_{50} of 560 μM. Although (3R)-1-octan-3-yl β-D-glucopyranoside **(37)** showed the weakest activity, it was however a great discovery as this was the first time an anti-histaminic activity from an aliphatic glycoside was recorded (Yamamura et al., 1998).

In 2007, Cariddi et al. investigated the anti-allergic activity of *Minthostachys verticillata*. The experiment was conducted using basophils from allergic patients pretreated with essential oil extracted from the stems and leaves of *M. verticillata* and followed by β-hexosaminidase assay. Results suggested that the essential oil showed a promising suppressive effect on β-hexosaminidase release with percentage inhibition ranging from 32.15% to 39.72%, which was comparable to dexamethasone (39.75%) and theophylline (41.63%) (Cariddi et al., 2007). A few years later, Cariddi et al. (2011) carried out an in-depth study on the components of the essential oil extracted from *M. verticillata* to identify the constituent responsible for anti-allergic effect. Limonene **(38)**, pulegone **(39)** and menthone **(40)** were found to be present in the essential oil. Results suggested that limonene **(38)** appeared to be the most effective compound in the inhibition of β-hexosaminidase release from human basophils as compared to the other two compounds, pulegone **(39)** and menthone **(40)**. It was shown that 20 μg/ml of limonene **(38)** was able to achieve inhibitory effect while the other two compounds both required a higher concentration of 40 μg/ml to reach the desired inhibition. The inhibitory effect could be achieved at a lower concentration (10 μg/ml) when the compounds were used in combination (Cariddi et al., 2011). This can be explained with the synergistic effect of the three compounds.

Jeon et al. (2014) highlighted that luteolin **(3)** isolated from the leaves of *P. frutescens* potently ($P < 0.05$ to $P < 0.001$) suppressed histamine release from compound 48/80-stimulated RPMC at 5, 10 and 20 μM in a dose-related manner. The expression and production of TNF-α and IL-1β were significantly ($P < 0.05$ to $P < 0.001$) reduced in PMACI-stimulated HMC-1 cells, with inhibition rates of 31.9%–76.8% and 27.3%–81.2% respectively, at the range of 5–20 μM of luteolin **(3)** (Jeon et al., 2014). Likewise, Ueda et al. (2002) also reported a similar activity on the TNF-α production with a dose range of 1–1,000 μg of luteolin **(3)** in sensitized mice. A the same time, *in vivo* studies conducted on ICR mice demonstrated that compound 48/80- or serotonin-induced scratching behavior and vascular permeability were dose-dependently decreased at concentrations of 5, 10 and 20 mg/kg of luteolin **(3)** (Ueda et al., 2002). According to a study conducted by Heo et al. (2011) on the anti-atopic effect of *P. frutescens*, the aqueous fraction demonstrated a promising outcome whereby the DNFB-sensitized mice experienced a 35% reduction in ear swelling symptoms with the administration of 100 μg/ml. In light of the study carried out by Zhu et al. (2014), rosmarinic acid methyl ester **(41)** was found to be able to produce a stronger β-hexosaminidase release inhibition than that of rosmarinic acid **(1)** extract prepared from *P. frutescens* leaves using supramolecular technique, in which the IC_{50} values were marked with 9.9 ± 0.8 and 52.9 ± 6.7 μg/ml respectively. Furthermore, Kamei et al. (2017) suggested that PDMF **(4)** significantly ($P < 0.05$) inhibited histamine release from RBL-2H3 cells with an IC_{50} value of 68.5 μM which was much more potent than other polyphenols. Hence, this finding deduced that PDMF **(4)** is a newly emerged potent anti-allergic component.

In light of a study by Ryu et al. (2000), 2α,3α-dihydroxyurs-12-en-28-oic acid **(42)** isolated from the methanolic extract of *Prunella vulgaris* possessed a significant ($P < 0.01$) inhibitory activity on β-hexosaminidase release from RBL-2H3 cells with IC_{50} of 57 μM. Another histamine release assay conducted recently by Choia et al. (2016) intended to provide more evidence on the anti-allergic effect of *P. vulgaris*. The results suggested that three triterpenoids isolated from the spike of *P. vulgaris* exhibited significant inhibitory effects on histamine release from HMC-1 cells. The active principles represented by β-amyrin **(43)**, 2α,3α,23-trihydroxyursa-12,20(30)-dien-28-oic acid **(44)** and euscaphic acid **(45)** demonstrated a percentage inhibition of 46.7%, 57.9% and 54.2% respectively. It is noteworthy that 2α,3α,23-trihydroxyursa-12,20(30)-dien-28-oic acid **(44)** was isolated for the first time and possessed promising activity against histamine release (Choia et al., 2016). Putting all the evidence together, *P. vulgaris* is experimentally proven to have high potential to be developed into efficacious anti-allergic agent. On the other hand, Mizushina et al. (2014) highlighted that carnosic acid **(5)** from *Rosmarinus officinalis* leaves significantly inhibited the release of β-hexosaminidase from RBL-2H3 cells at 10 μM.

Likewise, Choi and Kim (2004) and Ryu et al. (1999) reported that 15,16-dihydrotanshinone-I **(7)** and cryptotanshinone **(9)** isolated from the root of *S. miltiorrhiza* showed to have IC_{50} values of 16 ± 2.4 and 36 ± 3.7 μM on β-hexosaminidase release. Additionally, the ethanolic extract of *S. miltiorrhiza* also inhibited other chemical mediators, such as leukotriene C_4 with a IC_{50} value of 2.6 μg/ml, COX-1 and COX-2-dependent prostaglandin D_4 with IC_{50} values of 3.96 and 21.54 μg/ml respectively (Yang et al., 2008). With the aid of findings from Yang et al. (2008), Li et al. (2015) successfully identified 15,16-dihydrotanshinone-I **(7)** as the compound responsible for the inhibition of intracellular calcium level, hence suppressing the downstream release of leukotriene C_4 and prostaglandin D_4 from activated bone marrow-derived mast cells (BMMC). The inhibition percentage achieved was as high as 90% with a dose of 20 μM of 15,16-dihydrotanshinone-I **(7)** (Li et al., 2015).

In addition, the four active constituents isolated from *Scutellaria baicalensis* root, wogonin (WG) **(10)**, ganhuangenin (GHG) **(11)**, wogonoside (WGS) **(12)**, 3,5,7,2',6'-pentahydroxyflavanone (PHF) **(13)**, all showed a significant suppression of histamine ($P < 0.01$) and leukotriene B_4 ($P < 0.01$) release from A23817-induced rat peritoneal exudate cells (PEC) at a concentration of 100 μM (Lim, 2003). Moreover, Kim et al. (2010) reported that baicalin **(46)** isolated from the roots of *S. baicalensis* possessed a significant ($P < 0.001$) inhibitory activity of histamine and leukotriene release from OVA-sensitized guinea pig lung mast cells at 10, 30 and 60 μg. The histamine inhibition rates recorded with 47.1%, 59.4% and 61.5% while leukotriene production remarkably suppressed by 37.9%, 47.3% and 50.4% with the pretreatment of the three different doses of baicalin **(46)**. Interestingly, studies showed that the standardized extract of baicalin **(46)** at high dose produced a greater inhibition on both histamine and leukotriene production as compared to pure baicalin **(46)**. Results revealed that 70.4% of histamine suppression and 78% of leukotriene inhibition ($P < 0.001$) were found at high dose (60 μg) of standardized extract. However, the percentage inhibitions produced by low and medium dose standardized extract treatments were smaller than that of pure baicalin **(46)** (Kim et al., 2010). In a very interesting study conducted by (Trinh et al., 2010b), baicalin **(46)** isolated from rhizome of *S. baicalensis* was metabolized into baicalein **(47)** and oroxylin A **(14)** followed by oral administration. All three compounds possessed inhibitory activity against histamine-induced scratching behavior and vascular permeability in *in vivo* ICR mice model at doses of 20 and 50 mg/kg. However, the metabolite, oroxylin A **(14)** showed the most potent inhibition instead of its parent compound, baicalin **(46)**. In the study of Li et al. (2014), it was suggested that ethanol extract potently inhibited ($P < 0.01$) 4-AP-induced allergic skin pruritus, histamine-induced mice paw swelling and cutaneous anaphylactic-ear swelling at 1.42 g/kg. For instance, ear swelling inhibition produced by 1.42 g/kg of ethanol extract (47.10%) was superior to that of the positive control, 0.1 g/kg of cromolyn sodium (32.43%) (Li et al., 2014). In a food allergy study, 25 mg/kg of ethanol extract of *S. baicalensis* conferred 60% protection to food allergy anaphylactic death in OVA-sensitized mice (Shin et al., 2014b). Henceforth, these promising experimental outcomes make the anti-allergic potential of *S. baicalensis* worthwhile to be further explored.

REGULATION OF T CELL RESPONSES

It has been long recognized that allergic sensitization is led by lymphocytes (Zhang et al., 2014). There are various types of T lymphocytes, such as $CD4^+$, $CD8^+$, and natural killer T cells, in which each population produces response to allergens with different capacities. Among the different types of T lymphocytes, $CD4^+$ T cells are predominantly implicated in the pathogenesis of allergy. With the expression of major histocompatibility complex (MHC) class II molecules and allergen specific T-cell receptors (TCR), $CD4^+$ T cells are able to recognize peptide antigen presented by antigen-presenting cells (APCs). Antigen recognition is then led to the activation of downstream immune responses, such as allergic inflammatory cascade (Kallinich et al., 2007; Woodfolk, 2007).

Naive $CD4^+$ T cells can be differentiated towards T_H1, T_H2, T_H17 and Treg phenotypes (Zhang et al., 2014). T_H2 subsets are particularly renowned as the major contributors to the immunopathology of allergy. Late-phase allergic responses are provoked by the persistent existence of allergens, leading to T-cell activation (Palomares et al., 2010). Activated T_H2 lymphocytes play a critical role in the production of T_H2 cytokines (IL-4, IL-5, IL-9 and IL-13) (Deo et al., 2010; Ozdemir et al., 2010). IL-4 and IL-13 are crucial to the development of T_H2 cells and induction of IgE isotype switching from B cells, which are the major risk factor for the development of allergic asthma (Steinke and Borish, 2001; Woodfolk, 2007). IL-5 mainly mediates eosinophil recruitment and increases eosinophil survival while IL-9 stimulates mast cells and basophils (Woodfolk, 2007; Ozdemir et al., 2010). In contrast, T_H1 cells differentiation occurs in response to microbial activation of APC under the influence of IL-12 (Deo et al., 2010). These cells orchestrate the production of IL-2, IFN-γ and TNF-β (Romagnani, 2004). T_H1 cytokines tend

to produce pro-inflammatory responses which are important in killing phagocytosed microbes and perpetuating autoimmune responses (Berger, 2000). Recent evidence demonstrate that minimal microbial exposure in early life causes the T_H1/T_H2 balance in the immune system to skew towards the pre-allergic T_H2 response (Berger, 2000; Deo et al., 2010).

Recently, Treg cells emerged as the key component in the sensitization stage of allergy (Zhang et al., 2014). The transcriptional factor, $FOXP_3$ serves as a lineage specification factor of Treg cells which is required for the differentiation of Treg cells (Rudensky, 2011; Noval Rivas and Chatila, 2016). $FOXP_3$, which is dominantly expressed by Tregs, inhibits T_H2 cells activation, thus reducing the production of T_H2 cytokines, which is essential during the effector phase of allergic reactions (Albano et al., 2013). Treg cells also suppress allergic inflammation through direct action on mast cells, basophils and eosinophils (Palomares et al., 2010; Noval Rivas and Chatila, 2016). It has been shown that constitutive $FOXP^+$ Treg controls the symptomatic phase of mast cell activation and IgE-dependent anaphylaxis in mice (Kanjarawi et al., 2013). Apart from that, T_H17 cells and their corresponding cytokine, IL-17, are also highlighted for their involvement in the progression of T_H2-mediated allergic disorders (Oboki et al., 2008). A balance between T_H17 and Tregs is essential for immune homeostasis. Excessive or exaggerated T_H17 function and elevated T_H17 cells together with a defect in Treg function or reduction in Treg population lead to the development of allergic disorders, like allergic asthma and rhinitis. Restoring the balance between T_H17 and Treg can promote the resolution of allergic disorders, such as allergic inflammation seen in allergic asthma (Albano et al., 2013).

Natural compounds with the ability to regulate T cell responses have a great potential in the development of novel anti-allergic therapeutic agents. In this review, there are several plants described to have compelling effects on the down-regulation of T_H2 cytokines, re-establishment of T_H1/T_H2 balance, T_H17 inhibition and promotion of Treg cells functions. For example, 100 mg/kg of ethanolic extract of *Mentha haplocalyx* was reported to exhibit significant ($P < 0.05$) inhibition on the expression and production T_H2 cytokines (IL-4 and IL-5) in BALF (Lee et al., 2011a). Besides, Costa et al. (2012) evaluated the effectiveness of *Ocimum gratissimum* in alleviating allergic asthma. Results revealed that *Blomia tropicalis* mite-immunized and challenged mice receiving 100 mg/kg of *O. gratissimum* methanolic extract had a significant reduction ($P < 0.05$) in IL-4 level in BALF in relation to those of the untreated group (Costa et al., 2012). In addition, Trinh et al. (2010a) stated that tanshinone I **(6)**, 15,16-dihydrotanshinone I **(7)**, tanshinone IIA **(8)** isolated from *Salvia miltiorrhiza* possessed inhibitory activity on IL-4 and TNF-α expression in IgE-antigen complex-stimulated RBL-2H3 cells at a dose of 50 μM. The ability of *S. miltiorrhiza* in inhibiting IgE-switching cytokine, IL-4 and pro-inflammatory cytokine, TNF-α is thought to be a spring for allergic reactions improvement (Trinh et al., 2010a). Another species from the same genus, *S. plebeia,* was reported to reduce symptoms of atopic dermatitis through regulation of T cells responses (Choi et al., 2014). At a concentration of 100 mg/kg of ethanol extract of *S. plebeia*, the expression of T_H1, T_H2 and T_H17 cytokines was significantly reduced ($P < 0.05$) in *Der-f*-induced atopic dermatitis-like skin lesions (Choi et al., 2014). On the other hand, *Schizonepeta tenuifolia* demonstrated a significant reduction ($P < 0.05$) of IFN-γ, IL-4 and IL-13 in IgE-induced allergic model of RBL-2H3 cells with 100 μg/ml of aqueous extract. In the same study, *S. tenuifolia* aqueous extract showed no cytotoxicity even at a higher concentration up to 1,000 μg/ml (Lin et al., 2018). However, a more thorough and detailed toxicological investigation is required to develop a more evidence-based safety profile.

In another account, *Perilla frutescens* also suppressed T_H2 responses which then contributed to anti-allergic effect (Sanbongi et al., 2004). Results demonstrated that the expression and production of T_H2 cytokines (IL-4 and IL-5) were potently inhibited by daily oral administration of 1.5 mg of rosmarinic acid **(1)** from *P. frutescens* ($P < 0.05$) in *in vivo* house mite-challenged murine model (Sanbongi et al., 2004). Meanwhile, Chen et al. (2015) reported that IL-5 and IL-13 were diminished in OVA-induced allergic asthma murine model with ethanolic extract of *P. frutescens* leaves. Interestingly, aqueous fraction of *P. frutescens* leaves showed its effectiveness in alleviating atopic dermatitis through balancing of T_H1 and T_H2 cells (Heo et al., 2011). The effect was achieved by suppressing the release of IL-31, which is a T_H2 cytokine that promotes allergic symptoms like pruritus and allergic skin disorders (Heo et al., 2011; Meng et al., 2018). At the same time, the T-bet protein expression was augmented with 100 μg/ml of *P. frutescens* aqueous fraction (Heo et al., 2011). T-bet protein expression is essential for T_H1 cell differentiation (Chatila et al., 2008). Therefore, the combination of suppressed IL-31 and augmented T-bet protein expression resulted in T_H1/T_H2 balance and hence alleviation of allergic symptoms. Besides, Komatsu et al. (2016) suggested that the $CD4^+/CD8^+$ ratio in splenic T lymphocytes obtained from *Der f*-induced atopic dermatitis NC/Nga mice was decreased from 1.82 ± 0.32% to 1.50 ± 0.07% after receiving treatment of *Perilla* leaves extract. This suppression is believed to be associated with the T_H1 and T_H2 balance (Komatsu et al., 2016). Additionally, Liu et al. (2013) employed an *in vitro* assay to investigate the effect of *P. frutescens* extract on *Der p* 2-challenged human bronchial epithelial cells BEAS-2B cells. Result displayed that the pro-allergic cytokines (IL-4, IL-5, IL-13) and GM-CSF productions were all dose-dependently reduced ($P < 0.05$) in *Der p* 2-stimulated BEAS-2B cells with treatment of 5–50 μg/ml of methanolic extract of *P. frutescens* leaves (Liu et al., 2013).

In a recent work by Bae et al., 2016, *Scutellaria baicalensis* showed to be effective in ameliorating ovalbumin-induced food allergy murine model through the regulation of Treg cells with its natural flavonoid compound, baicalein **(47)**. Results revealed that baicalein **(47)** was able to increase Treg cells population through induction of $CD4^+$ $FOXP_3^+$ T cell differentiation without causing any cytotoxicity at concentrations smaller than 10 μmol/L (Bae et al., 2016). In light of a study by Jung et al. (2017), linoleic acid **(50)** in hexane fraction from ethanol extract of *S. baicalensis* roots was reported to cause a significant suppression ($P < 0.05$) in the production of pro-allergic cytokines (IL-4, IL-5, IL-10 and IL-13) and enhancement of secretion of T_H1 cytokines (IFN-γ ($P < 0.05$)

and IL-12 ($P < 0.01$)). The fact that a fatty acid from a plant can contribute anti-allergic effect through restoration of T_H1/T_H2 balance is a new discovery (Jung et al., 2017). A similar outcome was noted in a food allergy murine model conducted by Shin et al. (2014b) with *S. baicalensis* ethanol extract. Interestingly, the ethanol extract also down-regulated the IL-17 level produced by T_H17 and hence produced effective prevention to food allergy (Shin et al., 2014b). Within the same year, the same group of researcher successfully discovered that the active compounds isolated from *S. baicalensis*, represented by wogonin (WG) **(10)**, baicalin **(46)** and baicalein **(47)**, all inhibited the T_H2 cytokines (IL-4, IL-5, IL-10 and IL-13) and IFN-γ at dose of 50 μmol/L in an *ex vivo* study. However, only WG **(10)** was able to produce inhibition without affecting cell viability as compared to the other two active compounds (Shin et al., 2014a). This finding suggests that WG **(10)** could be a safer anti-allergic compound at a higher dose, but its toxicity profile should be further investigated with more extensive toxicity testings. Kim et al. (2010) focused on the therapeutic potential of *S. baicalensis* on atopic dermatitis. In the experiment, the male NC/Nga mice were orally fed with aqueous extract of *S. baicalensis* after atopic dermatitis-like skin lesion was conventionally developed in the mice. The feeding process was continued for 6 weeks. After 6 weeks, blood was drawn from the mice and analyzed to measure the level of cytokine release. Interestingly, the results revealed that *S. baicelensis* aqueous extract only exhibited specific inhibition on IL-5 ($P < 0.05$) at a dose of 50 mg/kg. There was no significant change in the level of IL-4 and IL-10 between *S. baicalensis* treatment group and control group (Kim et al., 2010). However, in the study by Kim et al. (2016), it was proposed that topical application of aqueous extract of *S. baicalensis* onto the DNCB-induced contact dermatitis can significantly ($P < 0.05$) reduce the level of IL-4 and IFN-γ in BALB/c mice skin cells. Zhou et al. (2016) reported that oroxylin A **(14)** isolated from *S. baicalensis* was effective against allergic asthma with significant inhibition ($P < 0.01$) of IL-4, IL-5 and IL-13 production at doses of 15, 30 and 60 mg/kg of oroxylin A **(14)**. In conclusion, *S. baicalensis* has good therapeutic potential in allergic diseases, such as atopic dermatitis, food allergy and allergic asthma through regulation of T cell responses.

With the intention to explore the effectiveness of 2-isopropyl-5-methylphenol (thymol) **(15)** isolated from *Thymus vulgaris* in alleviating allergic asthma, Zhou et al. (2014) employed an *in vivo* allergic murine model of asthma. Results revealed that thymol **(15)** effectively reduced the symptoms of allergic asthma through dose-dependent inhibition of IL-4, IL-5 and IL-13 production at 4, 8 and 16 mg/kg in OVA-challenged mice. Particularly, a potent reduction ($P < 0.01$) of OVA-induced T_H2 cytokines was recorded at 16 mg/kg of thymol **(15)** (Zhou et al., 2014). Apart from thymol **(15)**, carvacrol **(51)** from *T. vulgaris* was reported to effectively inhibit the production of IL-2, IFN-γ, IL-4 and IL-17 cytokines as well as T-bet expression in the *ex vivo* splenocytes cultures (Gholijani and Amirghofran, 2016). On the contrary, both thymol **(15)** and carvacrol **(51)** led to an increase in the level of IL-10 and TGF-β in mice splenocytes cultures (Gholijani and Amirghofran, 2016). Henceforth, the regulation of T cell responses that was shown by thymol **(15)** and carvacrol **(51)** suggests that these two compounds potentially benefit allergic disorders. Likewise, Lee et al. (2009) also highlighted that the active constituent of *Vitex rotundifolia*, 1H,8H-pyrano [3,4-c] pyran-l,8-dione (PPY) **(16)** potently suppressed the IL-5 production in BALF by 34%. Furthermore, hydro-ethanolic extract prepared from *Zataria multiflora* seeds was reported to restore T_H1/T_H2 balance by enhancing the ratio of IFN-γ/IL-4 in *in vivo* and *in vitro* assays (Boskabady et al., 2013). Another study conducted by Kianmehr et al. (2017) further supported the above finding. In addition to enhanced IFN-γ/IL-4 ratio, *Z. multiflora* hydro-ethanolic extract also caused potentiation of T_H1 and suppression effect on T_H2 and T_H17 cells, which led to therapeutic effect on allergic asthma in ovalbumin-sensitized BALB/c mice. Results showed that the number of Treg cells ($P < 0.001$), T_H1/T_H2 ratio ($P < 0.001$), IFN-γ/IL-4 ratio ($P < 0.01$), IFN-γ ($P < 0.05$) and $FOXP_3$ ($P < 0.001$) expression were increased significantly. Meanwhile, T_H2 and T_H17 cells ($P < 0.01$ to $P < 0.001$), IL-4, IL-17 and TGF-β ($P < 0.05$ to $P < 0.001$) expressions were significantly reduced in sensitized mice spleen cells (Kianmehr et al., 2017). In conclusion, *Z. multiflora* is able to produce anti-allergic therapeutic effects on type I hypersensitivities like allergic rhinitis, allergic asthma and urticarial by regulating T cell responses.

SUPPRESSION OF EOSINOPHILS MIGRATION

Eosinophils are long-lived circulating granulocytes which play a central role in promoting allergic reactions. They arise and differentiate in bone marrow and are then widely distributed in the blood, lungs, thymus, uterus, adipose tissues, spleen, etc and lastly readily migrate to the allergic sites (Fulkerson and Rothenberg, 2013; Wen and Rothenberg, 2016). They migrate to the target sites with the influence of chemoattractant, eotaxin and particularly, IL-5 for eosinophil proliferation, survival and priming (Wen and Rothenberg, 2016). Unlike mast cells and basophils with extensive expression of high affinity IgE receptors, eosinophils have a minimal expression of FcεRI. Nevertheless, they express a great range of cell surface molecules, such as receptors for IgG and IgA, complement receptors, cytokine receptors, whose aggregation can trigger eosinophils activation and development (Rigoni et al., 2018). Often, the number of eosinophils is greatly elevated in circumstances associated with allergic disorders, such as allergic rhinitis, allergic asthma and atopic dermatitis (Stone et al., 2010). Henceforth, natural products with the ability to suppress eosinophil recruitment or infiltration to allergic sites can be the noteworthy pharmacological therapeutic options for allergic disorders.

Several Lamiaceae plant species have demonstrated their suppressive effects on the eosinophil recruitment. Vadnere et al. (2007) showed that intraperitoneally administration of 100 mg/kg leaf aqueous extract of *Clerodendron phlomidis* produced a potent antagonizing effect towards milk-induced eosinophilia by showing a marked reduction in blood eosinophil count in mice with the value of 13.8 ± 2.4 as compared to the control group (43.1 ± 1.25). This reduction is indicative of the effectiveness of *C. phlomidis* in conferring anti-allergic effect. Another species under the same genus, *C. serratum*, was evaluated by Bhangare et al. (2012) to study the anti-allergic effect of its aqueous extract

prepared from root and stem using different concentrations. Results demonstrated that all extracts used possessed inhibitory activity on milk-induced leukocytosis. However, the most potent activity was shown by high dose (260 mg/kg) aqueous root extract as compared to low dose (130 mg/kg) or any doses of aqueous stem extracts. This extract accorded a more significant protection than the standard used, dexamethasone from milk-induced leukocytosis (Bhangare et al., 2012). This finding suggests that *C. serratum* might be a potential candidate for anti-allergic therapy. Moreover, Lee et al. (2011b) reported that acteoside **(22)** isolated from *C. trichotomum* at 25 mg/kg significantly ($P < 0.05$) reduced total leukocytes in BALF from $31.25 \pm 6.12 \times 10^5$ to $25.23 \pm 4.72 \times 10^5$. At 50 mg/kg, 29.70% of eosinophil infiltration suppression was achieved in guinea pigs model (Lee et al., 2011b). Similar outcome was seen with 200 µl/kg of *Mentha arvensis* essential oil (Sharma et al., 2018) and 100 mg/kg of ethanolic extract of *M. haplocalyx* in OVA-challenged mice (Lee et al., 2011a). In an attempt to investigate the effect of *Ocimum gratissimum* in alleviating respiratory allergy, Costa et al. (2012) carried out an experiment through *in vivo* model using male A/J mice. The mice were orally fed with 25, 50 or 100 mg/kg of methanolic leaves extract after subcutaneously injecting *Blomia tropicalis* antigen for respiratory allergy induction. Results revealed that *O. gratissimum* significantly suppressed the eosinophil counts ($P < 0.05$) in BALF as well as eosinophil peroxidase ($P < 0.01$) level at a dose of 100 mg/kg. The same concentration of methanolic extract *O. gratissimum* also attenuated mucus hypersecretion in airway, which is one of the features of allergic asthma (Costa et al., 2012). Henceforth, this suggests that *O. gratissimum* is effective against respiratory allergy disorders, like asthma.

Perilla frutescens is a Lamiaceae plant species which has a global distribution, but is especially concentrated in Asian countries, like China, Japan, Korea and Vietnam among others. In an effort to explore the isolate from *P. frutescens* with anti-allergic activity on respiratory allergy, Sanbongi et al. (2004) evaluated the isolate from the leaves of *P. frutescens*. Results from this study suggested that the isolated compound, rosmarinic acid **(1)**, can bring about a significant reduction in eosinophil infiltration ($P < 0.05$), BALF eosinophil counts ($P < 0.01$) and eotaxin expression ($P < 0.05$) in the *Der f*-challenged C3H/He mice (Sanbongi et al., 2004). Parallel activity was seen in OVA-sensitized BALB/c mice with its ethanolic extract in the study of Chen et al. (2015). On top of that, in an anti-atopic study carried out by Heo et al. (2011), the immunohistochemistry test showed that the eosinophil count was remarkably reduced by 73.7% and the MMP-9 expression was also decreased significantly ($P < 0.05$) at a 100 µg/ml of *P. frutescens* aqueous fraction (Heo et al., 2011). *Scutellaria baicalensis* is another well-known Chinese herb which is widely used as an anti-allergic herb (Li et al., 2014). In light of the study of Zhou et al. (2016), it was proposed that oroxylin A **(14)** from *S. baicalensis* was effective to alleviate allergic asthma. Results demonstrated that oroxylin A **(14)** at doses of 15, 30 and 60 mg/kg were able to inhibit eosinophil infiltration in BALF and consequently led to reduced airway hyperresponsivess in OVA-challenged BALB/c mice (Zhou et al., 2016). In the same year, Kim et al. (2016) evaluated the efficacy of topical treatment of aqueous extract of *S. baicalensis* on contact dermatitis. Results revealed that topical application was effective in suppressing leukocytes infiltration and hence ameliorated contact dermatitis symptoms (Kim et al., 2016).

In addition, Zhou et al. (2014) reported that 2-isopropyl-5-methylphenol (thymol) **(15)** from *Thymus vulgaris* produced a dose-dependent reduction of eosinophils infiltration in BALF across concentrations of 4, 8 and 16 mg/kg. A remarkable inhibition ($P < 0.01$) was noted at a dose of 16 mg/kg which was comparable to 2 mg/kg of dexamethasone standard. Consequently, mucus hypersecretion and goblet cell hyperplasia in lung tissues as well as the airway hyperresponsiveness in OVA-sensitized mice in response to methacholine were drastically reduced (Zhou et al., 2014). In 2011, Patel and Deshpande identified the mast cell stabilization potential of *Vitex negundo*. In 2013, they conducted another experiment on *V. negundo*'s leaves to study their anti-asthmatic activity. They successfully isolated a flavonoid compound, 5-hydroxy-3,6,7,3',4'-pentamethoxyflavone **(52)** from the leaves and found that this compound potently suppressed eosinophil count in BALF (9.50 ± 1.5044; $P < 0.05$) in egg albumin-sensitized guinea pigs. This result was remarkable as the inhibition portrayed was comparable to that of dexamethasone control (7.50 ± 0.5014; $P < 0.05$) at a dose of 200 mg/kg (Patel and Deshpande, 2013). This is indicative of the potential of *V. negundo* in the treatment of allergy diseases like asthma. Meanwhile, Lee et al. (2009) highlighted that 1H,8H-pyrano [3,4-c] pyran-l,8-dione (PPY) **(16)** isolated from *V. rotundifolia* fruits remarkably reduced eosinophil migration and eotaxin production by 48% and 70% respectively at 10 µg/ml in human type II-like epithelial lung cells (A549 cell media). In *in vivo* model, the eosinophil count in BALF was also shown to decrease significantly ($P < 0.05$) by 82% (Lee et al., 2009). In 2011, Koh et al. discovered another new compound, casticin **(53)**, isolated from the fruits of *V. rotundifolia* which also showed to inhibit 63% of eosinophil infiltration at 10 µg/ml while eotaxin production decreased dose-dependently with 0.1–1 µg/ml of casticin **(53)** (Koh et al., 2011). In conclusion, the species mentioned above have the potential to fill the knowledge gap of anti-allergy with the inhibition of eosinophil functions.

TOXICOLOGY

Undeniably, in the past decades, the medicinal potential of Lamiaceae species has captured the attention and interest of many researchers to conduct extensive exploration on their pharmacological properties. Nonetheless, the toxicological aspects of the use of Lamiaceae species have not been studied in-depth. To date, there are several toxicological studies available on Lamiaceae species. For instance, Qi et al. (2009) carried out a toxicological investigation on wogonin **(10)**, the active constituent of *Scutellaria baicalensis*, through acute toxicity testing and sub-chronic toxicity testing in murine models. In the acute toxicity testing, the mice were intravenously administered with 350, 315, 283.5, 255.5 and 229.64 mg/kg of wogonin **(10)** respectively. The mice were observed for their general behavior for an hour after the administration and intermittently observed for 24 h for signs of toxicity up to 14-day duration. In the first hour after treatment, the mice showed

decreased locomotor activity, muscle relaxation, catatonia, loss of body-righting reflex and bradypnoea. However, these symptoms diminished within 2 h after treatment. The LD_{50} value of wogonin **(10)** determined was 286.15 mg/kg. Meanwhile, in the sub-chronic toxicity study, doses of 30, 60 and 120 mg/kg of wogonin **(10)** were administered daily to the rats through intravenous route and the rats were put under observation for 90 days. Results revealed that a dose of 120 mg/kg of wogonin can cause heart injury with a long period of intravenous administration **(10)** (Qi et al., 2009).

In another account, the essential oil of *Thymus vulgaris* was evaluated on its toxicological profile (Fachini-Queiroz et al., 2012). Acute toxicity was assessed by administering single oral doses of 2,000, 3,000 and 4,000 mg/kg of *T. vulgaris* essential oil and monitoring for any signs of toxicity for seven consecutive days. There were no apparent behavioral side effects observed in the study and the median lethal dose, LD_{50}, of *T. vulgaris* essential oil determined was 4,000 mg/kg. The relatively high LD_{50} value suggested that *T. vulgaris* essential oil is non-toxic and relatively safe for use (Fachini-Queiroz et al., 2012). Haq et al. (2012) evaluated *Vitex negundo* in an acute neurotoxicity study. Results suggested that no signs of neurotoxicity were seen with doses of 250, 500, 1,000 and 2,000 mg/kg in the tested mice. The LD_{50} value determined was greater than 5,000 mg/kg, which indicates the extract of *V. negundo* has a good safety profile (Haq et al., 2012). In term of behavioral symptoms, symptoms started to show at a dose of 2,000 mg/kg, which presented with abdominal contraction, ataxia, reduced spontaneous activity and reduced alertness (Haq et al., 2012).

In China, people have been widely employing *Salvia miltiorrhiza* as a traditional herb for treating a range of cardiovascular disorders (Wang et al., 2012). Therefore, Wang et al. (2012) designed a toxicological study to assess the acute toxicity and sub-chronic toxicity of *S. miltiorrhiza* aqueous extract (Wang et al., 2012). In the acute toxicity investigation, the rats received two doses of 32 g/kg of aqueous extract of *S. miltiorrhiza* through intravenous administration. The sub-chronic toxicity was evaluated by assessment of hematological and biochemical parameters after administering 1.92, 5.76 and 19.20 g/kg of aqueous extract for 13 weeks. At acute doses, no deaths, weight gain or abnormal behavioral changes were observed. The LD_{50} value was deduced to be greater than 64 g/kg. At the same time, sub-chronic toxicity demonstrated that there was a significant reduction ($P < 0.05$) in haemoglobin concentration in high dose male rats and a significant decrease ($P < 0.05$) in platelet count, plateletocrit in low dose female rats. The total bilirubin level was also elevated significantly ($P < 0.05$) in all doses received by rats. As for histopathological findings, focal inflammation was observed at the injection site and the severity increased dose-dependently. Other vital organs showed no abnormalities (Wang et al., 2012).

Despite the widespread interest for the use of *Perilla frutescens*, there were only few toxicological studies documenting the toxic potential of *P. frutescens* (Yu et al., 2017). In spite of the diverse medicinal uses of *P. frutescens*, it is actually a medicinal plant with toxic potential, given that certain plant parts contain high concentrations of toxic phytochemicals. According to Kerr et al. (1986), a high concentration of perilla ketone was accumulated in the flowers and seed parts of *P. frutescens*. Perilla ketone has been reported as a potent pulmonary toxin associated with atypical interstitial pneumonia (Wilson et al., 1977; Guerry-Force et al., 1988; Muller-Waldeck et al., 2010). Furthermore, the toxicological profile of *Mentha arvensis* leaves ethanolic extract was also assessed. The toxic potential of ethanolic leaves extract of *M. arvensis* was tested using brine shrimp cytotoxicity assay. The extracts were serially diluted to concentrations of 1,000, 250, 125, 100 and 75 μg/ml and each extract was added to the tubes containing *Artemia salina*. The number of survivors in each tube was counted after 24 h from the treatment to determine the lethal concentration of ethanolic extract of *M. arvensis* leaves. Result demonstrated that 100 μg/ml as the LD_{50} dose caused a significant cytotoxic activity against the brine shrimp, *Artemia salina* (do Nascimento et al., 2009).

CONCLUSION AND FUTURE PERSPECTIVES

Undeniably, in recent years, there has been an increase in the global demand for natural products for healthcare supplementation. Lamiaceae species have been well known for their culinary values. Nonetheless, they are also a valuable plant family widely employed as medicinal herbs in traditional practices to treat a wide range of allergic inflammatory conditions, such as allergic skin diseases and allergic asthma. Therefore, this plant family may prove to be a diverse source of natural compounds for the development of novel therapeutic agents for allergic disorders. This review conveys deep insights into the botanical features, distribution, medicinal uses, phytochemistry, pharmacology and toxicological investigations conducted, with a particular focus on the anti-allergic activity of Lamiaceae species. A critical analysis of the relationship between phytoconstituents of Lamiaceae species and corresponding anti-allergic activity was done and documented. Currently available *in vitro*, *in vivo* and *ex vivo* studies have provided evidences to support the traditional uses of Lamiaceae species against allergic disorders. Moreover, after a comprehensive summarization, a total of 53 isolated constituents were identified as natural compounds that contribute to anti-allergic effects. In the present review, numerous compounds demonstrated promising anti-allergic activity, such as flavonoids, flavonoid glycosides, diterpenes and phenolics. Some of these constituents were shown to possess comparable anti-allergic activity to the positive control, including carnosic acid (polyphenol), rosmarinic acid, luteolin, oroxylin A, hispidulin and thymol (phenolic), marrubiin (diterpene) and acteoside (phenylpropanoid glycoside). Nevertheless, in the current review, it was found that many studies were conducted using plant extracts. Although the extracts were proven to exhibit anti-allergic effect, the active constituent responsible for the effect is yet to be identified. Therefore, further studies should be carried out on the plant extracts or fractions which exhibited promising experimental results in order to elucidate the exact active principles responsible for anti-allergic activity. With the availability of the information regarding phytochemicals, other studies such as the standardization of extracts and pharmacological studies as well as toxicological investigations of isolated bioactives can be conducted. Meanwhile, the natural compounds that have been

successfully isolated should be further explored and scrutinized for their therapeutic potential to establish a more evidence-based clinical profile. Henceforth, the development of lead molecules for drug discovery can be quicken and accelerated.

AUTHOR CONTRIBUTIONS

SLY obtained the literatures and wrote the manuscript, while NZAR and KH edited the manuscript.

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Cameroonian medicinal plants: pharmacology and derived natural products

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Many developing countries including Cameroon have mortality patterns that reflect high levels of infectious diseases and the risk of death during pregnancy and childbirth, in addition to cancers, cardiovascular diseases and chronic respiratory diseases that account for most deaths in the developed world. Several medicinal plants are used traditionally for their treatment. In this review, plants used in Cameroonian traditional medicine with evidence for the activities of their crude extracts and/or derived products have been discussed. A considerable number of plant extracts and isolated compounds possess significant antimicrobial, anti-parasitic including antimalarial, anti-proliferative, anti-inflammatory, anti-diabetes, and antioxidant effects. Most of the biologically active compounds belong to terpenoids, phenolics, and alkaloids. Terpenoids from Cameroonian plants showed best activities as anti-parasitic, but rather poor antimicrobial effects. The best antimicrobial, anti-proliferative, and antioxidant compounds were phenolics. In conclusion, many medicinal plants traditionally used in Cameroon to treat various ailments displayed good activities *in vitro*. This explains the endeavor of Cameroonian research institutes in drug discovery from indigenous medicinal plants. However, much work is still to be done to standardize methodologies and to study the mechanisms of action of isolated natural products.

Keywords: medicinal plants, ethnopharmacology, Africa

PUBLIC HEALTH CONCERN AND DISEASES IN CAMEROON

Health care is a basic service essential in any effort to combat poverty, and is often promoted with public funds in Africa to achieve this aim (Castro-Leal et al., 2000). Nevertheless, curative health spending is not always well targeted to the poorest, representing about 50.5% of Cameroonian (Edmondson, 2001). Many developing countries including Cameroon have mortality patterns that reflect high levels of infectious diseases and the risk of death during pregnancy and childbirth, in addition to cancers, cardiovascular diseases and chronic respiratory diseases that account for most deaths in the developed world (WHO, 2009). In Cameroon, 3 out of 20 patients are able to buy prescribed drugs in hospitals and one out of every 1000 patients are able to see a specialist. Health care activities are coordinated by the Ministry of Public Health which receives the second highest budgetary allocation per ministry each year (Speak Clear Association of Cameroon, 2004). Health facilities are either run as government services or private services managed by the various churches and other private individuals. There are also traditional doctors that play a great role as far as the provisions of health care services are concerned. The major diseases associated with high degree of risk within the population include food or waterborne diseases (bacterial and protozoal diarrhea, hepatitis A and E, and typhoid fever), vector borne diseases (malaria and yellow fever), water contact diseases (schistosomiasis), respiratory diseases (meningococcal meningitis), and animal contact diseases (rabies) (Index mundi, 2008). Very often, there is a coexistence of many infectious diseases. Ammah et al. (1999) demonstrated that high proportion of patients (33%) had malaria coexisting with typhoid (*Salmonella typhimurium*, *Salmonella paratyphi*, and *Salmonella typhi* infections). In the Cameroonian population, the lifetime risk of developing active tuberculosis once infected, in absence of HIV infection, is about 10%, meanwhile this increases tenfold in HIV infected individuals (Noeske et al., 2004). Malaria remains the leading cause of morbidity in Cameroon, and among the top five causes of mortality. Malaria represents approximately 45–50% of health consultations, and 23% of admissions (Edmondson, 2001). The unsatisfactory management of all diseases throughout the continent as well as in Cameroon, which allows partially treated and relapsed patients to become sequentially resistant, may play a significant role in the development of resistance for infectious diseases (Jones et al., 2008; McGaw et al., 2008). Effective treatment of diseases is challenging for various reasons, including lack of accessibility and elevated expense of drugs and low adherence owing to toxicity of second-line drugs. It is all too likely that the emergence of resistance will be experienced in the future, exhausting the current arsenal of chemical defenses at our disposal. For this purpose, new drugs are urgently needed, and research programs into alternative therapeutics including medicinal plants investigations should be encouraged.

BIODIVERSITY AND PROTECTED AREA IN CAMEROON

The biodiversity of Cameroon in term of protected land area, number of plant and some animals groups with threatened species are summarized in **Table 1** and **Figure 1**.

Cameroon has a rich biodiversity, with about 8,620 plants species and several animal groups (EarthTrends, 2003), encountered in both protected (about 8 %), and unprotected areas. About 155 plant species are classified by the International Union for the Conservation

Table 1 | Biodiversity and protected area in Cameroon, Sub-Saharan Africa, and the World (Source: EarthTrends, 2003).

	Cameroon	Sub-Saharan Africa	World
Total land areas	47,544	2,429,241	13,328,979
PROTECTED AREA (000 HA)			
Extent of protected areas by IUCN Category (000 ha), 2003			
Total protected area (Categories I–V)	3,741	264,390	1,457,674
Marine and Littoral protected areas[a]	389	–	417,970
Protected areas as a percent of total land area	8.0%	10.9%	10.8%
Biosphere reserves in 2002			
Number of sites	3	46	408
Total area (000 ha)	876	–	439,000
NUMBER AND STATUS OF SPECIES			
Higher plants			
Total known species (number)	8,260	–	–
Number of threatened species	155	–	5,714
Mammals			
Total known species (number)	409	–	–
Number of threatened species	40	–	1,137
Breeding birds			
Total known species (number)	165	–	–
Number of threatened specie	15	–	1,192
Reptiles			
Number of total known species	210	–	–
Number of threatened species	1	–	293
Amphibians			
Number of total known species	171	–	–
Number of threatened species	1	–	157
Fish			
Number of total known species	138	–	–
Number of threatened species	27	–	742

IUCN, International Union for the Conservation of Nature and Natural Resources; Categories I, Nature Reserves, Wilderness, Areas; Categories II, National Parks; Category III, Natural monument; Category IV, Habitat/species management area; Category V, Protected landscape/seascape.
[a]Marine and littoral protected areas are not included in the "Total area protected" above; [b]Includes IUCN categories I–V, marine and littoral protected areas are excluded from these totals.
(–): data not available.

of Nature and Natural Resources (IUCN) as threatened species. Threatened species used as medicinal plants include *Thecacoris annobonae* Pax & K. Hoffm (Euphorbiaceae) (Cheek, 2004), *Pausinystalia johimbe* (K. Schum) (Rubiaceae) (Ngo Mpeck et al., 2004), *Prunus africana* (Hook. f.) Kalkm (Rosaceae) (Focho et al., 2009). *Ancistrocladus korupensis* D. W. Thomas & Gereau (Ancistrocladaceae), *Carpolobia lutea* G.Don (Polygalaceae), *Dacryodes edulis* (G. Don) H. J. Lam. (Burseraceae), *Enantia chlorantha* Oliv (Annonaceae), *Garcinia mannii* Oliv. (Clusiaceae), *Garcinia cola* Heckel (Clusiaceae), *Gnetum africanum* Welw. (Gnetaceae), *Invingia gabonensis* Baill. (Irvingiaceae), *Massularia acuminata* (G. Don) Bullock (Rubiaceae), *Pentaclethra macrophylla* Benth. (Leguminosae), *Baillonella toxisperma* Pierre var. obovata Aubrév. & Pellegr. (Sapotaceae), *Calamus deeratus* Mann & Wendl. (Palmae), *Cola acuminata* (P.Beauv.) Schoot et Endl. (Sterculiaceae), *Eremospatha macrocarpa* (Mann & Wendl.) Wendl. (Palmae), *Raphia regalis* Becc. (Palmae), *Raphia vinifera* P.Beauv. (Palmae) and *Ricinodendron heudelotti* (Baill.) Pierre (Euphorbiaceae) (Koné, 1997). Protected zones include both land (3,741 ha) and marine areas (389 ha) (EarthTrends, 2003).

ETHNOBOTANICAL USES OF MEDICINAL PLANTS IN CAMEROON

Traditional healing plays an integral role in black African culture as it provides primary health care needs for a large majority (about 80%) of the population (WHO, 2002). In Cameroon, there is a rich tradition in the use of herbal medicine for the treatment of several ailments. Unfortunately, the integration of traditional medicine in the health system is not yet effective, due to its disorganization (Nkongmeneck et al., 2007). However, the government strategies of health envisage the organization of traditional medicine in order to provide the main trends for the development and its integration (Anonymous, 2006). Adjanohoun et al. (1996) provided a useful review of the traditional use of medicinal plants in Cameroon, although much work remains to be done regarding the documentation of existing ethnobotanical knowledge. Jiofack et al. (2010) also documented the traditional use of 289 plants species belonging to 89 families against 220 pathologies. Sixty eight percent of the documented plants are used to treat more than twenty important diseases. They are used as decoction, infusion, maceration, powder, powder mixtures, plaster, calcinations, and squeeze in water, boiling, cooking with young cock or sheep meat or groundnut paste, direct eating, juice, fumigation, and sitz bath (Jiofack et al., 2010). The most recurrent diseases or disorders treated are typhoid, male sexual disorders, malaria, gonorrhea, gastritis, rheumatism, fever, dysentery, diarrhea, dermatitis, boils, cough, wounds, syphilis, sterility, sexually transmitted diseases, ovarian cysts, and amoebiasis, with more than two hundred plants being used to cure these diseases or disorders (Jiofack et al., 2010).

INVESTIGATION OF THE PHARMACOLOGICAL POTENTIAL OF MEDICINAL PLANTS OF CAMEROON

ANTIMICROBIAL ACTIVITY

Plants are widely used traditionally for the treatment of microbial infections. A review of the antimicrobial potential of Cameroon medicinal plants (Kuete, 2010a) reported more than 58 species *in vitro* active extracts or isolated compounds. Cut-off points for activity in term of IC_{50}-values were set to 100 μg/ml for extract and 25 μM for compounds

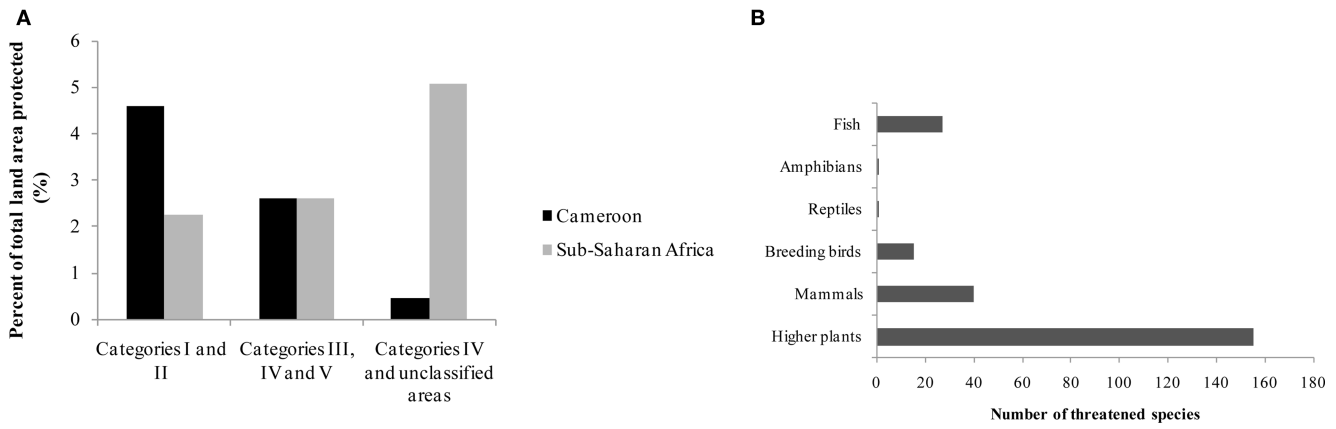

FIGURE 1 | Portion of land area protected (A) by IUCN Category (2003) and threatened species **(B)** (2002–2003) in Cameroon (Source: EarthTrends, 2003). IUCN, International Union for the Conservation of Nature and Natural Resources; Categories I, nature reserves, wilderness, areas; Categories II, national parks; Category III, natural monument; Category IV, habitat/species management area; Category V, protected landscape/seascape.

(Cos et al., 2006). However, in the case of antimicrobial evaluation of extracts and compounds, determination of IC_{50} is not the optimal parameter for significance, most of the reported data being given as MIC values. Kuete (2010a) also set the bar as follows for extract: significant (MIC < 100 μg/ml), moderate (100 < CMI ≤ 625 μg/ml) or weak (CMI > 625 μg/ml). For compounds, this stringent endpoints criteria were: significant (MIC < 10 μg/ml), moderate (10 < MIC ≤ 100 μg/ml), and low or negligible (MIC > 100 μg/ml) (Kuete, 2010a). More than 50 microorganisms were found to be sensitive to such extracts and significant activity with minimally inhibiting concentrations (MIC) of less than 100 μg/ml (Kuete, 2010a). Some of the extracts including those from *Bersama engleriana*, *Dorstenia angusticornis*, *Dorstenia barteri*, *Diospyros canaliculata*, *Diospyros crassiflora*, *Newbouldia laevis*, and *Ficus cordata* exhibited a wide range of activity on both bacteria and fungi (Kuete, 2010a).

Some of the bioactive compounds such as diospyrone (**23**), crassiflorone (**24**), newbouldiaquinone (**25**), newbouldiaquinone A (**26**), laurentixanthone A (**30**), laurentixanthone B (**31**), smeathxanthone B (**32**), cheffouxanthone (**33**), bangangxanthone A (**34**), globulixanthone C (**35**), D (**36**) and E (**37**), moracin T (**43**), and U (**44**), nkolbisine (**59**), norerythrosuaveolide (**60**), were isolated and characterized for the first time from Cameroonian medicinal plants (Kuete, 2010a). Other compounds such as plumbagin (**27**), lapachol (**28**) (found to be inactive *in vivo* in some cases), isobavachalcone (**47**), 4-hydroxylonchocarpin (**48**), kanzonol C (**49**) exhibited interesting activities and were suggested as potential candidates for new antimicrobial drug (Kuete, 2010a). Though compound **28** is known to possess good antimicrobial activity *in vitro*, it will be necessary to assess its *in vivo* efficacy. However this compounds was active *in vitro* against intracellular amastigotes of *Leishmania braziliensis* and inactive *in vivo* using hamster infected model (Lima et al., 2004). *Thecacoris cf. annobonae* Pax & K. Hoffm (Euphorbiaceae) exhibited significant antimicrobial (MIC < 10 μg/ml) activities against *Mycobacterium tuberculosis* H37Rv, *Bacillus cereus* and *Pseudomonas aeruginosa* (Kuete et al., 2010b). The extract from *T. annobonae* was reported to induce *E. coli* death through the inhibition of H^+-ATPase-mediated proton pumping (Kuete et al., 2010b). Investigations the mode of resistance of the microorganisms to bioactive compounds isolated from Cameroonian medicinal plants have shown that efflux by AcrAB-TolC pumps was one of the likely mechanisms of defense of Gram-negative bacteria to compounds **23** and **47** (Kuete et al., 2010c).

ANTIMALARIAL ACTIVITY

In Cameroon, several plant species are used to treat malaria. A review on traditionally used plants reported up to 217 species (Titanji et al., 2008). Some of these plants were screened *in vitro* for their activity against *P. falciparum* and more than 100 bioactive compounds were isolated (Titanji et al., 2008), most of which, however, showed only low or modest antimalarial activities. In the present review, we focus only on plant extract and compounds that exhibited considerably high activities. The proposed cut-off points for *in vitro* activity of antimalarial extracts based on their IC_{50} values can be categorized as follows: IC_{50} < 0.1 μg/ml (very good); 0.1–1 μg/ml (good); 1.1–10 μg/ml (good to moderate); 11–25 μg/ml (weak), 26–50 (very weak), >100 μg/ml (inactive) (Willcox et al., 2004a). The following inhibition percentages were proposed for *in vivo* activity of antimalarial extracts at a fixed dose of 250 mg/kg/day: 100–90% (very good activity); 90–50% (good to moderate); 50–10% (moderate to weak); 0% (inactive) (Willcox et al., 2004a). Several plant extracts from Cameroonian medicinal plants were reported for their antimalarial activities (**Table 2**), the most active (IC_{50} < 1 μg/ml) being that from *Enantia chlorantha* (Boyom et al., 2009).

Some isolated compounds were also reported for their antimalarial activities (**Table 2**). An IC_{50} of 1.5 μM was chosen as cut-off point for several compounds (Calas et al., 1997). The threshold for *in vitro* chloroquine resistance has been defined as IC_{50} > 100 nM (Ringwald et al., 1996). According to Mahmoudi et al. (2006), compounds with IC_{50} > 5 μM were considered as inactive against parasite development, compounds with IC_{50} between 0.06 and 5 μM being active, and values of IC_{50} < 0.06 μM implying the drugs to be very toward *P. falciparum*. We will take into consideration up to IC_{50} < 20 μg/ml to report on the activity of antimalarial compounds isolated from Cameroonian medicinal plants. So far, active compounds isolated belong to three main groups of secondary metabolites, terpe-

Table 2 | Plants used in Cameroon to treat malaria, with evidence of their activities.

Family	Species[a]	Traditional treatment	Plant part used	Bioactive (or potentially active) compounds[b]	Screened activity
Acanthaceae	*Thomandersia hensii* De Wild and Th. Dur (LB Th 0301)	Malaria, diarrhea, colitis, furuncles, abscesses, syphilis, ulcers, urogenital disorders, intestinal parasites, debility, tiredness, edema, rheumatism, eye inflammations (Letouzey, 1985; Ngadjui et al., 1994).	Bark, leaves, pulp, sap, roots	Not identified	IC_{50} < 30 μg/ml reported for hexane extract from the stem bark on *P. falciparum* W2 (Indochina I/CDC) chloroquine-resistant strain (Bickii et al., 2007b)
Annonaceae	*Uvariopsis congolana* (De Wild) Fries (37016/HNC)	Malaria (Boyom et al., 2009)	Bark, leaves	Not identified, but plants of this family were reported to contain acetogenins[c]	IC_{50} < 5 μg/ml reported for the crude extract from the leaves and bark on *P. falciparum* strain W2 (Boyom et al., 2009)
	Polyalthia oliveri Engl. (19416 SRF/Cam)	Malaria (Boyom et al., 2009)	Bark		IC_{50} < 5 μg/ml reported for the crude extract from the bark on *P. falciparum* strain W2 (Boyom et al., 2009)
	Enantia chlorantha Oliv. (32065/SRF/Cam)	Malaria (Boyom et al., 2009)	Bark, leaves	Not identified	IC_{50} < 1 μg/ml reported with the crude extract from the leaves and bark on *P. falciparum* strain W2 (Boyom et al., 2009)
Apocynaceae	*Picralima nitida* Stapf (LB Pn 0301)	Malaria, diarrhoea, intestinal worms, gonorrhoea, inflammation (Letouzey, 1985; Ezeamuzie et al., 1994; Fakeye et al., 2000)	Bark, roots, seeds; fruits	Not identified	IC_{50} < 30 μg/ml reported for the methanol and dichloromethane–methanol 1:1 extracts from the seeds and bark on *P. falciparum* W2 (Indochina I/CDC) chloroquine-resistant strain (Bickii et al., 2007b)
Euphorbiaceae	*Croton zambesicus* Muell. Arg. (8204/SRFCam)	Malaria (Boyom et al., 2009)	Bark	Not identified	IC_{50} < 10 μg/ml reported for the crude extract from the bark on *P. falciparum* strain W2 (Boyom et al., 2009)
	Neoboutonia glabrescens Müll. Arg. Prain (7433/SRFCam)	Malaria (Boyom et al., 2009)	Bark, leaves	Not identified	IC_{50} < 10 μg/ml reported for the crude extract from the leaves and bark on *P. falciparum* strain W2 (Boyom et al., 2009)
Guttiferae	*Symphonia globulifera* Linn f. (50788/HNC)	Stomach and skin aches, laxative for pregnant women, general tonic, Malaria (Aubreville, 1950; Irvine, 1961; Ngouela et al., 2006).	Bark	Gaboxanthone (**38**); symphonin (**39**); globuliferin (**40**); guttiferone A (**50**) (Ngouela et al., 2006).	IC_{50} <20 μM on *P. falciparum* reported for compounds **38**–**40** and **50** (Ngouela et al., 2006).
Lauraceae	*Beilschmiedia zenkeri* Engl.	Not reported	Bark	5-Hydroxy-7,8-dimethoxyflavone; pipyahyine; betulinic acid (Lenta et al., 2009)	IC_{50} <5 μM on chloroquine-resistant *P. falciparum* reported for pipyahyine (Lenta et al., 2009)
Meliaceae	*Entandrophragma angolense* Welwitsch C.D.C. (29933/HNC)	Malaria (Bickii et al., 2007a)	Bbark	22-Hydroxyhopan-3-one; 24-methylenecycloartenol (**8**); tricosanoic acid; methylangolensate; 7α-acetoxydihydronomilin (**9**); 7α-obacunylacetate (**10**) (Bickii et al., 2007a)	IC_{50} < 20 μg/ml on *P. falciparum* W2 strain reported for compounds **8**–**10**. The dichloromethane – methanol (1:1) extract of the stem bark of that plant exhibited IC_{50} of 18.8 μg/ml (Bickii et al., 2007a)

(Continued)

Table 2 | *Continued*

Family	Species[a]	Traditional treatment	Plant part used	Bioactive (or potentially active) compounds[b]	Screened activity
	Khaya grandifoliola C.D.C. (PM 098/95/HNC)	Malaria (Obih et al., 1985; Bray et al., 1990; Weenen et al., 1990).	Bark and seeds	Methylangolensate (**1**); 6-methylhydroxyangolensate (**2**); gedunin (**3**); catechin; 7-deacetylkhivorin (**4**); 1-deacetylkhivorin (**5**); swietenolide (**6**); 6-acetylswietenolide (**7**) (Bickii et al., 2000)	IC_{50} < 20 μg/ml on *P. falciparum* W2 strain reported for bark and seeds extracts; compounds **1–7**. Compound **3** exhibited an additive effect when combined with chloroquine (Bickii et al., 2000)
	Turreanthus africanus	Malaria and other fevers (Zhou et al., 1997)	Bark, seeds, leaves	16-oxolabda-8 (**17**), 12(*E*)-dien-15-oic acid; methyl-14, 15-epoxylabda-8 (**17**), 12(*E*)-diene-16-oate; turreanin A (Ngemenya et al., 2006)	None of the active compounds exhibited IC_{50} < 20 μg/ml on *P. falciparum* F 32, chloroquine sensitive strain (Ngemenya et al., 2006)
Moraceae	*Artocarpus communis* J.R. & G. Forst (43982 HNC)	Malaria (Boyom et al., 2009)	Bark, leaves	Not identified	IC_{50} < 10 μg/ml reported for the crude extract from the leaves and bark on *P. falciparum* strain W2 (Boyom et al., 2009)
	Dorstenia convexa De Wild (53450 HNC)	Malaria (Boyom et al., 2009)	Twigs	Not identified	IC_{50} < 10 μg/ml reported with the crude extract from the twigs on *P. falciparum* strain W2 (Boyom et al., 2009)
Zingiberaceae	*Aframomum zambesiacum* (Baker) K. Schum (37737HNY)	Malaria (Kenmogne et al., 2006)	Seeds	Aulacocarpin A (**11**); aulacocarpin B; 3-deoxyaulacocarpin A (**12**); methyl-14*n*,15-epoxy-3*b*-hydroxy-8(**17**),12-elabdadien-16-oate; galanolactone; zambesiacolactone A (**13**); zambesiacolactone B (**14**); aframodial (Kenmogne et al., 2006)	IC_{50} < 20 μM on *P. falciparum* reported for compounds **11–14** (Kenmogne et al., 2006)
	Reneilmia cincinnata (K. Schum.) Bak.	Malaria (Tchuendem et al., 1999)	Fruits	Oplodiol (**17**); 5*E*,10(14)-Germacradien-1β,4β-diol (**16**); 1(10)*E*,5*E*-germacradien-4β-ol (**15**) (Tchuendem et al., 1999)	IC_{50} < 5 μM reported on *P. falciparum* D6 and W2 strains for compounds **15–17** on *P. falciparum* D6 strain (Tchuendem et al., 1999

[a]*HNC or SRFK: Cameroon National herbarium code; LB, Laboratory of Botany, Yaoundé.*
[b]*Compounds characterized for the first time in Cameroonian medicinal plant are underlined.*
[c]*Annonaceous acetogenins are inhibitors of complex I (NADH: ubiquinone oxidoreductase) in mitochondrial electron transport systems (Lewis et al., 1993), and NADH oxidase of plasma membranes (Morré et al., 1995), two enzymes found in Plasmodium falciparum.*

noids (**Figure 2**), phenolics (**Figure 3**), and alkaloids (**Figure 4**). Antimalarial terpenoids including sesqui-, di-, and triterpenoids are the most frequently isolated compounds from Cameroonian plants. Several natural products were reported as being active against *Plasmodium falciparum*, with IC_{50} values below 20 μg/ml, including methylangolensate (**1**); 6-methylhydroxyangolensate (**2**); gedunin (**3**); 7-deacetylkhivorin (**4**); 1-deacetylkhivorin (**5**); swietenolide (**6**); 6-acetylswietenolide (**7**) (Bickii et al., 2000); 24-methylenecycloartenol (**8**); 7α-acetoxydihydronomilin (**9**); 7α-obacunylacetate (**10**) (Bickii et al., 2007a); aulacocarpin A (**11**); 3-deoxyaulacocarpin A (**12**); zambesiacolactone A (**13**), and B (**14**) (Kenmogne et al., 2006); 1(10)*E*,5*E*-germacradien-4β-ol (**15**); 5*E*,10(14)-germacradien-1β,4β-diol (**16**); oplodiol (**17**) (Tchuendem et al., 1999); IC_{50} < 5 μg/ml were obtained for compounds **3** (1.25 μg/ml), and **10** (2 μg/ml) (Bickii et al., 2000, 2007a), while values below 5 μM were recorded for compounds **12** (4.97 μM) (Kenmogne et al., 2006), **15** (1.54 μM); **16** (1.63 μM) and **17** (4.17 μM) (Tchuendem et al., 1999).

Phenolic compounds (**Figure 3**) such as gaboxanthone (**38**); symphonin (**39**); globuliferin (**40**); guttiferone A (**50**) (Ngouela et al., 2006) also exhibited antimalarial activities when tested on

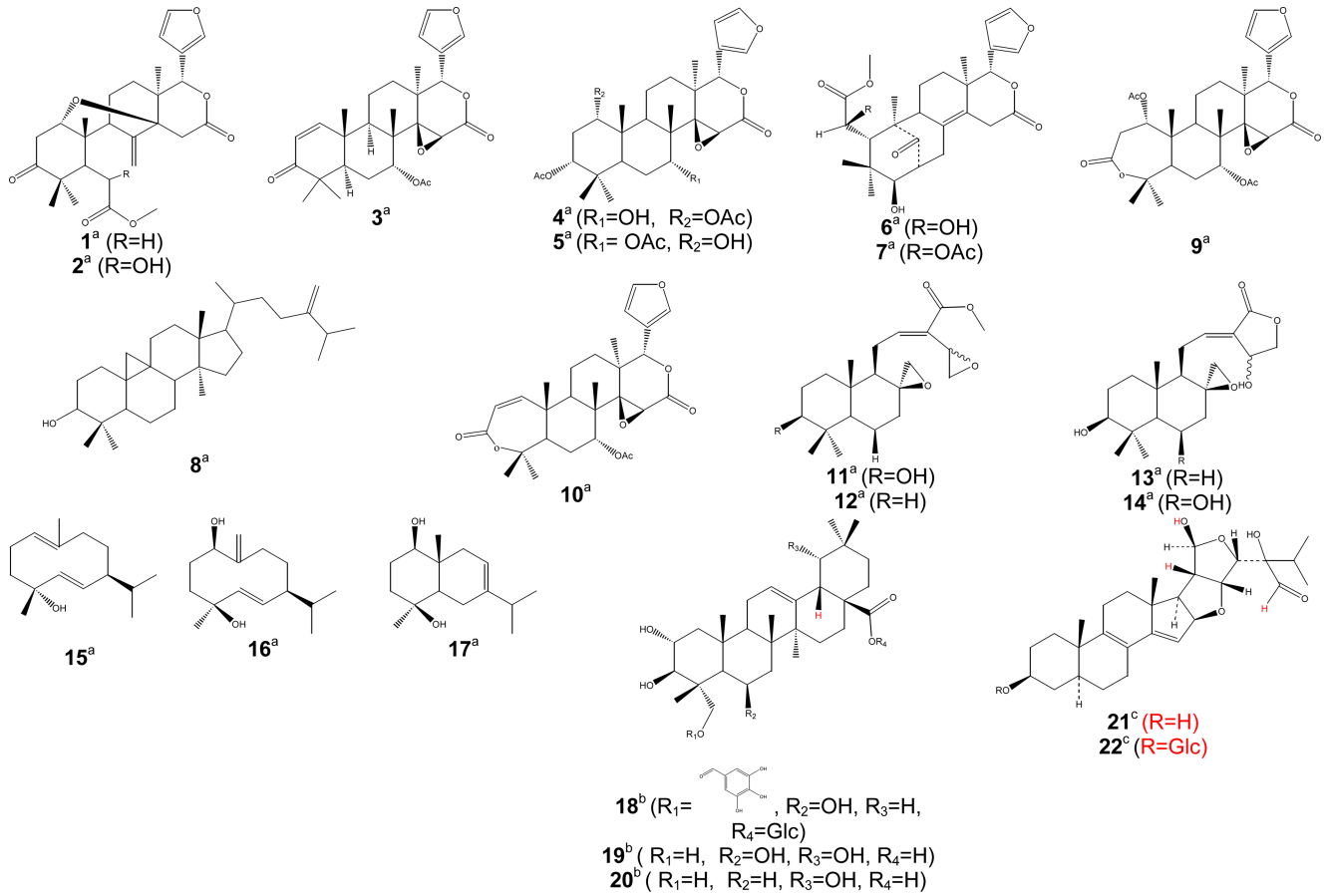

FIGURE 2 | Bioactive terpenoids. Activity [(a) antimalarial, (b) anti-inflammatory, (c) antitrypanosomal]; Glc, glucosyl group; Ac, acetyl group.

P. falciparum. IC_{50} values below 5 μg/ml were reported with compounds **38** (3.53 μM); **39** (1.29 μM); **40** (3.86 μM) and **50** (3.17 μM) (Ngouela et al., 2006). Few alkaloids from Cameroonian medicinal plants (**Figure 4**) were characterized. For example, pipyahyine (**61**) exhibited good activity ($IC_{50} < 5$ μM) toward chloroquine-resistant *P. falciparum* (Lenta et al., 2009).

Studies dealing with the mechanisms of action of antimalarial compounds are still limited. However, some of the extracts from Cameroonian medicinal plants such as those from *Annonaceae* species were suggested to exert their antiplasmodial activity by the inhibition of vital parasitic enzymes such as cysteine proteases (Boyom et al., 2009).

OTHER ANTI-PARASITIC ACTIVITIES

Parasitic trypanosomatids cause a number of important diseases, including human African trypanosomiasis, Chagas disease, and leishmaniasis. More than 60 million people living in 36 sub-Saharan Africa countries are at risk of contracting sleeping sickness, caused by *Trypanosoma brucei gambiense* and *T. b. rhodesience* (WHO, 2007). It is estimated that currently 300,000–500,000 people were infected in 2001, with 50,000 deaths annually (Fairlamb, 2003; WHO, 2007). *Leishmania* species cause a spectrum of disease ranging from self-healing cutaneous lesions to life-threatening visceral infections, with 2 million new cases occurring annually (WHO, 2005). It is estimated that about 200 million people worldwide are currently affected by schistosomiasis, a disease caused by flatworms belonging to the genus *Schistosoma*. The disease is usually chronic and debilitating, with severe consequences on the urinary tract where *S. haematobium* is the organism involved and major damage to the intestinal tract where *S. mansoni, S. intercalatum* or *S. japonicum* are involved (Jatsa et al., 2009). In humans, *Toxoplasma* infections are widespread and lead to severe diseases in individuals with immature or suppressed immune system. Consequently, toxoplasmosis became one of the major opportunistic infections of the AIDS epidemic (Luft and Remington, 1992). Toxoplasmosis also affects *T. gondii*-negative women during pregnancy and is a serious threat for embryos. Despite the huge impact of these parasitic diseases, the drugs used for their treatment are often toxic, marginally effective, administered by injection only, expensive, and/or compromised by the development of resistance (Ouellette et al., 2004; Croft et al., 2005). Only few researchers in Cameroon focused on antitrypanosomal and antileishmanial compounds from medicinal plants. Available published data on traditionally used medicinal plants are compiled in **Table 3**. Herein, similar cut-off points as indicated above for antimalarials have been considered for activities against trypanosomal, leishmanial and schistosomal pathogens. Compounds with good antileishmanial activities were isolated from *Garcinia lucida* (Clusiaceae), with IC_{50}

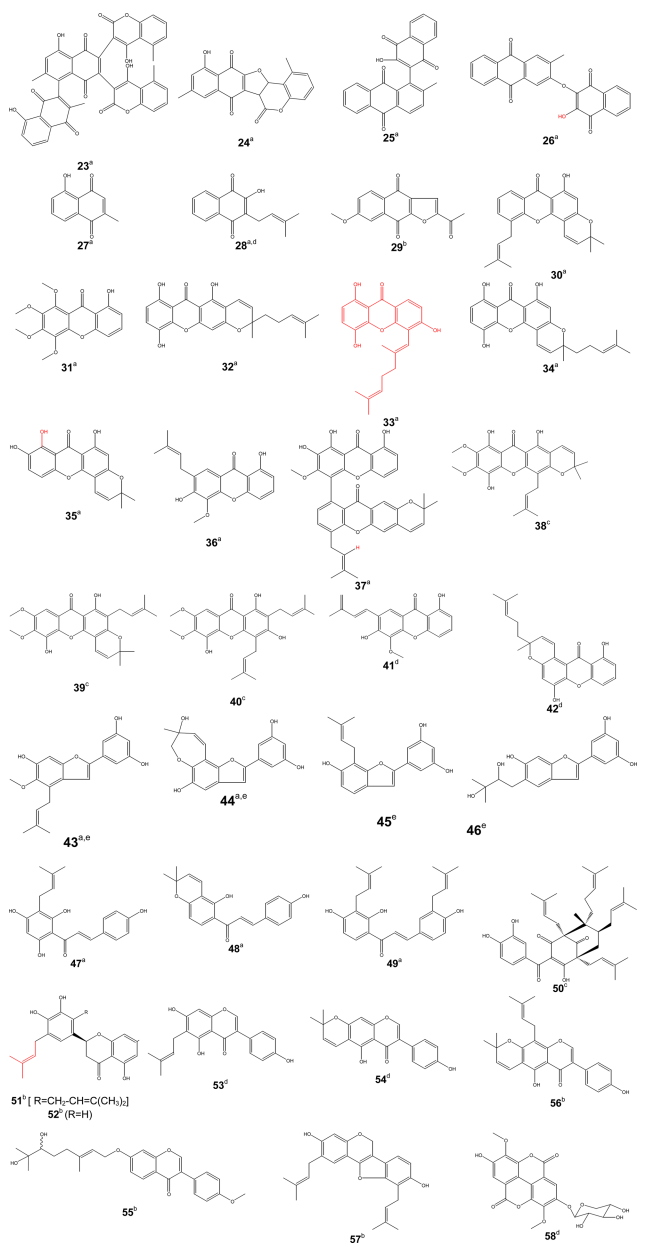

FIGURE 3 | Bioactive phenolics [quinones (23–29), xanthones (30–42), arylbenzofurans (43–46)]. Activity [(a) antimicrobial, (b) anti-inflammatory, (c) antimalarial, (d) anti-proliferative, (e) antioxidant]. [Chalcones (**47–49**), benzophenone (**50**), flavone (**51–52**), isoflavones (**53–56**), pterocarpene (**57**), ellagic acid derivative (**58**)]. Activity [(a) antimicrobial, (b) anti-inflammatory, (c) antimalarial, (d) anti-proliferative, (e) antioxidant].

values of 2.0 and 6.6 μg/ml, respectively, for dihydrochelerythrine (**62**) and 6-acetonyldihydrochelerythrine (**63**) against *L. donovani* (Fotie et al., 2007). Significant antitrypanosomal activities were also reported for stigmastane derivatives, vernoguinosterol (**21**) and vernoguinoside (**22**) (**Figure 2**) isolated from *Vernonia guineensis* (Asteraceae), against bloodstream trypomastigotes of *Trypanosoma brucei rhodesiense* with IC_{50} values ranging from 3 to 5 μg/ml (Tchinda et al., 2002).

ANTI-PROLIFERATIVE ACTIVITY

Screenings of medicinal plants used as anticancer drugs has provided modern medicine with effective cytotoxic pharmaceuticals. More than 60% of the approved anticancer drugs in United State of America (from 1983 to 1994) were from natural origin (Stévigny et al., 2005; Newman and Cragg, 2007). The diversity of the biosynthetic pathways in plants has provided a variety of lead structures that have been used in drug development. In this last decade, investigations on natural compounds have been particularly successful in the field of anticancer drug research. Early examples of anticancer agents developed from higher plants are the antileukemic alkaloids (vinblastine and vincristine), which were both obtained from the Madagascar periwinkle (*Catharanthus roseus*) (Voss et al., 2005). The development of the highly automated bioassay screening based on colorimetric methods that quantified the proliferation of cell culture (Mosmann, 1983) of a huge number of plants extracts have permitted to find that many plant families (Guittiferae, Rubiaceae, Apocynaceae, Euphorbiaceae, Solanaceae, etc.) exhibited a great potential of anti-proliferative activity (Hostettmann et al., 2000; Whelan and Ryan, 2003). A large number of plant extracts have shown the *in vitro* and *in vivo* antitumor activities (Hostettmann et al., 2000). In the US NCI plant screening program, a crude extract is generally considered to have *in vitro* cytotoxic activity if the IC_{50} value following incubation between 48 and 72 h, is less than 20 μg/ml, while it is less than 4 μg/ml for pure compounds (Boik, 2001). This cut-off point for good cytotoxic compound has also been defined as 10 μM (Brahemi et al., 2010). Despite the exceptional biodiversity of Africa, few scientific studies have been carried out in the continent regarding the anti-proliferative properties of medicinal plants. However, some Cameroonian plants and derived natural products were tested for their anti-proliferative effects. Five medicinal plants widely used in cancer treatment, *Sida acuta, Sida cordifolia*, *Sida rhombilifolia*, *Urena lobata*, *Viscum album,* were recently screened for their cytoxicity against Hep G2 hepatocarcinoma cells rather showed moderate anti-proliferative effects (Pieme et al., 2010). However, studies based on the inhibition of Crown Gall tumors revealed pronounced tumor reducing activity of extracts from the roots and leaves from *Bersama engleriana* (Kuete et al., 2008). An IC_{50} of 27.16 μg/ml was reported for *Antiaris africana* in DU-145 prostate cancer cells, while even better activity (IC_{50} of 13.84 μg/ml) was recorded in Hep G2 hepatocarcinoma cells (Kuete et al., 2009). One of the most active compounds isolated from *A. africana,* 3,39-dimethoxy-49-*O*-β-D-xylopyronosylellagic acid (**58**) (**Figure 3**) exhibited considerable anti-proliferative activities toward HepG2 (IC_{50} of 3.84 μg/ml) and DU-145 (IC_{50} of 6.24 μg/ml) cells (Kuete et al., 2009). Compound **28**, the main constituent of a Cameroonian medicinal plant, *Newbouldia leavis* (Bignoniaceae) was found to be very active against DU-145 cells with an IC_{50} of 64.59 nM (Eyong et al., 2008). Wighteone (**53**) and alpinumisoflavone (**54**) isolated from *Erythrina indica* (Leguminosae) were reported to be cytotoxic (effective dose of 0.78 and 4.13 μg/ml, respectively) when tested against KB nasopharyngeal cancer cells (Nkengfack et al., 2001). Globulixanthones A (**41**) and B (**42**) isolated for the first time in the Cameroonian medicinal plant, *Symphonia globulifera* L. f. (Clusiaceae), showed good anti-proliferative activities against human KB cells, with IC_{50} values of 2.15 and 1.78 μg/ml, respectively (Nkengfack et al., 2002). Compound

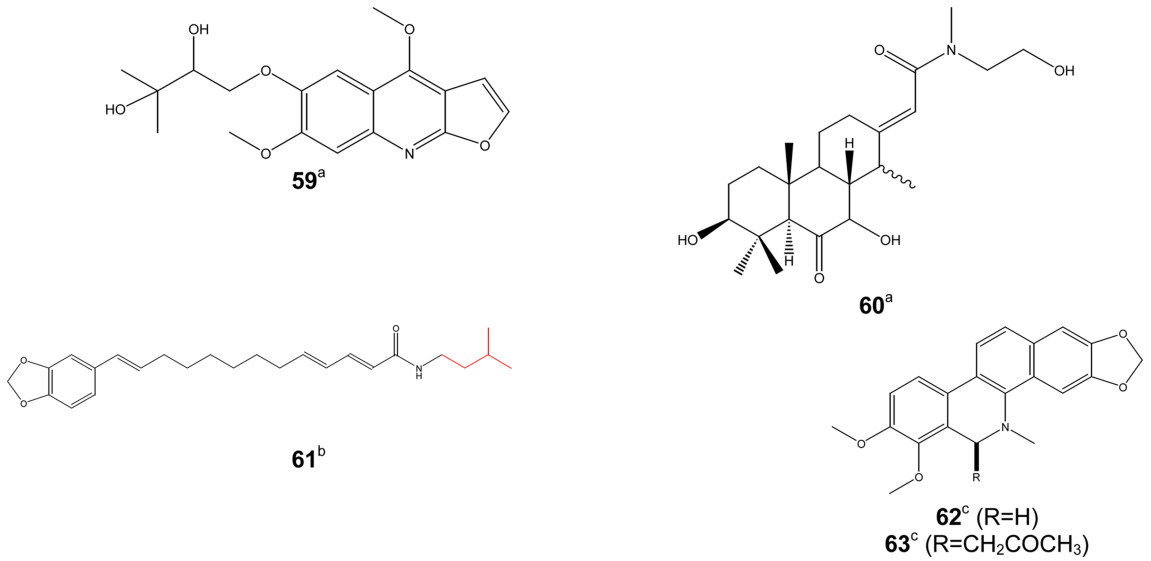

FIGURE 4 | Bioactive alkaloids. Activity [(a) antimicrobial, (b) antimalarial, (c) antileishmanial].

47 isolated from *Dorstenia barteri* (Ngameni et al., 2007) and *D. turbinata* (Ngameni et al., 2009) was cytotoxic toward a wide spectrum of tumor cell lines, including ovarian carcinoma OVCAR-8 cells, prostate carcinoma PC3 cells, breast carcinoma MCF-7 cells, and lung carcinoma A549 cells (Jing et al., 2010). Compound **47** significantly ablated Akt phosphorylation at Ser-473 and Akt kinase activity in cells, which subsequently led to inhibition of Akt downstream substrates and evoked significant levels the mitochondrial pathway of apoptosis (Jing et al., 2010). Nishimura et al. (2007) demonstrated that compound **47** induced apoptotic cell death with caspase-3 and -9 activation and Bax upregulation in neuroblastoma cell lines. Compound **47** inhibited MMP-2 secretion from U87 glioblastoma cells (Ngameni et al., 2007). Compounds **48** and **49** also isolated from *Dorstenia turbinata* (Moraceae), inhibited the matrix metalloproteinase (MMP)-2 secretion from brain tumor-derived glioblastoma cells (Ngameni et al., 2006).

ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES

Pain is one common health problem with substantial socioeconomic impact because of its high incidence. It is a symptom of many diseases and it is estimated that 80–100% of the population experience back pain at least once in the life time (Jain et al., 2002). The treatment of pain requires analgesics including inflammatory products. Hence, most of the non-steroidal anti-inflammatory agents also have analgesic activity. The inhibition of prostaglandin E2 (PGE2) and nitric oxide (NO) production has been proposed as a potential therapy for different inflammatory disorders (Nowakowska, 2007). Although, many analgesics and anti-inflammatory agents are present on the market, modern drug therapy is associated with some adverse effects like gastrointestinal irritation (Jain et al., 2002; Osadebe and Okoye, 2003), fluid retention, bronchospasm, and prolongation of bleeding time. Therefore, it is necessary to search for new drugs with less adverse effects. Medicinal plants have been used for the development of new drugs and continue to play an invaluable role for the progress of drug discovery (Raza et al., 2001). Plant extracts can be an important source of safer drugs for the treatment of pain and inflammation. Several medicinal plants and derived products were screened for their anti-inflammatory and analgesic properties (**Table 4**). Bark extract as well as terpenoids from *Combretum molle* (Combretaceae), β-D-glucopyranosyl 2α,3β,6β-trihydroxy-23-galloylolean-12-en-28-oate (**18**); combregenin (**19**); arjungenin (**20**) (**Figure 2**) showed good activities against carrageenan-induced paw edema in rat (Ponou et al., 2008). A naphthoquinone, 2-acetyl-7-methoxynaphthol[2,3-b]furan-4,9-quinone (**29**), isolated from the anti-inflammatory crude extract of *Millettia versicolor* inhibited 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced acute ear edema and phospholipase A_2 (PLA_2) acute mouse paw edema (Fotsing et al., 2003). Isoflavones, griffonianone D (**55**) (**Figure 3**) isolated from *Millettia griffoniana* (Yankep et al., 2003), warangalone (**56**) isolated from the bark of *Erythrina addisoniae* (Talla et al., 2003), and erycristagallin (**57**) isolated from the root of *Erythrina mildbraedii* (Njamen et al., 2003), showed marked effectiveness as an anti-inflammatory on PLA_2-induced paw edema and on TPA-induced ear edema in mice (Njamen et al., 2003; Talla et al., 2003). Flavonoids sigmoidin A (**51**) and B (**52**) (**Figure 3**) isolated from *Erithrina sigmoidea*, and compound **57** were also effective against TPA-induced ear edema (Njamen et al., 2003, 2004).

ANTI-DIABETIC ACTIVITY

Diabetes mellitus is a group of metabolic disorders with one common manifestation, hyperglycemia (WHO, 1980, 1985). Chronic hyperglycemia causes damage to eyes, kidneys, nerves, heart, and blood vessels (Mayfield, 1998). It is caused by inherited and/or acquired deficiency in insulin production of the pancreas, or by unresponsiveness toward insulin. It results either from inadequate secretion of hormone insulin, an inadequate response of target cells to insulin, or a combination of these factors (Malviya et al., 2010). Diabetes is projected to become one of the world's main disablers and killers within the next 25 years (Malviya et al., 2010). The management of diabetes is a global problem and a successful treatment

Table 3 | Plants used in Cameroon to treat some parasitic infections with evidence of their activities.

Family	Species[a]	Traditional treatment	Plant part used	Bioactive (or potentially active) compounds[b]	Screened activity[c]
Annonaceae	*Polyalthia suaveolens* Engl. & Diels (1227/SRFK)	Rheumatic pains (Surville, 1955)	Not specified	Polyveoline; 3-*O*-acetyl greenwayodendrin; POLYSIN; greenwayodendrin-3-one (Ngantchou et al., 2010)	Antitrypanosomal activity: weak activity for polyveoline (IC_{50}: 32 μM); 3-*O*-acetyl greenwayodendrin (IC_{50}: 54 μM); mixture of polysin and greenwayodendrin-3-one (IC_{50}: 18 μM) against *T. brucei* (Ngantchou et al., 2010)
Asteraceae	*Vernonia guineensis* Benth. (BUD 301)	Anthelmintic, anti-poison, malaria, jaundice (Iwu, 1993)	Leaves	Vernoguinosterol (**21**); vernoguinoside (**22**) (Tchinda et al., 2002)	Antitrypanosomal activity: significant for compounds **22** and **23** against four strains of bloodstream trypomastigotes *T. b. rhodesiense* with IC_{50} values in the range 3–5 mg/ml (Tchinda et al., 2002)
Guttiferae	*Garcinia lucida* Vesque (5768/HNC)	Gastric infections, anti-poison (Nyemba et al., 1990)	Bark	Dihydrochelerythrine (**62**); 6-acetonyldihydrochelerythrine (**63**); lucidamine A (Fotie et al., 2007)	Antileishmanial activity: Significant activity for compounds **62** and **63** and moderate for lucidamine A against *L. donovani*. Also, 100% Inhibition of promastigote at 100 μg/ml were reported for all the above compounds (Fotie et al., 2007)
Meliaceae	*Turraeanthus africanus* (Welw. ex C.D.C.) Pellegr (8233/HNC)	Asthma, stomachache, intestinal worms, and inflammatory diseases (Ekwalla and Tongo, 2003)	Aerial parts, roots	Turraeanthin C; sesamin (Vardamides et al., 2008)	Antitoxoplamal activity: Moderate activity for turraeanthin C and low activity for crude bark extract and sasamin. Inhibition of parasite growth at 10 μg/ml was found to be 55% for turraeanthin C, 20% for sesamin and 40% for crude extract (Vardamides et al., 2008)
Verbenaceae	*Clerodendrum umbellatum* Poir (7405/HNC)	Epilepsy, headache, intestinal helminthiasis, irregular menstruation, infective dermatitis, asthma, metaphysical powers, whitlow, vulvovaginitis (Adjanohoun et al., 1996; Jatsa et al., 2009)	Not specified	Not identified but, flavonoids, saponins, saponosides, tannins, and triterpenes were detected in the leaves aqueous extract (Jatsa et al., 2009)	Antischistosomal activity: 100 % reduction rate reported for mice infected with *S. mansoni* when treated with 160 mg/kg body weight of aqueous leaves extract (Jatsa et al., 2009)

[a]*HNC or SRFK: Cameroon National herbarium code; BUD, Herbarium of the Botany Department of the University of Dschang, Cameroon).*
[b]*Compounds characterized for the first time in Cameroonian medicinal plant are underlined.*
[c]*Screened activity: Leishmania donovani (L. donovani); Schistosoma mansoni (S. mansoni); Toxoplasma gondii (T. gondii); Trypanosoma brucei rhodesiense (T. b. rhodesiense); Trypanosoma brucei (T. brucei).*

has not yet been discovered. The total number of persons affected globally is projected to rise from 246 million in 2007 to 380 million in 2025, if prevention measures will not be scaled up (Bennett, 2007). In Africa, the number of diabetic patients in 2006 was 10.4 million, and it is expected to increase to 18.7 million in 2025. The annual mortality linked to diabetes worldwide is estimated to be above one million. In Cameroon, the prevalence of diabetes increased from 2% in 1998 (in a study supported by the World Diabetes Foundation, WDF) to 5% in 2003 and 6.5% in 2007 (in another WDF study, Walgate, 2008). Medicinal plants have been

Table 4 | Plants used in Cameroon as anti-inflammatory and analgesic agents, with evidence of their activities.

Family	Species[a]	Traditional treatment	Plant part used	Bioactive (or potentially active) compounds[b]	Screened activity[c]
Acanthaceae	*Acanthus montanus* (Nees) T. Anderson (1652/SRF61CAM)	Cough, hypertension, skin infection, boil, witches, dysmenorrhoea, pain, epilepsy, miscarriages, heart troubles, rheumatic pain, syphilis (Burkill, 1985; Adjanohoun et al., 1996; Babu et al., 2001; Noumi and Fozi, 2003; Igoli et al., 2005; Nana et al., 2008)	Leaves	Not identified	Leave extract showed analgesic and anti-inflammatory properties and the proposed mechanism was the inhibition of the prostaglandins pathway at 200 mg/kg in rats. Also, this extract at 200 mg/kg body weight in rats reduced carrageenan-induced edema, and formalin-induced pain (Asongalem et al., 2004).
Anacardiaceae	*Sclerocarya birrea* (A. Rich.) Hochst (7770/HNC)	Boils and blood circulation problems, rheumatism, infectious diseases, inflammation (Mojeremane and Tshwenyane, 2004; Fotio et al., 2009)	Bark	Not identified	Bark extract inhibited albumin-induced paw edema (Ojewole, 2004), Formalin- or Freund's adjuvant (CFA)-carrageenan-, histamine, or serotonin-induced paw edema (Fotio et al., 2009) in rats
Caesalpiniaceae	*Erythrophleum suaveolens*, Guillemin & Perrottet (HN001AD)	Anti-poison, dermatitis, infectious disease, convulsion, inflammation due to snake bite, cardiac problems, headaches, migraines edema, rheumatism, asthma (Dalziel, 1937; Bouquet, 1969; Leiderer, 1982; Neuwinger, 1998)	Bark	Not identified	Extract from the bark and fractions at 19.2 μg/ml showed inhibition of carrageenin-induced paw edema in rats; Hexane fraction inhibited the 5-lipoxygenase activity (Dongmo et al., 2001)
Combretaceae	*Combretum molle* R.Br. ex G.Don (6518/SRF/CAM)	Fever, abdominal pains, convulsion, worm infections, AIDS (Bessong et al., 2004)	Bark	β-D-glucopyranosyl 2α,3β,6β-trihydroxy-23-galloylolean-12-en-28-oate (**18**); combregenin (**19**); arjungenin (**20**), arjunglucoside I, combreglucoside (Ponou et al., 2008)	Bark extract, compounds **18**–**20** showed good activity against carrageenan-induced paw edema in rat (Ponou et al., 2008)
Crassulaceae	*Kalanchoe crenata* Andr. (50103/YA/HNC)	Earache, smallpox, headache, inflammation, pain, asthma, palpitation, convulsion, general debility (Dimo et al., 2006)	Not specified	Not identified	*n*-Butanol fraction inhibited carrageenani-, histamine-, serotonin-, and formalin-induced paw edema in rats (Dimo et al., 2006)
Euphorbiaceae	*Bridelia scleroneura* (42088/HNC)	Abdominal pain, contortion, arthritis, inflammation (Watt and Breyer-Brandwijk, 1962; Théophile et al., 2006)	Bark, roots	Not identified	Crude bark extract showed peripheral and central analgesic and anti-inflammatory activity against acute inflammation processes in rats (Théophile et al., 2006)
Euphorbiaceae	*Uapaca guineensis* (41501/HNC)	Fever, inflammation, pain, skin diseases, and sexual dysfunction (Vivien and Faure, 1996)	Not specified	Not identified	Bark crude extract showed analgesic activity, and inhibited carrageenan-induced inflammation in rats (Nkeh-Chungag et al., 2009)

(Continued)

Table 4 | *Continued*

Family	Species[a]	Traditional treatment	Plant part used	Bioactive (or potentially active) compounds[b]	Screened activity[c]
Guttiferae	*Allanblackia monticola* Staner L.C. (61168/HNC)	Amoebic dysentery, diarrhea, indigestion, pulmonary infections, skin diseases, headache, inflammation, and generalized pain (Raponda-Waker and Sillans, 1961)	Bark	Betulinic acid, lupeol, and amangostin (Nguemfo et al., 2009)	Crude extract from the bark, lupeol, betulinic acid, and a-mangostin inhibited paw carrageenan-induced edema rat (Nguemfo et al., 2007, 2009)
Leguminosae	*Erythrina addisoniae* Hutchinson & Dalziel (41617/HNC)	Dysentery, asthma, venereal diseases, boils, and leprosy (Talla et al., 2003)	Bark	Warangalone (**56**) (Talla et al., 2003)	Bark extract and compound **56** showed an anti-inflammatory on the PLA_2-induced paw edema and 12-*O*-tetradecanoylphorbol 13-acetate-induced ear edema in mice (Talla et al., 2003)
	Erythrina mildbraedii Harms (50452/HNC)	Dysentery, stomach pains, venereal diseases, asthma, female sterility, ulcers, boils and various types of inflammations (Oliver-Bever, 1986)	Bark, roots	Erycristagallin (**57**) (Njamen et al., 2003)	Root bark extract inhibited the carrageenan-induced mouse paw whilst compound **57** inhibited the PLA_2-induced mouse paw edema and mouse ear edema induced by 2-*O*-tetradecanoylphorbol 13-acetate (Njamen et al., 2003)
	Erythrina sigmoidea Hua	Female infertility, stomach pain, and gonorrhea (Giner-Larza et al., 2001)	Bark	Sigmoidin A (**51**) and B (**52**) (Njamen et al., 2004)	Compound **51** inhibited PLA_2-induced paw edema in mice, while both compounds **51** and **52** were found to be effective 12-*O*-tetradecanoylphorbol 13-acetate-induced ear edema (Njamen et al., 2004)
	Millettia versicolor Welw. (32315/HNC)	Intestine parasitosis, rheumatism, pain, infertility (Adjanohoun et al., 1988; Bouquet, 1969)	Not specified	2-acetyl-7-methoxynaphthol[2,3-b] furan-4,9-quinone (**29**) (Fotsing et al., 2003)	CH_2Cl_2 fraction from methanol crude bark extract inhibited carrageenan-induced paw edema and TPA-induced acute ear edema in mouse as well as compound **29** (Fotsing et al., 2003)
	Millettia griffoniana Baill. (32315/SRF/HNC)	Boils, insects bits, inflammatory affections like pneumonia, and asthma, infertility, amenorrhea, menopausal disorders (Sandberg and Cronlund, 1977)	Bark, roots	griffonianone D (**55**) (Yankep et al., 2003)	Extract of the root bark and compound **55** showed anti-inflammatory effects via inhibition of PLA_2-induced mouse paw edema and TPA-induced acute mouse ear edema (Yankep et al., 2003)
Solanaceae	*Solanum torvum Swartz.* (21103/HNC)	Fever, wounds, tooth decay, haemostatic properties, pain, anti-inflammation (Henty, 1973; Ndebia et al., 2007)	Leaves	Not identified	Crude extract from the leaves inhibits both acetic acid- and pressure-induced pain at 300 mg/kg body weight of rats, and also anti-inflammatory activity on carrageenan-induced paw edema (Ndebia et al., 2007

[a]*HNC or SRFK: Cameroon National herbarium code.*
[b]*Compounds characterized for the first time in Cameroonian medicinal plant are underlined.*
[c]*Screened activity: TPA (2-O-tetradecanoylphorbol 13-acetate); PLA_2 (phospholipase A_2).*

reported to be useful in diabetes worldwide and have empirically been used as anti-diabetic and anti-hyperlipidemic remedies. Anti-hyperglycemic effects of these plants were attributed to their ability to restore the function of pancreatic tissues by increasing insulin output, inhibiting the intestinal absorption of glucose, or enhancing metabolism of insulin-dependent processes. Several plant preparations were traditionally used in Cameroon to treat diabetes. Some of them were screened for their bioactivity, but most of the studies were not pursued until the isolation of active principles. Plants with hypoglycaemic activities include *Anacardium occidentale, Sclerocarya birrea, Ageratum conyzoides, Ceiba pentandra*, *Kalanchoe crenata, Bridelia ndellensis*, *Irvingia gabonensis, Bersama engleriana* and *Morinda lucida* (**Table 5**).

ANTIOXIDANT ACTIVITIES

The common link between oxidants and inflammatory reactions, infections, cancer, and other disorders has been well established (Mongelli et al., 1997; Wang et al., 1999). However, this may not really be of therapeutic relevance, but more of a preventive medicine. In chronic infections and inflammation as well as in other disorders, release of leukocytes and other phagocytic cells readily defends the organism from further injury. The cells do this by releasing free oxidant radicals, and these by-products are generally reactive oxygen species (ROS) such as super oxide anion, hydroxyl radical, nitric oxide, and hydrogen peroxide that result from cellular redox processes (Ames et al., 1993; Mongelli et al., 1997). At low or moderate concentrations, ROS exert beneficial effects on cellular responses and immune function. At high levels, however, free radicals and oxidants generate oxidative stress, a deleterious process that can damage cell structures, including lipids, proteins, and DNA (Pham-Huy et al., 2008). Oxidative stress plays a major role in the development of chronic and degenerative ailments such as cancer, autoimmune disorders, rheumatoid arthritis, cataract, aging, cardiovascular, and neurodegenerative diseases (Willcox et al., 2004b; Pham-Huy et al., 2008). Antioxidants act as free radical scavengers by preventing and repairing damages caused by ROS and, therefore, can enhance the immune defense and lower the risk of cancer and degenerative diseases (Ames et al., 1993; Pham-Huy et al., 2008). In recent years, there is an increasing interest in finding antioxidant phytochemicals, because they can inhibit the propagation of free radical reactions, and thereby protect the human body from diseases (Terao and Piskula, 1997). Several medicinal plants of Cameroon were screened for their antioxidant properties and a number of bioactive compounds was isolated (**Table 6**). Omisore et al. (2005) considered the cut-off point for antioxidant activity as 50 μg/ml. Samples with $IC_{50} > 50$ μg/ml were classified as being moderately active, while samples with $IC_{50} < 50$ μg/ml were judged as having high antioxidant capacity. In the present paper, samples will be considered to have high or significant antioxidant capacity with $IC_{50} < 50$ μg/ml (extract) or $IC_{50} < 10$ μg/ml (compounds), moderate antioxidant capacity with $50 < IC_{50} < 100$ μg/ml (extract) or $10 < IC_{50} < 20$ μg/ml (compounds) and low antioxidant capacity with $IC_{50} > 100$ μg/ml (extract) or $IC_{50} > 20$ μg/ml (compounds). Extracts from 42 medicinal plants of Cameroon used for the treatment of anemia, diabetes, AIDS, malaria, and obesity were recently screened for antioxidant properties, with a considerable number showing good activities (Agbor et al., 2007). Many of them exhibited high inhibition percentages on the basis of Folin, Ferric reducing antioxidant power (FRAP), and DPPH (1,1-diphenyl-2-picrylhydrazyl) assays. Plants with good activities included *Alchornea cordifolia* (Euphorbiaceae), *Dacryodes edulis* (Burseraceae), *Ocimum basilicum* (Lamiaceae), *Harungana madagascariensis* (Hypericaceae), *Cylicodiscus gabunensis* (Mimosaceae), *Coleus coprosifolius* (Lamiaceae) (Agbor et al., 2007). Arylbenzofurans isolated from the bark of *Morus mesozygia* (Moraceae), moracin T (**43**), moracin U (**44**), moracin S (**45**); moracin R (**46**) also showed strong DPPH scavenging capability with IC_{50} values of 4.12, 5.06, 6.08, and 7.17 μg/ml, respectively (Kapche et al., 2009). The activity of the crude extract of this plant was also reported as significant (IC_{50}: 5.92 μg/ml), by means of the DPPH scavenging assay (Kapche et al., 2009).

OTHER ACTIVITIES

Other studies involving Cameroon medicinal plants include their action on human fertility and enzymatic activities. However, few studies have focused on these activities, explaining the scarcity of published data.

Some plants with positive effects on the reproductive system based on studies using experimental rats have been reported. They include *Aloe buettneri* (Liliaceae), *Justicia insularis* and *Dicliptera verticillata* (Acanthaceae) and *Hibiscus macranthus* (Malvaceae), locally used to regulate the menstrual cycle and to treat dysmenorrhea or infertility in women (Telefo et al., 1998); *Basella alba* (Basellaceae) (Moundipa et al., 2005), and *Mondia whitei* (Periplocaceae) traditionally claimed to increase libido (Watcho et al., 2001).

Some compounds from Cameroonian plants were investigated for the ability to interfere with the activity of some enzymes such as xanthine oxidase, phosphodiesterase I, or prolyl endopeptidase. Xanthine oxidase catalyzes the oxidative hydroxylation of hypoxanthine or xanthine using oxygen as a cofactor, and the resulting end products are superoxide anion ($O_2^{\cdot-}$) and uric acid. The inhibitors of xanthine oxidase enzyme can prevent the generation of excess superoxide anions (Chung et al., 1997). Phosphodiesterase I successively hydrolyzes 5′-mononucleotides from 3′-hydroxyl-terminated ribo- and deoxyribo-oligonucleotides. The enzyme has been widely utilized as a tool for structural and sequential studies of nucleic acids. The 5′-nucleotide phosphodiesterase isozyme-V test is useful in detecting liver metastatis in breast, gastrointestinal, lung, and various other forms of cancers (Lei-Injo et al., 1980). Prolyl endopeptidase catalyzes the hydrolysis of peptide bonds at the L-proline carboxy terminal and, thus, plays an important role in the biological regulation of proline-containing neuropeptides and peptide hormones, which are recognized to be involved in learning and memory (Szeltner et al., 2000). The stilbene glycosides isolated from *Boswellia papyrifera* (Del.) Hochst (Burseraceae), *trans*-4′,5-dihydroxy-3-methoxystilbene-5-*O*-{α-L-rhamnopyranosyl-(1-2)-[α-L-rhamnopyranosyl-(1-6)]-β-D-glucopyranoside and *trans*-4′,5-dihydroxy-3-methoxystilbene-5-*O*-[α-L-rhamnopyranosyl-(1-6)]-β-D-glucopyranoside exhibited significant inhibition of phosphodiesterase I and xanthine oxidase (Atta-ur-Rahman et al., 2005). Triterpenes such as 3-α-acetoxy-27-hydroxylup-20(29)-en-24-oic acid, 11-keto-β-boswellic acid, β-elemonic

Table 5 | Plants used in Cameroon to treat diabetes, with evidence of their activities.

Family	Species[a]	Traditional treatment	Plant part used	Bioactive (or potentially active) compounds	Screened activity[b]
Anacardiaceae	*Anacardium occidentale* L. (41935/HNC)	Diabetes mellitus (Kamtchouing et al., 1998)	Leaves	Not identified	Leaves extract showed anti-diabetes activity through protective role against the diabetogenic action of STZ and hypoglycemic effects in rats (Kamtchouing et al., 1998; Sokeng et al., 2007)
	Sclerocarya birrea [(A. Rich.) Hochst] (7770/HNC)	Diabetes, diarrhea, dysentery, gangrenous rectitis, fevers, stomach disorders, ulcers, sore eyes (Watt and Breyer-Brandwijk, 1962; Bryant, 1966; Gelfand et al., 1985; Dieye et al., 2008)	Leaves, bark, roots	Not identified	Bark extracts have been reported to exert hypoglycemic in rats following acute and chronic treatments (Ojewole, 2003; Dimo et al., 2007; Gondwe et al., 2008), acting directly on insulin-secreting cells (Ndifossap et al., 2010)
Asteraceae	*Ageratum conyzoides* L. (19050/SFR/Cam)	Cough, fever, skin disease, diabetes, bleeding due to external wounds, furuncle, eczema, carbuncle, headaches (Lavergne and Véra, 1989; Tsabang et al., 2001)	Whole plant	Not identified	Leaves extract showed hypoglycemic and anti-hyperglycemic activities in STZ-induced diabetic rats (Nyunaï et al., 2009)
Bombacaceae	*Ceiba pentandra* (L) Gaertner (43623/HNC)	Diuretic, diabetes, hypertension, headache, dizziness, constipation, mental trouble, fever, peptic ulcer, rheumatism, leprosy (Noumi et al., 1999; Ngounou et al., 2000; Noumi and Dibakto, 2000; Noumi and Tchakonang, 2001; Ueda et al., 2002)	Bark, leaves, roots	Not identified	Roots extract reduced hyperglycemia in STZ-induced diabetic rats (Dzeufiet et al., 2006)
Crassulaceae	*Kalanchoe crenata* (WEKC) (50103/YA/HNC)	Inflammatory diseases, diabetes (Kamgang et al., 2008)	Whole plant	Not identified but terpenoids, tannins, polysaccharides, saponins, flavonoids and alkaloids were identified from the leaves (Kamgang et al., 2008)	Ethanol extract of the whole plant was found to possess significant hypoglycemic effect in normal rats by lowering blood glucose levels and anti-hyperglycemic effect by lowering and maintaining glycemia at normal levels in diabetic rats (Kamgang et al., 2008)
Euphorbiaceae	*Bridelia ndellensis* Beille (9676/HNC)	fever, rheumatism, diarrhea, and diabetes (Addae-Mensah and Achenbach, 1985; Onunkwo et al., 1996; Sokeng et al., 2005)	Not specified	Not identified	Ethyl acetate and dichloromethane extracts and fractions of the bark significantly lowered blood glucose levels in type 2 diabetic rats (Sokeng et al., 2005)
Irvingiaceae	*Irvingia gabonensis* (Aubry Lecomte ex O'Rorke) Baill. (28054/HNC)	Gonorrhea, gastrointestinal and hepatic disorders, wounds infection, diabetes, analgesis (Ngondi et al., 2005)	Bark, fruits, leaves, roots	Not identified	Seeds extract showed modulatory effect on diabetes induced dyslipidemia (Dzeufiet et al., 2009) in rats
Melianthaceae	*Bersama engleriana* Gurke (24725/HNC)	Cancer, spasms, infectious diseases, male infertility, diabetes (Watcho et al., 2007)	Leaves, Stem bark, roots	Not identified but flavonoids, phenols, triterpenes, saponins, and anthraquinones were detected in all parts of the plan (Kuete et al., 2008)	Leaves extract showed hypoglycemic properties (Njike et al., 2005)
Rubiaceae	*Morinda lucida* Benth	Uncontrolled adult cases of diuresis not necessarily associated with diabetes but linked to general body weakness and rapid loss of weight (Kamanyi et al., 1994)	Not specified	Not identified	Root extract showed potent hypoglycemic effects in both normoglycemic and alloxan-induced diabetic mice (Kamanyi et al., 1994)

[a]*HNC or SRFK: Cameroon National herbarium code.*
[b]*Screened activity: streptozotocin (STZ).*

Table 6 | Plants used in Cameroon with evidence of their as antioxidant activities.

Family	Species[a]	Traditional treatment	Plant part used	Bioactive (or potentially active) compounds[b]	Screened activity[c]
Ebenaceae	*Diospyros sanza-minika* A. Chevalier (9649/SRFCam)	Epilepsy, paralysis, convulsions, spasm, pains (Burkill, 1985)	Leaves	11-*O*-*p*-hydroxybenzoylnorbergenin; 4-*O*-(30-methylgalloyl) norbergenin; 4-*O*-syringoylnorbergenin; norbergenin; 4-*O*-galloylnorbergenin; quercitol (Tangmouo et al., 2009)	DPPH scavenging activity: significant for 4-*O*-galloylnorbergenin, moderate for norbergenin, 11-*O*-*p*-Hydroxybenzoylnorbergenin, 4-*O*-(30-Methylgalloyl)norbergenin and 4-*O*-Syringoylnorbergenin (Tangmouo et al., 2009)
Guttiferae	*Garcinia polyantha* Oliv (1337/SRF/Cam)	Dressing for wounds (Bouquet, 1969)	Sap	Bangangxanthone A; bangangxanthone B; 2-hydroxy-1,7-dimethoxyxanthone; 1,5-dihydroxyxanthone (Lannang et al., 2005)	DPPH scavenging activity: bangangxanthone A isolated from the bark showed the best activity with an IC_{50} = 87.0 μM while the standard value for BHA was IC_{50} = 42.0 μM (Lannang et al., 2005)
	Garcinia afzelii Engl.	Bacterial infections, dental caries (Adu-Tutu et al., 1979; Waffo et al., 2006)	Leaves; flowers	Afzeliixanthones A; afzeliixanthones B (Waffo et al., 2006)	DPPH scavenging activity: Significant for the crude extract and moderate for Afzelii xanthones A and B (Waffo et al., 2006)
Hypericaceae	*Harungana madagascariensis* Lam. (32358/HNC)	Diarrhea, dysentery, indigestion, poor pancreatic function (Berhaut, 1975; Prajapati et al., 2003)	Not specified	Harunmadagascarins A and Harunmadagascarins B, harunganol B and harungin anthrone (Kouam et al., 2005)	DPPH scavenging activity: IC_{50} of 60.97; 64.76 were recorded with harunmadagascarin and harunganol B respectively (Kouam et al., 2005)
Meliaceae	*Carapa grandiflora* sprsgue	Arthritis, general fatigue, skin diseases and as febrifuge (Ayafor et al., 1994)	Seeds	Quercitrin (Omisore et al., 2005)	DPPH scavenging activity: low for quercetin (Omisore et al., 2005)
Mimosaceae	*Entada rheedii* Spreng (19966/SRI/CAM)	Jaundice (Nzowa et al., 2010)	Seeds	Rheediinoside A; rheediinoside B (Nzowa et al., 2010)	ABTS·+ scavenging activity: moderate for rheediinoside B; low for rheediinoside A; DPPH scavenging activity: low activity for rheediinoside A and rheediinoside B (Nzowa et al., 2010)
Moraceae	*Dorstenia convexa* De Wild (53450 HNC)	Malaria (Boyom et al., 2009)	Twigs	Bartericins A; stigmasterol; isobavachalcone (Omisore et al., 2005)	DPPH scavenging activity: low bartericin A and isobavachalcone and stigmasterol (Omisore et al., 2005)
	Dorstenia barteri Bureau (44016/HNC)	Snakebite, rheumatic, infectious diseases, arthritis (Tsopmo et al., 1999)	Whole plant	Bartericins A, and B; stigmasterol; isobavachalcone; 4-hydroxylonchocarpin (Omisore et al., 2005)	DPPH scavenging activity: significant for twigs extract (Omisore et al., 2005)
	Dorstenia mannii Hook. f. (2135/HNC)	Rheumatism, stomach disorders (Bouquet, 1969)	Leaves	Dorsmanin F; 6,8-diprenyleridictyol (Omisore et al., 2005)	DPPH scavenging activity: low for 6,8-diprenyleriodictyol, and dosrmanin F (Omisore et al., 2005)
	Morus mesozygia Stapf. (4228/SRFK)	Arthritis, rheumatism, malnutrition, debility, pain-killers, stomach disorders, wound infections, gastroenteritis, peptic ulcer, infectious diseases (Burkill, 1985; Noumi and Dibakto, 2002)	Bark	Moracin R (**46**); moracin S (**45**); moracin T (4**3**); moracin U (**44**) (Kapche et al., 2009)	DPPH scavenging activity: significant for bark crude extract, compounds **43**–**46** (Kapche et al., 2009)

(Continued)

Table 6 | *Continued*

Family	Species[a]	Traditional treatment	Plant part used	Bioactive (or potentially active) compounds[b]	Screened activity[c]
Piperaceae	*Piper umbellatum* Linn (6516/SRF/CAM)	Poisoning, pitting edema, fetal malpresentation, filariasis, rheumatism, hemorrhoids, dysmenorrheal, general pains (Tabopda et al., 2008)	Whole plant	Piperumbellactams A; piperumbellactams B; piperumbellactams C; *N-p*-coumaroyl tyramine (Tabopda et al., 2008)	DPPH scavenging activity: Moderate activity reported for piperumbellactams A and low activities for piperumbellactams B; C; *N-p*-coumaroyl tyramine (Tabopda et al., 2008)

[a]*HNC or SRFK: Cameroon National herbarium code.*
[b]*Compounds characterized for the first time in Cameroonian medicinal plant are underlined.*
[C]*Screened activity: DPPH or 1,1-diphenyl-2-picryl hydrazyl radical assay (evaluates the ability of antioxidants to scavenge free radicals; Hydrogen-donating ability is an index of the primary antioxidants; these antioxidants donate hydrogen to free radicals, leading to non-toxic species and therefore to inhibition of the propagation phase of lipid oxidation [Lugasi et al., 1998]); ABTS+: 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium radical cation; BHA: 3-t-butyl-4-hydroxyanisole; Significant activity (IC_{50} < 50 µg/ml), moderate activity (50 < IC_{50} < 100 µg/ml), low activity (IC_{50} > 100 µg/ml).*

acid, 3 α-acetoxy-11-keto-β-boswellic acid, and β-boswellic acid also exhibited prolyl endopeptidase inhibitory activities (Atta-ur-Rahman et al., 2005).

CONCLUSION

The present review presents an overview of medicinal plants research in Cameroon and is intended to serve as scientific baseline information for the documented plants as well as a starting point for future studies. The paper draws attention on some active metabolites and plant extracts, with the potential for new drugs or improved plant medicines. The review inevitably shows the richness of the Cameroon flora as medicinal resource and demonstrates the effectiveness of numerous traditionally used plants. Presently, there is an urgent necessity for standardization of plant-derived drugs, as their use is still empirical. There is also an urgent requirement to standardize methods and cut-off points for describing their bioactivities. Other recommendations include parallel screenings by using cytotoxicity tests to preclude non-specific cytotoxicity from being interpreted as efficient following *in vitro* screening. The elucidation of the mechanisms of action of biologically active extracts and compounds should be strengthened and given priority in future investigations as already shown for natural products from other parts of the world (Kong et al., 2009; Youns et al., 2010; Konkimalla and Efferth, 2010).

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REVIEW
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Berberine: Botanical Occurrence, Traditional Uses, Extraction Methods, and Relevance in Cardiovascular, Metabolic, Hepatic, and Renal Disorders

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Berberine-containing plants have been traditionally used in different parts of the world for the treatment of inflammatory disorders, skin diseases, wound healing, reducing fevers, affections of eyes, treatment of tumors, digestive and respiratory diseases, and microbial pathologies. The physico-chemical properties of berberine contribute to the high diversity of extraction and detection methods. Considering its particularities this review describes various methods mentioned in the literature so far with reference to the most important factors influencing berberine extraction. Further, the common separation and detection methods like thin layer chromatography, high performance liquid chromatography, and mass spectrometry are discussed in order to give a complex overview of the existing methods. Additionally, many clinical and experimental studies suggest that berberine has several pharmacological properties, such as immunomodulatory, antioxidative, cardioprotective, hepatoprotective, and renoprotective effects. This review summarizes the main information about botanical occurrence, traditional uses, extraction methods, and pharmacological effects of berberine and berberine-containing plants.

Keywords: berberine, botanical occurrence, traditional uses, extraction methods, biological activities

INTRODUCTION

Berberine

Berberine(5,6-dihydro-9,10-dimethoxybenzo[g]-1,3-benzodioxolo[5,6-a]quinolizinium) **Figure 1**, is a nonbasic and quaternary benzylisoquinoline alkaloid, a relevant molecule in pharmacology and medicinal chemistry. Indeed, it is known as a very important natural alkaloid for the synthesis of several bioactive derivatives by means of condensation, modification, and substitution of functional groups in strategic positions for the design of new, selective, and powerful drugs (Chen et al., 2005).

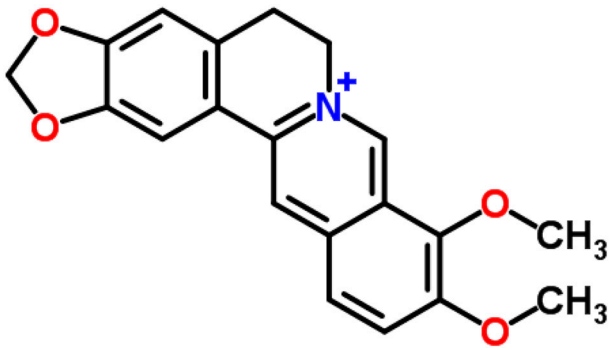

FIGURE 1 | Berberine structure (according to ChemSpider database).

TRADITIONAL USE OF BERBERINE-CONTAINING SPECIES

In the Berberidaceae family, the genus *Berberis* comprises of ∼450–500 species, which represent the main natural source of berberine. Plants of this genus are used against inflammation, infectious diseases, diabetes, constipation, and other pathologies (Singh A. et al., 2010). The oldest evidence of using barberry fruit (*Berberis vulgaris*) as a blood purifying agent was written on the clay tablets in the library of Assyrian emperor Asurbanipal during 650 BC (Karimov, 1993). In Asia, the extensive use of the stem, stem bark, roots, and root bark of plants rich in berberine, particularly *Berberis* species, has more than 3000 years of history. Moreover, they have been used as raw material or as an important ingredient in Ayurvedic and traditional Chinese medicine (Birdsall, 1997; Kirtikar and Basu, 1998; Gupta and Tandon, 2004; Kulkarni and Dhir, 2010). In Ayurveda, *Berberis* species have been traditionally used for the treatment of a wide range of infections of the ear, eye, and mouth, for quick healing of wounds, curing hemorrhoids, indigestion and dysentery, or treatment of uterine and vaginal disorders. It has also been used to reduce obesity, and as an antidote for the treatment of scorpion sting or snakebite (Dev, 2006). Berberine extracts and decoctions are traditionally used for their activities against a variety of microorganisms including bacteria, viruses, fungi, protozoa, helminthes, in Ayurvedic, Chinese, and Middle-Eastern folk medicines (Tang et al., 2009; Gu et al., 2010).

In Yunani medicine, *Berberis asiatica* has multiple uses, such as for the treatment of asthma, eye sores, jaundice, skin pigmentation, and toothache, as well as for favoring the elimination of inflammation and swelling, and for drying ulcers (Kirtikar and Basu, 1998). Decoction of the roots, and stem barks originating from *Berberis aristata*, *B. chitria,* and *B. lycium* (Indian *Berberis* species), have been used as domestic treatment of conjunctivitis or other ophthalmic diseases, enlarged liver and spleen, hemorrhages, jaundice, and skin diseases like ulcers (Rajasekaran and Kumar, 2009). On the other hand, the use of decoction of Indian barberry mixed with honey has also been reported for the treatment of jaundice. Additionally, it has been reported the use of decoction of Indian barberry and *Emblic myrobalan* mixed with honey in the cure of urinary disorders as painful micturition (Kirtikar and Basu, 1998). Numerous studies dealing with its antimicrobial and antiprotozoal activities against different types of infectious organisms (Vennerstrom et al., 1990; Stermitz et al., 2000; Bahar et al., 2011) have been assessed so far. Moreover, it has been used to treat diarrhea (Chen et al., 2014) and intestinal parasites since ancient times in China (Singh and Mahajan, 2013), and the Eastern hemisphere, while in China it is also used for treating diabetes (Li et al., 2004).

Nowadays, a significant number of dietary supplements based on plants containing berberine (Kataoka et al., 2008) are used for reducing fever, common cold, respiratory infections, and influenza (Fabricant and Farnsworth, 2001). Another reported use for berberine-containing plants is their application as an astringent agent to lower the tone of the skin. Also, positive effects were observed on the mucous membranes of the upper respiratory tract and gastrointestinal system with effects on the associated ailments (Chen et al., 2014; Yu et al., 2016).

In southern South America leaves and bark of species of the genus *Berberis* are used in traditional medicine administered for mountain sickness, infections, and fever (San Martín, 1983; Houghton and Manby, 1985; Anesini and Perez, 1993).

Furthermore, there are other genera which contain berberine. The genus *Mahonia* comprises of several species that contain berberine. Within them, *M. aquifolium* has been traditionally used for various skin conditions. Due to its main alkaloid (berberine), is known to be used in Asian medicine for its antimicrobial activity. *Coptidis rhizoma* (rhizomes of *Coptis chinensis*), another plant which contains berberine, is a famous herb very frequently used in traditional Chinese medicine for the elimination of toxins, "damp-heat syndromes", "purge fire", and to "clear heat in the liver" (Tang et al., 2009). **Table 1** gathers a synthesis of the main traditional uses of species containing berberine.

BOTANICAL SOURCES OF BERBERINE

Berberine has been detected, isolated, and quantified from various plant families and genera including Annonaceae (*Annickia*, *Coelocline*, *Rollinia,* and *Xylopia*), Berberidaceae (*Berberis*, *Caulophyllum*, *Jeffersonia*, *Mahonia*, *Nandina*, and *Sinopodophyllum*), Menispermaceae (*Tinospora*), Papaveraceae (*Argemone*, *Bocconia*, *Chelidonium*, *Corydalis*, *Eschscholzia*, *Glaucium*, *Hunnemannia*, *Macleaya*, *Papaver,* and *Sanguinaria*), Ranunculaceae (*Coptis*, *Hydrastis*, and *Xanthorhiza*), and Rutaceae (*Evodia*, *Phellodendron*, and *Zanthoxyllum*) (**Table 2**). The genus *Berberis* is well-known as the most widely distributed natural source of berberine. The bark of *B. vulgaris* contains more than 8% of alkaloids, berberine being the major alkaloid (about 5%) (Arayne et al., 2007).

Berberine is also widely present in barks, leaves, twigs, rhizomes, roots, and stems of several medicinal plants species, including *Argemone mexicana* (Etminan et al., 2005), *Berberis aristata*, *B. aquifolium*, *B. heterophylla*, *B. beaniana*, *Coscinium fenestratum* (Rojsanga and Gritsanapan, 2005), *C. chinensis*, *C. japonica*, *C. rhizome*, *Hydratis canadensis* (Imanshahidi and

TABLE 1 | Traditional uses of berberine-containing species.

Family	Scientific name	Traditional uses	References
Annonaceae	*Annickia chlorantha* (Oliv.) Setten & Maas (ex-*Enantia chlorantha* Oliv.)	Treat jaundice, hepatitis A, B, C, and D, conjunctivitis, leishmaniasis, medicine for cuts and infected wounds, sores and ulcers, antipyretic for various fevers, tuberculosis, vomiting of blood, urinary tract infections, treatment of fatigue, rheumatism, treat malaria symptoms, aches, wounds, boils, vomiting, yellow bitter, chills, sore, spleen in children and body pains, skin ailments, intercostal pain and to promote conception, intestinal worms, intestinal spasms, malaria and sexual asthenia, treat coughs and wounds; rickettsia fever, treat of sleeping sickness and dysentery, hemostatic and rickettsia, treat yellow fever and typhoid fever, treat diabetes, treat syphilis, and other infectious diseases, poliomyelitis, treat hypertension, treat HIV and prostate cancer	Oliver, 1960; Sandberg, 1965; Bouquet, 1969; Hamonniere et al., 1975; Onwuanibe, 1979; Burkill, 1985; Gill and Akinwumi, 1986; Gbile et al., 1988; Vennerstrom and Klayman, 1988; Vennerstrom et al., 1990; Adjanohoun et al., 1996; Nguimatsia et al., 1998; Kayode, 2006; Odugbemi et al., 2007; Ehiagbonare and Onyibe, 2008; Jiofack et al., 2008, 2009; Kadiri, 2008; Ogbonna et al., 2008; Olowokudejo et al., 2008; Betti and Lejoly, 2009; Ndenecho, 2009; Adeyemi et al., 2010; Noumi, 2010; Noumi and Anguessin, 2010; Noumi and Yumdinguetmun, 2010; Bele et al., 2011; Din et al., 2011; Ngono Ngane et al., 2011; Oladunmoye and Kehinde, 2011; Gbolade, 2012; Musuyu Muganza et al., 2012; Tsabang et al., 2012; Betti et al., 2013; Borokini et al., 2013; Fongod, 2014; Ishola et al., 2014; Ohemu et al., 2014
	Annickia pilosa (Exell) Setten & Maas (ex-*Enantia pilosa* Exell)	Medicine for cuts	Versteegh and Sosef, 2007
	Annickia polycarpa (DC.) Setten & Maas ex I.M.Turner (ex-*Enantia polycarpa* (DC.) Engl. & Diels)	Treat cuts, antiseptic to treat sores, stomach ulcers, leprosy and ophthalmia, treatment of skin infections and sores, treat jaundice, and treat fever including malaria and to promote wound healing, against intestinal problems	Irvine, 1961; Bouquet and Debray, 1974; Ajali, 2000; Govindasamy et al., 2007; Versteegh and Sosef, 2007
	Rollinia mucosa (Jacq.) Baill.	Treat of tumors	Hartwell, 1982
	Xylopia polycarpa (DC.) Oliv.	Treat wounds, ulcers, leprosy, rheumatism, stomach and gall-bladder problems, eye diseases, for conception, diarrhea, malaria, fevers and sleeping disorders	Neuwinger, 1996
Berberidaceae	*Berberis actinacantha* Mart.	Antipyretic	San Martín, 1983
	Berberis aquifolium Pursh	Skin conditions, treat eczema, acne, conjunctivitis and herpes, alleviate the symptoms of psoriasis, treat diarrhea and in higher doses to treat constipation, improvement of blood flow to the liver, stimulate intestinal secretions and bile flow, treat jaundice, hepatitis, cirrhosis and general digestive problems, treatment of gall bladder disease, hemorrhages and a few forms of cancer, fungal infections, dysentery, anti-inflammatory properties, stomach problems, sore womb following childbirth and/or menstruation	King, 1898; Ritch-Krc et al., 1996
	Berberis aristata DC.	Treat allergies, metabolic disorders, ophthalmia, and other eye diseases, treat bleeding piles, anti-osteoporosis, treat skin diseases, menorrhagia, fever, diarrhea, dysentery, cholera, jaundice, ear and urinary tract infections, anti-bacterial, anti-fungal, anti-inflammatory, analgesic, anti-pyretic, jaundice, piles, malaria, laxative, anti-scorbutic, anti-diabetic, and anti-hepatopathic	Bhattacharjee et al., 1980; Duke and Beckstrom-Sternberg, 1994; Küpeli et al., 2002; Acharya and Rokaya, 2005; Chhetri et al., 2005; Kunwar and Adhikari, 2005; Sharma et al., 2005; Joshi and Joshi, 2007; Meena et al., 2009; Shahid et al., 2009; Phondani et al., 2010; Saraf et al., 2010; Tiwary et al., 2010; Sati and Joshi, 2011; Yogesh et al., 2011
	Berberis asiatica Roxb. ex DC.	Jaundice, diabetes mellitus, wound healing, asthma; drying unhealthy ulcers, anti-inflammatory, swelling, treat pneumococcal infections, eye (conjunctivitis) and ear diseases, rheumatism, fever, stomach disorders, skin disease (hyperpigmentation), malarial fever, laxative, teeth problems (toothache), and headache	Watt, 1883; Kirtikar and Basu, 1933; Samhita, 1963; Hashmi and Hafiz, 1986; Bhandari et al., 2000; Shah and Khan, 2006; Uniyal et al., 2006; Uprety et al., 2010; Maithani et al., 2014

(Continued)

TABLE 1 | Continued

Family	Scientific name	Traditional uses	References
	Berberis buxifolia Lam.	Treat infections	Anesini and Perez, 1993; Mølgaard et al., 2011
	Berberis chitria Buch.-Ham. ex Lindl.	Treat skin disease, jaundice, rheumatism, affection of eyes (household treatment for conjunctivitis, ophthalmic, bleeding piles), ulcers, skin diseases, enlarged liver and spleen	Watt, 1883; Kirtikar and Basu, 1933; Sir and Chopra, 1958
	Berberis darwinii Hook.	Antipyrectic, anti-inflammatory, treat stomach pains, indigestion, and colitis	Montes and Wilkomirsky, 1987
	Berberis empetrifolia Lam.	Treat mountain sickness	San Martín, 1983
	Berberis integerrima Bunge.	Antipyretic, treat diabetes, bone fractures, rheumatism, radiculitis, heart pain, stomach aches, kidney stones, tuberculosis, chest pain, headaches, constipation, and wound	Khalmatov, 1964; Khodzhimatov, 1989; Baharvand-Ahmadi et al., 2016
	Berberis jaeschkeana C. K. Schneid.	Treat eye diseases	Kala, 2006
	Berberis koreana Palib.	Antipyretic, treat gastroenteritis, sore throats, and conjunctivitis	Ahn, 2003
	Berberis leschenaultia Wall. ex Wight & Arn.	Antipyretic, cold and complications during post-natal period	Rajan and Sethuraman, 1992
	Berberis libanotica Ehrenb. ex C. K. Schneid.	Treat rheumatic and neuralgic diseases, anti-inflammatory, treat arthritis and muscular pain	El Beyrouthy et al., 2008; Esseily et al., 2012
	Berberis lycium Royle	Treat eye diseases, febrifuge, jaundice, diarrhoea, menorrhagia, piles, backache, dysentery, earache, fracture, eye ache, pimples, boils, wound healing, cough and throat pain, intestinal colic, diabetes, throat pain, scabies, bone fractures, sun blindness, against stomachache and intestinal problems	Zaman and Khan, 1970; ul Haq and Hussain, 1993; Bushra et al., 2000; Kaur and Miani, 2001; Hamayun et al., 2003; Ahmed et al., 2004; Abbasi et al., 2005, 2009, 2010; Shah and Khan, 2006; Zabihullah et al., 2006; Hussain et al., 2008; Sood et al., 2010
	Berberis microphylla G. Forst. (ex-*Berberis heterophylla* Juss. ex Poir.)	Febrifuge, anti-inflammatory and treat diarrhea	Muñoz, 2001
	Berberis oblonga (Regel) C. K. Schneid	Heart tonic, treat neurasthenia, antipyretic, antidiarrheal, treat rheumatism, eye diseases and wounds of the mouth, jaundice, stomach aches, back pain and arthralgia	Khalmatov, 1964; Sezik et al., 2004; Pak, 2005
	Berberis petiolaris Wall. ex G. Don	Treat malarial fever, diarrhea, conjunctivitis, and jaundice	Karimov, 1993
	Berberis pseudumbellata R. Parker	Diuretic, treat jaundice, intestinal disorders, eye diseases, oxytocic and throat ache, stomach problems and ulcers	Kala, 2006; Khan and Khatoon, 2007; Singh et al., 2009; Khan et al., 2016
	Berberis thunbergii DC.	Anti-inflammatory	Küpeli et al., 2002
	Berberis tinctoria Lesch.	Antimicrobial for skin disease, jaundice, affection of eyes, treat menorrhagia, diarrhea, and rheumatism	Fyson, 1975; Satyavati et al., 1987
	Berberis umbellata Wall. ex G. Don	Treating fever, jaundice, nausea, eye disorders and skin problems, tonic	Singh et al., 2012
	Berberis vulgaris L.	Antiarrhythmic, sedative, anticancer, heal internal injuries, remove kidney stones, treat sore throat and fever	Tantaquidgeon, 1928; Chaudhury et al., 1980; Zovko Koncić et al., 2010
	Caulophyllum thalictroides (L.) Michaux	Menstrual cramps, relieve the pain of childbirth, promote prompt delivery, treat colics, cramps, hysteria, rheumatism, uterine stimulant, inducer of menstruation, and antispasmodic	Castleman, 1991; Hutchens, 1992
	Jeffersonia diphylla (L.) Pers.	Antispasmodic, diuretic, emetic, expectorant, treat diarrhea, dropsy, gravel and urinary problems, emetic, expectorant, treat sores, ulcers and inflamed parts	Uphof, 1959; Duke and Ayensu, 1985; Foster and Duke, 1990; Coffey, 1993; Moerman, 1998; Lust, 2014

(Continued)

TABLE 1 | Continued

Family	Scientific name	Traditional uses	References
	Mahonia fortunei (Lindl.) Fedde	Anticancer, febrifuge, antiodontalgic, treat testicular swelling and arthritic pain	Duke and Ayensu, 1985; He and Mu, 2015
	Mahonia napaulensis DC.	Diuretic, demulcent, treat dysentery and inflammations of the eyes	Chopra et al., 1986; Manandhar, 2002
	Nandina domestica Thunb.	Antitussive, astringent, febrifuge, stomachic and tonic, treat of fever in influenza, acute bronchitis, whooping cough, indigestion, acute gastro-enteritis, tooth abscess, pain in the bones, muscles and traumatic injuries, and antirheumatic	Kariyone and Koiso, 1971; Duke and Ayensu, 1985; Fogarty, 1990
	Sinopodophyllum hexandrum (Royle) T. S. Ying	Regulate menstruation, promote the circulation of blood, treat amenorrhea, difficult labor and retention of dead fetus or placenta	Kong et al., 2010
Menispermaceae	*Tinospora sinensis* (Lour.) Merr (ex-*Tinospora cordifolia* (Willd.) Miers)	Tonic, antiperiodic, anti-spasmodic, anti-inflammatory, antiarthritic, anti-allergic, anti-diabetic, improve the immune system, antistress, anti-leprotic and anti-malarial activities	Singh et al., 2003
Papaveraceae	*Argemone albiflora* Hornem (ex-*Argemone alba* F. Lestib.)	Anthydropic, cathartic, diaphoretic, diuretic, demulcent, emetic, purgative, treat jaundice, skin ailments, colds, colics and wounds	Smyth, 1903; Foster and Duke, 1990
	Argemone mexicana L.	Analgesic, antispasmodic, sedative, treat warts, cold sores, cutaneous affections, skin diseases, itches, treat cataracts, treat dropsy, jaundice, treat chronic skin diseases, expectorant, treat coughs and chest complaints, demulcent, emetic, expectorant, laxative and antidote to snake poisoning	Uphof, 1959; Pesman, 1962; Usher, 1974; Stuart and Smith, 1977; Emboden, 1979; Chopra et al., 1986; Coffey, 1993; Chevallier, 1996
	Argemone platyceras L.	Treat respiratory ailments as asthma, cough, bronchitis and pneumonia	Emes et al., 1994
	Bocconia frutescens L.	Treat skin conditions (ulcers and eruptions) and respiratory tract infections (bronchistis and tuberculosis)	Martinez, 1977, 1984
	Chelidonium majus L.	Treat ophthalmic diseases (remove films from the cornea of the eye), mild sedative, antispasmodic, relaxing the muscles of the bronchial tubes and intestines, treat warts, alterative, anodyne, antispasmodic, cholagogue, diaphoretic, diuretic, hydrogogue, narcotic, purgative, treat bronchitis, whooping cough, asthma, jaundice, gallstones and gallbladder pains, anticancer, analgesic, treat stomach ulcer, treat get rid of warts, ringworm and corns	Launert, 1981; Grieve, 1984; Phillips and Foy, 1990; Phillips and Rix, 1991; Chevallier, 1996; Lust, 2014
	Corydalis solida subsp. *brachylova* *Corydalis solida* subsp. *slivenensis* (Velen.) Hayek (ex-*Corydalis slivenensis* Velen.)	Anodyne, antibacterial, antispasmodic, hallucinogenic, calm the nerves, sedative for insomnia, CNS stimulant, painkiller, treat painful menstruation, lowering the blood pressure, traumatic injury and lumbago	Launert, 1981; Bown, 1995
	Corydalis solida subsp. *tauri cola*		
	Corydalis turtschaninovii Besser (ex-*Corydalis ternata* (Nakai) Nakai)	Treat memory dysfunction, treat gastric, duodenal ulcer, cardiac arrhythmia disease, rheumatism and dysmenorrhea	Tang and Eisenbrand, 1992; Kamigauchi and Iwasa, 1994; Orhan et al., 2004; Houghton et al., 2006

(Continued)

TABLE 1 | Continued

Family	Scientific name	Traditional uses	References
	Eschscholzia californica Cham.	Sedative, diuretic, relieve pain, relax spasms, promote perspiration, treat nervous tension, anxiety, insomnia, urinary incontinence (especially in children), narcotic, relieve toothache, antispasmodic, analgesic and suppress the flow of milk in lactating women	Coffey, 1993; Bown, 1995; Chevallier, 1996; Moerman, 1998
	Glaucium corniculatum (L.) Rud. subsp. *corniculatum*	Reduce warts, antitusive, treat CNS disturbances, sedative, cooling, and mild laxative	Al-Douri, 2000; Al-Qura'n, 2009; Hayta et al., 2014
	Macleaya cordata (Willd.) R.Br.	Analgesic, antioedemic, carminative, depurative, diuretic, treat insect bites, and ringworm	Grieve, 1984; Duke and Ayensu, 1985
	Macleaya microcarpa (Maxim.) Fedde	Treat some skin diseases and inflammation	Deng and Qin, 2010
	Papaver dubium L.	Sudorific, diuretic, expectorant and ophthalmia	Chopra et al., 1986
	Papaver dubium var. *lecoquii*		
	Papaver rhoeas L. var. *chelidonioides*	Ailments in the elderly and children, mild pain reliever, treat irritable coughs, reduce nervous over-activity, anodyne, emollient, emmenagogue, expectorant, hypnotic, slightly narcotic, sedative, treat bronchial complaints and coughs, insomnia, poor digestion, nervous digestive disorders and minor painful conditions, treat jaundice, fevers, and anticancer	Uphof, 1959; Launert, 1981; Grieve, 1984; Duke and Ayensu, 1985; Phillips and Foy, 1990; Bown, 1995; Chevallier, 1996
	Papaver hybridum L.	Treat dermatologic diseases, anti-infective, diuretic, sedative, and antitussive	Rivera Núñez and Obon de Castro, 1996; Ali et al., 2018
Ranunculaceae	*Coptis chinensis* Franch.	Control of bacterial and viral infections, relax spasms, lower fevers, stimulate the circulation, treat diabetes mellitus, analgesic, locally anaesthetic, antibacterial, antipyretic, bitter, blood tonic, carminative, cholagogue, digestive, sedative, stomachic, vasodilator, treat diarrhoea, acute enteritis and dysentery, treat insomnia, fidget, delirium due to high fever, leukaemia and otitis media, treat conjunctivitis, skin problems (acne, boils, abscesses and burns whilst), mouth, tongue ulcers, swollen gums, and toothache	Uphof, 1959; Usher, 1974; Duke and Ayensu, 1985; Yeung, 1985; Bown, 1995
	Coptis japonica (Thunb.) Makino	Control of bacterial and viral infections, relax spasms, lower fevers, stimulate the circulation, locally analgesic and anaesthetic, anti-inflammatory, stomachic, treat conjunctivitis, intestinal catarrh, dysentery, enteritis, high fevers, inflamed mouth and tongue	Kariyone and Koiso, 1971; Usher, 1974; Grieve, 1984; Bown, 1995
	Coptis teeta Wall.	Control of bacterial and viral infections, relaxes spasms, lowers fevers and stimulate the circulation, locally analgesic, anaesthetic, ophthalmic and pectoral diseases, effective antibacterial, treat dysentery	Stuart and Smith, 1977; Duke and Ayensu, 1985; Bown, 1995
	Hydrastis canadensis L.	Treat disorders of the digestive system and mucous membranes, treat constipation, antiperiodic, antiseptic, astringent, cholagogue, diuretic, laxative, stomachic, tonic, treat disorders affecting the ears, eyes, throat, nose, stomach, intestines, and vagina	Uphof, 1959; Weiner, 1980; Grieve, 1984; Mills, 1985; Foster and Duke, 1990; Coffey, 1993; Bown, 1995; Chevallier, 1996; Lust, 2014
	Xanthorhiza simplicissima Marshall	Treat mouth ulcers, stomach ulcers, colds, jaundice, treat piles, and digestive disorders	Weiner, 1980; Foster and Duke, 1990; Moerman, 1998

(Continued)

TABLE 1 | Continued

Family	Scientific name	Traditional uses	References
Rutaceae	*Phellodendron amurense* Rupr.	Treat gastroenteritis, abdominal pain and diarrhea, antiinflammator, immunostimulator and treat cancer (antitumor activities)	Uchiyama et al., 1989; Park et al., 1999
	• *Phellodendron chinense* C. K. Schneid. • *Phellodendron chinense* var. *glabriusculum* C. K. Schneid. • (ex-*Phellodendron wilsonii* Hayata & Kaneh.)	Act strongly on the kidneys, detoxicant for hot damp conditions, treat meningitis, conjunctivitis, antibacterial, antirheumatic, aphrodisiac, bitter stomachic, cholagogue, diuretic, expectorant, febrifuge, hypoglycaemic, treat ophtalmia, skin, vasodilator and tonic, treat acute diarrhoea, dysentery, jaundice, vaginal infections (with *Trichomonas vaginalis*), acute urinary tract infections, enteritis, boils, abscesses, night sweats and skin diseases, and expectorant	Kariyone and Koiso, 1971; Usher, 1974; Stuart and Smith, 1977; Grieve, 1984; Yeung, 1985; Bown, 1995; Chevallier, 1996
	Zanthoxylum monophyllum Tul.	Treat eye infections and dark vomitus	Hirschhorn, 1981; Eric Brussell, 2004

Hosseinzadeh, 2008), *Phellodendron amurense*, *P. chinense*, *Tinospora cordifolia* (Khan et al., 2011), *Xanthorhiza simplicissima* (Bose et al., 1963; Knapp et al., 1967; Sato and Yamada, 1984; Steffens et al., 1985; Inbaraj et al., 2001; Liu et al., 2008a; Srinivasan et al., 2008; Vuddanda et al., 2010). Several researches found that berberine is widely distributed in the barks, roots, and stems of plants, nevertheless, bark and roots are richer in berberine compared to other plant parts (Andola et al., 2010a,b). In the Papaveraceae family, *Chelidonium majus* is another important herbal source of berberine (Tomè and Colombo, 1995). An important number of plants for medicinal use, such as *Coptidis rhizoma* and barberry, are the natural sources with the highest concentration of berberine. Barberries, such as *Berberis aristata*, *B. aquifolium*, *B. asiatica*, *B. croatica*, *B. thunbergii,* and *B. vulgaris*, are shrubs grown mainly in Asia and Europe, and their barks, fruits, leaves, and roots are often widely used as folk medicines (Imanshahidi and Hosseinzadeh, 2008; Kosalec et al., 2009; Andola et al., 2010c; Kulkarni and Dhir, 2010). Different research groups have reported that maximum berberine concentration accumulates in root (1.6–4.3%) and in most of the *Berberis* species, plants that grow at low altitude contain more berberine compared to higher altitude plants (Chandra and Purohit, 1980; Mikage and Mouri, 1999; Andola et al., 2010a). However, a correlation could not be established within the results of berberine concentration regarding to species and season of the year (Srivastava et al., 2006a,c; Andola et al., 2010c; Singh et al., 2012). Comparative studies of berberine concentration contained in different species of the same genus have been reported, e.g., higher berberine content in *B. asiatica* (4.3%) in comparison to *B. lycium* (4.0%), and *B. aristata* (3.8%). Meanwhile, Srivastava et al. (2004) documented a higher berberine content in root of *B. aristata* (2.8%) compared with *B. asiatica* (2.4%) (Andola et al., 2010a). Seasonal variation of berberine concentration has been reported, e.g., the maximum yield of berberine for *B. pseudumbellata* was obtained in the summer harvest, and was 2.8% in the roots and 1.8% for the stem bark, contrary to that reported in the roots of *B. aristata*, where the berberine concentration (1.9%) is higher for the winter harvest (Rashmi et al., 2009). These variations may be caused to multiple factors, among which stand out: (i) the intraspecific differences, (ii) location and/or, (iii) the analytical techniques used. **Table 2** gathers a synthesis of the main species containing berberine.

EXTRACTION METHODS

Berberine, a quaternary protoberberine alkaloid (QPA) is one of the most widely distributed alkaloid of its class. Current studies suggest that isolation of the QPA alkaloids from their matrix can be performed using several methods. The principles behind these methods consist of the interconversion reaction between the protoberberine salt and the base. The salts are soluble in water, stable in acidic, and neutral media, while the base is soluble in organic solvents. Thus during the extraction procedure, the protoberberine salts are converted in their specific bases and further extracted in the organic solvents (Marek et al., 2003; Grycová et al., 2007).

In the case of berberine, the classical extraction techniques like maceration, percolation, Soxhlet, cold or hot continuous extraction are using different solvent systems like methanol, ethanol, chloroform, aqueous, and/or acidified mixtures. Berberine's sensitivity to light and heat is the major challenge for its extraction. Hence, exposure to high temperature and light could lead to berberine degradation and thus influencing its matrix recovery. In his study Babu et al. (2012) demonstrated that temperature represent a crucial factor in both extraction and drying treatments prior extraction. The yield of berberine content in *C. fenestratum* stem tissue samples was higher in case of samples dried under the constant shade with 4.6% weight/weight (*w/w*) as compared to samples dried in oven at 65°C (1.32% *w/w*) or sun drying (3.21% *w/w*). As well hot extraction procedure with methanol or ethanol at 50°C gave lower extraction yields when compared with methanol or ethanol cold extraction at −20°C. Thus, berberine content in

TABLE 2 | Botanical sources of berberine.

Family	Scientific name	Common name	Used part	References
Annonaceae	*Annickia chlorantha* (Oliv.) Setten & Maas (ex-*Enantia chlorantha* Oliv.)	African whitewood, african yellow wood Epfoué, Péyé, Nfol, Poyo	Bark	Mell, 1929
	Annickia pilosa (Exell) Setten & Maas (ex-*Enantia pilosa* Exell)	–	Bark	Buzas and Egnell, 1965
	Annickia polycarpa (DC.) Setten & Maas ex I. M. Turner (ex-*Enantia polycarpa* (DC.) Engl. & Diels)	African yellow wood	Bark	Buzas and Egnell, 1965
	Coelocline polycarpa A.DC.	Yellow-dye tree of Soudan	Bark	Henry, 1949
	Rollinia mucosa (Jacq.) Baill.	Biriba, wild sweet sop, wild cashina	Fruit	Chen et al., 1996
	Xylopia macrocarpa A.Chev.	Jangkang	Stem bark	Willaman and Schubert, 1961
	Xylopia polycarpa (DC.) Oliv.	–	Stem bark	Willaman and Schubert, 1961
Berberidaceae	*Berberis aetnensis* C.Presl	–	Roots	Bonesi et al., 2013
			Leaves	Musumeci et al., 2003
			Root	Henry, 1949
	Berberis amurensis Rupr.	Barberry	Stem & roots	Tomita and Kugo, 1956
	Berberis aquifolium Pursh	Oregon grape	Roots	Parsons, 1882
	Berberis aristata DC.	Tree turmeric	Bark	Chakravarti et al., 1950
			Roots	Singh A. et al., 2010
			Stem	
			Raw herb	Singh R. et al., 2010
			Extract	
			Fruit	Kamal et al., 2011
			Roots	Andola et al., 2010a,c
			Roots	Rashmi et al., 2009
			Roots	Singh and Kakkar, 2009
			Roots	Srivastava et al., 2004
			Roots	Srivastava et al., 2001
			Bark	Willaman and Schubert, 1961
	Berberis asiatica Roxb. ex DC.	Chutro, rasanjan (Nep); marpyashi (Newa); daruharidra, darbi (Sans)	Roots	Andola et al., 2010b
			Roots	Andola et al., 2010c
			Roots	Srivastava et al., 2004
			Roots, stem, bark	Willaman and Schubert, 1961
	Berberis barandana Vidal.	–	ND	Willaman and Schubert, 1961
	Berberis beaniana C. K. Schneid.	Kang song xiao bo (pinyin, China)	–	Steffens et al., 1985
	Berberis chitria Buch.-Ham. ex Lindl.	Chitra, indian barberry	Whole plant	Hussaini and Shoeb, 1985
			Roots	Srivastava et al., 2006a,b,c
	Berberis concinna Hook.f.	Barberry	Stem bark	Tiwari and Masood, 1979
	Berberis congestiflora Gay	Michay	Leaves and stem	Torres et al., 1992
	Berberis coriaria Royle ex Lindl.	–	Stem bark	Tiwari and Masood, 1979
	Berberis croatica Mart. ex Schult. & Schult.f.	Croatian barberry	Roots	Končić et al., 2010
			Roots	Kosalec et al., 2009
	Berberis darwinii Hook.	Michai, calafate	Roots	Richert, 1918
			Leaves	Urzúa et al., 1984
			Stem-bark	Habtemariam, 2011
	Berberis densiflora Raf.	–	Leaves	Khamidov et al., 1997b
	Berberis floribunda Wall. ex G.Don	Nepal barberry	Roots	Chatterjee, 1951
	Berberis fortunei Lindl.	Fortune's Mahonia	Wood	Willaman and Schubert, 1961
	Berberis guimpelii K. Koch & C. D. Bouché	–	Roots	Petcu, 1965a
	Berberis heteropoda Schrank	–	Root bark	Willaman and Schubert, 1961
	Berberis himalaica Ahrendt	–	Stem-bark	Chatterjee et al., 1952

(Continued)

TABLE 2 | Continued

Family	Scientific name	Common name	Used part	References
	Berberis horrida Gay	–	Leaves and stem	Torres et al., 1992
	Berberis iliensis Popov	–	Young shoots	Karimov and Shakirov, 1993
			Roots	Dzhalilov et al., 1963
	Berberis integerrima Bunge.	–	Root	Karimov et al., 1993
			Leaves	Karimov et al., 1993; Khamidov et al., 1996c, 1997b
	Berberis jaeschkeana C. K. Schneid.	Jaeschke's Barberry	–	Rashid and Malik, 1972
	Berberis jamesonii Lindl (ex-*Berberis glauca* Benth)	–	–	Willaman and Schubert, 1961
	Berberis japonica R.Br	Japanese Mahonia	Wood, root	Willaman and Schubert, 1961
	Berberis kawakamii Hayata	–	Roots	Yang and Lu, 1960a
	Berberis koreana Palib.	Korean barberry	Bark of the stem	Petcu, 1965b
			Bark of the roots	
			Seeds	
			Stem	
			Roots	
			–	Kostalova et al., 1982
			Roots	Yoo et al., 1986
			Leaves	
	Berberis lambertii R. Parker	–	Roots	Chatterjee and Banerjee, 1953
	Berberis laurina Thunb	Laurel barberry	Roots	Gurguel et al., 1934; Willaman and Schubert, 1961
	Berberis leschenaultii Wall. ex Wight & Arn (ex-*Mahonia leschenaultii* (Wall. ex Wight & Arn.) Takeda)	–	Bark	Willaman and Schubert, 1961
	Berberis libanotica Ehrenb. ex C. K. Schneid.	–	Root	Bonesi et al., 2013
	Berberis lycium Royle	Boxthorn barberry	Roots	Andola et al., 2010c
	Berberis microphylla G. Forst. (ex-*Berberis heterophylla* Juss. ex Poir. *Berberis buxifolia* Lam.)	Patagonian barberry, magellan barberry, calafate	Roots	Freile et al., 2006
			–	Rashid and Malik, 1972
	Berberis mingetsensis Hayata	–	Roots	Yang and Lu, 1960b
	Berberis nummularia Bunge	Nummular barberry	Young shoots	Karimov et al., 1993
	Berberis morrisonensis Hayata	–	Roots	Yang, 1960a,b
			Stem	
			–	
	Berberis nepalensis Spreng. (ex-*Mahonia acanthifolia* Wall. ex G.Don)	–	–	Willaman and Schubert, 1961
	Berberis nervosa Pursh	Dwarf Oregon-grape	–	Willaman and Schubert, 1961
	Berberis oblonga (Regel) C. K. Schneid	Oblong barberry	Stem	Karimov and Lutfullin, 1986; Gorval' and Grishkovets, 1999
			Leaves	Khamidov et al., 2003
			Roots	Tadzhibaev et al., 1974
	Berberis petiolaris Wall. ex G. Don	Chochar	Roots	Huq and Ikram, 1968
	Berberis pseudumbellata R. Parker	–	Roots	Andola et al., 2010b
			Stem bark	
			–	Pant et al., 1986
	Berberis repens Lindl.	Creeping mahonia, creeping Oregon grape, creeping barberry, or prostrate barberry	–	Willaman and Schubert, 1961
	Berberis sargentiana C. K. Schneid.	Sankezhen	–	Liu, 1992
	Berberis swaseyi Buckley	–	–	Willaman and Schubert, 1961
	Berberis thunbergii DC.	Japanese barberry	Stem	Khamidov et al., 1997a
			Leaves	Khamidov et al., 1997a
	Berberis tinctoria Lesch.	Nilgiri barberry	Roots	Srivastava and Rawat, 2007

(Continued)

TABLE 2 | Continued

Family	Scientific name	Common name	Used part	References
	Berberis trifolia (Cham. & Schltdl.) Schult. & Schult.f.	–	Root, stem	Willaman and Schubert, 1961
	Berberis turcomanica Kar. ex Ledeb.	–	Leaves	Khamidov et al., 1996a,b,c
	Berberis umbellata Wall. ex G.Don	Himalayan barberry	Roots	Singh et al., 2012
	Berberis vulgaris L.	Barberry	Stems and roots	Imanshahidi and Hosseinzadeh, 2008
			Roots	Končić et al., 2010
			Roots	Kosalec et al., 2009
	Berberis waziristanica Hieron.	–	Root bark	Atta-ur-Rahma and Ahmad, 1992
	Caulophyllum thalictroides (L.) Michaux (ex-*Leontice thalictroides* L.)	Blue cohosh	–	Willaman and Schubert, 1961
	Jeffersonia diphylla (L.) Pers.	Twinleaf	–	Willaman and Schubert, 1961
	Mahonia borealis Takeda	–	–	Willaman and Schubert, 1961
	Mahonia fortunei (Lindl.) Fedde	Fortune's Mahonia	wood	Willaman and Schubert, 1961
	Mahonia napaulensis DC. (ex-*Mahonia griffithii*; ex-*Mahonia manipurensis* Takeda; *Mahonia sikkimensis* Takeda)	Nepal Barberry	bark	Willaman and Schubert, 1961
	Mahonia simonsii Takeda	–	–	Willaman and Schubert, 1961
	Nandina domestica Thunb.	Nandina, heavenly bamboo or sacred bamboo	bark, root	Willaman and Schubert, 1961
	Sinopodophyllum hexandrum (Royle) T.S.Ying	Himalayan May Apple, Indian may apple	Root, rhizome	Willaman and Schubert, 1961
Menispermaceae	*Tinospora sinensis* (Lour.) Merr. *(ex-Tinospora cordifolia)* (Willd.) Miers	Gulbel, indian tinospora	Stem	Srinivasan et al., 2008
			–	Singh et al., 2003
Papaveraceae	*Argemone albiflora* Hornem. (ex-*Argemone alba* F.Lestib.)	White prickly poppy, Bluestem pricklypoppy	Aerial part and roots	Slavikova et al., 1960
				Foote, 1932
				Israilov and Yunusov, 1986
	Argemone hybrida R.Otto & Verloove	–	Leaves and stem	Israilov and Yunusov, 1986
	Argemone mexicana L.	Prickly poppy	Apigeal parts, seeds	Haisova and Slavik, 1975; Israilov and Yunusov, 1986; Fletcher et al., 1993
			Leaves	Bapna et al., 2015
			Seeds	Fletcher et al., 1993
			–	Singh, 2014
			–	Majumder et al., 1956; Hakim et al., 1961; Misra et al., 1961
			Superterranean parts	Slavikova and Slavik, 1955
			Roots	
			–	Santos and Adkilen, 1932; de Almeida Costa, 1935; Misra et al., 1961; Doepke et al., 1976; Abou-Donia and El-Din, 1986; Monforte-Gonzalez et al., 2012
			Roots	Pathak et al., 1985; Kukula-Koch and Mroczek, 2015
			Leaves and capsules	Schlotterbeck, 1902
			Whole plant	Bose et al., 1963; Haisova and Slavik, 1975
			Latex	Santra and Saoji, 1971

(Continued)

TABLE 2 | Continued

Family	Scientific name	Common name	Used part	References
	Argemone ochroleuca Sweet	Chicalote	Seeds	Fletcher et al., 1993
	Argemone platyceras L.	Chicalote poppy, crested poppy	Leaves and stem	Israilov and Yunusov, 1986
	Argemone subintegrifolia Ownbey	–	Aerial part	Stermitz et al., 1974
	Argemone squarrosa Greene	Hedgehog pricklypoppy	Aerial part	Stermitz, 1967
	Bocconia frutescens L.	Plume poppy, tree poppy, tree celandine, parrotweed, sea oxeye daisy, john crow bush	Leaves	Slavik and Slavikova, 1975
			Roots, stalks, leaves	Taborska et al., 1980
	Chelidonium majus L.	Celandine poppy	Roots	Jusiak, 1967
	Corydalis chaerophylla DC.	Fitweed	Roots	Jha et al., 2009
	Corydalis ophiocarpa Hook. f. *et* Thoms	Fitweed		Manske, 1939
	Corydalis solida subsp. *brachyloba*	Fitweed	Aerial parts	Sener and Temizer, 1988, 1991
	Corydalis solida subsp. *slivenensis* (Velen.) Hayek (ex-*Corydalis slivenensis* Velen.)	Fitweed	–	Kiryakov et al., 1982a,b
	Corydalis solida subsp. *tauricola*	Fitweed	–	Kiryakov et al., 1982b
			Rhizome	Sener and Temizer, 1990
	Corydalis turtschaninovii Besser. (ex-*Corydalis ternata* (Nakai) Nakai)	Fitweed	Tubers	Lee and Kim, 1999
	Eschscholzia californica Cham.	Californian poppy	Roots	Gertig, 1964
	Glaucium corniculatum (L.) Rud. subsp. *corniculatum*	Blackspot Hornpoppy	Aerial parts	Doncheva et al., 2014
			–	Slavik and Slavikova, 1957
	Glaucium grandiflorum Boiss. & A.Huet	Red Horned Poppy, Grand-flowered Horned Poppy	Aerial part	Phillipson et al., 1981
	Hunnemannia fumariifolia Sweet	Mexican Tulip Poppy, Golden Cup	Roots	Slavikova and Slavik, 1966
	Macleaya cordata (Willd.) R.Br.	Plume poppy	–	Kosina et al., 2010
	Macleaya microcarpa (Maxim.) Fedde	Poppy	Roots	Pěnčíková et al., 2011
	Papaver dubium L.	Long-Head Poppy	Roots	Slavik et al., 1989
	Papaver dubium var. lecoquii	Long-Head Poppy	Latex	Egels, 1959
	Papaver rhoeas L. var. *chelidonioides*	Corn Poppy	Roots	Slavík, 1978
	Papaver hybridum L.	Poppy	Aerial part	Phillipson et al., 1981
	Sanguinaria canadensis L.	Bloodroot		Greathouse, 1939
Ranunculaceae	*Coptis chinensis* Franch.	Chinese goldthread	Roots	Jin and Shan, 1982
			Roots	Lou et al., 1982
	Coptis japonica (Thunb.) Makino	Japanese goldthread	Rhizome	Kubota et al., 1980
	Coptis teeta Wall.	Gold thread	Rhizome	Chen and Chen, 1988
			Rhizome	Zhang et al., 2008
			Roots	
	Hydrastis canadensis L.	Goldenseal	–	Baldazzi et al., 1998
			–	Leone et al., 1996
	Xanthorhiza simplicissima Marshall	Yellowroot	Root, stem, and leaves	Okunade et al., 1994
Rutaceae	*Evodia meliaefolia (*Hance ex Walp.) Benth.	–	Bark	Perkin and Hummel, 1895
	Phellodendron amurense Rupr.	Amur cork tree	Bark	Chiang et al., 2006
			Root bark	Zhang et al., 2008
			Trunk bark	
			Perennial Branch bark	
			Annual branches	
			Leaves	

(Continued)

TABLE 2 | Continued

Family	Scientific name	Common name	Used part	References
	Phellodendron chinense C. K. Schneid.	Chinese cork tree	Bark	Chan et al., 2007
	Phellodendron chinense var. *glabriusculum* C. K. Schneid. (ex-*Phellodendron wilsonii* Hayata & Kaneh.)	Chinese cork tree	Bark, branch, leaf and heartwood	Chen, 1981
			–	Tan et al., 2013
			Bark	Chen, 1982
	Phellodendron lavallei Dode	Lavalle corktree	Bark	Yavich et al., 1993
	Zanthoxylum monophyllum (Lam.) P. Wilson	Palo rubio	Stem and branches	Stermitz and Sharifi, 1977
	Zanthoxylum quinduense Tul.	–	–	Ladino and Suárez, 2010

the shade-dried samples was 4.6% (*w/w*) for methanolic cold extraction and 1.29% (*w/w*) for methanolic hot extraction (Babu et al., 2012).

Along with extraction temperature, the choice of solvents is considered a critical step in berberine extraction as well (**Figure 2**). As seen in **Table 3**, methanol, ethanol, aqueous or acidified methanol or ethanol are the most used extraction solvents. The acidified solvents (usually with the addition of 0.5% of inorganic or organic acids) are used to combine with free base organic alkaloids and transform them in alkaloid salts with higher solubility (Teng and Choi, 2013). The effect of different inorganic acids like hydrochloric acid, phosphoric acid, nitric acid, and sulfuric acid as well as the effect of an organic acid like acetic acid were tested on berberine content and other alkaloids in rhizomes of *Coptis chinensis* Franch by Teng and Choi (2013). In this case, 0.34% phosphoric acid concentration was considered optimal. Moreover, when compared to other classical extraction techniques like reflux and Soxhlet extraction, the cold acid assisted extraction gave 1.1 times higher berberine yields.

Large solvent volumes and long extraction time represent other drawbacks of conventional extraction methods (Mokgadi et al., 2013). For example, Rojsanga and Gritsanapan (2005) used maceration process to extract 100 g of *C. fenestratum* plant material with a total volume of 3,200 mL solvent (80% ethanol) over a period of 416 h. Furthermore, in a different study, Rojsanga et al. (2006) used several classical extraction techniques like maceration, percolation, and Soxhlet extraction to extract the berberine from *C. fenestratum* stems. This time even if the extracted plant material was in a lower amount than the previous study (30 vs. 100 g), large solvent volumes (2,000 mL for maceration, 5,000 mL for percolation, and 600 mL for Soxhlet extraction) over long time periods (7 days for maceration and 72 h for Soxhlet extraction) were employed (Rojsanga and Gritsanapan, 2005; Rojsanga et al., 2006).

Large solvent volumes are characteristic for other conventional methods too. Shigwan et al. (2013) extracted berberine from *Berberis aristata* and *B. tinctoria* powdered stem bark (800 g) using hot extraction (50°C for 3 h) with 2,500 mL methanol (Shigwan et al., 2013).

Even though conventional methods are widely used in berberine extraction, a number of other different methods have been developed lately. This led to an improved extraction efficiency, a decreased extraction time and solvents' volumes used in the extraction. Thus, ultrasound assisted solvent extraction (USE), microwave-assisted solvent extraction (MAE), ultrahigh pressure extraction (UPE), and supercritical fluid extractions (SFE), pressurized liquid extraction (PLE) have been successfully used as alternative extraction techniques with better results when compared with classical extraction methods.

Ultrasonically and microwave-assisted extraction are considered green, simple, efficient, and inexpensive techniques (Alupului et al., 2009).

Teng and Choi (2013) extracted berberine from *Rhizome coptidis* by optimized USE. Using response surface methodology, they identified that the optimal extraction conditions were 59% ethanol concentration, at 66.22°C within 46.57 min. A decrease in the extraction time (39.81 min) was obtained by Chang (2013). He used the combination of ionic liquids solutions as green solvents with USE to extract berberine from *Coptis chinensis* in order to apply an environmentally friendly approach (Chang, 2013). Moreover, in their study, Xu et al. (2017) compared several extraction tehniques like USE, distillation, and Soxhlet extraction in order to establish an high-efficient method for phellodendrine, berberine, and palmatine extraction from fresh *Phellodendron* bark (*Cortex phellodendri*). In the case of berberine, the combination of simple or acidified solvent (water, ethanol, and methanol) with the adjustment of the specific setting characteristics to each extraction type enabled them to determine the highest extraction yield. They concluded that the use of USE and hydrochloric acid-acidified methanol were the most efficient in extracting berberine. The USE extraction yield was significantly higher when compared to distillation and Soxhlet extraction, with values of ∼100 mg/g toward 50 and 40 mg/g berberine, respectively (Xu et al., 2017).

The important reduction in organic solvent and extraction time determined the increasing interest in MAE, too. Lately, MAE was used as a green and cost-effective alternative to conventional methods. Using central composite design, Satija et al. (2015) successfully optimized the MAE parameters in terms

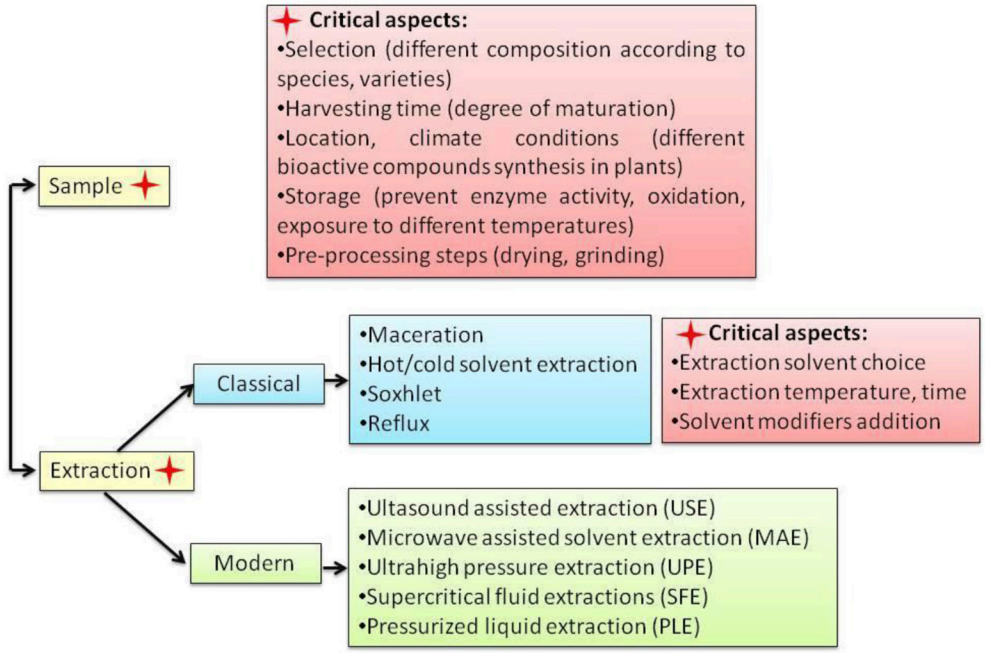

FIGURE 2 | Short view on berberine extraction methods.

of irradiation power, time, and solvent concentration to extract berberine form *Tinospora cardifolia*. They compared two classical extraction techniques like maceration and Soxhlet extraction with MAE under optimized conditions (60% irradiation power, 80% ethanol concentration, and 3 min extraction time). The results showed that MAE extraction had the highest yield of berberine content with 1.66% (*w/w*) while Soxhlet and maceration had 1.04 and 0.28% (*w/w*), respectively. Their study is emphasizing the dramatic time reduction in case of MAE (3 min) when compared with Soxhlet extraction (3 h) and maceration (7 days) together with solvent and energy consumption (Satija et al., 2015).

Another novel extraction technique considered to be environmentally friendly is UPE. The interest toward this extraction technique is increasing because it presents several advantages toward classical extraction techniques like increased extraction yields, higher quality of extracts, less extraction time, and decreased solvent consumption (Xi, 2015). These are achieved at room temperature by applying different pressure levels (from 100 to 600 MPa) between the interior (higher values) and the exterior of cells (lower values) in order to facilitate the transfer of the bioactive compounds through the plant matrices in the extraction solvent (Liu et al., 2006, 2013). In the study regarding berberine content in *Cortex phellodendri*, Guoping et al. (2012) made a comparison between UPE, MAE, USE, and heat reflux extraction techniques. They observed that the higher extraction yield and the lower extraction time was obtained in case of UPE with 7.7 mg/g and 2 min extraction time toward reflux, USE and MAE with 5.35 mg/g and 2 h, 5.61 mg/g and 1 h. and 6 mg/g and 15 min, respectively (Guoping et al., 2012).

Super critical fluid extraction is another environmentally friendly efficient technique used in phytochemical extraction. Because the extraction is performed in the absence of light and oxygen, the degradation of bioactive compounds is reduced. Also, the inert and non-toxic carbon dioxide used as a main extraction solvent in combination with various modifiers (e.g., methanol) and surfactants (e.g., Tween 80) at lower temperatures and relatively low pressure, allows the efficient extraction of bioactive compounds (Liu et al., 2006; Farías-Campomanes et al., 2015). In case of berberine extraction from the powdered rhizome of *Coptis chinensis* Franch, the highest recovery of berberine was obtained when 1,2-propanediol was used as a modifier of supercritical CO_2 (Liu et al., 2006).

Pressurized liquid extraction, also known as pressurized fluid extraction, pressurized solvent extraction, and accelerated solvent extraction (ASE) is considered a green technology used for compounds extraction from plants (Mustafa and Turner, 2011). Compared with conventional methods, PLE increases the extraction yield, decreases time and solvent consumption, and protects sensitive compounds. In their study, Schieffer and Pfeiffer (2001) compared different extraction techniques like PLE, multiple USE, single USE, and Soxhlet extraction in order to extract berberine from goldenseal (*Hydrastis canadensis*). When compared in terms of extraction yield the results are comparable, ∼42 mg/g berberine, except single USE with slightly lower content (37 mg/g berberine). Big differences were observed in the extraction time, PLE requiring only 30 min for a single sample extraction compared to 2 h for multiple extraction techniques or 6 h for Soxhlet extraction (Schieffer and Pfeiffer, 2001).

TABLE 3 | Extraction and detection methods for berberine in different herbal and biological matrixes.

Sample (weight)	Extraction method	Detection method	References
Dried stem powder *Coscinium fenestratum* (Gaertn.) (1 g)	**Extraction solvents (ES):** water, methanol–water (1:1. *v/v*), and methanol **Sonication** (15 min, room temperature) Centrifugation (2,800 rpm, 15 min) Filtration and evaporation Extracts resolubilization (methanol:water, 9:1 *v/v*)	**HPLC - DAD** Column: ODS, Chromolith, RP-18e,100 × 4.6 mm Mobile Phases: Methanol/Deionized Water (90:10, *v/v*) Flow: 0.5 mL/min, Temperature: 25°C **UV Spectrophotometric Analysis**	Akowuah et al., 2014
C. fenestratum (Gaertn.) (10 g)	**ES:** methanol **Hot extraction:** sample refluxed with ES for 3 h Filtration and evaporation. Extracts resolubilization (methanol) **Cold extraction:** sample extraction with ES for 24 h Filtration and evaporation. Extracts resolubilization (methanol)	**TLC** Adsorbent: Silica Gel GF $_{254}$ Solvent system: n-Butanol: Ethyl acetate: Acetic acid (2.5:1.5:1, *v/v/v*) Detection: 254 and 366 nm	Arawwawala and Wickramaar, 2012
Dried C. *fenestratum* (0.1 g)	**ES**: absolute methanol **Cold extraction:** sample extraction at −20°C **Hot extraction:** water bath sample extraction at 50°C **ES**: absolute ethanol **Cold extraction** sample extraction at −20°C **Hot extraction:** water bath sample extraction at 50°C Samples centrifugation (10 min at 10°C after cooling down) Samples filtration	**HPLC** Column: C18, 250 × 4.6 mm, 5 μm Mobile Phases: Acetonitrile/0.1% Trifluro-acetic acid (50:50, *v/v*) Detection: 344 nm Flow: 0.8 mL/min	Babu et al., 2012
C. fenestratum (1,000g) *Capsules* (containing 62.5 mg *C. fenestratum*)	**ES**: petroleum ether, chloroform, methanol (1L each) **Soxhlet extraction**: with each ES for 3 days at (30–40°C) **ES:** methanol (10 mL) Extraction for 1 h Filtration and evaporation Resolubilisation in methanol (5 mL)	**HPLC** Column: Luna C18, 150 × 4.6 mm, 5 μm, Phenomenex Mobile Phases: (A) Potassium dihydrogen phosphate (pH −2.5) and (B) Acetonitrile Detection: 220 nm Flow: 1 mL/min **HPTLC** Adsorbent: Silica Gel 60F $_{254}$ Solvent system: n-Butanol: Glacial acetic acid: Water (8:1:1, *v/v/v*) Detection: 350 nm for all measurements	Jayaprakasam and Ravi, 2014
Tinospora cordifolia (Willd.), *Tribulus terrestris* (L.), *Emblica officinalis* (Gaertn.) (3 g)	**ES**: chloroform Dried sample trituration with ammonia solution Drying at room temperature Extraction with ES for 1h Chloroform phase extraction with 5% sulfuric acid (x 3) Basification of acid extract with sodium carbonate (pH −9) Extraction of basified solution with chloroform (X 3) Evaporation of chloroform phase (temperature under 50°C) Residue solubilization with methanol	**UV-VIS** UV absorbance: 348 nm	Joshi and Kanaki, 2013
Cortex phellodendri (2 g)	**Ultrahigh pressure extraction (UPE)** Optimal parameters: ES: ethanol (69.1%), liquid-solid ratio−31.3, extracting pressure−243.30 MPa, extraction time−2 min	**HPLC** Column: Hypersil ODS C18, 250 × 4.6 mm, 5 μm Mobile Phases: (A) 0.3% triethanolamine aqueous solution (pH − 3.5) Detection: 265 nm Temperature: 30°C Flow: 1 mL/min	Guoping et al., 2012
Rhizome of *Coptis chinensis* Franch (1 g)	**Supercritical fluid extraction** Extraction time: up to 3 h Temperature: 60°C Pressure: from 200 to 500 bar Flow-rate of carbon dioxide (gaseous state): 1 L/min Flow-rate of modifier: 0.4 mL/min. Organic solvent modifier systems: ethanol-modified supercritical carbon dioxide, methanol-modified supercritical carbon dioxide,	**HPLC** Column: Diamonsil C18, 250 × 4.6 mm, 5 μm Mobile Phases: 33 mM Potassium dihydrogen phosphate : acetonitrile (70:30, *v/v*) Detection: 345 nm Flow: 1 mL/min	Liu et al., 2006

(Continued)

TABLE 3 | Continued

Sample (weight)	Extraction method	Detection method	References
	1,2-propanediol-modified supercritical carbon dioxide, 5% Tween 80 in methanol-modified supercritical carbon dioxide, 5% Tween in ethanol-modified supercritical carbon dioxide **Soxhlet extraction** **ES**: hydrochloric acid: methanol (1: 100, *v/v*) Time: 8 h		
Cortex pellodendri amurensis (1 g)	**Ultrahigh pressure extraction** **ES**: ethanol (50 %), liquid-solid ratio −30: 1, extracting pressure −400 MPa, extraction time −4 min, extraction temperaturte −40°C **Ultrasonic extraction** **ES**: 70% ethanol Sample soaking for 24 h in 40 ml ES Sonic extraction for 60 min at 30°C **Heat reflux extraction** **ES**: 70% ethanol Sample soaking for 24 h in 40 ml ES Sample extraction for 4 h at boiling state **Soxhlet extraction** **ES**: 70% ethanol Sample soaking for 24 h in 40 ml ES Sample extraction: 4 h	**HPLC- DAD** Column: Daisopak SP-120-5-ODS_BP, 250 × 4.6 mm, 5 μm Mobile Phases: (A) acetonitrile and (B) phosphoric acid: water (0.7:100, *v/v*) Detection: 345 nm Temperature: 25°C Flow: 1 mL/min	Liu et al., 2013
Goldenseal *(Hydrastis canadensis* L.) (2, 5, 5 g)	**Pressurized hot water extraction** **ES:** water at 140°C, Optimal parameters: pressure: 50 bars and flow rate: 1 mL/min, Time: 15 min **Reflux extraction** **ES:** methanol (200 mL) Sonication: 4 h at 80°C **Ultrasonic extraction** **ES:** methanol (50 mL) Reflux: 6 h with continuous stirring	**HPLC-DAD** Column: Zorbax eclipse Plus C 18, 75 x 4.6 mm, 3.5 μm Mobile phases: (A) 0.1 % Formic Acid (pH 2.7) and (B) methanol Detection: 242 nm Temperature: 35°C Flow: 1 mL/min **MS** Detection: ESI (+) Capillary temperature: 200°C, Sheath gas: 80, Capillary voltage: 20 V, Tube lens voltage: 5V	Mokgadi et al., 2013
Berberis aristata DC (1.5 g), *Berberis aristata* herb extract (0.1 g), Ayurvedic form (6 g)	**Crude herb reflux extraction** **ES**: methanol (100 mL) for 1 h in a water bath Filtratio Reextraction with ES (50 mL) for 30 min (×2) Filtrates combination and concentration to 50 mL **Herb extracts ultrasonic extraction** **ES**: methanol (up to 10 mL) Sonication Filtration **Ayrvedic form ultrasonic extraction** **ES**: methanol (up to 25 mL) Sonication	**HPLC** Column: Zorbax ODS II, 250 x 4.6 mm, 5 μm Mobile phase: potassium hydrogen phosphate buffer (pH 2.5)/ acetonitrile Detection: 346 nm Temperature: 40°C Flow: 1 mL/min	Singh R. et al., 2010
Berberis aristata DC root	**Soxhlet extraction** **ES**: ethanol **Berberine isolation** Ethanolic extract concentration to obtain a syrup mass Dissolvation in hot water and filtration Acidification (36.5% w/v hydrochloric acid) Cool: ice bath - 30 min, overnight in refrigerator	**HPTLC** Stationary phase: precoated silica gel 60GF254 Mobile phases: n-butanol: glacial acetic acid: water (12:3:4 *v/v/v*) Temperature: 33 ± 5°C Detection: 350 nm	Patel, 2013
Mahonia manipurensis (Takeda) stem bark (100 g)	**Cold extraction** **ES**: 80% methanol (1,000 mL) Stirring at room temperature Extract concentration	**TLC** Stationary phase: precoated silica gel G F254 Mobile phase: hexane: ethyl acetate: methanol (56:20:5) Fraction purification: positive test using Dragendroff's reagent Further analysis of purified fraction Mobile phase: chloroform: ethyl acetate: diethylamine: methanol: 20% ammonium hidroxide (6:24:1.5:6:0.3)	Pfoze et al., 2014

(Continued)

TABLE 3 | Continued

Sample (weight)	Extraction method	Detection method	References
		HPLC Column: Water Symmetry C18, 250 x 4.6 mm, 5 μm Mobile phase: methanol/ formic acid buffer (0.1%, *v/v*) Detection: 346 nm Flow: 1 mL/min **UV-VIS** UV spectra: 200–500 nm **ESI-MS**	
Coscinium fenestratum (100 g)	**Maceration** **ES:** 80% ethanol (500 ml), 160 h Shaken: 80 h (200 rpm), stand: 80 h Reextraction: 48 h, shaken: 24 h, stand: 24 h Combined extracts concentration Evaporation to dryness (dry extract) Resolubilisation in 80% ethanol (10 mg dry extract/mL)	**TLC** Stationary phase: Silica gel GF254 Mobile phase: ethyl acetate : butanol : formic acid : water (50:30:12:10); Detection: 366 nm	Rojsanga and Gritsanapan, 2005
Argemone mexicana	**Soxhlet extraction** **ES**: methanol Evaporation to dryness Resolubilisation in methanol (known concentration)	**HPTLC** Stationary phase: precoated silica gel 60F254 Mobile phases: toluene: ethyl acetate (9:3, *v/v*). Detection: 266 nm	Samal, 2013
Tinospora cordifolia (20 g)	**Microwave assisted extraction (MAE)** **ES**: 80% ethanol Irradiation power: 60%, Extraction time: 3 min **Soxhlet extraction** **ES**: ethanol, for 3 h Filtration Concentration **Maceration** **ES**: ethanol (200 mL), 7 days, occasional stirring	**HPTLC** Mobile phases: methanol: acetic acid: water (8: 1: 1, *v/v/v*). Detection: 366 nm	Satija et al., 2015
Berberis aristata, Berberis tinctoria (800 g)	**Hot extraction** **ES**: methanol (2.5 L) (X2) Extraction time: 3 h Temperature: 50°C Extract concentration under vacuum	**HPLC** Column: Unisphere C18, 150 x 4.6 mm, 5 μm Mobile phase: (A) 0.1% trifluoroacetic acid and (B) acetonitrile (60:40, *v/v*) Detection: 350 nm Temperature: 30°C Flow: 1 mL/min	Shigwan et al., 2013
Coptis chinensis Franch. (1g)	**Acid assisted extraction** **ES**: several inorganic acids (hydrochloric acid, phosphoric acid, nitric acid, and sulfuric acid) and one organic acid (acetic acid) Extraction time:1–8 h, Acid concentrations: 0–1% Solvent to sample ratios: 20–60 mL/g Maceration at 25°C Filtration Dilution to 100 mL final volume **Soxhlet extraction** **ES**: 50% ethanol (100 mL), 4 h at 70°C Extract evaporation to dryness Resolubilization in ES (up to 100 mL final volume) **Heating reflux extraction** **ES**: 50% ethanol Soaked for 1 h Extraction: 4 h at 70°C (heated water bath) Filtration Dilution (up to 100 mL final volume)	**HPLC** Column: XTerra C18, 250 x 4.6 mm Mobile phase: (A) acetonitrile and (B) 25 mmol/L potassium dihydrogen phosphate,(27:75, *v/v*) Detection: 345 nm Temperature: 30°C	Teng and Choi, 2013
Rabbit plasma (100 μl)	Mixing 100 μl sample with 3% formic acid in acetonitrile (200 μl) Vortex: 30 s Centrifugation: 10 min at 4°C Evaporation of supernatant: under nitrogen stream at 40°C	**LC-ESI-MS** HPLC system Column: Capcell Pakc_{18} MG, 100 × 2.1 mm, 5 μm with Security Guard C18, 4 × 2 mm, 5 μm Mobile Phases: (A) 0.4% formic acid solution and (B) 0.2 % formic acid solution in methanol (60:40, *v/v*)	Liu et al., 2011

(Continued)

TABLE 3 | Continued

Sample (weight)	Extraction method	Detection method	References
	Residue solubilization: in 100 μl of 20% methanol	Temperature: 25°C Flow: 0.4 mL/min MS detection: Source: ESI (+) Quantification: MRM mode	
Rat plasma	**Solid phase extraction (SPE)** Cartridges: Oasis HLB (1 cc, 30 mg) Pre-conditioning: 2 mL methanol Equilibrtating:	**UPLC-MS/MS** UPLC system Column: 120 EC-C18, 50 × 4.6 mm, 2.7 μm with Security Guard C18, 4 × 2 mm, 5 μm Mobile Phases: (A) 10 mM ammonium acetate in water (pH- 4.5) and (B) acetonitrile Temperature: 35°C Flow: 0.8 mL/min MS detection: Source: ESI (+) Quantification: MRM mode	Liu M. et al., 2015
Rat plasma Rat tissue	**Rat plasma** **ES**: methanol Mixing sample (200 μl) with internal standard (40 μl) and ES (560 μl) Vortex: 20 s Centrifugation: 10 min, 12,000x *g* Filtration **Rat tissue** Grinding: 3 mL physiological saline with 600 mg tissue Centrifugation: 10 min, 12,000x *g*, 4°C Mixing supernatant (200 μl) with internal standard (40 μl) and ES (560 μl) Vortex: 20 s Centrifugation: 10 min, 12,000x *g* Filtration	**UPLC-MS/MS** UPLC system Column: Acquity BEH C18, 50 × 2.1 mm, 1.7 μm Mobile Phases: (A) acetonitrile and (B) formic acid: water (0.1:99.9, *v/v*) Flow: 0.25 mL/min MS detection: Source: ESI (+) Quantification: MRM mode	Wang et al., 2016
Rat plasma	Evaporation of 10 ul IS in the working tube Mixing sample (200 μl) with internal evaporated standard Vortex: 1 min Mixing sample with 10 μl 1% formic acid and 200 μl acetone Vortex: 2 min Centrifugation: 10 min, 10,000 rpm Mixing supernatant with 200 μl methanol Vortexing, centrifugation Mixing supernatant wit 400 μl acetonitrile Vortexing, centrifugation Evaporation to dryness (37°C, under nitrogen stream) Resolubilization in methanol	**LC-MS/MS** LC system Column: Zorbax Eclipse XDB-C18, 150 × 2.1 mm, 3.5 μm Mobile Phases: (A) acetonitrile and (B) water with 1% acetic acid and 0.001 mol/L ammonium acetate Flow: 0.2 mL/min MS detection: Source: ESI (+) Quantification: MRM mode	Xu et al., 2015
Rat plasma	**ES**: 90% methanol Mixing sample (100 μl) with internal standard (10 μl) and ES (100 μl) Vortex: 1 min Centrifugation: 10 min, 12,000 rpm, 4°C Supernatant evaporation to dryness under nitrogen stream Resolubilization (100 μl ES)	**UPLC-MS/MS** UPLC system Column: Acquity UPLC BEH C18, 50 × 2.1 mm, 1.7 μm Mobile Phases: (A) formic acid: water (0.1:99.9, *v/v*) and (B) acetonitrile Flow: 0.4 mL/min MS detection: Source: ESI Quantification: MRM mode	Yang et al., 2017

When referring to berberine extraction from biological samples, the extraction process is relatively simple and involves several steps like sample mixing with extraction solvents (e.g., methanol, acetone, acetonitrile), vortex, centrifugation followed by supernatant evaporation under nitrogen stream (**Table 3**). Other extraction techniques like solid phase extraction (SPE) can also be applied.

ANALYTICAL TECHNIQUES

After extraction and purification, the separation and quantification of berberine are commonly resolved by chromatographic methods. According to literature studies, berberine determination in plants was predominantly performed using methods like UV spectrophotometry (Joshi and Kanaki, 2013), HPLC (Babu et al., 2012; Akowuah et al., 2014), HPTLC and TLC (Rojsanga and Gritsanapan, 2005; Arawwawala and Wickramaar, 2012; Samal, 2013), capillary electrophoresis (Du and Wang, 2010), while berberine content in biological fluids was mainly achieved by using LC-MS (Deng et al., 2008; Feng et al., 2010), UPLC-MS (Liu M. et al., 2015; Liu L. et al., 2016), UHPLC/Q-TOF-MS (Wu et al., 2015).

UV-Vis spectrophotometry can be considered as one of the most rapid detection methods for berberine quantitative analysis from plant extracts. Based on the Beer-Lambert law, berberine concentration can be determined according to its absorption maxima at 348 nm. Joshi and Kanaki (2013) quantified berberine in *Rasayana churna* samples in the range of 2–20 μg/mL, the interference with other compounds being avoided by the specific isolation of the alkaloid fraction (Joshi and Kanaki, 2013).

Next, high-performance liquid chromatography (HPLC) is a versatile, robust, and widely used technique for the qualitative and quantitative analysis of natural products (Sasidharan et al., 2011). This approach is widely used in berberine identification and quantification. Generally, the choices of stationary phase in berberine separation are variants of C18-based silica column (**Table 3**) with a mobile phase consisting of simple or acidified solvents like water, methanol, or acetonitrile, used as such or in combination with phosphate buffers. Normally, the identification and separation of berberine can be accomplished using either isocratic or gradient elution system. Berberine identification is further accomplished using high sensitivity UV or DAD (diode array detectors) detectors. For example, Shigwan et al. (2013) developed in his study a reverse phase HPLC method with photodiode array detection (PDA) to quantify berberine from *Berberis aristata* and *B. tinctoria*. They used a Unisphere-C18 column (5 μm, 4.6 × 150 mm) with an isocratic gradient of acidified water (with 0.1% trifluoroacetic acid) and acetonitrile (60:40, *v/v*) to elute berberine within 5 min. The developed method was reproducible, validated, precise, and specific for berberine quantification (with a concentration range between 0.2 and 150 μg/mL; Shigwan et al., 2013).

Two other commonly used techniques in berberine quantification are thin layer chromatography (TLC) and high performance thin layer chromatography (HTPLC). Sometimes, these methods are preferred over HPLC, offering the possibility of running several samples simultaneously along with the use of small amount of both samples and mobile phases (Samal, 2013). For these reasons, Samal (2013) used an HPTLC method to quantify berberine from *A. mexicana* L. using toluene and ethyl acetate (9:3, *v/v*) as mobile phases, and a silica gel plate as stationary phase, they developed a simple, rapid, and cost-effective method for berberine quantification. The LOD (0.120 μg) and LOQ (0.362 μg) of the method are in accordance with high-quality requirements.

Following the same principles (small sample volume, high separation efficiency, and short analysis time), capillary electrophoresis (CE) was successfully used in berberine analysis. Du and Wang (2010) used CE with end-column electrochemiluminescence (ECL) detection for berberine analysis in both tablets and *Rhizoma coptidis*. Using a 4 min analysis time, a small sample volume (3.3 nL) and a LOD of (5×10^{-9} g/mL), the developed method proved to be highly sensitive and with good resolution (Du and Wang, 2010).

Besides UV, HPLC, HTPLC, TLC, and CE, other detection methods like liquid chromatography coupled with mass spectrometry (LC/MS) are currently employed to quantify berberine in biological fluids. Generally, it is considered a powerful technique for the analysis of complex samples because it offers rapid and accurate information about the structural composition of the compounds, especially when tandem mass spectrometry (MS^n) is applied. For example, Xu et al. (2015) developed a sensitive an accurate LC-MS/MS method to determine berberine and other seven components in rat plasma using multiple reactions monitoring (MRM) mode. Compounds separation was optimized using six different types of reverse-phase columns, and two different mobile phases (methanol–water and acetonitrile–water with different additives). Additives like formic acid, acetic acid, and ammonium acetate were added in different concentrations as follows: 0.1, 0.5, 1, and 2% for formic acid, 0.1, 0.5, 1, and 2% for acetic acid and 0.0001, 0.001, 0.01 mol/L for ammonium acetate. The method was also tested in terms of specificity, linearity, lower limit of quantification (LLOQ), precision, accuracy, and stability (Xu et al., 2015).

ANTIOXIDANT EFFECT

Under normal conditions, the body maintains a balance between the antioxidant and pro-oxidant agents (reactive oxygen species—ROS and reactive nitrogen species—RNS; Rahal et al., 2014).

The imbalance between pro and antioxidants occurs in case of increased oxidative stress (Bhattacharyya et al., 2014).

The oxidative stress builds up through several mechanisms: an increase in the production of reactive species, a decrease in the levels of enzymes involved in blocking the actions of pro-oxidant compounds, and/or the decrease in free radical scavengers (Pilch et al., 2014).

An experimental study demonstrated the effect of berberine on lipid peroxidation after inducing chemical carcinogenesis in small animals (rats). An increase in LPO (lipid peroxidation) was observed after carcinogenesis induction, but also its significant reversal after berberine administration (30 mg/kg). Berberine shows therefore at least partial antioxidant properties, due to its effect on lipid peroxidation (Thirupurasundari et al., 2009).

Other mechanisms involved in the antioxidant role of berberine are: ROS/RNS scavenging, binding of metals leading to the transformation/oxidation of certain substances, free-oxygen removal, reducing the destructiveness of superoxide ions and nitric oxide, or increasing the antioxidant effect of some endogenous substances. The antioxidant effect of berberine was comparable with that of vitamin C, a highly-potent antioxidant (Shirwaikar et al., 2006; Ahmed et al., 2015).

The increase in blood sugar leads to oxidative stress not by generating oxygen reactive species but by impairing the antioxidant mechanisms. Administration of berberine to rats with diabetes mellitus increased the SOD (superoxide dismutase) activity and decreased the MDA (malondialdehyde) level (marker of lipid peroxidation). This antioxidant effect of berberine could explain the renal function improvement in diabetic nephropathy (Liu et al., 2008b).

The oxidative stress plays an important role in the pathogenesis of many diseases. The beneficial effect of berberine is presumed to reside mostly in its antioxidant role.

CARDIOVASCULAR EFFECTS OF BERBERINE

Effect on Cardiac Contractility

The beneficial effect of berberine in cardiac failure was demonstrated in a study on 51 patients diagnosed with NYHA (New York Heart Association) III/IV cardiac failure with low left ventricular ejection fraction (LVEF) and premature ventricular contractions and/or ventricular tachycardia. These patients received tablets containing 1.2 g berberine/day, together with conventional therapy (diuretics, ACEI—angiotensin-converting-enzyme inhibitors, digoxin, nitrates) for 2 weeks. An increase in LVEF was observed in all patients after this period, but also a decrease in the frequency and complexity of premature ventricular contractions. The magnitude of the beneficial effect was in direct proportion with the plasma concentration of berberine (Zeng, 1999).

The Cardioprotective Effect During Ischemia

Berberine can provide cardio-protection in ischemic conditions by playing various roles at different levels: modulation of AMPK (AMP—activated kinase) activity, AKT (protein kinase B) phosphorylation, modulation of the JAK/STAT (Janus kinase/signal transducers and activators of transcription) pathway and of GSK3β (glycogen synthase kinase 3β; Chang et al., 2016). AMPK is an important enzyme playing an essential role in cellular metabolism and offering protection in ischemic conditions by adjusting the carbohydrate and lipid metabolism, the function of cell organelles (mitochondria, endoplasmic reticulum) and the apoptosis (Zaha et al., 2016).

Berberine activates the PI3K (phosphoinositide 3-kinase)/AKT pathway which is considered a compensatory mechanism limiting the pro-inflammatory processes and apoptotic events in the presence of aggressive factors. The activation of this pathway is associated with a reduction of the ischemic injury through the modulation of the TLR4 (toll-like receptor 4)-mediated signal transduction (Hua et al., 2007).

Several supporting data indicate that the JAK2/STAT3 signaling plays an important role in cardioprotection against ischemia-reperfusion injury (Mascareno et al., 2001).

GSK3β is a serine/threonine protein-kinase, an enzyme involved in reactions associated to important processes at the cellular level: metabolization, differentiation, proliferation, and apoptosis. Berberine inhibits this kinase, thereby exercising its cardioprotective effect (Park et al., 2014).

EFFECTS ON THE ENDOTHELIUM

Berberine induces endothelial relaxation by increasing NO production from arginine through the activity of eNOS (endothelial nitric oxide synthase) which is considered a key element in the vasodilation process. Besides increasing the NO level, it also up-regulates eNOS mRNA. Furthermore, berberine facilitates the phosphorylation of eNOS and its coupling to HSP 90 (heat shock proteins), which consequently increases NO production (Wang et al., 2009).

Moreover, berberine reduces endothelial contraction by reducing COX-2 expression. Any imbalance in COX 1 or 2 activity may alter the ratio between prothrombotic/antithrombotic and vasodilator/vasoconstrictor effects (Liu L. et al., 2015).

The beneficial effect of berberine on the TNFα-induced endothelial contraction was also recorded, as well as an increase in the level of PI3K/AKT/eNOS mRNA (Xiao et al., 2014).

THE ROLE OF BERBERINE IN ATHEROSCLEROSIS

Atherogenesis is a consequence of high blood lipid levels and is associated with inflammatory changes in the vascular wall. Berberine interferes with this process by up-regulating the expression of SIRT1 (silent information regulator T1) and by inhibiting the expression of PPARγ (peroxisome proliferator-activated receptor-γ). SIRT1 is a NAD-dependent deacetylase. The SIRT1 enzyme has many targets (PPARγ, p53), all playing different roles in atherogenesis (Chi et al., 2014).

The Role of Berberine in Lipid Metabolism

The effects of berberine on lipid metabolism are also the consequence of its effects on LDL cholesterol receptors. On one hand, these receptors are stabilized by an extracellular signal-regulated kinase (ERK)-dependent pathway, and on the other, berberine increases the activity of LDL receptors through the JNK pathway (Cicero and Ertek, 2009).

Moreover, berberine has an effect on ACAT (cholesterol acyltransferases), a class of enzymes that transform cholesterol into esters, thus playing an essential role in maintaining cholesterol homeostasis in different tissues. There are two types of ACAT enzymes, ACAT1, and ACAT2. ACAT1 is a ubiquitous enzyme, while ACAT2 can be found only in hepatic cells and enterocytes. Berberine influences the activity of ACAT2 without an effect on ACAT1, therefore reducing the intestinal absorption of cholesterol and decreasing its plasmatic level (Chang et al., 2009; Wang et al., 2014).

The hypolipidemic effect of berberine is also a result of its action on PCSK9 (proprotein convertase subtilisin kexin 9). This enzyme can attach itself to LDL receptors, leading to a decrease in LDL metabolization and an increase in its blood level (Xiao et al., 2012).

In a clinical trial, 63 patients with dyslipidemia were randomly divided in three groups. The first group was treated with berberine (1,000 mg/day), the second with simvastatin (20 mg/day) and the third with a combination of berberine and simvastatin. The authors reported a 23.8% reduction in LDL-C levels in patients treated with berberine, a 14.3% reduction in those treated with simvastatin and a 31.8% LDL-C reduction in the group treated with both simvastatin and berberine. This result demonstrates that berberine can be used alone or in association with simvastatin in the treatment of dyslipidemia (Kong et al., 2008).

THE ROLE OF BERBERINE IN GLUCOSE METABOLISM

Many studies demonstrated that berberine lowers blood sugar, through the following mechanisms:

- Inhibition of mitochondrial glucose oxidation and stimulation of glycolysis, and subsequently increased glucose metabolization (Yin et al., 2008a).
- Decreased ATP level through the inhibition of mitochondrial function in the liver, which may be the probable explanation of gluconeogenesis inhibition by berberine (Xia et al., 2011).
- Inhibition of DPP 4 (dipeptidyl peptidase-4), a ubiquitous serine protease responsible for cleaving certain peptides, such as the incretins GLP1 (glucagon-like peptide-1) and GIP (gastric inhibitory polypeptide); their role is to raise the insulin level in the context of hyperglycemia. The DPP4 inhibition will prolong the duration of action for these peptides, therefore improving overall glucose tolerance (Al-masri et al., 2009; Seino et al., 2010).

Berberine has a beneficial effect in improving insulin resistance and glucose utilization in tissues by lowering the lipid (especially triglyceride) and plasma free fatty acids levels (Chen et al., 2011).

The effect of berberine (1,500 mg day) on glucose metabolism was also demonstrated in a pilot study enrolling 84 patients with type 2 diabetes mellitus. The effect, including on HbA1c, was comparable to that of metformin (1,500 mg/day), one of the most widely used hypoglycemic drugs. In addition, berberine has a favorable influence on the lipid profile, unlike metformin, which has barely any effect (Yin et al., 2008b).

HEPATOPROTECTIVE EFFECT OF BERBERINE

The hepatoprotective effect of berberine was demonstrated on lab animals (mice), in which hepatotoxicity was induced by doxorubicin. Pretreatment with berberine significantly reduced both functional hepatic tests and histological damage (inflammatory cellular infiltrate, hepatocyte necrosis; Zhao et al., 2012).

The mechanism by which berberine reduces hepatotoxicity was also studied on CCl_4 (carbon tetrachloride)-induced hepatotoxicity. Berberine lowers the oxidative and nitrosamine stress and also modulates the inflammatory response in the liver, with favorable effects on the changes occurring in the liver. Berberine prevents the decrease in SOD activity and the increase in lipid peroxidation and contributes to the reduction in TNF-α, COX-2, and iNOS (inducible nitric oxide synthase) levels. The decrease in transaminase levels supports the hypothesis according to which berberine helps maintain the integrity of the hepatocellular membrane (Domitrović et al., 2011).

NEPHROPROTECTIVE EFFECT OF BERBERINE

The chronic kidney damage occurring in time in patients with HT (hypertension) and DM (diabetes mellitus) is well known; it is mainly due to the atherosclerosis of the renal artery, caused by inflammation and oxidative stress. The protective effect of berberine on kidneys was studied on 69 patients suffering from both HT and DM, with blood pressure and blood sugar levels controlled with conventional medication. The patients received 300 mg berberine/day for 24 months, with 2-week interruptions every 5 months. The authors recorded lower CRP (C-reactive protein), MDA and SOD levels after treatment, but without significant changes in creatinine, arterial pressure, or glycaemia levels. These results support the renal protective effect of berberine through its anti-inflammatory and antioxidant effects (Dai et al., 2015).

Another animal study tested the renoprotective effect of berberine after administration of $HgCl_2$ (mercury chloride). This substance induces hepato-renal damage by increasing the oxidative stress (increases lipid peroxidation and NO levels, and lowers glutathione and SOD levels as well as the activity of other protective enzymes). Administration of $HgCl_2$ increased the AST (aspartate aminotransferase), ALT (alanine aminotransferase), and ALP (alkaline phosphatase) levels, compared to the control group. However, pretreatment with berberine lowered these enzymes significantly. In addition, both urea and creatinine levels were significantly increased in the $HgCl_2$ group vs. the control group, and again pretreatment with berberine prevented these changes. Additionally, the authors recorded higher pro-oxidant and lower antioxidant levels in the intervention group. These data support the hepatic and renal protective effects of berberine. Other studies performed on animal models with CCl_4–induced hepatotoxicity demonstrated the same effect (Othman et al., 2014).

In addition, berberine can lower the nephrotoxicity caused by cisplatine. In an animal study, berberine was administered in progressive doses of 1, 2, 3 mg/kg, orally, for 2 successive days, starting 2 days after cisplatine administration. After the last doses of berberine, the animals were sacrificed and the kidneys were examined by the pathologist. The results showed significant histological improvement and a reduction in NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells), TNF α, COX2 an iNOS levels, all of which support the anti-inflammatory effect of berberine (Domitrović et al., 2013).

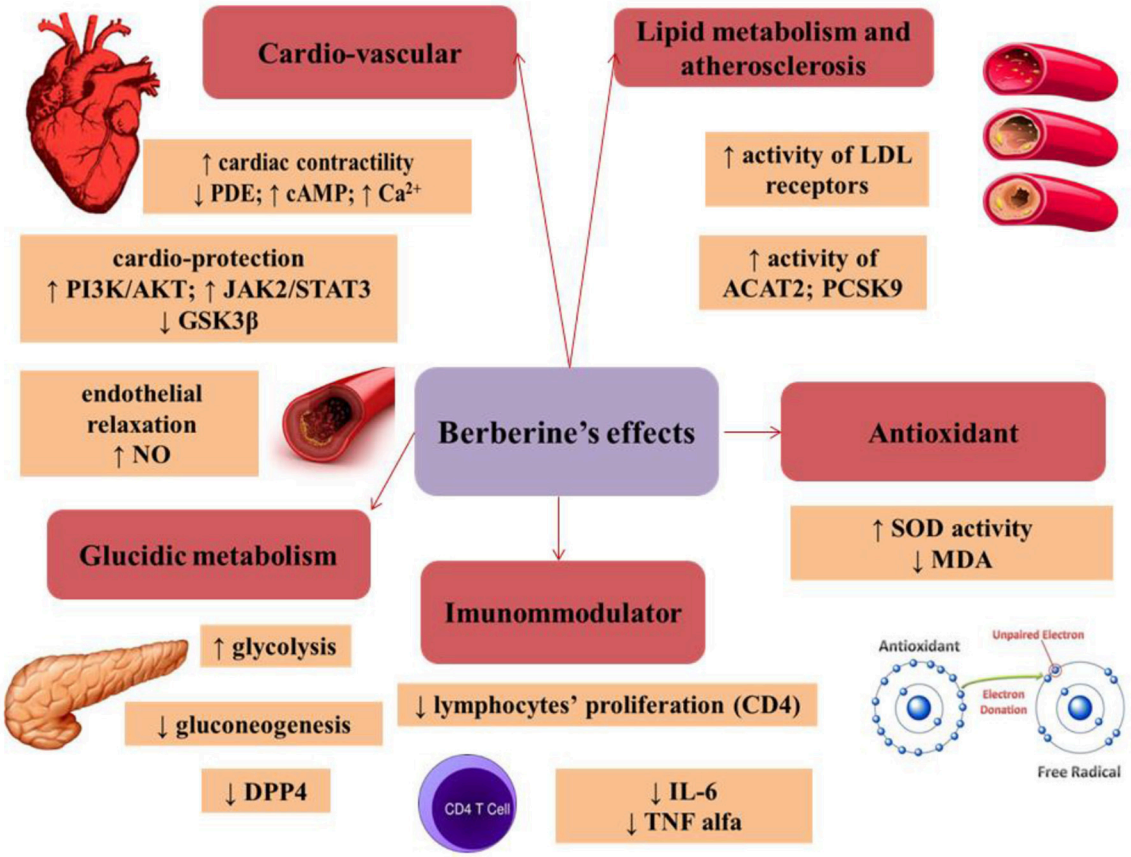

FIGURE 3 | Therapeutic effects of berberine *in vivo*. Mechanisms of berberine in regulation of metabolism, immunity and oxidative reactions. Phosphodiesterase (PDE), cyclic 3′,5′-adenosine monophosphate (cAMP), phosphoinositide 3-kinase/protein kinase B (PI3K/AKT), Janus kinase/signal transducers and activators of transcription (JAK/STAT), glycogen synthase kinase 3β (GSK3β), superoxide dismutase (SOD), malondialdehyde (MDA), nitric oxide (NO), cholesterol acyltransferases (ACAT2), dipeptidyl peptidase-4 (DPP 4), proprotein convertase subtilisin kexin 9 (PCSK9).

IMMUNOMODULATORY EFFECT OF BERBERINE

The immunomodulatory effect of berberine was demonstrated in many experimental and clinical contexts.

In an experimental autoimmune myocarditis model, berberine contributed to mitigate the cardiac damage by: limiting the rise in anticardiac myosin antibodies, modulating the activity of certain STATs and blocking Th1 and Th2 cell differentiation, which play an important role in the pathogenesis of myocarditis (Liu X. et al., 2016).

Experimental autoimmune neuritis is an experimental animal model equivalent to the Guillain-Barre syndrome in humans. This neurologic syndrome is characterized by autoimmune injury of the peripheral nervous system. The beneficial effect of berberine on this animal model resided in its influence on cellular and humoral immunity through the inhibition of lymphocyte proliferation (especially CD4), and the decrease in pro-inflammatory cytokines (IL-6 and TNF α; Li et al., 2014).

Experimental autoimmune encephalomyelitis is an established model of multiple sclerosis. Multiple sclerosis is a one of the most common diseases of the central nervous system (CNS) and involves neurodegenerative and inflammatory processes, and autoimmune demyelination (Ransohoff et al., 2015). The blood-brain barrier permeability and changes in matrix metalloproteinase (MMP) levels in the cerebrospinal fluid and brain were studied using this model (Ma et al., 2010). MMPs may be involved in demyelination and their activity in tissues depends on the balance between their level and their tissue inhibitors. MMP2 and MMP9 are the main endoproteinases involved in the migration of lymphocytes in CNS and in altering the BBB (blood brain barrier) (Avolio et al., 2003). Berberine has a beneficial effect in experimental autoimmune encephalomyelitis by inhibiting the activity of MMP9, reducing BBB permeability and, consecutively, by decreasing the inflammatory cellular infiltration of the CNS (Ma et al., 2010).

The current therapy used for inflammatory bowel diseases, including glucocorticoids and immunosuppressive agents, has a low level of safety. The effect of berberine was studied in combination with 5-ASA (5-aminosalicylic acid) vs. 5-ASA alone using an experimental animal model with DSS (dextran sulfate sodium)-induced colitis. The authors analyzed the level

of proinflammatory cytokines in the animal gut. A decrease in COX2, IL6, and IL23 mRNA levels was observed in animals treated only with 5-ASA, whereas animals treated with both 5-ASA and berberine had a reduction in mRNA levels for COX2, IL6, IL23 as well as for TNF alfa and IL12b. This beneficial effect could partially be attributed to the inhibition of NF-kB and the reduction in JAK2 phosphorylation (through the influence on the JAK/STAT pathway) by both 5-ASA and berberine (Li et al., 2015; **Figure 3**).

Another study demonstrated that berberine increases the corticosteroid level in rats with experimentally-induced colitis. This engendered the theory that its beneficial effect may also be attributed to the increase in endogenous glucocorticoid levels, compounds with well-known therapeutic effect in inflammatory bowel disease (Minaiyan et al., 2011).

CONCLUSION

A review of the available scientific literature shows that the traditional medical uses of berberine-containing plants have been evaluated by modern pharmacological studies. Different species of berberine-rich plants have multiple pharmacological and therapeutic actions, such as antioxidant and immunomodulatory effects, protective action on the cardiovascular system, liver and kidney, endothelial relaxation, regulator on glucose metabolism and atherosclerosis, which can all be explained by the presence of berberine as well as other phyto constituents (when dealing with berberine-containing plant extracts). Moreover, the effects of berberine vary according to its origin (different plants or pharmaceutical products) and its concentration, depending on the very diverse extraction and detection techniques already described. Over time, modern extraction techniques were increasingly preferred to classical ones. Since classical methods are generally time- and solvent-consuming processes, modern extraction techniques such as USE, MAE, UPE, SFE, and PLE are seen as better alternatives to overcome these limitations. Furthermore, berberine, due to its antioxidant and anti-inflammatory effects, has several clinical applications in many disorders, from inflammatory conditions to the metabolic syndrome. However, there are some traditional uses that have not yet been completely elucidated, and further studies are needed. Therefore, extensive studies on the potential of plants containing berberine that have shown aforementioned pharmacological activities should go through additional *in vitro* and *in vivo* studies.

AUTHOR CONTRIBUTIONS

MN, AM, JE, and RP have conceived and designed the structure of the manuscript, data collection, and drafting, as well as its revision. CB, GC, and AB have critically reviewed the manuscript. All authors have seen and agreed on the final version of the manuscript.

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REVIEW
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Application and Mechanisms of Triptolide in the Treatment of Inflammatory Diseases—A Review

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Bioactive compounds from medicinal plants with anti-inflammatory and immunosuppressive effects have been emerging as important sources of drugs for the treatment of inflammatory disorders. Triptolide, a diterpene triepoxide, is a pharmacologically active compound isolated from *Tripterygium wilfordii* Hook F (TwHF) that is used as a remedy for inflammatory and autoimmune diseases. As the most promising bioactive compound obtained from TwHF, triptolide has attracted considerable interest recently, especially for its potent anti-inflammatory and immunosuppressive activities. Over the past few years, an increasing number of studies have been published emphasizing the value of triptolide in the treatment of diverse inflammatory disorders. Here, we systematically review the mechanism of action and the therapeutic properties of triptolide in various inflammatory diseases according to different systematic organs, including lupus nephritis, inflammatory bowel disease, asthma, and rheumatoid arthritis with pubmed and Embase. Based on this review, potential research strategies might contribute to the clinical application of triptolide in the future.

Keywords: triptolide, inflammatory disorders, anti-inflammation, immunosuppression, pubmed, Embase

INTRODUCTION

Complementary and alternative medications, including traditional Chinese medicines (TCMs), have long been used to treat inflammatory disorders, and they are generally well tolerated by patients (Goldrosen and Straus, 2004). The use of TCMs has necessitated urgent research into the mechanisms of action of natural products. One TCM, *Tripterygium wilfordii Hook F* (TwHF), has been used in folk medicine for the treatment of a variety of inflammatory disorders for many centuries (Tao et al., 1991; Goldbach-Mansky et al., 2009; Lv et al., 2015). TwHF belongs to celastraceae family and *Tripterygium* genus. It has been collected in Southern China and its roots have been used in various preparations to "relieve stasis and internal warmth," among many other conditions diagnosed by TCM practitioners. TwHF was used to deal with rheumatoid arthritis and psoriasis in ancient China. In addition, TwHF was also used as a method of birth control in men. Previous studies demonstrated TwHF exhibited multiple pharmacological activities including antitumor, immune modulation, anti-inflammatory, and antifertility effects. Especially in RA, TwHF was found to have anti-inflammatory and cartilage protective effects (Zhou et al., 2018). However, TwHF might have significant side effects and severe toxicity, which limits the clinical application.

Triptolide is a major bioactive compound derived from *T. wilfordii Hook F* (Kupchan et al., 1972). It is a diterpene triepoxide containing three epoxy groups, a C-14-hydroxyl group and a lactone ring (**Figure 1**).

With pubmed and Embase, we systematically review the therapeutic properties of triptolide in inflammatory diseases according to different systematic organs and illustrate its potential clinical applications.

POTENTIAL EFFECTS OF TRIPTOLIDE ON INFLAMMATORY DISEASES

The therapeutic potential of triptolide has been tested in various inflammatory and autoimmune disorder models, including nephritis, asthma, arthritis, and neurodegenerative disorders, and triptolide has been found to modulate a wide variety of inflammatory mediators. These disorders and their inflammatory mediators will be discussed in brief below.

Renal Diseases

Membranous Nephropathy

Membranous nephropathy (MN) is one of the major causes of nephrotic syndrome in adults and is characterized by subepithelial deposition of immune complexes (Cattran and Brenchley, 2017). Globally, the overall incidence of membranous nephropathy (MN) is estimated as 1/100,000. Immune-mediated podocyte injury is considered to underlie the proteinuria in MN. Asymptomatic proteinuria and generalized edema are clinical presentations of MN. Researchers found that triptolide could reduce podocyte injuries in MN to reduce proteinuria and alleviate inflammatory response in animal model of MN.

Chen et al. (2010) demonstrated that 200 μg/kg/day triptolide could effectively reduce proteinuria and inhibit immune-mediated injuries in an experimental rat model of MN. The recovery of podocyte injuries was promoted after triptolide treatment, accompanied by a reduction in glomerular complement component 5b-9 (C5b-9) deposits. In addition, Triptolide also suppressed reactive oxygen species (ROS) generation and p38 mitogen-activated protein kinase (MAPK) activation in the podocytes induced by C5b-9.

Later, Zhou et al. (2016) showed that 200 μg/kg/day triptolide attenuated the inflammatory response in MN rats *via* suppression of the nuclear factor-kappa B (NF-κB) signaling pathway. Interestingly, they also found that triptolide treatment could significantly decrease malondialdehyde (MDA) levels while enhancing superoxide dismutase (SOD) activity in the serum to reduce oxidative stress and inflammatory responses.

More recently, Chen et al. (2017) revealed that 100 and 200 μg/kg/day triptolide reduces podocyte injury by inhibiting podocyte apoptosis in an experimental rat model of MN. Cleaved caspase-3 and cleaved poly ADP-ribose polymerase (PARP) were markedly decreased after triptolide treatment. Triptolide inhibited C5b-9-induced MAPK activation in podocytes by decreasing p-JNK and p-ERK expression.

So, triptolide could alleviate membranous nephropathy by inhibiting inflammatory signaling pathways including NF-κB and MAPK pathways. Oxidative stress and apoptosis were also involved in the mechanism of triptolide against MN. However, the researchers have not investigated the triptolide cytotoxicity in the organ of animal model of MN. Only Chen et al. (2017) studied aminotransferase in the serum.

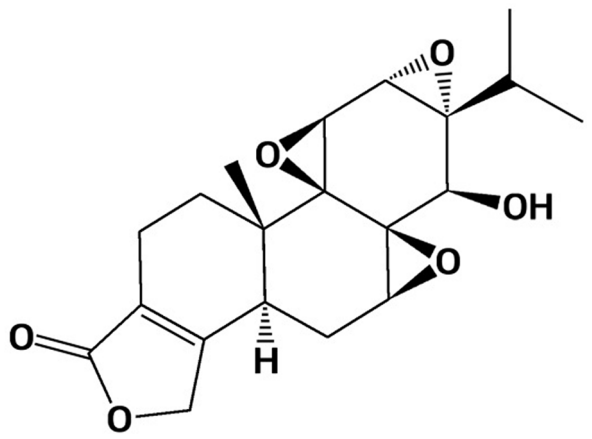

FIGURE 1 | Chemical structure of triptolide.Triptolide has been shown to possess a broad spectrum of anti-inflammatory and immunosuppressive properties in treating various inflammatory disorder models. Based on potent anti-inflammatory biological activities, triptolide has increasingly drawn attention worldwide in recent decades.

Lupus Nephritis

Lupus nephritis is inflammation of the kidney caused by systemic lupus erythematosus and is characterized by increased production of cytokines and autoantibodies, deposition of immune complexes, and infiltration of leukocytes (Lech and Anders, 2013). Lupus nephritis (LN) influences almost 40% of patients with systemic lupus erythematous (SLE).

Tao et al. (2008) discovered that triptolide with 15 weeks administration could increase the survival rate, reduce disease severity and decrease cytokine production in mice with lupus nephritis (LN). They indicated that the survival rate of mice was significantly higher in the triptolide group (87.5%) than in the vehicle group (35.7%). Triptolide could decrease proteinuria and blood urea nitrogen levels were significantly reduced in the triptolide-treated mice compared with the control mice throughout the treatment period. Histopathologic analysis showed that triptolide-treated mice had less severe kidney disease, with significantly diminished glomerular and interstitial disease. In this study, the NZB/NZWF1 mice were used as the animal model of LN. There are some shortcomings of NZB/NZWF1 mice. These mice do not possess some clinical manifestations of lupus such as arthritis and rash. Another drawback of this strain is the long disease incubation time almost 6 months which is the long disease incubation time.

Kidney Transplantation

Due to allograft rejection, the fate of long-term grafts has not changed significantly over the past decades. Allograft rejection,

characterized by the activation of various inflammatory cells, cytokines, chemokines, and adhesion molecules, is one of the leading causes of graft loss in clinical transplantation (Ingulli, 2010). Triptolide could prolong the survival of kidney transplantation by inhibiting inflammatory activities.

In 2009, an experimental study demonstrated that triptolide with 250 and 500 μg/kg/day treatment effectively prolongs allograft survival (Zhang et al., 2009). Brown Norway rat kidneys were transplanted into Lewis recipients to generate allograft groups, and graft recipients were treated with 0.25 and 0.5 mg/kg/day triptolide for 14 days. The average median survival time (MST) was 18 and 19.8 days in the 0.25 and 0.5 mg/kg/day triptolide treatment groups, respectively. There had some flaws in this study. The researchers have not evaluated the cytotoxicity of triptolide in the healthy organs of kidney transplantation animal model.

Later, Zhang et al. (2013) demonstrated that complex phenotypic and allostimulatory functional changes induced in rat bone marrow-derived dendritic cells (DCs) by 1-10 nM triptolide may account for the prolonged allograft survival. They also found that triptolide-conditioned DCs could induce allospecific T-cell regulation and prolong renal graft survival.

Crews et al. (2005) demonstrated that downregulation of vascular cell adhesion molecule 1 (VCAM-1) and transforming growth factor beta (TGF-β) are associated with successful 500 μg/kg/day triptolide with 10 days treatment of chronic allograft rejection in rats. The gene expression levels of VCAM-1 and TGF-β exhibited significant associations with the CADI scores of the experimental groups.

Importantly, Hong et al. (2002) showed triptolide is a potent suppressant of C3, CD40 and B7h expression in activated human proximal tubular epithelial cells (PTECs). Triptolide exerts inhibitory effects on C3, CD40, and B7h expression in TNF-α-stimulated PTECs.

Therefore, triptolide might have a beneficial effect on kidney transplantation by inhibiting inflammatory molecules such as VCAM-1, TGF-β, C3, and CD40.

Renal Fibrosis

Renal fibrosis is associated with a decline in renal excretory function, and unresolved inflammation promotes progressive renal fibrosis, which can culminate in end-stage renal disease (Zhou et al., 2016). It has been found that 0.6 mg/kg per day triptolide attenuates renal interstitial fibrosis by decreasing α-SMA and TGF-β1 expression and interstitial collagen deposition in the kidney (Yuan et al., 2011). Triptolide could also inhibit macrophage and myofibroblast infiltration.

Gastrointestinal Disease

Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) includes ulcerative colitis and Crohn's disease (CD) and is characterized by chronic inflammation of the gastrointestinal tract (Abraham and Cho, 2009). An estimated 1.6 million Americans are affected by IBD, and approximately 2.5–3 million people in Europe currently have IBD. Previous studies found that triptolide could deal with IBD in mouse model and patients.

Two kinds of inflammatory bowel disease animal models including dextran sulfate sodium (DSS) induced colitis mice and interleukin-10 gene-deficient (IL-$10^{-/-}$) mice were adopt in these studies. 0.07 mg/kg/day Triptolide with 8 weeks treatment alleviated diarrhea, edema, hyperemia, and inflammatory cells infiltration in the animal models (Wei et al., 2008). In addition, researchers also investigated the mechanism of triptolide in the treatment of IBD. Li et al. (2010) found that triptolide ameliorates colitis by suppressing the IL-6/signal transducer and activator of transcription 3 (STAT3) signaling pathway and downregulating IL-17. Zhang et al. (2014) found that 0.6 mg/kg/day triptolide administration ameliorates intestinal injury by inhibiting the expression of secondary lymphoid tissue chemokine chemokine (C-C motif) ligand 21 (CCL21), which has chemotactic effects on T cells, B cells, NK cells, and DCs. However, they only investigated the expression of CCL21, which is not sufficient to demonstrate triptolide influence the chemotactic effects of T cells, B cells, NK cells, and DCs.

Inflammatory bowel disease is associated with an increased risk of developing colorectal cancer. Wang et al. demonstrated that triptolide with 0.1, 0.3, or 1 mg/kg/day for 20 weeks inhibited colitis-related colon cancer progression *in vivo via* downregulating Rac1 and the Janus kinase (JAK)/STAT3 pathway (Wang et al., 2009). *In vitro* cell cycle analysis revealed that triptolide inhibits the proliferation, migration and colony formation of colon cancer cells. Triptolide could reduce the secretion of IL6 and levels of JAK1 and IL6R by interrupting the IL6R-JAK/STAT pathway. The shortcoming in this study was that the high dose group was 1 mg/kg/day which might lead to organ damage. However, Wang et al. have not investigated the organ damage in this group.

Intestinal Fibrosis

Intestinal fibrosis is a common complication of inflammatory bowel disease that is characterized by abnormal deposition of extracellular matrix proteins produced by activated myofibroblasts in the intestine (Rieder and Fiocchi, 2008). Tao et al. (2015) discovered that 45 mg/kg per day triptolide ameliorates colonic fibrosis in an experimental rat model. Triptolide could reduce collagen production and extracellular matrix deposition in the colon. Collagen I protein and collagen Iα1 transcript expression were also inhibited after treatment in the isolated subepithelial myofibroblasts of rats with colonic fibrosis.

Liver Fibrosis

Liver fibrosis is the excessive accumulation of extracellular matrix that occurs in most types of chronic liver diseases (Bataller and Brenner, 2005). Chong et al. (2011) found that 20 μg/kg triptolide exerts antihepatofibrotic effects in animal model of liver fibrosis. Triptolide inhibited the NF-κB signaling pathway in hepatic stellate cells. In addition, triptolide treatment reduced hepatic fibrosis scores *in vivo*.

Respiratory Disease

Asthma

Asthma is a common long-term inflammatory airway disorder. Airway smooth muscle cells, goblet cells and eosinophils

contribute to asthmatic airway inflammation (Cohn et al., 2004). Approximately 8% of the adult population in worldwide are diagnosed asthma. Triptolide could inhibit asthma airway remodeling by suppressing the inflammatory signaling pathway, interfering with the production of pro-inflammatory chemokines and cytokines.

In the ovalbumin (OVA)—sensitized asthma mice model, Chen et al. showed that 40 μg/kg/day triptolide treatment may function as an inhibitor of asthma airway remodeling (Chen et al., 2011; Chen et al., 2015b).Triptolide could inhibit mucous gland hypertrophy, goblet cell hyperplasia and collagen deposition through the suppression of TGF-β1/Smad and NF-κB signaling pathways in airways. However, they only tested p-P65 protein in NF-κB signaling pathway which was not sufficient to demonstrated NF-κB signaling pathway was involved in the mechanism of triptolide. Furthermore, they revealed that triptolide inhibits the proliferation and migration of rat ASMCs (Chen et al., 2015a). Triptolide exerts a time- and dose-dependent inhibition of TGF-β1-induced ASMC proliferation by blocking S and G2/M phases without apparent cytotoxic effects.

Apart from signaling pathways, researchers also focused on the inflammatory cells in asthma. Ji et al. (2015) have shown that triptolide modulates the $CD4^+$ T cell balance in an asthmatic animal model. Triptolide could regulate Th1/Th2 balance and Th17/Treg equilibrium. Inflammatory cytokines in the bronchoalveolar lavage fluid (BALF), such as IL-10, IL-13, IL-17, TNF-α, and TGF-β, are downregulated after treatment. Mao et al. (2008) found that 2 weeks of 40 μg/kg/day triptolide treatment alleviated eosinophil recruitment in BALF by inhibiting bone marrow eosinophilopoiesis. The number of eosinophils in the peripheral blood and bone marrow were significantly reduced after triptolide treatment

Acute Lung Injury

Acute lung injury (ALI) is a disorder of acute inflammation consisting of acute hypoxemic respiratory failure (Rubenfeld et al., 2005). Triptolide was found to alleviate ALI in LPS-induced mouse model and chlorine exposure mouse model.

In mouse models of chlorine gas-induced acute lung injury, triptolide ranged 100–1,000 μg/kg/day administration showed anti-inflammatory effects by decreasing neutrophils infiltration to the lung lavage fluid and lung tissue (Hoyle et al., 2010). In the LPS-induced ALI mouse model, 1–50 μg/kg/day triptolide ameliorated ALI by inhibiting the NF-κB signaling pathway (Wang et al., 2014). Triptolide inhibited chemokines such as macrophage inflammatory protein alpha (MIP-1α), MIP-1β, regulated upon activation normal T cell express sequence (RANTES) in the lung tissue and inflammatory cytokines such as TNF-α, IL-1β, IL-6 in the BALF of mice with ALI. Suppressed expression of p-IκB-α and p-NF-κB p65 showed that triptolide inhibits the NF-κB signaling pathway. Moreover, in the same animal model, Wei et al. found that triptolide with 5, 10, and 15 μg/kg treatment attenuated the LPS-induced inflammatory response by inhibiting the MAPK signaling pathway (Wei and Huang, 2014). Triptolide inhibits the phosphorylation of p38, JNK, and ERK. However, there have some significant disadvantages in LPS-induced ALI mouse model. LPS preparations might be contaminated with bacterial materials and other bacterial lipoproteins. LPS does not induce injury of epithelial and endothelial cells occurring in acute respiratory distress syndrome of humans.

Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) is an incurable disease characterized by increased blood pressure in the arteries of the lungs (Farber and Loscalzo, 2004). There is an increasing appreciation of inflammation in the pathogenesis of PAH with an accumulation of inflammatory cells and elevated cytokines. Triptolide could attenuate the development of pulmonary hypertension by down-regulating expression of functionally related genes.

In a rat experimental pulmonary arterial hypertension model, it has been shown that 0.25 mg/kg/day triptolide can effectively attenuate the development of pulmonary hypertension (Faul et al., 2000). By Day 35, the mean pulmonary arterial pressure was diminished in triptolide-treated rats compared with vehicle-treated rats. Triptolide-treated rats also showed significantly less pulmonary arterial neointimal formation and right ventricular hypertrophy. Another study analyzed longitudinal transcriptional expression in pulmonary hypertension rats administered 0.25 mg/kg/day triptolide treatment for 30 days (Vaszar et al., 2004). Transcriptional analysis with total lung RNA was performed for every experimental time point. They found that a group of functionally related genes, such as matrix metalloproteinase (MMP) and mast cell chymases, were significantly coexpressed with the development of PAH. The global gene expression pattern also resembled that seen in intermediate stages of severity. Functionally related genes were downregulated in response to triptolide treatment. Monocrotaline (MCT)-induced pulmonary hypertension (MCTP) was used as animal model in these two studies. Compared with chronic hypoxia PAH animal model, MCTP is easily to be therapeutically improved owning to the acute nature, which is not alike the characteristics of PAH in human.

Pulmonary Fibrosis

Pulmonary fibrosis is a chronic, debilitating and lethal lung disorder. Pulmonary fibrosis is estimated to have prevalence of 13 to 20 per 100,000 people worldwide. It has been demonstrated that 0.25 mg/kg triptolide effectively reduced radiation-induced lung fibrosis (Yang et al., 2015; Chen et al., 2016). Triptolide improved the pulmonary function by inhibiting myofibroblast activation, collagen deposition and ROS production in lung tissues. Triptolide also mitigated pulmonary fibrosis partly by downregulating nicotinamide adenine dinucleotide phosphate-oxidase 2 (NOX2) through the NF-κB pathway.

Endocrine Diseases

Diabetic Nephropathy

Diabetic nephropathy (DN) is a serious complication in those with diabetes mellitus. Podocyte injuries, such as decreased density of podocytes due to chronic inflammation and oxidative stress, are observed in the development of diabetic glomerular injury (Navarro-González and Mora-Fernández, 2008). Triptolide exerted protective effects on DN of DN animal model.

In an *in vitro* model of db/db diabetic mice with increased albuminuria, it has been revealed that triptolide markedly attenuates albuminuria. It has been shown that 50 μg/kg/day triptolide with 12 weeks treatment attenuates inflammation in the kidneys accompanied by alleviated podocyte injury. Triptolide could reduce the expression of desmin protein, MCP-1 protein and CD68-positive macrophages in db/db diabetic mouse kidneys (Gao et al., 2010).

Apart from db/db diabetic animal model, investigators also used streptozocin-induced DN model to reveal the mechanism of triptolide against DN. db/db diabetic animal model was used to be type 2 diabetes model, while streptozocin-induced DN model was used to be type 1 diabetes model.

Ma et al. demonstrated that 8 weeks of 100 μg/kg/day triptolide administration significantly reduces the expression of TGF-β1 and osteopontin to alleviate inflammation in the kidney. (Ma et al., 2013). Later, Guo et al. discovered that 4 weeks of 6, 12, or 24 mg/kg/day triptolide improves DN by regulating Th cell balance and macrophage infiltration (Guo et al., 2016). Triptolide could inhibit proinflammatory cytokines and increase anti-inflammatory cytokines by regulating the Th1/Th2 cell balance. Triptolide also inhibits macrophage infiltration and macrophage-mediated inflammation in the kidneys. Han et al. (2017) confirmed that triptolide significantly inhibits mesangial cell proliferation by suppressing the PDK1/Akt/mTOR pathway. More recently, Dong et al. (2017) reported that triptolide alleviates oxidative stress in the kidneys of DN. Renal homogenate SOD and MDA were significantly downregulated after treatment.

So, Th cell balance, macrophage, oxidative stress might be involved in the mechanism of triptolide in treating DN.

Diabetic Cardiomyopathy

Diabetic cardiomyopathy is one of the leading cardiovascular complications in diabetic patients. Chronic inflammation plays an important role in diabetic cardiomyopathy (Frati et al., 2017).

Studies have suggested that triptolide ranged 100, 200, or 400 μg/kg/day improves cardiac diastolic and systolic function in diabetic rats (Wen et al., 2013; Guo et al., 2016). Triptolide treatment prevents myocardial fibrosis and collagen accumulation in diabetic myocardium by decreasing the expression of cardiac inflammatory mediators including TGF-β1, α-SMA, TNF-α, IL-1β, and vimentin. Triptolide treatment also inhibits the recruitment of macrophages and T lymphocytes in diabetic rat hearts. The inhibitory effect of triptolide on diabetic cardiomyopathy might be mediated by the suppression of the NF-κB immune pathway. More recently, Liang et al. (2015) detected that 100, 200, or 400 μg/kg/day triptolide improves cardiac function and increases cardiac energy metabolism by activating the MAPK signaling pathway.

Thus, triptolide could inhibit inflammatory cells recruitment and cytokines expression to reduce myocardial fibrosis, apoptosis and necrosis in diabetic cardiomyopathy. The shortcomings of these studies were that the researchers only tested N-κB p65 in NF-κB signaling pathway and p38 MAPK protein in MAPK signaling pathway when they studied the related pathways. Only one protein in the inflammatory signaling pathway was not persuasive to demonstrate the related pathways were involved in the mechanism.

Rheumatic Diseases

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a systemic inflammatory autoimmune disorder in which genetic and environmental risk factors contribute to disease development (Collison, 2016). Descriptive epidemiology studies of RA showed a population prevalence of 0.5–1% in worldwide. The disease is characterized by inflammation of synovial joints leading to the destruction of articular cartilage and erosion of the bone. Fibroblast-like synoviocytes (FLS), T cells, macrophages, DCs, osteoclasts, and chondrocytes play important roles in the pathogenesis of RA. Previous studies have observed antirheumatic properties of triptolide. In an animal model of RA, triptolide ameliorates the severity of arthritis (Gu and Brandwein, 1998). The anti-inflammatory effects of triptolide in dealing with RA have thus far been attributed to the aspects described in **Figure 2**.

Fibroblast-Like Synoviocytes

Fibroblast-like synoviocytes, also known as synovial fibroblasts, are special cells that play a crucial role in the pathogenesis of RA (Bartok and Firestein, 2010). In RA, FLSs are the most common cell type at the cartilage–pannus junction and perpetrate inflammation through their massive production of inflammatory cytokines, chemokines, and matrix-degrading molecules and through immigration and invasion of joint cartilage (Bustamante et al., 2017).

Triptolide has been found to inhibit inflammation of fibroblast-like synoviocytes in several studies. Triptolide with

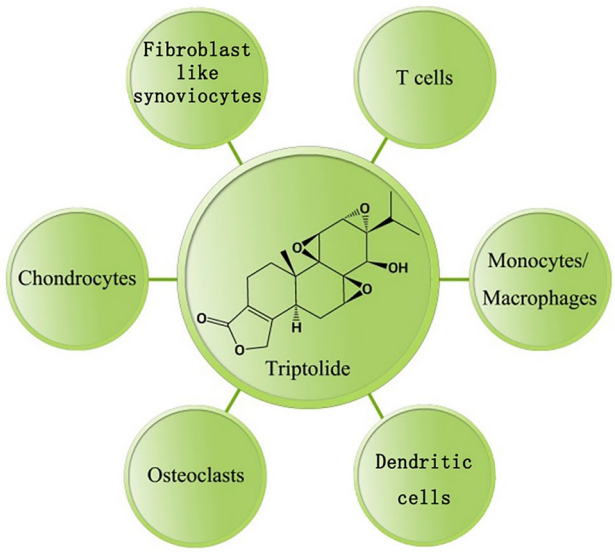

FIGURE 2 | Anti-inflammatory effect(s) of triptolide on RA.Triptolide has a broad array of cellular mechanisms and potential therapeutic applications (**Table 1**).

TABLE 1 | Cellular target(s) modulated by triptolide in rheumatoid arthritis.

Type of cells	Mechanism(s) of action
Fibroblast-like synoviocytes	Inhibits viability, proliferation, migration and invasive capacities. Affects cytoskeletal rearrangement. Arrests the cell cycle. Induces apoptosis. Reduces inflammatory mediators, including IL-18, prostaglandin E2 (PGE-2), cyclooxygenase-2 (COX-2), pro-MMP1 and 3. Inhibits the NF-κB and MAPK pathways.
T cells	Inhibits proliferation and differentiation. Induces apoptosis. Reduces inflammatory cytokines, including IL-2, IL-13, IL-17 and IFN-γ. Inhibits the NF-κB and MAPK pathways.
Monocytes/ Macrophages	Reduces inflammatory cytokines, including IL-1β, IL-6, IL-8, IL-12, IL-37, TNF-α and IFN-γ. Reduces inflammatory chemokines, including CCL-1, 2, 5, 7, and 12 as well as chemokine (C-X-C motif) ligand (CXCL)-10 and 11. Reduces the expression of costimulatory molecules CD80 and CD86. Reduces production of nitric oxide (NO), superoxide anion and ROS. Induces apoptosis. Inhibits the NF-κB pathway and TAK1 kinase activity.
Dendritic cells	Inhibits differentiation, maturation, allostimulatory capacities, chemotactic responses and migration. Induces apoptosis. Reduces inflammatory mediators, including MIP-1α, MIP-1β, MCP-1, PGE-2 and COX-2. Reduces the expression of costimulatory molecules CD1a, CD40, CD80 and CD86. Inhibits the PI3-K/Akt and NF-κB pathways.
Osteoclasts	Inhibits differentiation and osteoclastogenesis. Regulates the RANKL/RANK/osteoprotegerin signaling pathway. Inhibits NF-κB activation.
Chondrocytes	Reduces inflammatory mediators, including TNF-α, IL-6, PGE-2, COX-2, MMP-3 and MMP-13. Inhibits aggrecanase-1 expression. Inhibits NF-κB activation.

100 ng/ml concentration could inhibit inflammatory cytokine production of FLS and inhibit invasive capacities of FLS. Lu et al. (2008) demonstrated that triptolide inhibits the expression of IL-18 and its receptor in PMA-stimulated FLSs in a dose-dependent manner by suppressing NF-κB activity. However, Lu et al. (2008) only analyzed one inflammatory cytokine produced by FLS. Lin et al. (2001) demonstrated that 28–140 nM triptolide suppresses the production of proMMP1 and 3 in IL-1α-induced FLSs. In addition, triptolide downregulates the expression of PGE-2 in activated FLSs by selectively suppressing the production of COX-2. Kusunoki et al. (2004) revealed that 100nM triptolide decreases viability, inhibits proliferation, and induces apoptosis of FLSs in a concentration-dependent manner at very low concentrations. Later, Yang et al. (2016) discovered that 10–50 nM triptolide reduces the migratory and invasive capacities of FLSs by affecting the cytoskeletal rearrangement of FLSs. They also indicated that triptolide blocks the activation of the JNK MAPK pathway in FLSs. Thus, triptolide suppresses the inflammatory mediators including IL-18, COX-2, proMMP1 and 3 in activated FLS. NF-κB and MAPK pathways might be involved in the mechanism of inhibiting FLS inflammatory activities.

T Cells

T cells take center stage in the pathogenesis of rheumatoid arthritis (Cope et al., 2007). The predominance of T cells in lymphocytic infiltrates in the tissue of patients with RA has been defined.

Previous studies have demonstrated the immunosuppressive effects of triptolide in regulating T cell function. Inflammatory cytokine production, apoptosis, cell proliferation, and inflammatory pathways were involved in the effects of 10–500 nmol triptolide on T cells. Inflammatory cytokines production of T cells including IL-2, IFN-γ, IL-13, IL-17 were inhibited by triptolide (Chan et al., 1999; Dai et al., 2013). Qiu et al. (1999) reported that triptolide inhibits IL-2 expression in T cells at the level of purine-box/nuclear factor of activated T cells and NF-κB transcriptional activation triggered by all stimuli examined. Dai et al. (2013) demonstrated that triptolide inhibits IL-13 expression by inhibiting GATA3 and nuclear factor of activated T cells 1 (NFAT1) nuclear translocation and their binding rates to the IL-13 gene promoter region. However, Dai et al. have not revealed the relationship between IL-13 and GATA3 in T cells clearly. They only examined the expression of IL-13 and GATA3 after triptolide administration.

In the aspects of apoptosis and signaling pathways, Yang et al. (1998) discovered that 10–100 ng/ml triptolide induces T cell apoptosis accompanied by increased caspase activity and degradation of the caspase substrate PARP. Liu et al. (2000) showed that 2–10 μg/L triptolide attenuates T cell NF-κB activation by upregulating IκBα mRNA expression. In addition, Ho et al. (2013) discovered that 50–200 ng/ml triptolide attenuates both the NF-κB and MAPK signaling pathways.

Monocytes/Macrophages

Monocytes/macrophages play an important role in the pathogenesis of rheumatoid arthritis (Davignon et al., 2013). A number of monocytes/macrophages infiltrate into the rheumatoid synovium. They are a potent source of cytokines, chemokines and ROS that participate in the initiation, maintenance, and resolution of inflammation. The number of macrophages in the inflamed synovial membrane and cartilage-pannus junction are correlated with cartilage and bone destruction in RA (Kinne et al., 2007). As such, monocytes and macrophages are viewed as relevant therapeutic targets in treating RA.

Triptolide has been found to inhibit inflammation of monocytes and macrophages. Triptolide inhibited inflammatory cytokines and costimulatory molecules in monocytes. Triptolide suppressed the production of IL-12 in the THP-1 human monocytic leukemia cell line at dosage of 2.5–0.625 μg/L (Liu et al., 2005). Triptolide also suppressed expression of the costimulatory molecules CD80 and CD86 on LPS-activated THP-1 cells. In addition, 5–25 nM triptolide prompted THP-1 apoptosis by inhibiting the NF-κB pathway and activating the MAPK pathway (Park and Kim, 2013). Interestingly, MAPK pathway was activated in this study, which is different with other studies. Activated MAPK pathway might contribute to the apoptosis of monocytes induced by triptolide.

Apart from monocytes, triptolide inhibits the inflammatory activities of macrophages through multiple mechanisms. First, 5–40 ng/ml triptolide inhibits some key inflammation-related cytokines in LPS-activated macrophages, including TNF-α, IL-1β, IL-6, IL-8, and IFN-γ (Wu et al., 2006; Yang et al., 2010). Some proinflammatory chemokines, including CCL-1, 2, 5, 7, and 12, as well as CXCL-10 and 11, are attenuated after triptolide

administration with concentrations as low as 10–50 nM (Matta et al., 2009). Second, several studies have revealed that 5–25 ng/ml triptolide suppresses nitric oxide (NO) and ROS production in activated macrophages (Wang et al., 2004; Bao et al., 2007). Kim et al. (2004) discovered that triptolide abrogates inducible NO synthase (iNOS) gene expression and inhibits NO production in a dose-dependent manner. Wu et al. (2006) found that triptolide inhibits superoxide anion and ROS production in murine peritoneal macrophages. Third, triptolide down-regulates NF-κB activation and JNK phosphorylation. Kim et al. (2004) revealed that triptolide significantly inhibits the DNA binding activity of NF-κB, whereas Premkumar et al. (2010) showed that myeloid differentiation primary response 88 (MyD88)-dependent and independent pathways of toll-like receptors (TLRs) are engaged in the biological activity of triptolide. So, triptolide could influence the inflammatory cytokines production, oxidative stress, and related inflammatory signaling pathways in the macrophages to alleviate inflammation.

Dendritic Cells

Dendritic cells play important roles in the induction of immunity and in mediating immune tolerance as professional antigen-presenting cells (Khan et al., 2009). In the synovial fluid of RA joints, DCs amplify immune responses by ingesting and presenting antigens to T cells (Bell et al., 2017).

It has been found that triptolide could suppress the migration and differentiation of dendritic cells DCs. Chen et al. (2005) found that triptolide is a potent suppressor of DCs maturation and trafficking. The allostimulatory capacities and chemotactic responses of DCs are inhibited by 2.5–10 nM triptolide over a pharmacologic concentration range. Zhu et al. (2005) discovered that 20 ng/ml triptolide prevents the differentiation of immature DCs by inhibiting CD1a, CD40, CD80, CD86 and HLA-DR expression. Thus, triptolide could inhibit the trafficking, maturation, and differentiation of DCs and. In another article, triptolide inhibits DC-mediated chemoattraction of neutrophils and T cells by inhibiting DC production of CC and CXC chemokines, including MIP-1α, MIP-1β, and MCP-1, in response to LPS (Liu et al., 2006). However, they have not revealed the mechanisms of triptolide inhibiting DC-mediated chemoattraction of other inflammatory cells. Later, Liu et al. (2007) revealed that 10–100 ng/ml triptolide impairs DC migration by inhibiting the phosphatidylinositol-3 kinase (PI3-K)/Akt and NF-κB pathways. Triptolide-mediated suppression of the NF-κB pathway, STAT3 phosphorylation and an increase in suppressor of cytokine signaling 1 (SOCS1) expression in DCs may be involved in the inhibitory effect of triptolide. (Liu et al., 2004).

Osteoclasts

Extensive bone destruction is a feature of patients with rheumatoid arthritis, leading to severe deformity of the affected joints. As a result, ameliorating bone destruction is a very important issue in the treatment of RA.

Triptolide has been shown to efficiently ameliorate the progression of bone destruction in rheumatoid arthritis by inhibiting osteoclast activities. The related signaling pathways might include receptor activator of the nuclear factor kappa-B ligand (RANKL)/RANK/osteoprotegerin (OPG) and NF-κB signaling pathways. Liu et al. (2013) showed that 8-32μg/kg/day triptolide prevents bone destruction and inhibits osteoclast formation in an animal model of RA by regulating the RANKL/RANK/OPG signaling pathway. Later, Huang et al. (2015) reported that 1.25nM triptolide inhibits osteoclastogenesis and osteoclastic bone resorption by suppressing RANKL-induced activation of NF-κB and p65 nuclear translocation in osteoclast-like cells. More recently, Xu et al. (2016) found that triptolide inhibits osteoclast differentiation and bone resorption by increasing expression of the immunosuppressive cytokines IL-10 and TGF-β1 by Tregs. Therefore, these data show that triptolide is effective in treating bone destruction by regulating osteoclast inflammatory activities, which is a hallmark of RA physiopathology.

Chondrocytes

Rheumatoid arthritis is characterized by synovitis in joints and destruction of cartilage. Cartilage is destroyed by enzymatic and mechanical processes. The chondrocytes themselves also synthesize cytokines and MMPs or respond to local cytokine release to accelerate articular cartilage destruction (Otero and Goldring, 2007).

Triptolide could reduce the expression of inflammatory mediators including TNF-α, IL-6, COX-2, and MMPs in chondrocytes to prevent cartilage damage. In 2005, Liacini et al. (2005) showed that triptolide at 125–250 nM concentrations possesses cartilage-protective effects by suppressing MMP expression in chondrocytes *in vitro*. 100nM triptolide inhibits proinflammatory cytokine-induced MMP-3 and MMP-13 expression in chondrocytes in a dose-dependent manner. Later, Lin et al. (2007) discovered that triptolide suppresses inflammation and cartilage destruction in RA mice. In 2009, Xiao et al. (2009) demonstrated that triptolide reduces the expression of the proinflammatory cytokines COX-2 and NF-κB in paw cartilage in arthritic rats. Immunohistochemistry staining revealed that triptolide inhibits the expression of TNF-α, IL-6, COX-2 and NF-κB in superficial cartilage. The flaw of this study was that they only test NF-κB protein in the tissue. Other proteins in the NF-κB signaling pathway have not been investigated.

Neurologic Diseases

In the last decade, many studies have demonstrated that triptolide is a promising neuroprotective agent and alleviates neuroinflammation in animal models of neurodegenerative diseases.

Alzheimer's Disease

Alzheimer's disease (AD) is a chronic neurodegenerative disease that devastates later decades of life. Increasing evidence suggests that the pathogenesis of AD is not restricted to the neuronal compartment but includes strong interactions with neuroinflammation in the brain (Heneka et al., 2015). The prevalence of AD in worldwide is approximately as high as 24 million. Triptolide could alleviate the immune-inflammatory

pathology including Aβ deposition, cytokines expression, and oxidative stress in the treatment of AD.

Triptolide treatment with 0.4 mg/kg/day administration of 18 days could reduce Aβ deposition and neuroinflammation in the hippocampal and cortical areas by upregulating the degradation pathway of Aβ in a transgenic Alzheimer's disease model (Cheng et al., 2014). Another study found that 20 μg/kg/day triptolide for 8 weeks exerted anti-inflammatory and antioxidative effects on the transgenic mouse brain (Wang et al., 2014). Triptolide inhibited the expression of proinflammatory markers TNFα and IL-1β in the hippocampus in the hippocampus. Recently, Li et al. (2016) demonstrated that 5 μg/kg/day triptolide treatment for 45 days inhibits the activation and proliferation of microglial cells and astrocytes in the hippocampus in a transgenic AD mouse model, reducing neuroinflammation in the brain. Additionally, Cui et al. (2016) found that triptolide with 20 μg/kg/day for 15 weeks alleviates neuroinflammation by suppressing MAPK activity. In the previous studies, APP transgenic mice were used as animal models. The brain of APP transgenic mice is similar to the brain pathology of AD patients with Aβ deposition and neuroinflammation.

Similar to what is observed in animal models of Alzheimer's disease, the protective effect of triptolide has been found *in vitro*. Xu et al. demonstrated that triptolide has a protective effect in PC12 cells against $A\beta_{25-35}$-induced cytotoxicity by inhibiting the autophagy pathway (Xu et al., 2015). Furthermore, they found that triptolide protects PC12 cells by reducing oxidative stress. Triptolide downregulated the expression of ROS, hydrogen peroxide and MDA induced by $A\beta_{25-35}$ (Xu et al., 2016). PC12 cell line was used to mimic AD *in vitro* model in these studies. Apart from PC12 cell line, human neuroblastoma and human induced pluripotent stem cells are also used as *in vitro* model for AD research. However, these cell lines are not really satisfactory *in vitro* model in order to mimic the features of AD.

Parkinson's Disease

Parkinson's disease (PD) is a chronic and progressive disorder. Chronic inflammation is a major characteristic of PD (Herrero et al., 2015). There were 6.1 million prevalent cases of PD globally. The worldwide the burden of Parkinson's disease has more than 6.1 million patients. Triptolide shows anti-inflammatory and neuroprotective effects *in vivo* and *in vitro*. Triptolide protected dopaminergic cells and reduced inflammatory cytokines expression in the brain of PD.

It has been demonstrated that triptolide protects dopaminergic neurons from inflammation-mediated damage. Moreover, 1–10 nM triptolide has been found to suppress LPS-induced activation of microglia and excessive production of TNFα and NO (Li et al., 2004). The administration of triptolide 1 or 5μg/kg for 24 days also attenuated the depletion of dopamine in the striatum and protected dopaminergic neurons from the injury induced by intranigral LPS injection (Zhou et al., 2005). Hu and colleagues have shown that 50 nM triptolide promotes the clearance of pathogenic proteins in neuronal cells using *in vitro* models of PD (Hu et al., 2017).

In addition, using an *in vitro* Parkinson's disease model of lipopolysaccharides -stimulated PC12 cells, it has been shown that 10–100 ng/ml triptolide downregulates inflammatory mediators such as COX-2 and PGE-2 by inhibiting the NF-κB and MAPK signaling pathways in LPS-stimulated PC12 cells (Geng et al., 2012). Triptolide with concentration more than 200ng/ml showed cytotoxicity effects on PC12 cells. PC12 cells were also used to mimic AD as *in vitro* model. So, this cell lines are not really satisfactory *in vitro* model in order to mimic the features of PD.

Multiple Sclerosis

Multiple sclerosis (MS) is a chronic inflammatory autoimmune disease, and experimental autoimmune encephalomyelitis (EAE) is considered an animal model for MS (Constantinescu et al., 2011). MS affects more than 2 million individuals in the worldwide. Triptolide alleviate MS in animal models by reducing inflammation and demyelination pathology.

Kizelsztein et al. (2009) discovered that 100 μg/kg/day triptolide for 2 weeks reduces cellular infiltration and tissue damage in experimental autoimmune encephalomyelitis mice. EAE-promoting cytokines, including IL-2, IL-6 and TNF-α, were reduced in inflamed brains. Molecular analysis revealed that triptolide suppressed NF-κB signaling pathway. Another study conducted by Wang et al. (2008) found that 100 μg/kg/day triptolide for 4 weeks modulates T-cell inflammatory responses and ameliorates EAE. Triptolide inhibited the mRNA expression of both Th1/Th(IL-17) and Th2 cytokines in spleen mononuclear cells and in spinal cord tissues. The levels of IFN-γ, TNF-α, IL-12, IL-6, IL-17 and IL-23 were significantly reduced in both spleen MNCs and spinal cord tissues after triptolide administration. EAE animal model was used to mimic MS in these studies. However, there are some limitations of EAE animal model. Firstly, EAE model offer little information on the progression of MS. EAE is an infectious disorder affecting white matter of spinal cord, whereas MS affects cerebral and cerebellar cortex.

CONCLUSION

With an increasing worldwide rate of inflammatory disorders, there is an urgent need for the improvement of the therapeutic activity of anti-inflammatory agents. The anti-inflammatory and immunosuppressive properties of triptolide make it a promising agent to treat inflammatory disorders. In this review, we systemically discussed the potential effects and mechanisms of triptolide in the treatment of different inflammatory diseases including MN, lupus nephritis, kidney transplantation, IBD, asthma, ALI, PAH, etc. Firstly, triptolide could reduce the production of inflammatory mediators such as TNF-α, IL-6, IL-17. Secondly, triptolide alleviated oxidative stress of damaged organs in animal model and related cell lines. Thirdly, triptolide could inhibit the activities of inflammatory cells such as T cells and macrophages. The most related signaling pathway involved in the mechanisms of triptolide was NF-κB and MAPK signaling pathways. Despite the great therapeutic potential of triptolide, there are still some shortcomings in the process of developing it as a new drug. The most studies were focused on the studies of animal models and cell lines. The successful track records of

real patients in randomized controlled trials seem very poor. The triptolide cytotoxicity in other healthy organs have not been investigated clearly, either. Hopefully, future stringent preclinical studies on triptolide will provide crucial information regarding its pharmacokinetics and dosage, allowing for further optimization of this compound.

AUTHOR CONTRIBUTIONS

KY, XL, QL, QZ, and HJ summarized the literature and drafted the manuscript. KY, XL, TW, GH, and AX revised and edited the manuscript. AX, TW, and GH supervised the work. KY, XL, TW, GH, and AX initiated, finalized, and submitted the manuscript.

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REVIEW
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Lycopene and Vascular Health

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Lycopene is a lipophilic, unsaturated carotenoid, found in red-colored fruits and vegetables, including tomatoes, watermelon, papaya, red grapefruits, and guava. The present work provides an up to date overview of mechanisms linking lycopene in the human diet and vascular changes, considering epidemiological data, clinical studies, and experimental data. Lycopene may improve vascular function and contributes to the primary and secondary prevention of cardiovascular disorders. The main activity profile of lycopene includes antiatherosclerotic, antioxidant, anti-inflammatory, antihypertensive, antiplatelet, anti-apoptotic, and protective endothelial effects, the ability to improve the metabolic profile, and reduce arterial stiffness. In this context, lycopene has been shown in numerous studies to exert a favorable effect in patients with subclinical atherosclerosis, metabolic syndrome, hypertension, peripheral vascular disease, stroke and several other cardiovascular disorders, although the obtained results are sometimes inconsistent, which warrants further studies focusing on its bioactivity.

Keywords: lycopene, arterial stiffness, endothelial function, intima-media thickness, cardiovascular risk

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INTRODUCTION

Cardiovascular disorders (CVD) are the leading mortality cause worldwide and prophylactic measures to combat it deserve special attention. Atherosclerosis is, in most cases, the main manifestation of CVD, and its progression may be clinical silent for a long time, up to a certain moment, when it directly leads into a severe adverse event (e.g., heart attack or stroke). Beneficial lifestyle changes are important and most cost-effective components of prevention or treatment of cardiovascular disorders. Compounds occurring in plants often display diverse bioactivities with therapeutic potential and different plants and plant-derived compounds have a long history of being reported to exhibit effects counteracting CVD (Atanasov et al., 2015; Waltenberger et al., 2016). Among lifestyle factors, a healthy diet is considered a cornerstone of cardiovascular disease prevention, and the inclusion of sufficient fruits and vegetables in the diet is regarded as especially important (Böhm, 2012; Piepoli et al., 2016). The prevalence of cardiovascular disorders is remarkably unevenly distributed in developed countries, and some areas, e.g., Southern Europe, seem to be protected by having significantly less prevalence of the disease. This effect has often been attributed to dietary factors, as e.g., the Mediterranean diet, with a lot of vegetables, including tomatoes, tomato products, and olive oil (Müller-Nordhorn et al., 2008; Krasinska et al., 2017). Tomatoes, tomato sauce, and watermelon are important sources of lycopene and may be a surrogate

for the Mediterranean diet to some degree (Sesso et al., 2003; Burton-Freeman and Sesso, 2014; Naz et al., 2014).

Dietary lycopene is considered to confer cardiovascular benefits, as e.g., consuming at least 7 servings/week of lycopene-based products significantly decreased cardiovascular risk within seven years in postmenopausal women, free from prior cardiovascular disorders and cancer (Sesso et al., 2003). A negative correlation between serum lycopene concentration and cardiovascular mortality was also found in a follow-up study with a large Japanese cohort (Ito et al., 2006). Many studies about the relationship between lycopene and cardiovascular risk have been conducted and, although, some of the results are inconsistent, overall dietary lycopene intake and high-serum concentration of lycopene, significantly reduced the risk of major cardiovascular events (Cheng et al., 2017; Song et al., 2017). Not all outcomes were positive, as exemplified in the Physicians Health Study, including 499 patients with cardiovascular disorders, not revealing any association of higher plasma lycopene and CVD (Müller et al., 2016). Within the Kuopio Ischaemic Heart Disease Risk Factor (KIHD), no relationship was reported between low serum lycopene and an increased CVD mortality and sudden cardiac death, respectively, in Finnish, middle-aged men (Karppi et al., 2012; Müller et al., 2016).

Vascular health depends on endothelial function, arterial stiffness, and the presence of atherosclerotic plaques. The following section will discuss the different methods to measure vascular health. *Endothelial dysfunction* enables development of the atherosclerotic plaque. The flow mediated dilatation of the brachial artery was traditionally used to assess endothelial function for a long period, but forearm plethysmography and reactive hyperemia—peripheral arterial tonometry have become the gold standards for assessing endothelial vascular function (Kim et al., 2011; Gajendragadkar et al., 2014). While hyperemia—peripheral arterial tonometry is less operator-dependent and non-invasive, while being as reliable as the traditional method, forearm plethysmography additionally provides mechanistic information related to nitric oxide synthesis, is a marker for cardiovascular risk, and can improve risk prediction (Kim et al., 2011; Gajendragadkar et al., 2014).

An increased *arterial stiffness* is one of the first structural and functional changes of the vessel wall and is mainly caused by arteriosclerosis, atherosclerosis and vessel wall calcification (Cavalcante et al., 2011). Arterial stiffness is associated with cardiovascular risk and predicts cardiovascular disorders and mortality (Vlachopoulos et al., 2010; Mozos et al., 2017c). Pulse wave velocity (PWV) and augmentation indices are simple, noninvasive, inexpensive and validated methods used to assess arterial stiffness and as screening methods for the detection of pre-clinical cardiovascular disorders (Vlachopoulos et al., 2010; Mozos et al., 2017a).

Carotid intima-media thickness (IMT) assessed non-invasively by B-mode ultrasonography, is a simple, validated and safe method used to measure the extent of subclinical atherosclerosis (Dwyer et al., 2004; Wood and Johnson, 2004; Hosseini et al., 2017; Kim and Youn, 2017). IMT is defined by the measurement of the dimension of the intima and media of the arterial wall, whereas a value >0.9 mm is considered abnormal (Cooney et al., 2015; Piepoli et al., 2016). Although IMT has been considered as a surrogate measure of cardiovascular risk, predicting cardiovascular events, especially myocardial infarction and stroke (O'Leary et al., 1999; Touboul, 2015; Piepoli et al., 2016; Hosseini et al., 2017; Pleskovic et al., 2017), both the American and the European guidelines on cardiovascular disease prevention do not recommend the systematic measurement of IMT to improve risk assessment (Goff et al., 2014; Piepoli et al., 2016). Major concerns of IMT include lack of standardization, its high variability and low intra-individual reproducibility (Piepoli et al., 2016). Additionally, a trained sonographer is required to limit variability of the results due to the patient or medical equipment (Touboul, 2015). There are also methods available that can better predict cardiovascular events than IMT, most prominently MRI (magnetic resonance imaging) that can be used to monitor atherosclerotic plaques. Several methods are stronger predictor of myocardial infarction than IMT, such as monitoring atherosclerotic plaques and exploring the carotid wall by MRI (because the adventitia is also included in the measurement of the wall thickness; Zhang et al., 2014). However, the resources needed to access wall thickness by MRI are manifold higher than using ultrasonography, but especially for patients with abnormal findings in IMT screenings, a subsequent MRI scan might be useful to provide a more accurate description of the atherosclerotic plaque and the risk of major cardiovascular events (Zhang et al., 2014).

Despite the inevitable influence of aging, vascular changes are at least partially reversible, and dietary changes may improve vascular function. Lycopene has several advantages, such as almost no adverse reactions, its wide availability and low cost (Gao et al., 2016). The present review aims to give a current overview of the mechanisms linking lycopene in the human diet and vascular changes, considering epidemiological data, clinical observational, retrospective, intervention and randomized studies, dietary and biomarker studies, *in vitro* and *in vivo* research and discussing preventive benefits of lycopene intake in context of cardiovascular disease prevention.

LYCOPENE

Lycopene is a lipophilic, biologically active, unsaturated, acyclic carotenoid, with the chemical formula $C_{40}H_{56}$ (**Figure 1**). In plants it is considered as an important intermediate of carotenoid synthesis, but in human nutrition lycopene plays no role as provitamin A precursor due to the absence of appropriate enzymes. Plant lycopene is usually present as all-trans isomer. Isomerization of the all-trans isomer into the more bioavailable cis-isomer, occurs under acidic conditions (e.g., gastric acid), due to exposure to light and thermal energy. Lycopene can be found in tomatoes, watermelon, papaya, red grapefruits, apricots, and guava, and gives their red color (Kong et al., 2010; Gajendragadkar et al., 2014; Mozos et al., 2017c). Lycopene content increases during different stages of ripening of fruits, e.g., for tomatoes there is a steady increase in lycopene content from the breaker to the red stage (Saini et al., 2017). Watermelon

pulp can also be used for lycopene extraction (Oberoi and Soqi, 2017) and is a rich source of cis-isomeric lycopene, abundant in higher concentrations than in tomatoes (Naz et al., 2014). Interestingly also the fungal plant pathogen *Blakeslea trispora* has been recognized as a commercial source to produce lycopene (Mantzouridou and Tsimidou, 2008). Several foods high in lycopene content are classified as functional foods (Naz et al., 2014). Tomato juice, paste, puree, ketchup, sauce or soup represent lycopene sources with improved bioavailability due to thermal treatment, but also because processing releases lycopene from the fibrous cell structure matrix (Basu and Imrhan, 2007; Thies et al., 2012; Burton-Freeman and Sesso, 2014).

Not only lycopene intake counts, but also its serum concentration may influence cardiovascular risk. Low serum and adipose tissue lycopene levels were correlated with early atherosclerosis, and major acute coronary and cerebrovascular events, and were found to be more reliable in risk assessment than the daily intake of lycopene (Agarwal and Rao, 2000; Rissanen et al., 2001; Kim et al., 2011). Oxidative stress and inflammation are responsible for a reduced level of antioxidants (Kim et al., 2010). Smoking is a potent oxidative stressor, able to impair arterial elasticity and endothelial function (Kim et al., 2010; Mozos et al., 2017b), and lycopene was the only major serum carotenoid able to reduce the atherosclerotic risk in current and former smokers according to the Rotterdam Study (Klipstein-Grobusch et al., 2000).

CARDIOVASCULAR PROTECTIVE MECHANISMS

Lycopene has several cardiovascular beneficial effects, such as an antioxidative, antiinflammatory, anti-atherogenic, cardioprotective, and antiplatelet effect, improving endothelial function (nitric oxide bioavailability and blood flow), the metabolic profile (by impairing cholesterol synthesis) and blood pressure control (**Figure 2**) (Klipstein-Grobusch et al., 2000; Heber and Lu, 2002; Ahuja et al., 2006; Basu and Imrhan, 2007; Verghese et al., 2008; Kim et al., 2010; Kong et al., 2010; Riccioni et al., 2010; Ried and Fakler, 2011; Böhm, 2012; Wolak and Paran, 2013; Gajendragadkar et al., 2014; Naz et al., 2014; Müller et al., 2016; Cheng et al., 2017; Milani et al., 2017).

Antioxidative and Protective Endothelial Effects

Lycopene is considered an effective singlet oxygen quencher in the carotenoids group (Kong et al., 2010; Viuda-Martos et al., 2014). It is a much more potent *antioxidant* than alpha-tocopherol (10 × more potent) or beta-carotene (twice as potent) (Kim et al., 2010; Kong et al., 2010). Lycopene modulates also the production of antioxidant enzymes, such as superoxide dismutase and catalase (Böhm, 2012; Pereira et al., 2017). Lycopene can also scavenge peroxynitrite, resulting oxidized lycopene products (Pisoschi and Pop, 2015).

Oxidative stress causes endothelial dysfunction due to uncoupling of the nitric oxide synthase and oxidative injury of the endothelial cells (Mozos and Luca, 2017). Both are associated with inflammation. By reducing oxidative stress and reactive oxygen species, lycopene increases the bioavailability of nitric oxide (NO), improves endothelium-dependent vasodilation and reduces protein, lipids, DNA, and mitochondrial damage (Hollman et al., 2011; Naz et al., 2014; Nakamura et al., 2017; Abdel-Daim et al., 2018).

Endothelial NO enables vasodilation, inhibits platelet functions, and adhesion and transmigration of white blood cells, and reduces smooth muscle cell proliferation (Opatrilova et al., 2017). Watermelon supplementation, due to L-citrulline content, increases plasma L-arginine, enabling NO production (Figueroa et al., 2017), because NO is synthesized from L-arginine by NO synthase in virtually all cell types (Jobgen et al., 2006). Lycopene supplementation improved endothelial mediated vasodilation in cardiovascular disease patients, but not in healthy controls (Gajendragadkar et al., 2014), suggesting the importance of lycopene in secondary cardiovascular prevention (Costa-Rodrigues et al., 2018).

In summary, lycopene scavenges both reactive oxygen and nitrogen species, increases the production of antioxidant enzymes and protects the endothelial cells from oxidative damage.

Anti-inflammatory Effect

Inflammation is related to atherosclerosis, arterial stiffness, and major cardiovascular events. The *anti-inflammatory* role of lycopene was demonstrated by several studies (Hung et al., 2008; Kim et al., 2010; Xu et al., 2012; He et al., 2016). Hung et al. revealed that lycopene can inhibit TNF-alpha induced NF-kappa B activation, expression of intracellular adhesion molecule-1 (ICAM-1), and interaction between monocytes and endothelial cells, which might explain the cardiovascular benefits of lycopene (Hung et al., 2008). In a different study with Korean women, lycopene levels were found to correlate with cytokines, but no correlation with acute phase reactants was found, probably due to lycopene's inhibitory effect on the formation of oxidized LDL (Kim et al., 2010). Xu et al. found an inverse association of lycopene with vascular cell adhesion protein 1 (VCAM-1), which enable adhesion of monocytes to the endothelial cells, but could not verify any association between serum lycopene concentration and atherosclerosis in their study as suggested by earlier studies (Xu et al., 2012). In the same context, Gianetti et al. reported no significant correlations between plasma lycopene and soluble adhesion molecules (Gianetti et al., 2002). Lycopene obtained from red guava exerts several anti-inflammatory effects besides modulation of inflammatory mediators, such as inhibition of leukocyte mobilization, stabilization of mast cells, and inhibition of genes which expression is involved in inflammation (Vasconcelos et al., 2017).

Lycopene can also reduce the secretion of metalloproteinases by macrophages and inhibit T lymphocyte activation (Thies et al., 2017). Recently, lycopene was found as an effective antiglycation agent, able to reduce the synthesis of advanced glycation end-products (AGE), downregulating the expression of their receptors (RAGE), which further contributes to vessel protection (Tabrez et al., 2015; Thies et al., 2017).

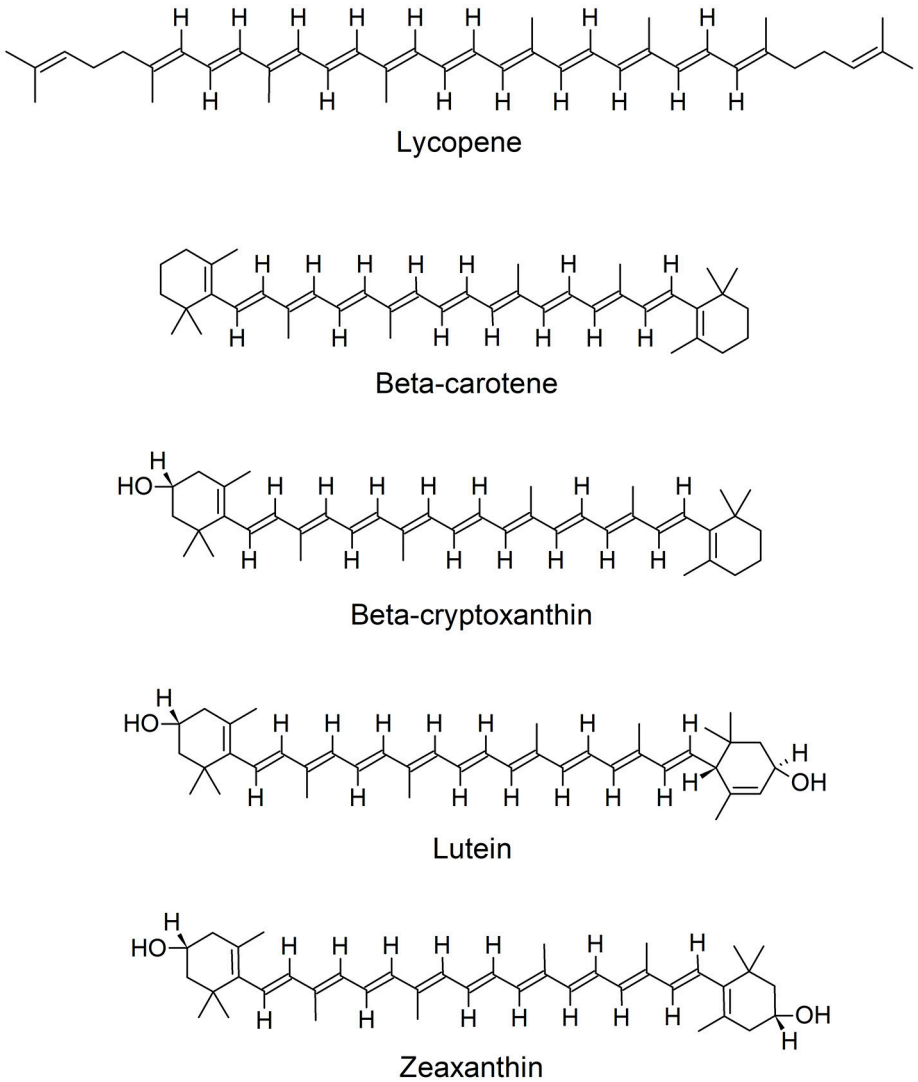

FIGURE 1 | Chemical structures of lycopene and other carotenoids.

Tomato products reduced oxidative stress related to postprandial lipemia and the associated inflammatory reaction in a study including normal weight participants (Burton-Freeman et al., 2012). He et al. reported the benefits of lycopene in preventing transplant vasculopathy, demonstrating that intimal hyperplasia and smooth muscle cell proliferation were reduced by the administration of lycopene and the infiltration of inflammatory cells in allograft vessels was reduced in an animal model (He et al., 2016). Lycopene can ameliorate allograft atherosclerosis via downregulating Rho-associated kinases and regulating the expression of key factors through NO/cGMP pathways (He et al., 2016). On the other hand, the benefits of the tomato-rich diet were not directly related to the anti-inflammatory effect according to a randomized study including 103 apparently healthy volunteers, after 300 g tomatoes daily for 1 month or placebo (Blum et al., 2007).

Watermelon was shown to reduce levels of inflammation by downregulation of the proinflammatory mediator cyclooxygenase 2 (COX-2), impairing prostaglandin E2 and I2 production, which reduces the progression of cardiovascular disorders (Sellers et al., 2010; Hong et al., 2015). Watermelon powder supplementation exerts an anti-inflammatory effect similar to COX-2 inhibitors or conventional non-steroidal anti-inflammatory drugs (Hong et al., 2015).

High mobility group box 1 (HMGB1), a non-histone DNA binding protein, produced by necrotic and immune cells, exposed to pro-inflammatory signals, has an important pro-inflammatory effect by attracting and activating inflammatory cells and mediators and binding to RAGE and toll-like receptors, related to fatal outcomes (Lee et al., 2012). Lee et al. demonstrated that lycopene inhibits adhesion molecules expression, which impair HMGB1—induced monocyte adhesion

FIGURE 2 | Cardiovascular benefits of lycopene [supported by *in vivo* (*) and/or *in vitro* (Φ) findings].

and transmigration (Lee et al., 2012). Lycopene has been also shown to inhibit lipopolysaccharide-induced HMGB1 release and HMGB1-mediated secretion of TNF-alpha and secretory phospholipase A2 (Lee et al., 2012).

Oxysterols, the result of cholesterol auto-oxidation, accumulate in the subendothelial arterial layer, exerting oxidative and pro-inflammatory roles and favoring the atherosclerotic process (Palozza et al., 2011). Lycopene impairs oxysterol-induced pro-inflammatory cytokines production in human macrophages and oxysterol-induced ROS production, limiting the formation of atherosclerotic plaque (Palozza et al., 2011).

Lycopene exerts a cardioprotective effect against atrazine induced cardiac injury due to its anti-inflammatory effect, by blocking the NF-kappa B pathway and NO production (Li et al., 2017).

Considering the mentioned anti-inflammatory mechanisms (**Figure 3**), including decrease of adhesion molecules, pro-inflammatory cytokines, inhibition of leukocyte migration and genes involved in inflammation, impaired monocyte-endothelium interaction, T lymphocytes activation and synthesis of AGE and RAGE and downregulation of cyclooxygenase 2, lycopene can be useful in the therapy for vascular inflammatory disorders.

Modulation of Lipids

Lycopene is transported in the circulation by lipoproteins and is actively taken up into adipocytes (McEneny et al., 2013). Lycopene is a *regulator of cholesterol* levels by inhibition of HMG-CoA reductase (like statins) and down-regulation of proprotein convertase subtilisin/kexin type 9 mRNA synthesis. These results suggest that lycopene supplementation could be especially beneficial for patients with statin intolerance (Sultan Alvi et al., 2017). Changes in hepatic gene expression, such as reduced expression in fatty acid synthase, responsible for fatty acid storage, were described after watermelon powder consumption in rats (Hong et al., 2015). Carbonic anhydrase III and adenylate kinase 2 were involved in the lipid-lowering and antioxidant effects of tomatoes (Hsu et al., 2008). On the other hand, NO stimulates fatty acid oxidation and lipolysis in adipose cells (Jobgen et al., 2006; Hong et al., 2015).

Lycopene is not able to increase HDL cholesterol, but was shown to improve the LDL/HDL ratio, HDL functionality and reduced the accumulation of cholesterol in the rabbit aorta,

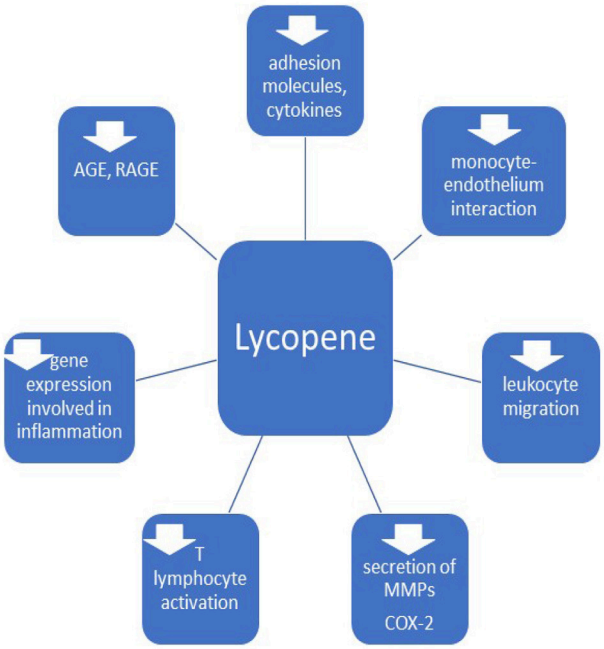

FIGURE 3 | Anti-inflammatory effects of lycopene. MMPs, matrix metalloproteinases; COX-2, cyclooxigenase 2; AGE, advanced glycation end-products; RAGE, receptors of AGE.

underlining the beneficial effects of lycopene during initial stages of atherosclerosis (Lorenz et al., 2012; Thies et al., 2017). However, a recent meta-analysis found that significant reductions in total and LDL cholesterol were revealed only at doses of, at least, 25 mg lycopene/day in human subjects, and the effects were comparable with low-dose statin medication, without concomitant side effects (Ried and Fakler, 2011; Lorenz et al., 2012). Fecal cholesterol excretion increases with levels of dietary lycopene due to decreased intestinal cholesterol absorption. This can be explained by the insight that increased fecal excretion impairs the enterohepatic circuit of bile acids, thus increasing the conversion of cholesterol to bile acids (Verghese et al., 2008). The lipid lowering properties of lycopene involve also an increased activity of LDL receptors in macrophages (Li et al., 2015; Cheng et al., 2017).

Lycopene can lower synthesis of dysfunctional HDL, modulating HDL functionality toward an antiatherogenic phenotype, with a low serum amyloid A level and beneficial changes of the activity of HDL remodeling enzymes (cholesterol ester transfer protein and lecithin cholesterol acyl transferase) (McEneny et al., 2013). A hypotriglyceridemic effect of tomato juice was seen only in subjects with initial high serum triglyceride levels (Li et al., 2015).

Lycopen can also regulate the hepatic lipid metabolism and counteracts the hepatic steatosis induced by a high-fat diet, due to sirtuins (SIRT1) induction and activation, being able to suppresses lipogenesis, to stimulate lipid catabolism in the liver and skeletal muscles and lipid mobilization in the white adipose tissue (Lomb et al., 2010; Li et al., 2015). Another mechanism able to ameliorate liver steatosis, by lycopene, was described in mice and is related to decrease of fatty acid binding protein 7 due to binding to microRNA-21 (Ahn et al., 2012; Li et al., 2015).

In summary, lycopene has lipid lowering properties, reducing the total and LDL cholesterol, triglyceride level, LDL oxidation, and synthesis of dysfunctional HDL.

Anti-aggregative Effect

Platelets are involved in the pathogenesis of the atherosclerotic plaque, development of acute thrombotic events and restenosis after endovascular procedures (Krasinska et al., 2017; O'Kennedy et al., 2017). Another cardiovascular beneficial effect of lycopene, protecting against myocardial infarction and stroke, is its *antiplatelet* activity, which is concentration dependent, and was demonstrated *in vivo* and *in vitro* (Sawardekar et al., 2016). Several mechanisms were considered in explaining the reversible antiplatelet effect of lycopene, such as the interaction with thromboxane, thrombin, collagen, von Willebrand factor, P-selectin and inflammatory mediators, the influence on calcium and cyclic guanosine monophosphate signaling and ADP-mediated aggregation (Sawardekar et al., 2016; Krasinska et al., 2017). It was noticed that lycopene can potentiate the antiplatelet effect of aspirin, which requires low lycopene diet in patients on secondary prophylaxis with aspirin due to the potential bleeding risk (Sawardekar et al., 2016). On the other hand, in high cardiovascular risk, aspirin (ASP) resistant patients, or those with ASP contraindications, high risk of complications after antiplatelet therapy or hyperactive platelets (obese, sedentary, hypertensive, diabetic, aging patients, and smokers), lycopene could have an important contribution in cardiovascular prophylaxis (Sawardekar et al., 2016; Krasinska et al., 2017; O'Kennedy et al., 2017).

Antihypertensive Effect

Lycopene has antihypertensive effects due to inhibition of the angiotensin converting enzyme (ACE) and due to its antioxidant effect, reducing oxidative stress induced by angiotensin-II and indirectly enhancing production of nitric oxide in the endothelium (Li and Xu, 2013; Belovic et al., 2016; Khan et al., 2016; Han and Liu, 2017). A study including 8,556 adult overweight and obese participants demonstrated association of lycopene and lycopene/uric acid ratio with lower prevalence of hypertension (Han and Liu, 2017). Paran et al. reported a decrease in both systolic and diastolic blood pressure in 54 patients with moderate hypertension, treated with ACE inhibitors or calcium channel blockers, after 6 weeks of tomato extract supplementation, suggesting a cause-effect relationship (Paran et al., 2009). Li et al. concluded, in a metanalysis, that lycopene supplementation (more than 12 mg/day) might significantly reduce systolic, but not diastolic blood pressure, in prehypertensive or hypertensive patients (Li and Xu, 2013).

Angiotensin II induces, besides direct vasoconstriction and oxidative stress, also vascular smooth muscle cells phenotypic transformation and production of inflammatory cytokines (Ren et al., 2017), and lycopene might impair the mentioned pathways, as well.

Anti-atherosclerotic Mechanisms

Besides improving endothelial function, oxidative stress (preventing oxidation of LDL) and metabolic profile, the anti-inflammatory and antiplatelet effect, lycopene has several other anti-atherosclerotic contributions (**Figure 4**), such as inhibition of vascular smooth muscle cell (VSMC) proliferation and foam cell formation (Napolitano et al., 2007; Wang et al., 2014). In general, not all studies confirm the relationship between lycopene and early atherosclerosis (Kim et al., 2010).

Contractile VSMC change to a proliferative and migratory phenotype during the atherosclerotic process, enabling migration of VSMC into the intima and production of the extracellular matrix of the plaque (Karagiannis et al., 2013). Such changes in phenotype are called "phenotypic modulation" (Manabe and Nagai, 2003) and play an important role in vascular remodeling, not only due to atherosclerosis, but also in hypertension and diabetic macroangiopathy (Ren et al., 2017). Lycopene can suppress VSMCs proliferation, due to inhibiting G1 phase cells entry into the S phase of the cell cycle, related to its antioxidative effect (Chen et al., 2010), and not due to the inhibition of matrix metalloproteinases (Lo et al., 2007). Minimally-oxidized LDL can induce phenotypic modulation of VSMC (Karagiannis et al., 2013), and synthesis of oxidized LDL may be impaired by lycopene (Kim et al., 2010). Lycopene may block VSMC migration and proliferation also by direct binding to platelet-derived growth factor (PDGF) and inhibiting PDGF-signaling (Lo et al., 2007), or due to it antioxidant effect, considering that reactive oxygen species accelerate the switch from the contractile to the synthetic phenotype (Sung et al., 2005).

Lycopene has also barrier integrity activity in the endothelial membrane, by blocking the activation of CD14 and Toll like receptor-4 expression (Bae and Bae, 2011) and impairs the apoptosis of endothelial cells *in vitro*, by disrupting the upregulation of p53 and caspase 3 mRNA (Tang et al., 2009).

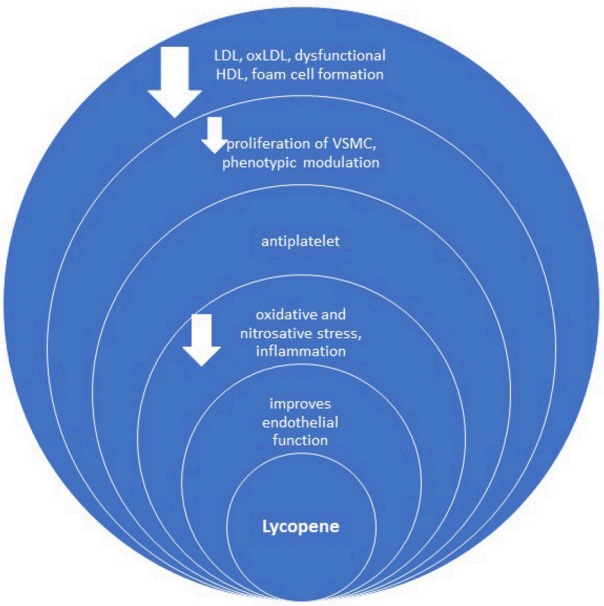

FIGURE 4 | Anti-atherosclerotic effect of lycopene. oxLDL, oxidized LDL; VSMC, vascular smooth muscle cells.

Cardioprotective Effects

Recent studies emphasized the cardioprotective effects of lycopene. An experimental study including rats with isoproterenol induced myocardial infarction, demonstrated improved ECG findings (shorter QT and RR intervals and QRS complexes, reduced ST segment elevation) if lycopene was previously administrated, related to its cell membrane stabilizing and antioxidant properties (Aman et al., 2012). Supplementation of lycopene in the same study also prevented alteration in hemodynamic parameters (systolic, diastolic, and mean blood pressure), biochemical and inflammatory markers, apoptotic changes, and reduced the size of myocardial infarction due to the antioxidant and anti-inflammatory properties of lycopene (Aman et al., 2012; Wong et al., 2017).

Tomato and lycopene supplementation attenuated cardiac-remodeling and improved diastolic dysfunction after myocardial infarction according to a study including male Wistar rats, enabled by impairing type I and type III collagen content in the left ventricle, reduced cardiomyocyte death, expression of miRNAs and by the anti-inflammatory effect and reduction of oxidative stress (Pereira et al., 2017). Wang et al. administered lycopene in infarcted rats for 28 days, revealing an increased ejection fraction compared to controls, associated with decreased collagen fraction in the peri-infarct zone, due to inhibition of p38 mitogen-activated protein kinase and matrix metalloproteinase 9 expression (Wang et al., 2014). Lycopene effect on interstitial collagen accumulation was not confirmed by Anjos Ferreira et al., in a study including male Wistar rats and testing the effect of lycopene on doxorubicin-induced cardiotoxicity (Anjos Ferreira et al., 2007). The anti-inflammatory effect of lycopene has also a contribution in reversing ventricular remodeling, by inhibiting the nuclear factor-κB signaling pathway (He et al., 2015).

Lycopene might also serve as a cardioprotective agent against several drugs. The cardioprotective effect of lycopene was demonstrated in the case of tulathromycin, a macrolide antibiotic, and diclofenac sodium, a non-steroidal anti-inflammatory drug, by Abdel-Daim et al., in a study including Swiss albino mice, and was attributed to its antioxidant activity (Abdel-Daim et al., 2018). Lycopene can also protect against the cardiotoxicity induced by doxorubicin (Karimi et al., 2005; Anjos Ferreira et al., 2007; Abushouk et al., 2017), isoproterenol (Aman et al., 2012; Mohamadin et al., 2012), and atrazine (Li et al., 2017).

Concluding, lycopene supplementation is beneficial for early and late prognosis in myocardial infarction. It may reduce myocardial infarction size and related electrocardiographic, hemodynamic, and biochemical changes (Aman et al., 2012). Lycopene also reverses ventricular extracellular matrix remodeling after an acute coronary event, by inhibiting myocardial fibrosis and preventing apoptosis and necrosis of cardiomyocytes, its anti-inflammatory effect and the ability to increase left ventricular function, preventing development of heart failure and increasing survival rates (Aman et al., 2012;

Wang et al., 2014; He et al., 2015). Considering difficulties and high cost of early revascularization and significant side effects of cardiovascular drugs, lycopene could represent a safe and effective option in controlling post infarction ventricular remodeling (Wang et al., 2014).

Lycopene and Endoplasmic Reticulum Stress (ERS)

The endoplasmic reticulum has several functions related to intracellular calcium storage, lipid and protein synthesis, and modification (Schönthal, 2012). Disturbance of those functions causes "endoplasmic reticulum stress" (ERS) involved in myocardial ischemia/reperfusion injury (Gao et al., 2016). The cell reacts to ERS by initiating an "unfolded protein response," including several mechanisms, enabling cellular adaptation and survival or, in severe cases, apoptosis (Schönthal, 2012).

Lycopene protects the cardiomyocytes by relieving ERS and preventing apoptosis, through stimulation of adenosine 5-monophosphate-activated proteinkinase, the transcription factor CHOP (C/EBP homologous protein), p-JNK and Caspase pathways (Xu et al., 2015; Gao et al., 2016). However, high lycopene levels might be toxic to the cardiomyocytes (Gao et al., 2016).

PRECLINICAL STUDIES

Lycopene supplementation for 4 weeks strongly reduced total and LDL serum cholesterol and the amount of cholesterol in the aorta, but the surface lipid accumulation in the aorta and IMT were not significantly reduced and no impairment of vasoreactivity or increase of pro-oxidant parameters were detected in New Zealand White rabbits after either a standard or a high-cholesterol diet (Lorenz et al., 2012). Although lycopene suppressed cholesterol uptake and intestinal absorption and increased fecal cholesterol excretion in rabbits, it did not decrease the expression of HMG-CoA reductase (Lorenz et al., 2012).

Hu et al. reported anti-atherosclerotic effects for both lycopene and fluvastatin, in the aorta, in rabbits, after a high-fat diet (Hu et al., 2008; He et al., 2009). Verghese et al. also revealed a significant decrease in the atherosclerotic plaque formation with the consumption of lycopene in a study including New Zealand male rabbits, receiving a high cholesterol diet. Further observed effects include an improved serum lipid profile as well as reduction in total cholesterol, LDL and triglycerides (Verghese et al., 2008). Hsu reported, besides reduction in total and LDL cholesterol and plasma malondialdehyde (MDA) levels, also an increase in HDL cholesterol in hamsters fed a 9% tomato paste containing 0.2% cholesterol, after 8 weeks (Hsu et al., 2008).

Watanabe Heritable Hyperlipidemic rabbits were used in a study by Frederiksen et al. demonstrating that lycopene did not influence cholesterol and triacylglycerol levels, lipoprotein fractions, oxidation of lipids, and aortic atherosclerosis evaluated biochemically and by microscopy (Frederiksen et al., 2007). The lack of response of lycopene in Watanabe Heritable Hyperlipidemic rabbits is, probably, related to their defective LDL receptors (Tanazawa et al., 1980; Lorenz et al., 2012), which would suggest the involvement of these receptors in the cardiovascular benefits of lycopene (Perera and Yen, 2007). Considering the bloodstream transport of lycopene in LDL particles, functional LDL receptors enable cardiovascular benefits of lycopene (Lorenz et al., 2012). Bansal et al. reported a beneficial cardioprotective effect of lycopene, due to the reduction of oxidative stress and myocardial injury, in an experimental model of myocardial ischemia-reperfusion injury in adult male albino Wistar rats (Bansal et al., 2006). Lycopene protects also endothelial progenitor cells, necessary to replace the injured vascular endothelium and for angiogenesis, in a microenvironment of advanced glycation end products (AGEs), which act as damage-causing agents (Zeng et al., 2017). Lycopene improved cell proliferation and regulated protective mechanisms of AGEs-induced autophagy in endothelial progenitor cells from diabetic rats, suggesting that supplementation with this compound might be a new therapeutic option for diabetic vascular complications (Zeng et al., 2017).

CLINICAL RESEARCH

Endothelial Function

Both cross-sectional and supplementation studies emphasized the benefits of tomato products on vascular function, mainly due to the antioxidative effects of lycopene (Kim et al., 2010; Thies et al., 2012; Xaplanteris et al., 2012; Gajendragadkar et al., 2014). However, several articles failed to show improvement of endothelial function after tomato consumption (Stangl et al., 2011; **Table 1**).

Gajendragadkar et al. concluded that lycopene supplementation can improve endothelial function in patients with cardiovascular disorders, but not in age-matched healthy volunteers (Gajendragadkar et al., 2014). Forearm responses to intraarterial infusions of acetylcholine were assessed using venous plethysmography, which resulted in an improvement by 53% of endothelium-dependent vasodilatation (EDV) in patients with cardiovascular disorders post-lycopene (Gajendragadkar et al., 2014). Endothelial function was improved regardless of traditional risk factors or inflammatory markers, and, even a modest increase in serum lycopene, further impaired endothelial function in atherosclerotic patients (Gajendragadkar et al., 2014). One conclusion of the mentioned study was that lycopene affects especially smaller vessels, such as resistance arteries, rather than larger vessels, as measured arterial stiffness remained unaltered in all study participants (Gajendragadkar et al., 2014).

Pulse Wave Velocity (PWV)

Kim et al. reported an independent, inverse association between circulating lycopene and brachial pulse wave velocity in 264 healthy women, regardless of age, body mass index, smoking and drinking habits, menopause, blood pressure, beta-carotene, alpha-tocopherol, markers of oxidative stress, and inflammation (Kim et al., 2010; **Table 1**). Reduced oxidative LDL changes may have an important contribution to arterial stiffness reduction due to lycopene (Kim et al., 2010). Another study, including 126 healthy men revealed the benefits of lycopene on oxidative stress and endothelial dysfunction, especially in subjects with an

TABLE 1 | Effects of lycopene on endothelial function and arterial stiffness.

Number of participants	Methodology	Results, conclusions	References
36 statin treated cardiovascular patients (mean age: 67–68 years) and 36 healthy volunteers (mean age: 61–68 years)	Double-blind trial: 7 mg lycopene or placebo daily for 2 months.	Lycopene supplementation improved endothelial function in patients with cardiovascular disorders on optimal secondary prevention, but not in healthy persons.	Gajendragadkar et al., 2014
225 overweight volunteers, aged 40–65 years	Participants were randomly assigned into 1 of 3 dietary intervention groups: control diet (low tomato content), a high-tomato-content diet, or a control diet with addition of lycopene capsules (10 mg/d) for 12 wk. Collected blood samples were tested for carotenoid and lipid profiles and inflammatory markers. Arterial stiffness and dietary intake were also monitored.	A relatively high daily consumption of tomato-based products (32–50 mg lycopene/day) or lycopene supplements (10 mg/day) was ineffective in reducing conventional cardiovascular risk markers, inflammatory markers, markers of insulin resistance and sensitivity, lipid profile and arterial stiffness in moderately overweight, healthy, middle-aged individuals.	Thies et al., 2012
25 study participants, mean age: 27 ± 8 years	Randomized, intervention model, crossover assignment. The participants consumed high-fat meals containing processed tomato products or non-tomato alternative.	Tomato products attenuate postprandial lipemia-induced oxidative stress and inflammatory response, with a modest improved flow-mediated dilatation (FMD).	Burton-Freeman et al., 2012
19 volunteers, 39 ± 13 years	Randomized, single-blind, crossover assignment.	Daily tomato paste consumption exerts a beneficial midterm effect on endothelial function.	Xaplanteris et al., 2012
299 Korean men	Subgrouped according to the number of metabolic syndrome risk factors; brachial-ankle pulse wave velocity (PWV), oxidative stress and antioxidants (including lycopene) were measured.	An inverse correlation was found between PWV and serum lycopene, considering blood pressure, insulin resistance and oxidative stress.	Yeo et al., 2011
19 healthy non-smoking postmenopausal women	Administration of 70 g tomato puree. Endothelial-dependent FMD and endothelial-independent nitro-mediated dilation of the brachial artery were measured with high-resolution ultrasound.	Acute and long-term (7 d) intake of tomato products, despite a significant increase in plasma lycopene had no effect on endothelial function.	Stangl et al., 2011
126 healthy men	Administration of placebo/6 mg/15 mg lycopene daily for 8 weeks. Endothelial function was assessed by reactive hyperemia peripheral arterial tonometry. Plasma superoxide dismutase was used to assess oxidative stress.	An inverse correlation between serum lycopene levels and arterial stiffness was found. An increased serum lycopene decreases oxidative stress, which might influence endothelial function.	Kim et al., 2011
264 healthy women, 31–75 years	The relationship between serum lycopene and brachial-ankle pulse wave velocity was assessed.	An independent, inverse relationship between circulating lycopene and brachial PWV was observed.	Kim et al., 2010

impaired endothelial function (Kim et al., 2011). After 15 mg/day lycopene supplementation, for 8 weeks, Kim et al. reported a decrease of systolic blood pressure and high sensitivity C reactive protein (Kim et al., 2011).

The main biological mechanism by which lycopene reduces the risk and mortality of the *metabolic syndrome*, include the antioxidant, anti-inflammatory and antiobesity effects, the ability to improve endothelial function, glycemic control, insulin sensitivity and lipid profile (Tsitsimpikou et al., 2014; Li et al., 2015; Han et al., 2016a,b). An inverse relationship was found by Yeo et al. between lycopene level and brachial-ankle pulse wave velocity (Yeo et al., 2011). PWV was significantly higher in patients with metabolic syndrome, with lower than median serum lycopene values (≤0.0294 mmol/l) compared to patients without metabolic syndrome; no statistically significant differences between the 2 groups were found when lycopene levels were high (Fantin et al., 2010; Yeo et al., 2011). Higher serum carotenoid levels were associated not just with a lower prevalence of the metabolic syndrome, but also with fewer abnormal metabolic syndrome components. A significant association between lycopene and the metabolic syndrome was described only for normal-weight and overweight participants, but not in obese patients, according to a study enrolling 13,196 subjects, probably related to an increased oxidative stress and decreased antioxidant ability, due to sequestration of lycopene in the adipose tissue and more important inflammation in obese (Han et al., 2016b). On the other hand, daily tomato juice intake reduced waist circumference, cholesterol, and monocyte chemotactic protein-1 (inflammatory adipokine) and increased adiponectin (anti-inflammatory adipokine) levels in 30 young, healthy Taiwanese females (Li et al., 2015). Lycopene was shown to impair pro-inflammatory cytokine production, such as IL-6, IL-1b, and TNF-α, preventing insulin resistance (Gouranton et al., 2011).

L-arginine or L-citrulline supplementation modulate the arginine-NO pathway, enabled by multiple cyclic guanosine-3′,5′-monophosphate-dependent pathways, with important prophylactic and therapeutic contributions in the metabolic syndrome (Jobgen et al., 2006).

A large study, including 225 middle-aged, overweight volunteers reported no changes of conventional cardiovascular risk factors, inflammatory tests, insulin resistance and sensitivity, lipid profile, oxidized LDL, von Willebrand factor, and arterial stiffness after high daily intake of lycopene, despite good compliance (Thies et al., 2012). One week of lycopene supplementation increased plasma lycopene, but not biomarkers of vascular oxidative stress and inflammation, or biomarkers of nitric oxide (plasma nitrate/nitrite) in healthy, active subjects, suggesting that the participants already possessed a robust antioxidant capacity and lycopene provided no additional benefit (Denniss et al., 2008).

Intima-Media Thickness (IMT)

Several studies reported an association between serum lycopene levels and intima-media thickness (Gianetti et al., 2002; Riccioni et al., 2009, 2011; Karppi et al., 2013; Zou et al., 2014), while other authors reported no association (Dwyer et al., 2004; **Table 2**). Zou et al. revealed a decrease in carotid artery intima-media thickness (IMT) after 12 months of lutein and lycopene supplementation (20 mg each) in 144 Chinese patients with subclinical atherosclerosis, demonstrating more effective results after the intake of both lutein and lycopene compared to lutein alone (Zou et al., 2014).

High serum levels of lycopene, alpha and beta-carotene were associated with a slow IMT progression during 7 years in a study including 840 middle-aged men from Eastern Finland (Karppi et al., 2013). The association between lycopene level and IMT was mentioned in the scientific literature also for elderly Finish subjects (Karppi et al., 2011).

Higher carotenoids levels (lutein, zeaxanthin, and beta-cryptoxanthin; **Figure 1**) were correlated with reduced IMT progression over 18 months, in a study with 573 middle-aged participants, free of cardiovascular symptoms at baseline. Beta-carotene and lycopene levels were not significantly associated with IMT progression (Dwyer et al., 2004).

An inverse correlation was found in women between lycopene levels and IMT, independent of conventional risk factors, in a large study including 1,111 subjects (McQuillan et al., 2001).

WHY CONFLICTING RESULTS?

Several studies revealed the anti-atherosclerotic effect of lycopene (McQuillan et al., 2001; Gianetti et al., 2002; Hu et al., 2008; Verghese et al., 2008; Riccioni et al., 2009; Kim et al., 2010; Gajendragadkar et al., 2014), but there are also studies available that report conflicting results related to the vascular effects of lycopene (McQuillan et al., 2001; Dwyer et al., 2004; Stangl et al., 2011; Thies et al., 2012). The possible reasons for this obvious discrepancy are manifold and include methodological differences in the study designs, such as different lycopene sources, the use of food-frequency questionnaires, different intervention times, the methodology used to assess vascular function, measurement of blood, adipose or dietary lycopene. Besides those, the use of unstandardized amounts of tomato food products, different modes of delivery, misclassification of overall tomato intake, combination of lycopene with other antioxidants, different processing procedures or eating behavior influenced by cultural and temporal patterns among different individuals, may influence the results (Sesso et al., 2003; Kong et al., 2010; Thies et al., 2012; Gajendragadkar et al., 2014). Other carotenoids extracted from tomatoes could be also partially responsible for the effects attributed to lycopene (Rao, 2002). This is underlined by a study that could not find beneficial effects for lycopene supplementation alone, but beneficial effects upon supplementation with tomato-based products (Sesso et al., 2003). Some studies did not consider dietary intake at all (Yeo et al., 2011). The interaction flavanone metabolites—lycopene is difficult to assess, considering the rapid metabolization of the mentioned metabolites (Habauzit et al., 2015). Duration of treatment, dose and bioavailability of lycopene, and vascular endpoint were also different in the studies published on this topic and might have influenced obtained results. Several factors influence the bioavailability of lycopene, such as season, the processing of tomatoes, their origin, dimensions, shape, and the way they are consumed (Gajendragadkar et al., 2014; Gammone et al., 2015). Absorption of lycopene may be reduced by diets rich in fibers and in elderly people (Kong et al., 2010) and is increased in the presence of oil.

The isomerization of lycopene is another source of variability. Fresh tomatoes contain lycopene in all-trans form (Shi and Le Maguer, 2000). Several factors, including high temperatures, light, oxygen, acids, and metal ions enable isomerization of lycopene (Kong et al., 2010). Lycopene degradation occurs during thermal processing, mainly isomerization of all- trans to cis forms and oxidation (Shi and Le Maguer, 2000). Dehydrated and powdered tomatoes have poor lycopene stability, depending of storage in a hermetically sealed atmosphere, and a significant increase of cis-isomers, giving the highest bioavailability of lycopene and higher ability to be incorporated in lipoproteins (Shi and Le Maguer, 2000; Kong et al., 2010). Uptake of cis lycopene is significantly higher than all trans-isomers (Kong et al., 2010).

Lycopene is very bioavailable in the presence of oil, especially in monounsaturated oils, other dietary fats and processed tomato products (Shi and Le Maguer, 2000; Basu and Imrhan, 2007; Kong et al., 2010; Gajendragadkar et al., 2014). Lycopene can increase the antioxidant properties of vitamin C, E, polyphenols and beta-carotene in a synergistic way (Kong et al., 2010; Karppi et al., 2013). Supplementation with tomatoes, containing lycopene (red tomatoes) or not (yellow tomatoes), showed a better antioxidant effect than lycopene alone, probably due to the synergistic effects of naturally occurring secondary metabolites in tomatoes (Basu and Imrhan, 2007; Gitenay et al., 2007). Generally, supplementation with whole fruits is often more beneficial than supplementing single food constituents: Watermelons contain, besides lycopene, hundreds of different compounds, including L-citrulline and ascorbic acid, both of which improve the L-arginine/NO pathway, endothelial function,

TABLE 2 | Lycopene and intima-media thickness (IMT).

Number of participants	Methodology	Results, conclusions	References
144 subjects, aged 45–68 years, with subclinical atherosclerosis	20 mg lutein ($n = 48$), 20 mg lutein + 20 mg lycopene ($n = 48$) or placebo ($n = 48$) were administrated for 12 months; carotid artery intima-media thickness (IMT) was measured using Doppler ultrasonography.	Lutein and lycopene supplementation significantly increased the serum concentration of lutein and lycopene with a decrease in carotid artery IMT.	Zou et al., 2014
840 middle-aged men from Eastern Finland	Ultrasonography of the common carotid arteries, serum levels of carotenoids	7-year change in maximum intima media thickness was inversely associated with serum levels of lycopene, alpha and beta-carotene, respectively. Elevated serum levels of carotenoids may have anti-atherosclerotic effect.	Karppi et al., 2013
1,212 elderly men from Eastern Finland	B-mode ultrasound (IMT of the common carotid artery); plasma levels of carotenoids	High plasma concentrations of lycopene, alpha-carotene and beta-cryptoxanthin are related to decreased carotid atherosclerosis in elderly patients.	Karppi et al., 2011
120 subjects without history of symptomatic carotid artery disease	Ultrasonic measurement of common carotid artery IMT, serum profile analysis of cholesterol (total and LDL), triglycerides and lycopene.	Carotid atherosclerosis was associated with lower plasma lycopene levels.	Riccioni et al., 2011
640 participants with asymptomatic carotid atherosclerosis	Carotid ultrasound investigation was performed; medical history and laboratory data were collected.	Participants with IMT $\geq$ 0.8 mm had significantly lower concentrations of vitamin A and E, lycopene, and beta-carotene compared to participants with no evidence of carotid atherosclerosis.	Riccioni et al., 2011
573 middle-aged women and men from an occupational cohort	Ultrasound examination of the common carotid arteries was performed, lipid profile and risk factors were assessed at baseline and 18-month follow-up. Plasma antioxidants were determined at baseline.	18-month change in IMT was inversely related to serum levels of some measured carotenoids, not including lycopene, regardless of cardiac risk factors and high-sensitivity C-reactive protein.	Dwyer et al., 2004
11 healthy controls, 11 patients with uncomplicated hypertension, 11 with essential hypertension and peripheral vascular disease	Patients were matched for age, sex, smoking habit and body mass index; IMT, adhesion molecules, LDL and antioxidants (including lycopene) were measured.	A statistically significant correlation was found between lycopene and IMT, independent of LDL, creatinine clearance, and plasma insulin. No significant correlation was found between lycopene and soluble adhesion molecules	Gianetti et al., 2002
1,111 subjects, aged 27–77 years	Dietary vitamin intake, fasting plasma levels of vitamins (A, C, and E), lycopene, alpha and beta-carotene, bilateral	There was an inverse association between carotid artery IMT and plasma lycopene in women, but not in men.	McQuillan et al., 2001

aortic systolic blood pressure, reduce arterial stiffness, and improve glycemic control, thus acting synergistic with lycopene (Wu et al., 2007; Figueroa et al., 2013, 2017). Grapefruits also include in their composition not just lycopene but also flavonoids, with several benefits, such as the anti-inflammatory and anti-atherogenic effect, improving vascular reactivity, reducing insulin resistance, decreasing arterial stiffness, LDL cholesterol, and blood pressure (Habauzit et al., 2015). These synergistic effects hamper assessment of quantitative and qualitative effects of lycopene as a dietary factor.

Several studies included healthy participants or subjects with different disorders and cardiovascular risk factors (Kong et al., 2010; Thies et al., 2012; Gajendragadkar et al., 2014). Enrolling volunteers with established elevated risk markers for cardiovascular disorders may increase the probability of detecting changes, especially in short time studies (Thies et al., 2012). Also, several other uncontrolled or unidentified lifestyle factors or dietary constituents associated with cardiovascular disorders, may provide alternative explanations for the different study results (Sesso et al., 2003). Genetic factors remain unconsidered at all in all of the reviewed publications, although they are reported to strongly influence circulating concentrations of lycopene in different ethnicities (Zubair et al., 2015). Furthermore, plasma, adipose, and dietary carotenoids are not sufficiently correlated to be interchangeably (Sesso et al., 2003).

FUTURE RESEARCH DIRECTIONS

Most of the studies considered only tomatoes and tomato products as lycopene source. It will be the aim of future human intervention studies to include other lycopene containing fruits such as watermelon, papaya, red grapefruits, and guava, and consider synergistic effects with other components and their importance in primary and secondary cardiovascular prophylaxis.

Benefits of lycopene should be especially considered in patients with high cardiovascular risk, statin intolerance, borderline hypertension, aspirin resistance, hyperactive platelets, vascular inflammatory diseases, metabolic syndrome and coronary heart disease, and its inclusion in combination therapies for the mentioned disorders, should be approached. Further mechanistic research is needed to identify new targets for prevention and complementary treatment of cardiovascular disorders.

CONCLUSIONS

The present review supports the importance of lycopene in improving vascular function and in the primary and secondary prevention of cardiovascular disorders. The demonstrated effects of lycopene in view of cardiovascular health comprise its general antioxidant and anti-inflammatory abilities, the antiplatelet, anti-apoptotic and antihypertensive properties, the ability to improve endothelial function, the metabolic profile and ventricular remodeling, reduction of arterial stiffness as well as reduction of size of atherosclerotic plaque. Lycopene exerts favorable effects in patients with subclinical atherosclerosis, metabolic syndrome, hypertension, peripheral vascular disease, and several other cardiovascular disorders, but sometimes conflicting results were obtained. Clearly, more and better-designed studies will be necessary to improve our understanding of the positive effects of lycopene on vascular health and to elucidate the involved mechanisms on a molecular level.

Future cardiovascular disease prevention strategies might include lycopene-enriched products, lycopene supplementation and new combinations including lycopene. Future studies focused on dietary lycopene and its synergistic effects with other dietary components in different study populations, with elevated cardiovascular risk, are highly warranted and might enable development of functional foods useful in prevention and complementary treatment of cardiovascular disorders.

AUTHOR CONTRIBUTIONS

IM is the author of the first draft of the manuscript. DS, AC, CM, JH, and AA contributed toward revising the paper and agree to be accountable for all aspects of the work. All authors agreed on the finally submitted version of the manuscript.

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REVIEW
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Tanshinones, Critical Pharmacological Components in *Salvia miltiorrhiza*

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Salvia miltiorrhiza Bunge, a member of the Lamiaceae family, is valued in traditional Chinese Medicine. Its dried root (named Danshen) has been used for hundreds of years, primarily for the treatment of cardiovascular and cerebrovascular diseases. Tanshinones are the main active ingredients in *S. miltiorrhiza* and exhibit significant pharmacological activities, such as antioxidant activity, anti-inflammatory activity, cardiovascular effects, and antitumor activity. Danshen dripping pill of Tianshili is an effective drug widely used in the clinical treatment of cardiovascular diseases. With the increasing demand for clinical drugs, the traditional method for extracting and separating tanshinones from medicinal plants is insufficient. Therefore, in combination with synthetic biological methods and strategies, it is necessary to analyze the biosynthetic pathway of tanshinones and construct high-yield functional bacteria to obtain tanshinones. Moreover, the biosynthesis of tanshinones has been studied for more than two decades but remains to be completely elucidated. This review will systematically present the composition, extraction and separation, pharmacological activities and biosynthesis of tanshinones from *S. miltiorrhiza*, with the intent to provide references for studies on other terpenoid bioactive components of traditional Chinese medicines and to provide new research strategies for the sustainable development of traditional Chinese medicine resources.

Keywords: tanshinones, *Salvia miltiorrhiza*, extraction and separation, pharmacological activities, biosynthesis

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INTRODUCTION

Salvia miltiorrhiza Bunge, an important Salvia species with terrific economic, social and medicinal benefits (Su et al., 2015), was first recorded in the *Shenlong Bencao Jing* (200–300 AD, Han Dynasty), the oldest medicine monograph in China. *Salviae miltiorrhizae* Radix et Rhizoma, dry roots of Danshen, has been widely applied in the clinical treatment of cardiovascular diseases (Chen and Chen, 2017), dysmenorrhea, amenorrhea, and hypertension, hepatocirrhosis, chronic renal failure and other diseases (Su et al., 2015). With the development of science and technology, the dosage forms containing Danshen have been gradually diversified. Tablet, injection solution, dripping pill, oral liquid, capsule, slow-release formulation, and soft gel are all dosage forms that have been prepared into medicines. Among these diverse preparations, composite Danshen droplet pills, used to treat angina pectoris and coronary heart disease, represent a star drug and, moreover, a demonstration of traditional Chinese medicine entering the international market. Currently,

advanced clinical tests on this drug are ongoing in the United States. The active ingredients in *S. miltiorrhiza* (**Table 1**) can be mainly divided into two categories: fat-soluble tanshinone compounds and water-soluble salvianolic acids. Both ingredients have significant pharmacological activities and are also representative active ingredients in traditional Chinese medicine (Ma et al., 2015), especially tanshinones.

As secondary metabolites, tanshinones mainly accumulate in the roots, but the yield is very low. Currently, tanshinones are obtained from roots by chemical separation and purification. However, because traditional methods have low efficiency, high energy consumption and unfriendliness to the environment and plant resources, it is meaningful and necessary to find new methods. With the successful applications of synthetic biology technology in the study of taxol, ginsenoside, and artemisinin (Huang et al., 2014), this approach could also provide new developments and approaches for the modern study of tanshinones (Shi et al., 2018). Based on research on tanshinones at home and abroad, this paper will systematically review the research results of extraction, purification, pharmacological activity and biosynthesis of the original plant *S. miltiorrhiza*.

THE PLANT *S. miltiorrhiza*

Salvia miltiorrhiza Bunge belongs to the genus Salvia of the Lamiaceae family, which is a perennial erect herb. Its dry root is used as Danshen and has the effects of promoting blood circulation, relieving pain, regulating heat, and calming the nerves.

Wild *S. miltiorrhiza* generally grows in central and northeastern China. Environmental and soil conditions affect the quality of Danshen and the content of active ingredients (Peng et al., 2014). The Chinese Flora reports that there are two varieties in the *S. miltiorrhiza* species, namely, the original variety (*S. miltiorrhiza* var. *miltiorrhiza*) and *S. miltiorrhiza* Bunge var. *charbonnelii*. The original variety can be divided into two forms, namely, the original form (*S. miltiorrhiza* var. *miltiorrhiza* f. *miltiorrhiza*) and *S. miltiorrhiza* var. *miltiorrhiza* f. *alba*. These forms mainly differ in plant morphology and geographical distribution. For example, the flower color of *S. miltiorrhiza* var. *miltiorrhiza* f. *alba* is white, and it originates from Shandong, China.

In recent years, due to the excessive harvesting of wild *S. miltiorrhiza*, the resources are already on the verge of extinction. With the reduction in wild resources, artificial domestication cultivation of *S. miltiorrhiza* has been carried out since the 1970s. The main producing areas of the current cultivated *S. miltiorrhiza* are Linyi Shandong Province, Jiaozuo Henan Province, Wanrong Shanxi Province, Shangluo Shaanxi Province, Zhongjiang Sichuan Province and other places. To date, *S. miltiorrhiza* from Sichuan Province is still considered to have the best quality. Due to the low yield of secondary metabolites and the long growth cycle of cultivated plants, the production of tanshinones from cultivated *S. miltiorrhiza* can no longer meet the rapidly growing market demand. It is essential to use modern biotechnology methods to increase

TABLE 1 | A list of abbreviations would be adequate to define all abbreviations used in the study.

Abbreviation	Full name
S. miltiorrhiza	Salvia miltiorrhiza Bunge
SFE	Supercritical fluid extraction
CUAE-HIUP	Continuous ultrasound-assisted extraction with high intensity ultrasonic probe
SPE	Solid-phase extraction
HPLC	High performance liquid chromatography
HSCCC	Semi-preparative high-speed countercurrent chromatography
DNA	Deoxyribonucleic acid
NADPH	Nicotinamide adenine dinucleotide phosphate
ROS	Reactive oxygen species
STS	Sodium tanshinone IIA silate
VSMC	Vascular smooth musle cell
AMPK	Adenosine 5′-monophosphate (AMP)-activated protein kinase
MI	Myocardial infarction
ox-LDL	Oxidized low density lipoprotein
AD	Alzheimer's disease
BBB	Blood-brain barrier
iNOS	Inducible nitric oxide synthase
IPP	Ispentenyl diphosphate
DMAPP	Dimethylallyl diphosphate
MEP	2-*C*-methyl-D-erythritol 4-phosphate
MVA	Mevalonate
IDS	Isoprenyl diphosphate synthase
GGPP	Geranylgeranyl diphosphate
TPS	Terpene synthases/cylases
HMGR	3-Hydroxy-3-methylglutaryl-CoA reductase
MeJA	Methyl jasmonate
AACT	Acetyl-CoA C-acyltransferase
HMGS	3-Hydroxy-3-methylglutaryl-coenzyme A synthase
MK	Mevalonate kinase
PMK	Phosphomevalonate kinase
MDC	Mevalonate diphosphate decarboxylase
Pyr	Pyruvate
G3P	Glyceraldehyde 3-phosphate
DXS	1-Deoxy-D-xylulose-5-phosphate synthase
DXP	1-Deoxy-D-xylulose 5- phosphate
DXR	1-Deoxy-D-xylulose 5- phosphate reductoisomerase
IDI	Isopentenyl diphosphate isomerase
GPPS	Geranyl diphosphate synthase
FPPS	Farnesyl diphosphate synthase
GGPPS	Geranylgeranyl diphosphate synthase
RACE	Rapid amplification of cDNA ends
CPS	Labdadienyl/Copalyl diphosphate synthase
KSL	Kaurene synthase-like
GA	Gibberellin
CYP	Cytochrome p450
CPR	Cytochrome p450 reductase
UV-B	Ultraviolet-B

the yield. Therefore, various *in vitro* culture systems of *S. miltiorrhiza*, including suspension cell (Zhao et al., 2010), callus (Wu et al., 2003), adventitious root and hairy root, as well as new techniques such as using endophytic fungi (Li et al., 2016; Zhou et al., 2018) and transgenic plants (Liu et al., 2017; Wei et al., 2018), can be used to accumulate tanshinone production.

TANSHINONES OF *S. miltiorrhiza*

In the 1930s, domestic and foreign scholars have begun to study the chemical constituents of *S. miltiorrhiza* (Xu et al., 2018). Japanese scholar Nakao first isolated tanshinone IIA from *S. miltiorrhiza* and identified its chemical structure in 1934. After the 1970s, with the development of clinical application of *S. miltiorrhiza* and the advancement of Chinese medicine extraction and separation technology, its chemical composition research has developed rapidly (Kakisawa et al., 1968, 1969). Tanshinones, also known as total tanshinone, are a general term for a class of lipophilic phenanthrene compounds that are rich in the roots of *S. miltiorrhiza* (Ma et al., 2015). What these compounds have in common is that they possess an *ortho*- or *fluorene* structure and can be reduced to a diphenol derivative, which is converted to hydrazine after oxidation. In this process, these molecules facilitate electron transfer, and can promote or interfere with various reactions of the body; accordingly, these compounds exhibit significant pharmacological properties such as anti-cancer, antibacterial and anti-viral effects. To date, more than 40 tanshinones (Pang et al., 2016) have been isolated from *S. miltiorrhiza*, including *ortho*-quinones (i.e., tanshinone I, tanshinone IIA, dihydrotanshinone, and cryptotanshinone) and *para*-quinones (i.e., isotanshinone I, isotanshinone IIA, and isocryptotanshinone). In addition, other diterpenoids and triterpenoids have been isolated from Danshen (Wu et al., 2016), such as danshenxinkun A/B/C/D and neo-tanshinlactone (Gao S. et al., 2012). With the increasing clinical demand for tanshinones, more research is currently being conducted to explore how to efficiently obtain tanshinones and enhance their pharmacological effects by exploring structure activity relationships (Pickens et al., 2011; Krivoruchko and Nielsen, 2015; Luo et al., 2015; **Figure 1**).

EXTRACTION AND SEPARATION METHODS OF TANSHINONES

Due to the clinical application of tanshinones, the extraction, and purification processes of these compounds were developed early in tanshinone research, and the research on associated extraction and separation process technologies is relatively extensive. The main extraction methods include alcohol extraction, ultrasonic extraction, supercritical CO_2 fluid extraction (Wang L. et al., 2015), pressurized liquid extraction (PLE) (Ong and Len, 2004; Li et al., 2007), microwave-assisted extraction (Pan et al., 2001), high-speed countercurrent chromatography, column chromatography, and vacuum liquid phase extraction.

Because terpenoids generally have low polarity, they are often extracted with chloroform, ethyl acetate or petroleum ether. For some slightly more polar compounds, the plant roots are usually first degreased and then extracted with a polar solvent such as acetone (Wang et al., 2012) and n-butanol (Pang et al., 2016). Although the alcohol extraction method is a relatively traditional extraction process, it is still widely used in the market for its simple and easy operation, and modern ideas have been incorporated to optimize the method. For example, by using orthogonal design, the best extraction and purification processes for total tanshinones were identified. The content of total tanshinones in crude extract increased from 18.23 to 57.29% by purification.

The ultrasonic extraction method does not require heating due to the short production cycle, thus avoiding the thermal degradation reaction of tanshinone IIA. Compared with traditional methods, such as methanol reflux extraction and organic solvents macerating at room temperature, ultrasonic-assisted extraction and microwave-assisted extraction of tanshinones have advantages such as extraction speed and high efficiency (Pan et al., 2002). Similarly, supercritical fluid extraction technology (SFE) (Wang L. et al., 2015) has a short production cycle and does not require heating, which avoids the degradation reaction of tanshinone IIA. Compared with the traditional alcohol extraction method, SFE can retain more tanshinone IIA, but due to the expensive equipment and high production cost, it still needs further promotion to enter the market.

In addition, the extraction of tanshinones has many new methods and new technologies, such as non-ionic surfactant-assisted extraction (Bi et al., 2011), infrared-assisted extraction (Chen et al., 2010), ionic liquid-based ultrahigh pressure extraction (Liu et al., 2013), and selectively modified microfluidic chips (Xuan et al., 2010). The combined application of methods can make extraction more efficient and productive, for example, ultrasound-assisted extraction combined with an amino-modified monolithic cartridge used as a solid-phase extraction sorbent to improve extraction efficiency (Tao et al., 2013); on-line continuous sampling combined with ionic liquid-based dynamic microwave-assisted extraction (Gao S. et al., 2015); a dynamic continuous ultrasound-assisted extraction with a high-intensity ultrasonic probe (CUAE-HIUP) combined with solid-phase extraction (SPE) (Qiao et al., 2007); and ionic liquid surfactant-mediated ultrasonic-assisted extraction combined with high performance liquid chromatography (HPLC) (Wu et al., 2015).

Chromatography is commonly used for the separation and purification of tanshinone monomers. The semipreparative high-speed countercurrent chromatography (HSCCC) technique (Sun et al., 2011) could successfully separate and purify the four major components of Danshen at high purities of over 95% (Tian et al., 2002). Furthermore, the high-efficiency separation of tanshinones could be achieved by the macroporous resin

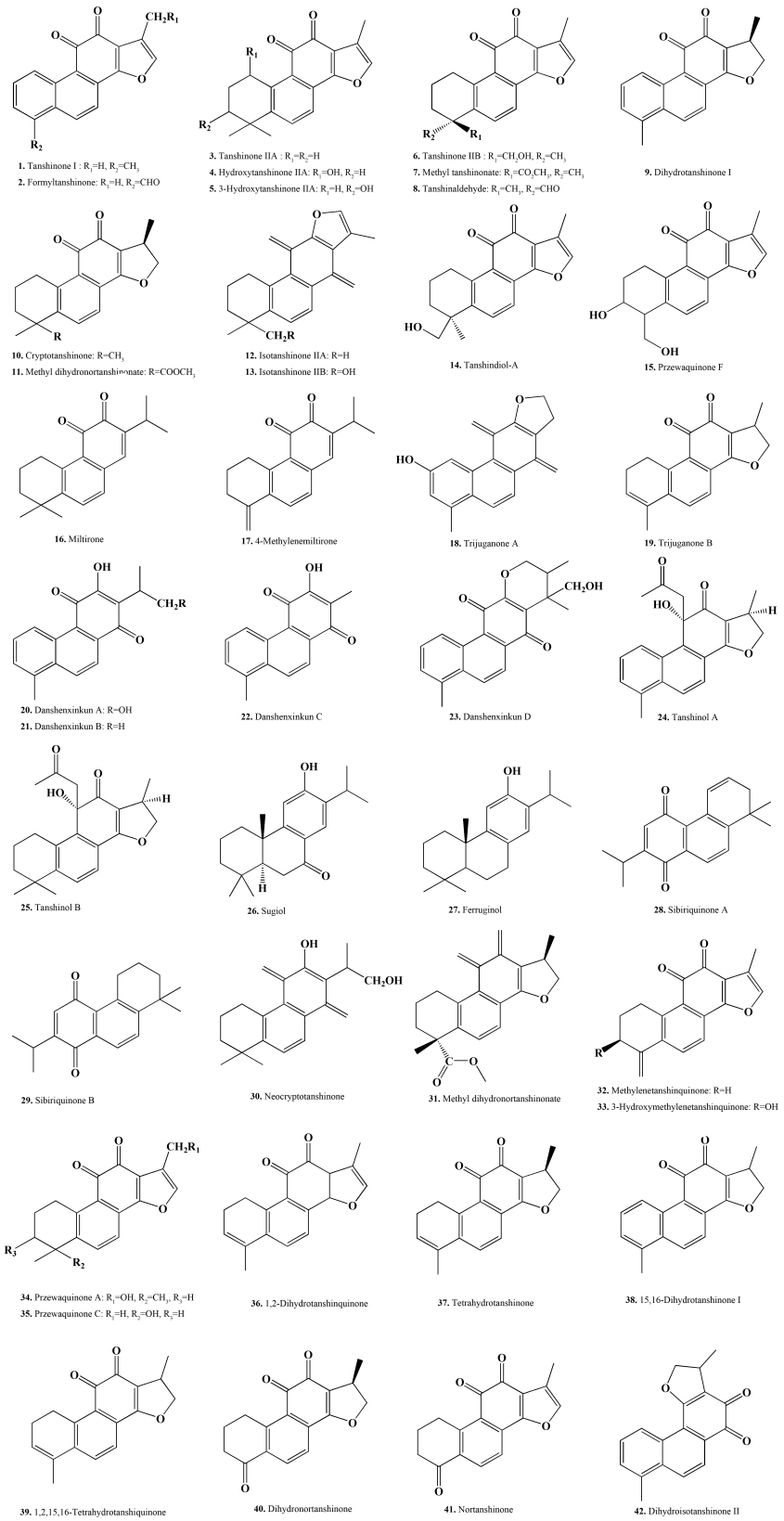

FIGURE 1 | Chemical structure of representative tanshinone compounds contained in *S. miltiorrhiza*.

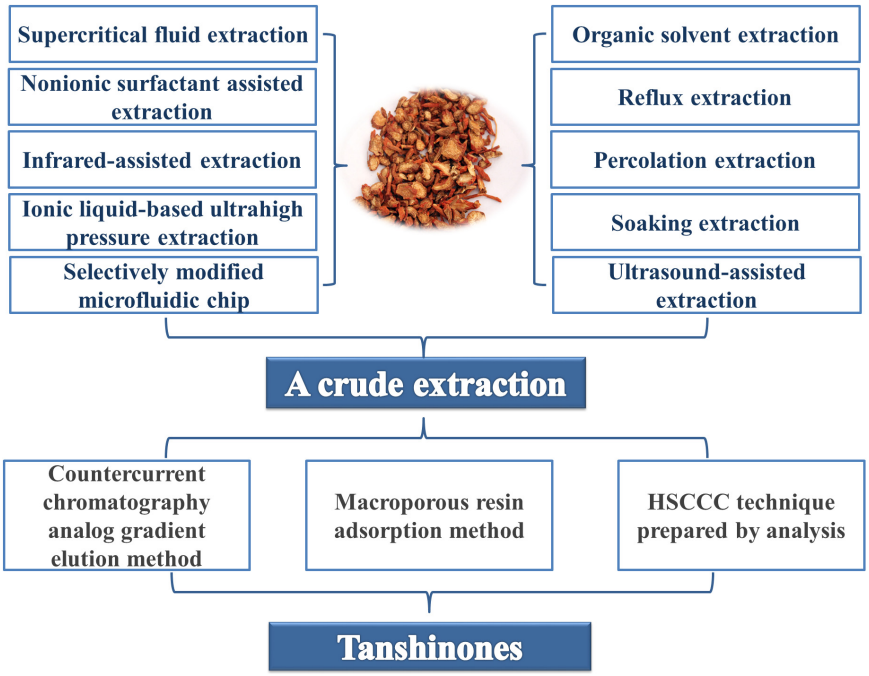

FIGURE 2 | A brief summary of the extraction and separation of tanshinones from the original plants of *S. miltiorrhiza*.

adsorption method and the countercurrent chromatography analog gradient elution method (Wu S. et al., 2012; **Figure 2**).

PHARMACOLOGICAL ACTIVITIES OF TANSHINONES

Tanshinones have received extensive attention due to their remarkable activities in the clinical treatment of cardiovascular diseases. With deepening research, these compounds have been found to possess a wide range of pharmacological activities, such as antibacterial, antioxidant, anti-inflammatory, and anti-tumor properties. A brief introduction to the pharmacological activity of tanshinones will follow (**Figure 3**).

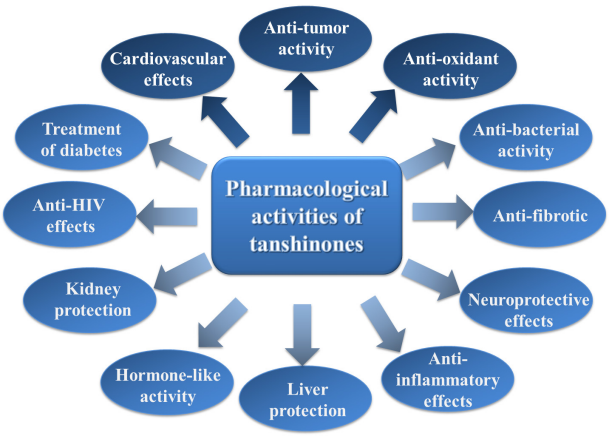

FIGURE 3 | The brief summary of the pharmacological activities of tanshinones. The boxes filled with dark blue indicate that the pharmacological activities are stronger, and the boxes filled with light blue indicate that the pharmacological activities are general.

Anti-tumor Activity

There are many kinds of anti-tumor drugs, and their mechanisms of action are also different. For example, some drugs can interfere with the anabolism of nucleic acids, inhibit mitosis, and inhibit protein synthesis. Currently, most of the applied chemotherapy drugs are cytotoxic and cannot only kill cancer cells, but also cause side effects (Yuan et al., 2003). The phenanthrene-quinone structure widely present in tanshinone compounds is a major cause of the cytotoxicity (Wu et al., 1991; Shi et al., 1997; Wang et al., 2004). During the antitumor reaction, the DNA molecule binds to the phenanthrene ring structure of the tanshinone, and the furan ring and steroid structure generate free radicals to hinder the synthesis of DNA in the tumor cells. Tanshinone IIA can regulate oxidative stress and reduce the mitochondrial membrane potential to induce apoptosis in cancer cells such as human lung cancer A549 cells (Chiu and Su, 2010); tanshinones inhibit the growth of human prostate cancer cell lines by arresting the cell cycle and inducing apoptosis *in vitro*. Among them, tanshinone I has the most significant activity with IC50 approximately 3–6 μM and its side effects on normal prostate epithelial cells are relatively small (Gong et al., 2011); cryptotanshinone induces tumor cell apoptosis by triggering cell cycle arrest, altering mitochondrial signaling pathways, and by other apoptosis-regulating proteins or death receptor pathways;

the methanol extract of *S. miltiorrhiza* induced apoptosis in non-small cell lung cancer through phosphatase and tensin homolog (PTEN)-mediated inhibition of the PI3K/Alct pathway (Ye et al., 2017); *S. miltiorrhiza* exerted clear cytotoxic effects, and strongly inhibited the proliferation of HepG2 cells (Liu et al., 2000); and tanshinone I inhibits tumor angiogenesis by the phosphorylation of reducing signal transducers and activators of transcription (STAT3) at TYR705 and hypoxia-induced HIF-1a accumulation in both endothelial and tumor cells (Wang Y. et al., 2015). In summary, tanshinones are expected to become a new type of anti-tumor drug with high efficiency and low toxicity.

Effects on the Cardiovascular System

Cardiovascular diseases refer to ischemic or hemorrhagic diseases in the heart, brain and whole body tissues caused by hyperlipidemia, blood viscous, atherosclerosis, and hypertension. *S. miltiorrhiza* and its compound preparations, such as composite Danshen droplet pills (Zhang et al., 2008) and Guanxin Danshen drop pills (Yao et al., 2018), have achieved good curative effects in the clinical treatment of cardiovascular diseases. Modern pharmacological research has found that *S. miltiorrhiza* has been widely used in the treatment of coronary artery, myocardial ischemia and myocardial infarction, improvement of microcirculation, and reduction of myocardial oxygen consumption.

Tanshinone IIA is a representative monomeric compound in *S. miltiorrhiza*. However, due to the poor water solubility, its water-soluble derivative, sodium tanshinone IIA silate (STS), is more widely used in the clinical treatment. Experimental results show that STS restrains the proliferation and migration of high glucose-induced vascular smooth muscle cells (VSMC), possibly through adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK) activation (Chen, 1984; Kyoko et al., 2002; Yang et al., 2007). In addition, STS has protective effects on myocardial apoptosis induced by acute myocardial infarction (MI) in adult rats (Yang et al., 2008) and inhibits cardiac fibrosis after pathological stimuli to the cardiovascular system (Le et al., 2009). The clinical efficacy results also show that tanshinone IIA sodium sulfonate injection is effective in the treatment of acute cerebral infarction, and can also be used as an additional treatment for patients with unstable angina pectoris (Zhang et al., 2014).

As the main lipophilic component of *S. miltiorrhiza*, tanshinone IIA also has cardiovascular protective effects. Its mechanisms of action mainly include the following: significantly reducing intimal thickening and restraining the proliferation and migration of VSMC, which is a significant event in the development of atherosclerosis (AS); by reducing the content of macrophages, reducing the uptake of oxidized low-density lipoprotein (ox-LDL) (Gong et al., 2009; Liu et al., 2014), promoting cholesterol excretion, and reducing the formation of foam cells, thereby regulating blood lipids and reducing body weight; inhibiting the development of left ventricular hypertrophy and reducing the level of apoptosis; significantly reducing blood viscosity, inhibiting thrombin activation, promoting fibrin degradation and inhibiting the formation of thrombosis (Wu L. et al., 2012). In summary, tanshinone IIA has broad application prospects in the prevention and treatment of cardiovascular diseases, but its specific mechanism remains to be further studied. In addition to tanshinone IIA, cryptotanshinone (Li et al., 2018), tanshinone I, dihydrotanshinone I (Wei Y. et al., 2016), etc., have proved to be effective coronary dilators, which can improve microcirculation and enhance vascular blood flow.

Antioxidant Activity

Oxidative stress can cause DNA oxidative damage and abnormal expression of proteins puts the body in a vulnerable state, and is intently connected with the occurrence and development of various diseases. Previous studies have shown that tanshinone I, tanshinone IIB, cryptotanshinone, dihydrotanshinone I, methylenetanshinquinone, and miltiradiene (Houlihan et al., 1985) were all found to act as antioxidants (Ke et al., 1990; Weng and Gordon, 1992). The specific antioxidant activity of tanshinone IIA is that it can effectively restrain the interaction between DNA and the intracellular lipid peroxidation products (Cao et al., 1996; Niu et al., 2000; Zhou et al., 2013) and protect DNA by eliminating lipid free radicals and blocking the chain reaction of lipid peroxidation. In addition, tanshinone IIA inhibits atherosclerosis calcification in rats by inhibiting oxidative stress (Tang et al., 2007). Latter researches have indicated that it can also inhibit the activity and gene expression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, as well as the production of reactive oxygen species (ROS) (Yang et al., 2010; Wang et al., 2011; Huang et al., 2018).

Anti-bacterial Activity

The pharmacological effects of *S. miltiorrhiza* are very extensive, and in addition to the anti-tumor activity, which has been explored deeply, it has antibacterial and anti-inflammatory effects. The main lipophilic components (Lee et al., 1999) that play the antibacterial roles in *S. miltiorrhiza* are cryptotanshinone, dihydrotanshinone I (Honda et al., 1988), hydroxyl tanshinone and tanshinone IIB. These compounds could significantly inhibit gram-positive bacteria, especially cryptotanshinone and dihydrotanshinone I exhibited strong antimicrobial activity. They could bring forth superoxide radicals, which are of great significance in the antibacterial action. In comparison, tanshinone IIA and tanshinone I were confirmed to have medium antimicrobial activity (Zhao et al., 2011).

Effects on Neurodegenerative Disorders

Neurodegenerative diseases are caused by the loss of neurons or their myelin, which over time, in succession causes degeneration of the structure and function of the central nervous system (Javed et al., 2016). Neurodegenerative diseases could be divided into two categories according to their phenotype: one that affects body movement, like Parkinson's disease, and one that affects memory and related dementia, like Alzheimer's disease (AD) (Prince et al., 2013; Ahmed et al., 2015; Orhan et al., 2015). Due to the good lipophilicity and small molecular weight, tanshinones are able to penetrate the blood-brain barrier (BBB). For example, tanshinone I (Jing et al., 2016), tanshinone IIA (Bi et al., 2008), and cryptotanshinone (Zhang F. et al., 2009;

Park et al., 2012) have been reported to exhibit significant neuroprotective effects. Tanshinone I selectively suppressed pro-inflammatory gene expression in activated microglia and prevented nigrostriatal dopaminergic neurodegeneration in a mouse model of Parkinson's disease (Wang S. et al., 2015). Tanshinone IIA protects neurons from the neurotoxicity of amyloid beta (Aβ) 25–35, increases neuronal viability and downregulates the expression of phosphorylated *tau* in AD. In addition, tanshinone IIA inhibits the transcription and expression of genes encoding inducible nitric oxide synthase (iNOS), metalloproteinase (MMP)-2, and human nuclear factor (NF-κBp65) pathway, thereby inhibiting the occurrence of neuroinflammation and reducing AD risk (Jiang P. et al., 2014).

Other Bioactivities

In addition to the above mentioned pharmacological activities, tanshinone compounds exhibit anti-inflammatory effects (Fan et al., 2009), liver protection, protection against kidney damage, hormone-like activity, and so on. Methyl tanshinonate, hydroxytanshinone, tanshinone IIA, and tanshinone IIB show anti-platelet aggregation activity. Tanshinone IIA has the potential to ameliorate bone resorption diseases *in vivo* by reducing both the number and activity of osteoclasts (Han et al., 2007). Cryptotanshinone and dihydrotanshinone I have been reported to have anti-cholinesterase activity (Ren et al., 2004). Due to their wide range of pharmacological activities, tanshinones are expected to contribute to the development of new drugs for clinical treatment.

BIOSYNTHESIS OF TANSHINONES IN *S. miltiorrhiza*

Terpenoids are derived from methylpentahydroxy acid and have a molecular skeleton of an isoprene unit (C5 unit) as a basic structure unit. Terpenoids are widely found in nature and are also important class of compounds in Chinese herbal medicine (Cheng et al., 2007). According to the number of C5 units, terpenoids can be divided into monoterpenes, sesquiterpenes, diterpenes, and triterpenes, in addition to the more specific structure of iridoids. The biosynthesis pathway of terpenoids has certain rules to follow. The common precursor iospentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) are generated by the 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway and/or the mevalonate (MVA) pathway. Under the action of prenyltransferase, IPP and DMAPP condense to form precursors of monoterpenes, sesquiterpenes, diterpenes, and similar compounds. These precursors form the basic skeleton of various terpenoids under the action of a quinone synthase, and then form a final product under the action of a modifying enzyme such as cytochrome P450 hydroxylase or glycosyltransferase (Hasan et al., 2014; Zi et al., 2014). Tanshinones are a group of diterpenoids, and the biosynthesis pathway in *S. miltiorrhiza* also follows the above rules. Interference experiments have shown that the MVA pathway plays a more critical role in the cell growth of *S. miltiorrhiza* hairy roots, while the MEP pathway is more committed to the production of tanshinones (Yang et al., 2012). Of course, the interrelationship and crosstalk between the two pathways together promote cell growth and metabolite production (**Figure 4**).

The MVA Pathway

Since the late 1950s, the MVA pathway has received extensive attention due to its involvement in the biosynthesis pathway of plant steroids. The MVA pathway begins with acetyl-CoA and ends with the production of IPP and DMAPP. 3-Hydroxy-3-methylglutaryl-CoA reductase (HMGR) (Dai et al., 2011), a rate-limiting enzyme in isoprenoid biosynthesis, catalyzes the conversion of HMG-CoA to MVA. There are four *SmHMGRs* (Cui et al., 2011) in the draft genome of *S. miltiorrhiza.*

The analysis of the tissue expression of the four genes is as follows: *SmHMGR1* has the highest tissue expression in flowers and root steles and the lowest expression in leaves; *SmHMGR2* is expressed in higher amounts in roots and stems; in addition to the low expression level in flowers, SmHMGR3 expressed the highest of the four homologous genes in other tissues (Zhang et al., 2012); in contrast, *SmHMGR4* has the highest tissue expression in flowers, but has the lowest expression in other tissues among the four homologous genes. Induced expression experiments show that *SmHMGR1, SmHMGR2* and *SmHMGR3* are sensitive to methyl jasmonate (MeJA) (Pan et al., 2009; Dai et al., 2011; Ma et al., 2012). Overexpression of the above three genes can significantly increase the content of tanshinones in hairy root culture.

In addition to *SmHMGR*s, the genes involved in the MVA pathway include *SmAACT, SmHMGS*, *SmMK*, *SmPMK*, and *SmMDC* (Cui et al., 2010; Lin et al., 2011; Ma et al., 2012). The proteins encoded by these genes each play a specific function, catalyzing the corresponding reactions and performing their duties; thus, IPP and DMAPP are finally obtained from the MVA pathway.

The MEP Pathway

The MEP pathway, also appearing the non-mevalonate pathway, uses pyruvate (Pyr) and glyceraldehyde 3-phosphate (G3P) as precursors to produce the two products (IPP and DMAPP) under the action of a series of enzymes. In plants, the MEP pathway is localized in the plastid and provides precursors of the synthesis of terpenoids, as well as side chains of chlorophylls and tocopherols.

Many genes in the MEP pathway have been cloned and identified (Wang et al., 2008; Gao W. et al., 2012; Ma et al., 2012; Cheng Q.Q. et al., 2013; Hao et al., 2013; Jiang D. et al., 2014). 1-Deoxy-D-xylulose-5-phosphate synthase (DXS) is the first enzyme in the MEP pathway and a key regulatory site for the pathway (Wei Z. et al., 2016). There are five DXSs (Ma et al., 2012) in the draft genome of *S. miltiorrhiza*, and *SmDXS2* expression was predominantly detected in roots than the other four (Kai et al., 2011; Wu et al., 2011), which suggested that *SmDXS2* is more likely to be involved in the biosynthetic pathway of tanshinones. Phylogenetic analysis showed that *SmDXS2* and *SmDXS3* were clustered into one branch, belonging to the DXS2 clade. *SmDXS3* also has high tissue expression in roots and root

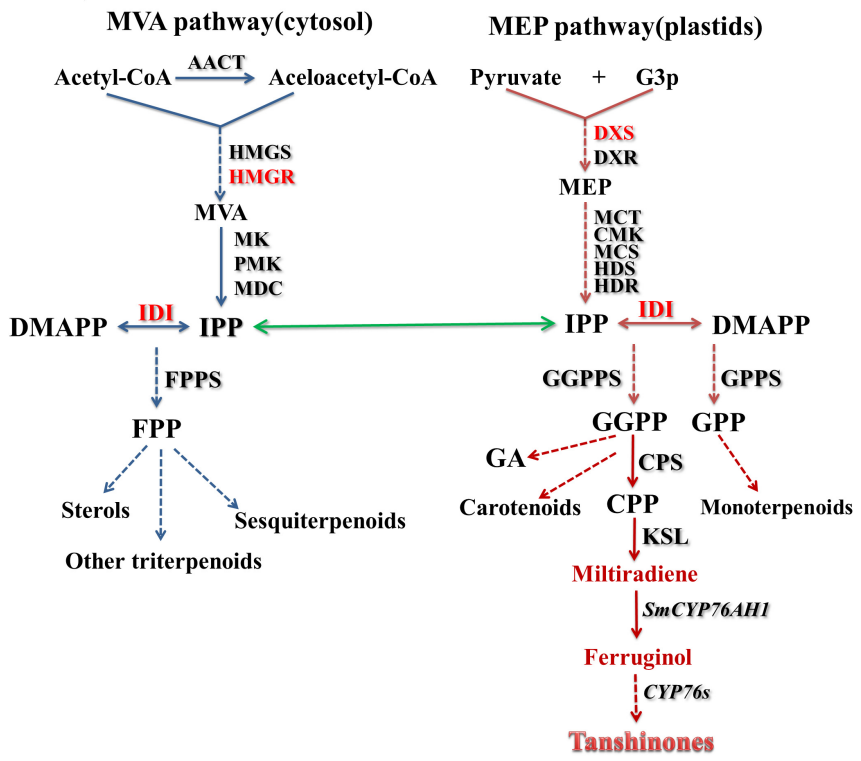

FIGURE 4 | Outline of the biosynthesis pathway of tanshinones in *S. miltiorrhiza.* The common precursors IPP and DMAPP of the universal isoprene precursors are generated by MEP and MVA pathways. TPSs catalyze the production of GGPP by IPP and DMAPP, and GGPP further produces tanshinones through a series of cyclization and hydroxylation reactions. The abbreviations used are listed in **Table 1**.

steles and is highly sensitive to MeJA, indicating that it may be involved in plant defense mechanisms.

SmDXR catalyzes 1-deoxy-D-xylulose 5- phosphate reductoisomerase (DXP) to generate MEP. *SmDXR* is expressed in various tissues and has the highest expression in roots. Under high-osmotic stress and yeast elicitors, the content of tanshinones in *S. miltiorrhiza* hairy roots increased significantly and RT-PCR results showed that the transcription level of *SmDXR* gene was also upregulated. The DXR genes may contribute to the control of tanshinone metabolic route and be a regulatory site for efficient heterogeneous production of tanshinones (Yan et al., 2009; Kai et al., 2011; Wu et al., 2011).

Genes Involved in the Miltiradiene Branch Pathway

Isopentenyl diphosphate isomerase (IDI), also known as isopentenyl pyrophosphate isomerase, catalyzes the conversion of the relatively unreactive IPP to the more-reactive electrophile DMAPP. Two IDI genes (*SmIDI1* and *SmIDI2*) have been found and cloned from *S. miltiorrhiza*. *SmIDI1* has been confirmed as a candidate gene involved in the biosynthesis of tanshinone (Cui et al., 2011; Yang et al., 2011). Tissue expression analysis revealed that *SmIDI1* has the higher expression in leaves than in the roots and stems. The relative expression of *SmIDI1* increased under the induction of Ag^{+} and MeJA. Color complementation experiments showed that *SmIDI1* promoted the accumulation of lycopene. Compared with *SmIDI1*, there are few studies about *SmIDI2* (Ma et al., 2012), which has a chloroplast-localized transit peptide, whereas *SmIDI1* does not have one. The tissue expression level of *SmIDI2* was lower than that of *SmIDI1* in each analyzed tissue. In general, *SmIDI1* may play a more important role in the biosynthesis process of tanshinones.

As universal precursors for the biosynthesis of terpeniods, both IPP and DMAPP condense to form precursors for monoterpene, diterpene, sesquiterpene and triterpenes, and so forth under the action of specific prenyltransferases. For example, one molecule of IPP and one molecule of DMAPP generate farnesyl diphosphate (FPP) under the catalysis of farnesyl diphosphate synthase (FPPS). FPPS belongs to family of short-chain prenyltransferases which also includes geranyl diphosphate synthase (GPPS), geranylgeranyl diphosphate synthase (GGPPS).

Analysis of the *S. miltiorrhiza* genome shows there are five GPPS genes (*SmGPPS* encoding the homomeric GPPS subunit; *SmGPPS.LSU*, *SmGPPS.SSUI*, *SmGPPS.SSUII.1,* and *SmGPPS.SSUII.2* encoding heteromeric GPPS subunits), one FPPS gene (*SmFPPS*), and three GGPPS genes (*SmGGPPS1*, *SmGGPPS2*, and *SmGGPPS3*) (Cui et al., 2011; Kai et al., 2011; Ma et al., 2012, 2015).

GPPS is generally considered to be regarded as the backbone of monoterpene constituents, but recent studies have found that it is also necessary in the biosynthesis pathway of some

diterpenoids, such as gibberellins (van Schie et al., 2010). To date, there are still few studies on the five GPPS genes in *S. miltiorrhiza*. The differences in homology and tissue specificity also make the research more difficult. The monoterpenes and sesquiterpenoids contained in many *Salvia* species are mainly enriched in the trichomes of the aerial parts. They also have a wide range of pharmacological activities, such as antimicrobial and antioxidant activities. There is still a need to further identify and characterize the *SmFPPS* and *SmGPPS* genes to explore the roles they played in the biosynthesis of essential oils in *S. miltiorrhiza* (Ma et al., 2012).

GGPPS catalyzes the condensation reaction between FPP and IPP to form GGPP. GGPP is a key precursor of biosynthesis of diterpenoids, chlorophylls, and carotenoids. *S. miltiorrhiza* contains three GGPPS genes. The full-length cDNA of *SmGGPPS1* has been isolated by rapid amplification of cDNA ends (RACE). Tissue expression pattern analysis showed that *SmGGPPS1* expression was higher in leaves and roots than stems. Functional identification experiments showed that *SmGGPPS1* can catalyze the production of carotenoids, and the introduction of *SmGGPPS1* gene into the transgenic hairy roots could significantly increase the content of tanshinones. So far, there are few studies on *SmGGPPS2* and *SmGGPPS3*, the functions of the two genes remain to be elucidated (Zhang L. et al., 2009; Kai et al., 2010; Hua et al., 2012; Ma et al., 2012; Yan et al., 2014).

Comparing the structures of GGPP and tanshinone compounds, the differences can be clearly observed and GGPP needs to undergo a cyclization to form tanshinones. The key enzymes involved in the process from GGPP to the production of miltiradiene are labdadienyl/copalyl diphosphate synthases (CPS) and kaurene synthase-like (KSL), which are class II diterpene cyclases. There are twelve *SmCPS* and nine *SmKSL* homologs reported in *S. miltiorrhiza* and based on the genome sequences (Ma et al., 2012), seven cDNA sequences encoding TPSs (including *SmCPS1* to *SmCPS5*, *SmKSL1,* and *SmKSL2*) have been cloned. Previous work has indicated that *SmCPS1* and *SmKSL1* participate in the tanshinone biosynthesis (Gao et al., 2009) and using the modular path engineering (MOPE) strategy, *SmCPS*, *SmKSL* and other related genes construction fusion proteins were expressed in the optimized yeast strain and finally the yield of miltiradiene in the 15 L bioreactor reached 365 mg/L (Zhou et al., 2012). Combining *SmCPS2* and *SmKSL1* expressed proteins and feeding GGPP as substrate also lead to production of miltiradiene. Phylogenetic analysis indicates that *SmCPS1* to *SmCPS3* are closely related to CPSs involved in the biosynthesis of CPP, while *SmCPS4* and *SmCPS5* are closely related to the CPSs generally for gibberellin biosynthesis (Ma et al., 2012; Cheng Q. et al., 2013; Cui et al., 2015). Compared with *SmKSL2*, *SmKSL1* has experienced a loss of the N-terminal g-domain, which indicates that *SmKSL1* involved in a more specific diterpenoid metabolic process. Phylogenetic analysis also indicated *SmKSL2* clusters with other dicotyledonous KSL genes, many of which have been identified to be involved in GA biosynthesis (Gao et al., 2009; Ma et al., 2012; Cui et al., 2015).

Under the action of TPS, GGPP undergoes cyclization reactions to form miltiradiene, which needs to be further modified to produce tanshinones. These modification reactions include decarboxylation, oxidation and reductions, and each step can be catalyzed by the corresponding specific enzyme.

Cytochromes P450 is an important mixed-function heme oxidoreductase in animals, plants and microorganisms, and is a gene superfamily (Ghosh, 2017). It can catalyze a variety of chemical reactions and plays an important role in protecting organisms from external aggression and in plant secondary metabolism (David and Danièle, 2011). CYP450s often form multi-enzyme complexes with other enzymes on the endoplasmic reticulum to act on the biosynthetic pathway of phenylpropanoid. With the development of modern sequencing technology and the analysis of plant genomes, more and more P450 genes involved in plant secondary metabolic activities have been discovered (Chen et al., 2014). The transcripts of the induction process of *S. miltiorrhiza* hair roots were analyzed to reveal 125 CYPs expressed therein (Gao et al., 2014). Guo et al. used a next-generation sequencing approach to identify six candidate CYP genes being related with the biosynthesis of tanshinones in both the rhizome and Danshen hairy roots. Among these six genes, they identified *SmCYP76AH1* could catalyze the conversion of miltiradiene to ferruginol *in vivo* and *in vitro*, making the biosynthesis pathway of tanshinone a critical step. And the ferruginol content reached 10.5 mg/L by heterologous expression of *SmCYP76AH1* and *phyto*-CYP reductase genes in yeast (Guo et al., 2013). With further research, they found other two *SmCYP*s, respectively, named *CYP76AH3* and *CYP76AK1*, exhibiting similar transcription profiles as *SmCYP76AH1* in elicited *S. miltiorrhiza* hairy roots (Guo et al., 2016). *SmCYP76AH3* oxidizes ferruginol at two different carbon centers to obtain 11-hydroxy ferruginol and 11-hydroxy sugiol. And then *SmCYP76AK1* could hydroxylate C-20 of two of the resulting intermediates. Together, sequential cooperation between two CYPs convert ferruginol into 11, 20-dihydroxy ferruginol and 11, 20-dihydroxy sugiol which may be a bifurcating pathway for the biosynthesis of tanshinones. After analyzing the structure of ferruginol and tanshinones, a putative biosynthetic pathway can be inferred, that is, ferruginol can be dehydrogenated to form cryptotanshinone, and then cryptotanshinone can be reduced by reductases to form tanshinone IIA (Cai et al., 2013; Guo et al., 2016).

Two full length cytochrome P450 reductase (CPR) genes have also been cloned from *S. miltiorrhiza* named *SmCPR1* and *SmCPR2*. Although there are relatively few studies on these two genes, they have a great role in supporting the CYP450 genes to play the catalytic functions (Schenkman and Ingela, 2003). The experiment shows that when importing *SmCYP76AH1* alone into the miltiradiene-producing yeast strain failed to produce ferruginol, in contrast yeast strains with plant CPRs after introducing *SmCYP76AH1* could produce ferruginol (Zhou et al., 2015).

The biosynthetic pathway from ferruginol to tanshinones remains to be further resolved. The discovery and comparison of structural features of intermediates that may occur in the tanshinone biosynthesis pathway represent a more effective way to find related functional enzymes, excavate functional genes, and analyze the complete pathway. Currently, the field

of gene sequencing is full of unknowns and expectations. The development and application of fourth-generation gene sequencing technology will definitely bring brighter prospects to the fields of scientific research and medicine.

SYNTHETIC BIOLOGY OF TANSHINONES

In recent years, the discovery of key genes for the biosynthesis of medicinal active ingredients and the use of synthetic biology strategies to design and engineer microbial strains to produce natural products have been considered a promising method for resource acquisition (Gao W. et al., 2015). Miltiradiene is a key precursor for the biosynthesis of tanshinones. At present, *Saccharomyces cerevisiae* is used as the chassis cell, the engineered strains with a high yield of miltiradiene were constructed by designing functional modules; the yield could reach 488 mg/L (Gao et al., 2009; Zhou et al., 2012). The cloning of the first modified enzyme gene *CYP76AH1* downstream of the tanshinone biosynthesis pathway successfully transformed miltiradiene into ferruginol, which is stable and biologically active. The highest yield of ferruginol reached 10.5 mg/L under shake flask fermentation conditions (Guo et al., 2016).

Increasingly more scholars have focused on not only functional genes in the biosynthetic pathway but also the effects of elicitors and regulatory genes on secondary metabolites in plants. Elicitors can directly or indirectly prompt the accumulation of plant secondary metabolites in plant tissues, cell culture and hairy root culture. According to their sources, these elicitors can be divided into biological inducers and abiotic inducers. Biological elicitors such as *Trichoderma atroviride* (Ming et al., 2013), *Chaetomium globosum* D38 (Zhai et al., 2017), *Alternaria sp.* A13 (Zhou et al., 2018) and other endophytic fungi, as well as some non-biological inducers such as MeJA, salicylic acid, yeast extract, Ag^+ (Xing et al., 2014), and ultraviolet-B (Wang et al., 2016), could induce the accumulation of active ingredients in *S. miltiorrhiza*.

CONCLUSION AND PROSPECTS

As a perennial cross-pollinating plant species, *S. miltiorrhiza* is strongly adaptive and widely distributed with generations having overlapping features and significant genetic diversity. Since ancient times, the single-component Danshen or its combination with other drugs has been used in the clinical treatment of various diseases exhibiting good effects. *S. miltiorrhiza* contains a variety of active ingredients, among which tanshinones have exhibited significant pharmacological properties, such as antibacterial, antioxidant, anti-inflammatory, and anti-tumor activities. In this paper, the extraction and purification methods of tanshinones are briefly introduced. Many new methods and new technologies have been applied, and it is worth mentioning that the combination of different methods can achieve high efficiency and low consumption and may have promising development prospects.

The traditional method of obtaining tanshinones has been unable to meet the clinical drug needs. In recent years by continuously investigating the functional genes in the tanshinone biosynthesis pathway, analyzing the complete pathway and reconstituting the biosynthetic pathway in heterologous hosts, the method of efficiently and heterologously producing tanshinones has become the focus of attention. To date, studies have shown that miltiradiene is an intermediate for the biosynthesis of tanshinones, and that it can be converted to tanshinones under the action of various modified enzymes. Therefore, more functional genes must be further discovered. Synthetic biology strategies and methods not only provide ideas for more efficient and convenient access to tanshinones, but also lay the foundation for the sustainable use of traditional Chinese medicine resources.

AUTHOR CONTRIBUTIONS

ZJ, WG, and LH contributed the conception and design of the study. ZJ organized the database and wrote the first draft of the manuscript. WG revised parts of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

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Diabetes and alpha lipoic acid

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Diabetes mellitus is a multi-faceted metabolic disorder where there is increased oxidative stress that contributes to the pathogenesis of this debilitating disease. This has prompted several investigations into the use of antioxidants as a complementary therapeutic approach. Alpha lipoic acid, a naturally occurring dithiol compound which plays an essential role in mitochondrial bioenergetic reactions, has gained considerable attention as an antioxidant for use in managing diabetic complications. Lipoic acid quenches reactive oxygen species, chelates metal ions, and reduces the oxidized forms of other antioxidants such as vitamin C, vitamin E, and glutathione. It also boosts antioxidant defense system through Nrf-2-mediated antioxidant gene expression and by modulation of peroxisome proliferator activated receptors-regulated genes. ALA inhibits nuclear factor kappa B and activates AMPK in skeletal muscles, which in turn have a plethora of metabolic consequences. These diverse actions suggest that lipoic acid acts by multiple mechanisms, many of which have only been uncovered recently. In this review we briefly summarize the known biochemical properties of lipoic acid and then discussed the oxidative mechanisms implicated in diabetic complications and the mechanisms by which lipoic acid may ameliorate these reactions. The findings of some of the clinical trials in which lipoic acid administration has been tested in diabetic patients during the last 10 years are summarized. It appears that the clearest benefit of lipoic acid supplementation is in patients with diabetic neuropathy.

Keywords: diabetes, alpha lipoic acid, neuropathy, nephropathy, antioxidant, oxidative stress

INTRODUCTION

Lipoic acid (LA) or α-lipoic acid (ALA) is a naturally occurring compound that is also known as 1,2-dithiolane-3-pentanoic acid or thioctic acid (Busby et al., 1999). It is synthesized enzymatically in plant and animal mitochondria from octanoic acid and cysteine (as a sulfur source). ALA acts as a cofactor for pyruvate dehydrogenase and α-keto-glutarate dehydrogenase activity (Schmidt et al., 1994), and is also required for the oxidative decarboxylation of pyruvate to acetyl-CoA, a critical step bridging glycolysis and the citric acid cycle (Reed, 1998). The presence of an asymmetric carbon produces two optical isomers R-LA and S-LA. Only the naturally occurring R isomer is bound to protein and acts as an essential cofactor in biological systems (Reed, 1998). However, synthetic LA is a racemic mixture of R and S isoforms, where S-LA can prevent the polymerization of R-LA to enhance its bioavailability (Shay et al., 2009). In cells containing mitochondria, ALA is reduced in an NADH-dependent reaction with lipoamide dehydrogenase to form dihydrolipoic acid (DHLA), whereas in cells that lack mitochondria, ALA can instead be reduced to DHLA via NADPH with glutathione (GSH) and thioredoxin reductases (Jones et al., 2002). Unlike GSH, for which only the reduced form is an antioxidant, both the oxidized and reduced forms of LA are powerful antioxidants whose functions include: (1) quenching of reactive oxygen species (ROS), (2) regeneration of exogenous and endogenous antioxidants such as vitamins C and E, and GSH, (3) chelation of metal ions, and (4) reparation of oxidized proteins (5) regulation of gene transcription (6) inhibition of the activation of nuclear factor kapp B (NF-κB; Biewenga et al., 1997; Packer, 1998; Zhang and Frei, 2001; **Figure 1**). This impressive array of cellular and molecular functions has raised considerable interest for the use of this substance as a nutritive supplement and also as a therapeutic agent. In view of this growing interest, this review provides an update on the therapeutic role(s) of this substance in ameliorating diabetes and diabetes-induced complications.

Abbreviations: AICAR, 5-aminoimidazole-4-carboxamide-1-beta-D-ribofurano side; ACC, acetyl-CoA carboxylase; ADMA, asymmetric dimethylarginine; AGEs, advanced glycation end products; ALA, alpha lipoic acid; ASK1, apoptosis signal-regulating kinase 1 (also known as MAP3K5, mitogen activated protein kinase kinase kinase 5); BH4, tetrahydrobiopterin; DHLA, dihydrolipoic acid; DDAH, dimethylarginine dimethylamino hydrolase; eNOS, endothelial nitric oxide synthase; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; GLUT4, glucose transporter 4; GSH, glutathione; IκB, inhibitor of kappa B; IKK α and β, TNF-α-inducible IκB kinase complex; Keap1, Kelch-like ECH-associated protein 1; LA, lipoic acid; LDL, low density lipoprotein; MAPKs, mitogen activated protein kinases; MEKs, MAPK kinases; MEKKs, MEK kinases; NADH, nicotinamide adenine dinucleotide; NF-κB, nuclear factor kapp B; NOS, nitric oxide synthase; Nrf2, nuclear factor erythroid 2-related factor 2; p38-MAPK, P38-mitogen activated protein kinases; PARP, poly (ADP-ribose) polymerase; PKC, protein kinase C; PPARs, peroxisome proliferator activated receptors; ROS, reactive oxygen species; TAK 1, transforming growth factor β activated kinase 1; TBARS, thiobarbituric acid reactive substances; CoQ10, ubiquinone.

BIOLOGICAL FUNCTION OF LIPOIC ACID

QUENCHING REACTIVE OXYGEN SPECIES

Based on the definition of an antioxidant provided by Halliwell and Gutteridge as being "any substance that when present at low

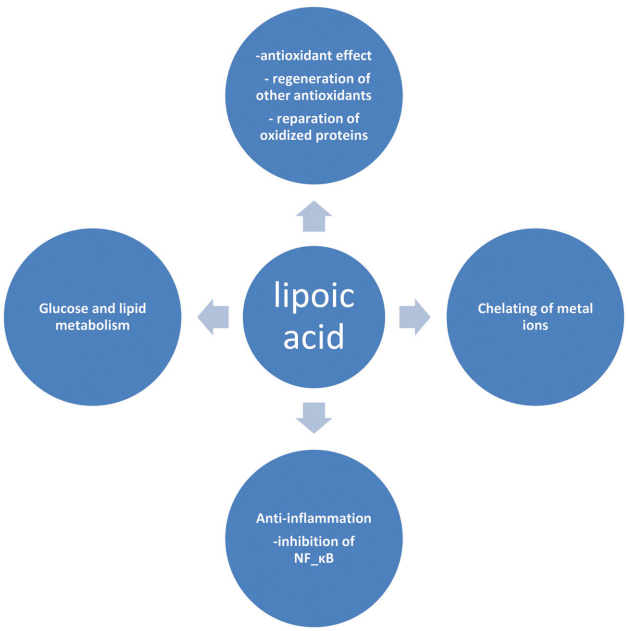

FIGURE 1 | Selected biological actions of lipoic acid.

concentrations compared to those of an oxidizable substrate significantly delays or prevents oxidation of that substrate," LA has several unique characteristics among other natural antioxidants to fulfill this criterion (Petersen Shay et al., 2008). Common antioxidants are either water-soluble or lipid soluble agents. In contrast, LA has both hydrophilic and hydrophobic properties. Being both water and fat-soluble means that ALA is widely distributed in plants and animals in both cellular membranes and in the cytosol (Wada et al., 1997). Therefore, it can elicit its antioxidant action in both the cytosol and plasma membrane in contrast to vitamin C (which is lipophobic) and vitamin E (which is lipophilic). Both LA and DHLA scavenge hydroxyl radicals and hypochlorous acid and prevent protein carbonyl formation. In addition DHLA may also be able to regenerate other endogenous antioxidants such as vitamins C and E, and has the beneficial property of neutralizing free radicals without itself becoming one in the process (Biewenga et al., 1997; Bast and Haenen, 2003).

REGENERATION OF OTHER ANTIOXIDANTS

When an antioxidant molecule reacts with an unstable free radical molecule, the antioxidant molecule itself becomes oxidized and loses its benefits until it is reduced again. DHLA has the ability of reducing the oxidized forms of other antioxidants such as vitamin C and E, and GSH.

Glutathione is one of the most important low molecular weight cellular antioxidants, buffering the thiol redox state. The level of GSH is strictly controlled by either substrate availability or transcriptional regulation of specific gene (Petersen Shay et al., 2008). A critical role has recently been described for a transcription factor "nuclear factor erythroid 2-related factor 2 (Nrf2)" against oxidative stress. Normally, Nrf2 is located in the cytoplasm and kept dormant by a cytoplasmic repressor Kelch-like ECH-associated protein 1 (Keap1). A variety of activators release and translocate Nrf2 into the nucleus, where it can regulate the expression of antioxidant enzymes (Lee et al., 2011). LA is one such inducer of Nrf-2-mediated antioxidant gene expression and by so doing is able to significantly increase the cellular capacity of GSH synthesis (Petersen Shay et al., 2008). Furthermore LA also elevates GSH levels through its ability to increase cysteine uptake (Busse et al., 1992). In a variety of cultured cells such as human Jurkat T cells human erythrocytes, C6 glial cells, NB41A3 neuroblastoma cells, and peripheral blood lymphocyte, DHLA also increases GSH synthesis by reducing the ratio of cystine to cysteine (as cysteine is the rate-limiting substrate for this reaction; Han et al., 1997).

Dihydrolipoic acid also regenerates vitamin E either as a result of the direct reaction with tocopheroxyl radical or indirectly by reducing dehydroascorbate, which in turn reduces alpha tocopherol. DHLA is also able to reduce ubiquinone (CoQ10) to ubiquinol, which is an important component of the mitochondrial electron transport chain (Bast and Haenen, 2003). DHLA also reduces the oxidized form of CoQ10, which can additionally reduce the alpha-tocopheroxyl radical (Smith et al., 2004).

CHELATION OF METAL IONS

Because of the presence of two thiol groups, LA and DHLA both have metal chelating properties. In fact, ALA is a potent chelator of divalent metal ions *in vitro* and forms stable complexes with Mn^{2+}, Cu^{2+}, Fe^{2+}, Pb^{2+}, and Zn^{2+} (Ou et al., 1995). Decreased iron uptake and its diminished cytosolic reactive pool have been shown in cultured lens epithelial cells following LA administration. These changes were associated with increased cell resistance to a H_2O_2 challenge, thus allowing LA to reduce the risk of iron induced oxidative stress (Goralska et al., 2003). In another animal study, R-LA prevented age related cortical iron accumulation and improved antioxidant status (Suh et al., 2005). DHLA also regenerates ascorbate, which in turn reduces iron (Bonomi et al., 1989).

REPARATION OF OXIDIZED PROTEINS

Accumulation of oxidized proteins is thought to be the hallmark of the cell's aging process (Berlett and Stadtman, 1997; Beckman and Ames, 1998). This can be due to either an increase in the rate of protein oxidation or a decrease in the rate of oxidized protein repair, or a combination of both. Cysteine and methionine, both sulfur-containing amino acids, are among the most sensitive to oxidation; however they are the only amino acids in which the oxidation products can be repaired by specific enzymatic systems (Mary et al., 2004).

Glutathione (GSH) as a versatile antioxidant that has a critical role in maintaining protein thiols in a reduced form and so in regulating the cell cycle (Dickinson and Forman, 2002a,b). Age related decreases in tissue GSH and/or reduced GSH/GSSG (oxidized glutathione) ratios occur in different tissues such as brain, heart (Suh et al., 2004), and liver (Hagen et al., 2000). LA reversed these age related changes and significantly boosted antioxidant system. The beneficial use of LA in reducing the age-associated alterations in GSH can be explained in different ways. First, delivery of exogenous GSH to tissues such as the heart and brain is impossible since it is not transported into these tissues. Second,

conventional cysteine delivery agents (e.g., *N*-acetylcysteine) have low bioavailability to the brain. However, LA is easily taken up into neural tissues and can thus modulate the age related alteration in GSH levels (McLellan et al., 1995; Suh et al., 2005).

REGULATION OF GENE TRANSCRIPTION

Further to the effects of LA on Nrf-2-mediated antioxidant gene expression, LA has also been shown to modulate peroxisome proliferator activated receptors (PPARs)-regulated genes. The PPARs are a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes (Michalik et al., 2006), and have essential roles in the regulation of cellular differentiation, development and metabolism (carbohydrate, lipid, protein), and tumorigenesis (Belfiore et al., 2009) of higher organisms (Berger and Moller, 2002). The discovery of the effects of thiazolidinediones and fibrates on PPAR-γ and PPAR-α respectively, confirms the importance of ligand-dependent modulation of gene transcription as a pharmacological target in human metabolic diseases. LA activates both PPAR-α (Butler et al., 2009) and -γ (McCarty et al., 2009). PPAR-α regulates the expression of carnitine palmitoyltransferase 1A and acetyl-CoA synthase and PPAR-γ increases the expression of fatty acid translocase/CD36, adipocyte fatty acid binding protein, and lipoprotein lipase. These enzymes are key regulators of glucose and lipid metabolism (Pershadsingh et al., 2005). Some of the metabolic effects of LA are secondary to its regulatory effect on these pathways.

INHIBITION OF THE ACTIVATION OF NF-κB

Nuclear factor kapp B is a protein complex that controls the transcription of DNA. NF-κB is found in almost all animal cell types and is involved in cellular responses to stimuli such as stress, cytokines, free radicals, ultraviolet irradiation, oxidized low density lipoprotein (LDL), and bacterial or viral antigens (Brasier, 2006; Gilmore, 2006). NF-κB has a key role in regulating the immune response to infection. Incorrect regulation of NF-κB has been linked to cancer, inflammatory and autoimmune diseases, septic shock, viral infection, and improper immune development. NF-κB has also been implicated in processes of synaptic plasticity and memory (Meffert et al., 2003; Tian and Brasier, 2003). The NF-κB protein complex is normally located in the cytoplasm in an inactive form by virtue of its binding to a family of inhibitor of NF-κB (IκB) proteins. Upon cell stimulation by a wide variety of stimuli, signal responsive IKK α and β (TNF-α-inducible IκB kinase complex also known as IKK1 and IKK2) are activated, resulting in the phosphorylation of IκB and its proteasomal degradation. IκB degradation liberates NF-κB, allowing it to translocate to the nucleus and induce gene expression (Baldwin, 1996; Barnes and Karin, 1997).

It is been shown that LA inhibits IκB degradation and NF-κB-dependent gene expression by inhibition of IKK2, suggesting that LA inhibits NF-κB activation independent of its antioxidant function (Ying et al., 2011). ALA inhibits NF-κB activation at the level of or upstream of, IKK-α and IKK-β, by modulating the MEKK1–MKK4–IKK pathway (Lee et al., 2008). This system is a signaling pathway of intracellular protein kinases that can transduce signals from the cell surface to changes in gene expression. Mitogen activated protein kinases (MAPKs) are the most important group of protein kinases in this regard (Zeigler et al., 1999). MAPKs are phosphorylated by upstream MAPK kinases (MEKs), which in turn are activated by MEK kinases (MEKKs; Widmann et al., 1999; Kyriakis and Avruch, 2001). The MEKK system is extraordinary diverse and complex and consists of several members, including MEKK1, 2, 3, 4, ASK1, TAK1, and MLK3. Among these MEKK1 is the best-characterized member and is present in spleen, heart, brain, lung, and kidney (Gardner et al., 1994). The hierarchy of these pathways varies depending on the extracellular stimuli, the specific enzymes expressed in the cell, and the cell types (Packer et al., 1995).

CLINICAL USES OF ALA

Alpha lipoic acid is effective in many pathological conditions where ROS have been implicated, including diabetes mellitus. Before considering these studies, however, it may be helpful to briefly discuss the pharmacokinetics of this compound.

In humans, LA is synthesized in liver and other tissues and is also obtained from both animal and plant sources in the diet. In animal tissue, higher concentrations of LA are found in the heart, liver, and kidney while spinach, broccoli, tomato, garden peas, brussel sprouts, and rice bran are among rich plant sources. Naturally occurring R-LA in foods covalently bound to lysine in proteins (lipoyllysine), while LA in supplements is free (Singh and Jialal, 2008). An oral dose of LA is rapidly absorbed from the gastrointestinal tract and appreciably increases plasma LA levels. Typically, 20–40% of orally administered racemic ALA is absorbed with peak plasma concentrations higher in the R isomer compared to the S-isomer (Gleiter et al., 1996; Hermann et al., 1996) suggesting better absorption in the former isomer. The amount of a given dose and the presence or absence of food may explain absorption variability. Rapid gastrointestinal uptake is followed by an equally rapid clearance, reflecting both transport into tissues as well as glomerular filtration and renal exertion. Experiments using radiolabeled ALA indicate that when 14C-labeled LA was administered to rats (intraperitoneal, oral, or by a nasogastric tube) 80% of the administered dose was either excreted or found in the tissues (Harrison and McCormick, 1974). LA is subject to extensive catabolism. The most common metabolites are bisnorlipoate, tetranorlipoate, and β-hydroxy-bisnorlipoate (Shay et al., 2009). In addition to its catabolism, some is rapidly reduced to DHLA, which is equally rapidly excreted from cells (Jones et al., 2002). In all animal models studied, LA and its metabolites are readily excreted, primarily in the urine (Biewenga et al., 1997). However, ALA is safe in patients with end stage renal failure as well as in liver disease (Bustamante et al., 1998; Teichert et al., 2005), and in fact, has been used in the treatment of several hepatic diseases with encouraging results (Berkson, 1999; Poh and Goh, 2009).

DIABETES AND OXIDATIVE STRESS

Diabetes is a chronic metabolic disorder that continues to be a major worldwide health issue. It is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action and is associated with chronic hyperglycemia and disturbances of carbohydrate, lipid, and protein metabolism. As a consequence of the metabolic derangements in diabetes, various complications develop including both macro- and micro-vascular dysfunctions

(Duckworth, 2001). Many studies have shown that diabetes mellitus (type 1 and 2) is associated with increased formation of free radicals and decreased antioxidant potential, leading to oxidative damage of cell components (Bashan et al., 2009). Direct evidence of oxidative stress in diabetes is provided by the measurement of oxidative stress markers such as plasma and urinary F2-isoprostane as well as plasma and tissue levels of nitrotyrosine and superoxide radicals ($O_2^{\bullet}-$; Ceriello et al., 2001; Oberg et al., 2004; Vega-Lopez et al., 2004). There are multiple sources of oxidative stress in diabetes, including non-enzymatic, enzymatic, and mitochondrial pathways.

Non-enzymatic sources of oxidative stress originate from the oxidative biochemistry of glucose. Hyperglycemia directly increases ROS generation since glucose undergoes autoxidation to generate •OH radicals (Turko et al., 2001). In addition, glucose reacts with proteins in a non-enzymatic manner leading to the development of Amadori products followed by formation of advanced glycation end products (AGEs). ROS is generated at multiple steps during this process. In hyperglycemia, there is enhanced metabolism of glucose through the polyol (sorbitol) pathway, which also results in enhanced production of $O_2^{\bullet}-$.

Enzymatic sources of augmented generation of ROS in diabetes include nitric oxide synthase (NOS), NAD(P)H oxidase, and xanthine oxidase (Guzik et al., 2000, 2002; Aliciguzel et al., 2003). All isoforms of NOS require five cofactors/prosthetic groups such as flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), heme, tetrahydrobiopterin (BH4), and Ca^{2+}-calmodulin. If NOS lacks its substrate L-arginine or one of its cofactors ("uncoupled" NOS), NOS produces $O_2^{\bullet}-$ instead of •NO (Aliciguzel et al., 2003; Maritim et al., 2003). A major source of $O_2^{\bullet}-$ production is NAD(P)H oxidase which is a membrane associated enzyme that consists of five subunits (Guzik et al., 2000, 2002; Etoh et al., 2003; Kitada et al., 2003). Guzik et al. (2002) investigated $O_2^{\bullet}-$ levels in vascular specimens from diabetic patients and probed sources of $O_2^{\bullet}-$ using inhibitors of NOS, NAD(P)H oxidase, xanthine oxidase, and the mitochondrial electron transport chain and reported that the enhanced production of $O_2^{\bullet}-$ in diabetic patients is predominantly mediated by NAD(P)H oxidase.

The mitochondrial respiratory chain is a non-enzymatic source of reactive species. During oxidative phosphorylation, electrons are transferred from the electron carriers NADH and FADH2, through four complexes in the inner mitochondrial membrane, to oxygen, and generate ATP in the process (Green et al., 2004). Under normal conditions, $O_2^{\bullet}-$ is immediately eliminated by natural antioxidant defense mechanisms. Cells have evolved highly complex antioxidant systems (enzymic and non-enzymatic), which work synergistically to protect cells and organ systems against free radical-induced damage. The most efficient enzymatic antioxidants involve glutathione peroxidase, catalase, and superoxide dismutase. Non-enzymatic antioxidants include vitamins E and C and thiol antioxidants (glutathione, thioredoxin; Golbidi and Laher, 2010). Each of these antioxidants can combine with reactive oxidants to produce other less reactive species that generally has reduced or no toxicity. Superoxide dismutase promotes the dismutation of the superoxide radical to form hydrogen peroxide (H_2O_2) and oxygen. Enzymes such as glutathione peroxidase (GPx) use reduced glutathione (GSH) as a reducing equivalent to reduce H_2O_2 to form oxidized glutathione and water. Furthermore, GSH can remove selected oxygen radicals directly and assist in the recycling of vitamin C and E. Catalase converts H_2O_2 to water and oxygen. The newly identified peroxiredoxin family, which has six isoforms in mammalian cells, represents a group of peroxidases that also catalyze the reduction of H_2O_2 (Cox et al., 2009). Among the six mammalian isoforms known so farm, peroxiredoxin III is synthesized with a mitochondrial targeting sequence, as is the case for MnSOD.

Hyperglycemia-induced generation of $O_2^{\bullet}-$ at the mitochondrial level is thought to be the major driver of the vicious cycle of oxidative stress in diabetes (Nishikawa et al., 2000; Brownlee, 2001). There is increased generation of ROS (especially $O_2^{\bullet}-$) when endothelial cells are exposed to clinically relevant hyperglycemic conditions. The augmented generation of pyruvate via accelerated glycolysis under hyperglycemic conditions is thought to flood the mitochondria and thus generates $O_2^{\bullet}-$ formation at the level of Complex II in the respiratory chain (Nishikawa et al., 2000).

Reactive oxygen species stimulates oxidation of LDL; ox-LDL is not recognized by the LDL receptor and is subsequently taken up by scavenger receptors in macrophages to form foam cells and so lead to atherosclerotic plaques (Boullier et al., 2001). Superoxide anions can activate several damaging pathways in diabetes including accelerated formation of AGE's, polyol pathway, hexosamine pathway, and protein kinase C (PKC), all of which are involved in micro and macrovascular complications. Both $O_2^{\bullet}-$ and H_2O_2 stimulate stress-related signaling mechanisms such as NF-κB, p38-mitogen activated protein kinases (p38-MAPK) and signal transducers and activators of transcription-Janus kinases (STAT-JAK), resulting in vascular smooth muscle cell migration and proliferation. In endothelial cells, H_2O_2 mediates apoptosis and pathological angiogenesis (Taniyama and Griendling, 2003). Furthermore, $O_2^{\bullet}-$ immediately reacts with •NO to generate cytotoxic peroxynitrite ($ONOO^-$) and this reaction itself has several consequences. First, $ONOO^-$ alters the function of biomolecules by protein nitration as well as by causing lipid peroxidation (Turko et al., 2001). For example, potassium channels, which regulate vasorelaxation, are inhibited by nitration (Liu and Gutterman, 2002; Liu et al., 2002). As reviewed by Turko et al. (2001) increased levels of nitrotyrosine are associated with apoptosis of myocytes, endothelial cells, and fibroblasts in diabetes. Importantly, $ONOO^-$ causes single-strand DNA breakage, which in turn activates nuclear enzyme poly(ADP-ribose) polymerase (PARP; a nuclear DNA-repair enzyme that is able to cause a depletion of NAD^+; Soriano et al., 2001). Additionally, $ONOO^-$ decreases •NO bioavailability causing impaired relaxation and inhibition of the anti-proliferative effects of •NO (Maritim et al., 2003). Furthermore, $ONOO^-$ oxidizes BH_4, an important cofactor for NOS, and causes uncoupling of NOS to produce $O_2^{\bullet}-$ instead of •NO (Maritim et al., 2003). ROS-induced peroxidation of membrane lipids alters the structure and the fluidity of biological membranes, which will have global effects that alter vascular function (Soriano et al., 2001; Liu and Gutterman, 2002; Liu et al., 2002; Griendling and FitzGerald, 2003; Maritim et al., 2003; Taniyama and Griendling, 2003).

ALA AS A THERAPEUTIC ADJUVANT

Lipoic acid has potential applications for many aspects of the pathology of diabetes. One important action of LA is on the expression of AMPK in the hypothalamus and peripheral tissues. The AMPK complex is evolutionally a well-conserved serine/threonine kinase that functions as a fuel sensor in the cell and is activated when cellular energy is depleted and AMP/ADP ratio rises (Kola et al., 2006). The result of AMPK activation is the inhibition of energy-consuming biosynthetic pathways and the activation of ATP producing catabolic pathways. AMPK can also affect transcription of specific genes involved in energy metabolism, thereby exerting long-term metabolic control (Winder, 2001). The heterotrimeric protein AMPK is formed by combination of the catalytic α subunit (with two isoforms α_1 and α_2) with β (two isoforms) and γ (three isoforms) subunits. The γ subunit includes four particular cystathionine beta synthase domains giving AMPK its ability to sensitively detect shifts in the AMP/ATP ratio (Cheung et al., 2000). The catalytic domain has a site of phosphorylation at a threonine residue (Thr172) that is an important site for AMPK activation by upstream kinases. Binding of AMP to the regulatory γ subunit of AMPK promotes (a) allosteric activation, (b) phosphorylation of Thr172 by upstream kinases and (c) inhibition of dephosphorylation by protein phosphatases. Cellular stresses that cause an increase in the AMP/ATP ratio such as hypoxia, oxidative stress, hypoglycemia, exercise, or nutrient deprivation can affect cellular metabolic conditions partially through this pathway (Kola et al., 2006). After the discovery of the effect of insulin-sensitizing anti-diabetic drugs such as metformin and thiazolidinediones on the activation of AMPK, much interest was generated toward targeting this pathway for the treatment of diabetes (Musi et al., 2002; Saha et al., 2004). *In vivo* and *in vitro* studies have shown that activation of AMPK leads to reduce glucose output form the liver (Viollet et al., 2009). Overexpression of hepatic AMPK also leads to mild hypoglycemia in normal mice and prevented hyperglycemia in diabetic mice (Foretz et al., 2005; Viana et al., 2006). This hypoglycemic effect of AMPK activation is accompanied by down regulation of gluconeogenic gene expression (e.g., phosphoenolpyruvate carboxykinase and glucose-6-phosphatase) and reduced glucose production in hepatocytes expressing activated MAPK or treated with AICAR (5-Aminoimidazole-4-carboxamide ribotide, metabolized to ZMP which is an analog of AMP; Lochhead et al., 2000; Foretz et al., 2005; Viana et al., 2006). Activation of AMPK in skeletal muscle, a major regulator of cellular energy metabolism, increases glucose uptake and fatty acid oxidation. AMPK stimulates GLUT4 translocation to the plasma membrane in an insulin independent manner and increases the expression of the GLUT4 gene through enhanced binding of the transcription factor MEF-2 (myocyte enhancer factor-2) to promoters in the GLUT4 gene (Konrad et al., 2001). Some studies have also shown that AMPK regulates glucose transport through GLUT1 (Xi et al., 2001).

Insulin sensitivity is also improved through reduced triglyceride accumulation by skeletal muscles (Evans et al., 2002). This occurs as a result of AMPK phosphorylating, and thus inactivation, of acetyl-CoA carboxylase (ACC), resulting in decreases in malonyl-coenzyme A (Winder and Hardie, 1996; Ruderman and Flier, 2001). ACC is an important rate-limiting enzyme for the synthesis of malonyl-CoA, which in turn is a critical precursor of fatty acids biosynthesis and a potent inhibitor of mitochondrial fatty acid oxidation. Decrease in malonyl-CoA content results in reduction of fatty acid synthesis and increases in fatty acid oxidation. Triglyceride accumulation in skeletal muscle contributes to insulin resistance in obesity associated insulin resistance and type 2 diabetes (Goodpaster and Kelley, 2002). Muscular AMPK is decreased in Obese Long Evans Tokushima Fatty rats, which are prone to diabetes (Lee et al., 2005a). When these rats are administered ALA, there is increased insulin stimulated whole body glucose disposal and also in skeletal muscle. ALA also increased fatty acid oxidation and stimulated AMPK in skeletal muscle (Lee et al., 2005a). Adenovirus mediated administration of dominant negative AMPK into skeletal muscle prevented the ALA induced increases in fatty acid oxidation and insulin stimulated glucose uptake. These results imply that ALA induced improvement of insulin sensitivity is mediated by activation of AMPK and reduced triglyceride accumulation in skeletal muscle (Lee et al., 2005a).

The exact mechanism underlying ALA stimulated AMPK activation remains to be clarified. To date, two upstream AMPK kinases (AMPKK) have been identified: LKB1 (serine–threonine kinase liver kinase B1, also known as STK11) and Ca/calmodulin dependent protein kinase kinase (CaMKK; Shackelford and Shaw, 2009). LKB1 was originally recognized as a tumor suppressor in humans, and may dictate most of the AMPK activation in the majority of tissues (except hypothalamic neurons Anderson et al., 2008, T cells Tamás et al., 2009, and endothelial cells Stahmann et al., 2006). Activation and phosphorylation of AMPK by LKB1 requires an increase in AMP. Binding of MAP to AMPK changes the conformation of AMPK, making it a better substrate for LKB1. Therefore, LKB1 mediates the prolonged and adaptive activation of AMPK following energy stress (Shackelford and Shaw, 2009). CaMKK phosphorylates and activates AMPK in an AMP independent manner; CAMKK is activated by a rise in intracellular Ca2+ concentrations (Hawley et al., 2005; Hurley et al., 2005). CaMKK has two isoforms, CaMKK-α (e.g., in skeletal muscle) and CaMKK-β (in neural tissues; Anderson et al., 1998; Jensen et al., 2007). In an attempt to clarify the mechanism(s) of ALA induced AMPK activation, Shen et al. (2007) reported that LKB1 may not be involved in this process, since ALA treatment of C2C12 myotubes increased the intracellular Ca2+ concentration, suggesting that ALA may activate AMPK by stimulating the CaMKK pathway. Chelation of intracellular free Ca2+ and inhibition of CaMKK abolished ALA induced AMPK activation, with ALA treatment increasing the association of AMPK with CaMKK.

AMPK is also expressed in the hypothalamus where it is implicated in the regulation of appetite (Andersson et al., 2004; Minokoshi et al., 2004). Activation of hypothalamic AMPK increases food intake and body weight, whereas inhibition of it is necessary for leptin to promote satiety and reduce food consumption (Andersson et al., 2004). Increases in energy expenditure, decreases hypothalamic AMPK activity and reduced plasma glucose, insulin, free fatty acid, and leptin have all been demonstrated in ALA treated rats (Kim et al., 2004; Lee et al., 2005b). Furthermore, intracerebroventricular injection of AICAR (an activator of AMPK) reversed the effects of ALA on food consumption. These

studies suggest that hypothalamic AMPK is involved in appetite regulation and ALA exerts anorexic, anti-obesity effects by suppressing hypothalamic AMPK activity. ALA suppresses AMPK phosphorylation and α_2 MAPK activity in the arcuate nucleus but not in the paraventricular nucleus or the lateral hypothalamic area (Kim et al., 2004). Recently, a report shows that ALA (1800 mg/day) reduced body weight in obese diabetic human subjects (Koh et al., 2011; **Table 1**).

As discussed earlier, some of the metabolic effects of LA could be secondary to its modifying effects on PPAR α/γ. Adenovirus mediated overexpression of PPAR coactivator-1α in the skeletal muscle increases mitochondrial respiration and elevates GLUT4 transcript levels (Michael et al., 2001). ALA increases mRNA expression of PPAR coactivator-1α, which might account for improved glucose homeostasis (Wang et al., 2010). It is also been shown that ALA prevented hyperglycemia-induced reduction of PPAR-γ, hyperinsulinemia, insulin resistance, systolic hypertension, and superoxide production (Midaoui et al., 2003; El Midaoui et al., 2006).

Alpha lipoic acid can have both detrimental and cytoprotective effects on pancreatic beta cells, based on the underlying pathophysiologic state and on the concentration of ALA. In type 2 diabetes, lowering of beta cell numbers is accompanied by a significant increase in beta cell apoptosis where oxidative stress is an important contributor (Butler et al., 2003; Marchetti et al., 2004). The prolonged AMPK activation by ALA leads to enhanced production of mitochondria-derived oxygen radicals and onset of an intrinsic mitochondrial apoptosis pathway (Targonsky et al., 2006; Cai et al., 2007). Using type 1 diabetic mice, Richards et al. (2005) reported that adenovirus mediated activation of AMPK in isolated mouse islets resulted in reduced glucose oxidation and insulin secretion and poorer glycemic control compared with islet cells infected with null or dominant negative AMPK viruses. However, a review of the literature indicates that ALA effects are concentration dependent (Bilska and Wlodek, 2005). For instance, in tumor cells LA induces cell proliferation at low concentrations (1 μmol/l) while it is anti-proliferative at higher concentrations (100 μmol/l; Dovinova et al., 1999). The concentration of ALA required for significant induction of AMPK activation in islet cells is at least 500 μmol/l (Targonsky et al., 2006), which is considerably greater than the plasma concentrations of ALA reached during treatment of diabetic neuropathy (Niebch et al., 1997; Chen et al., 2005). There are also several reports on the pro-oxidant effects of LA – which again, occur at different concentrations than those associated with its beneficial effects (Saris et al., 1998; Mottley and Mason, 2001; Dicter et al., 2002; Moini et al., 2002). However, it is reasonable to conclude that the beneficial/protective effects of ALA are most likely to occur under conditions of increased oxidative stress such as diabetes. For example, Lee et al. (2009) demonstrated dose-dependent, pro-apoptotic effects of LA in rat insulinoma cells, while in a related study, they also reported that pre-treatment with ALA reduced ROS production, mitochondrial membrane depolarization, and c-JNK activation in beta cells. It is believed that while ALA is detrimental to beta cells at high concentrations, it exerts cytoprotective effects on beta cells in diabetes at clinically relevant concentrations.

Another potential application for using LA as an adjuvant in the treatment of diabetes is related to its ability to inhibit glycation reactions. Although several mechanisms have been explained for the pathophysiology of chronic diabetic complications, protein glycation potentially can be an important underlying mechanism (Brownlee et al., 1988; Schalkwijk et al., 2002). Preventive effects of ALA in these reactions have been shown in different studies (Suzuki et al., 1992; Kawabata and Packer, 1994). Both LA and DHLA can protect albumin from glycation, suggesting that the preventive effect is independent of its redox state. It has been suggested that non-covalent hydrophobic binding to serum albumin is involved in this effect (Kawabata and Packer, 1994). Due to the presence of a hydrophobic carbon chain, it is likely that binding between albumin and α-lipoate is by hydrophobic interactions similar to that with fatty acids. This reaction leads to masking the glycation sites of serum albumin (Packer et al., 1995). Protective effects of ALA against LDL glycation have also been investigated in several studies (Kawabata and Packer, 1994; Schepkin et al., 1994). ALA was found not to protect LDL from glycation in the short term *in vitro* studies.

As stated earlier, ROS-induced vascular dysfunction is one of the main features of diabetic mellitus; this state of ROS accumulation is strongly associated with impaired endothelium dependent NO-mediated vasodilation. Activated PKC has a variety of effects on gene expression, such as decreased expression of eNOS and increases in the expression of endothelin, vascular endothelial growth factor, plasminogen activator inhibitor-1, transforming growth factor-β, NAD(P)H oxidases, and NFκB (which in turn activates many proinflammatory genes in the vasculature; Golbidi et al., 2011). LA improves the redox state of the plasma and endothelium dependent vasodilation (Heitzer et al., 2001; Sena et al., 2008). It is known that insulin receptor tyrosine kinase, phosphatidylinositol 3-kinase (PI 3-kinase), and Akt are essential components of insulin signaling pathways related to production of NO in vascular endothelium. Phosphorylation of endothelial nitric oxide synthase (eNOS) by Akt is also necessary for its activation by insulin (Montagnani et al., 2002). In aged endothelial cells, administration of LA partially restores the reductions in eNOS phosphorylation through Akt (Smith and Hagen, 2003). ALA also induces Akt phosphorylation in human umbilical vascular endothelial cells and the THP-1 human monocyte cell line (Artwohl et al., 2007; Zhang et al., 2007). These studies suggest that improved endothelial function due to ALA is at least partially attributed to recoupling of eNOS and increased NO bioavailability.

Asymmetric dimethylarginine (ADMA) is an endogenous NOS inhibitor that is produced by methylation of protein bound L-arginine in a reaction that is catalyzed by protein arginine methyltransferases (McBride and Silver, 2001). ADMA is excreted via the kidneys following metabolism by the enzyme dimethylarginine dimethylamino hydrolase (DDAH), which is sensitive to oxidative stress (Mittermayer et al., 2010). DDAH is expressed in different tissues such as kidneys, pancreas, brain, liver, lungs, endothelium, and myocardial cells (Kimoto et al., 1993; Nijveldt et al., 2003a,b). ADMA causes endothelial dysfunction in forearm resistance arteries (Calver et al., 1993) and increases the systemic vascular resistance and arterial blood pressure while decreasing cardiac output (Achan et al., 2003). An increased concentration

Table 1 | Selected clinical trials with ALA in diabetic patients during the last 10 years.

References #	Patient groups and characteristics	Treatment duration	Measured parameters	Outcome
METABOLIC, ANTI-INFLAMMATORY, AND ANTIOXIDANT EFFECTS				
Koh et al. (2011)	360 obese patients with DM, HT, or hypercholesterolemia randomized to: (a) ALA 1200 mg/day (b) ALA 1800 mg/day (c) Placebo	20 weeks	– BW, waist circumference, body fat, BP, FBS, TC, LDL, HDL, TG	– ALA 1800 mg/day led to a modest weight loss in obese subjects
Palacka et al. (2010)	59 patients with T2D in three groups: (a) PL ($n = 19$; 10 min, bid) (b) QALA ($n = 20$; 60 mg CoQ10 + 100 mg ALA + 200 mg Vit E) (c) PL + QALA	3 months	– CRP – LDH – MDA – Serum antioxidant level (CoQ10, α and τ-tocopherol, β-carotene) – Echocardiographic parameters of left ventricular function	– Combined therapy had better results in increasing antioxidant levels, decreasing MDA and improving left ventricular function
Mittermayer et al. (2010)	30 T2D patients (a) ALA (600 mg/day, iv) (b) Placebo	21 days	– Blood levels of ADMA (NOS inhibitor).	– ALA decreased plasma levels of ADMA.
Gianturco et al. (2009)	14 T2D patients (a) ALA (400 mg/day) (b) Placebo	4 weeks	– Markers of oxidative stress (assessed by commercially available test, d-ROMs) – BAP – Lipid profile, CRP	– ALA decreased markers of oxidative stress and HDL, had a borderline effect on BAP ($p = 0.06$) and LDL ($p = 0.07$) – No significant effect on CRP, TC, and TG
Huang and Gitelman (2008)	40 adolescents with T1D (a) Controlled release ALA (17 mg/kg/day) (b) Placebo	90 days	– 8-hydroxy-2′ deoxyguanosine -2-TBARS – Protein carbonyl – Total reactive antioxidant potential – HbA_{1c} – Urine albumin to creatinine ratio	– No significant differences in any of the measured parameters
Kamenova (2006)	– 12 T2D patients compared to 12 healthy subjects (a) ALA (600 mg/day)	4 weeks	– Insulin sensitivity	– ALA increased insulin sensitivity in diabetic patients
Ansar et al. (2011)	57 T2D patients (a) ALA (300 mg/day) (b) Placebo	2 months	– FBS – 2 h PPG – Serum insulin level – GH-Px	– Decreases in FBS and PPG, insulin resistance and GH-Px in treated group.
Zhang et al. (2011)	22 obese subjects with IGT (a) ALA (600 mg/day, iv, $n = 13$) (b) Placebo ($n = 9$)	2 weeks	– ISI – FFAs – LDL, sd-LDL, ox-LDL, VLDL, TG, TC – MDA, 8-iso-PG – TNF-α, IL-6	– In treated group ISI improved. Decreases in FFAs, TG, TC, LDL, sd-LDL, ox-LDL, VLDL, MDA, 8-iso-PG, TNF-α, and IL-6
de Oliveira et al. (2011)	102 T2D patients (a) ALA (600 mg/day, $n = 26$) (b) α-tocopherol (800 mg/day, $n = 25$) (c) α-tocopherol + ALA (d) Placebo	4 months	– Plasma α-tocopherol – Lipid profile – Glucose – Insulin – HOMA index	– Improved lipid fractions in the LA, vitamin E, and combined groups, HOMA index in LA group – All the above were not significant statistically

(Continued)

Table 1 | Continued

References #	Patient groups and characteristics	Treatment duration	Measured parameters	Outcome
Rahman et al. (2011)	40 diabetic patients with stage I hypertension (a) Quinapril (QUI) 40 mg/day (b) QUI + ALA (600 mg/day)	8 weeks	– BP – 24 h urinary albumin – Endothelial dependent FMD	– Urinary albumin decreased by 30% in the QUI group and 53% in QUI + ALA – FMD increased 58% in QUI group and 116% in QUI + ALA – QUI decreased BP by 10%, no further reduction in combined group
DIABETIC POLYNEUROPATHY				
Ziegler et al. (2006)	– 181 T2D patients (a) ALA (600 mg/day) (b) ALA (1200 mg/day) (c) ALA (1800 mg/day) (d) Placebo	5 weeks	– Evaluation of neuropathic pain based on TSS, neuropathy symptoms and change score, neuropathy impairment score, and patients' global assessment	– ALA improved neuropathic symptoms (600 mg/day, had the optimal risk to benefit ratio)
Tankova et al. (2004)	46 T1D patients with different forms of autonomic neuropathy (a) ALA (600 mg/day, i.v.) 10 days followed by ALA (600 mg/day, oral) 50 days (b) Control group	60 days	– Scoring different signs and symptoms of autonomic neuropathy – Laboratory parameters of oxidative stress	– ALA alleviated diabetic autonomic neuropathy – Increased serum antioxidant capacity
Hahm et al. (2004)	38 (out of 61) T2D with symptomatic polyneuropathy (a) ALA (600 mg/day)	8 weeks	– Primary efficacy parameter (TSS for neuropathic symptoms) – Secondary efficacy parameters (clinical neurological assessment, overall rating by the physician and patients at the end of treatment) – Laboratory measurements (HbA_{1c}, FBS)	– Improvement of polyneuropathy symptoms (decreased TSS score) – FBS and HbA_{1c} did not change
Ametov et al. (2003)	120 T2D patients (a) ALA (600 mg/day, i.v., 5 days a week for 14 doses) (b) Placebo	~3 weeks	– TSS – Score of neuropathy signs – Score of neuropathy symptoms and change – Quantitative sensation tests	– ALA significantly improved neuropathic symptoms
Gu et al. (2010)	236 T2D with polyneuropathy (a) ALA (600 mg/tid $n = 117$) (b) Placebo ($n = 119$)	12 weeks	– TSS – NCV – Individual symptom score – HbA1c and safety parameters	– TSS and individual symptom scores decreased significantly – No changes in NCV – Major side effect was burning sensation in esophagus.
Ametov et al. (2010)	T2D patients with myodiabetic polyneuropathy (a) ALA (600 mg/day, iv) (b) Placebo	3 weeks	– TSS – NIS-LL	– Decreases in TSS and NIS-LL score in treated individuals
Ziegler et al. (2011)	460 T2D patients with mild to moderate DSPN (a) ALA (600 mg/day, $n = 233$) (b) Placebo ($n = 227$)	4 years	– NIS and NIS-LL – NCV – QSTs	– Changes from baseline were better with ALA than placebo for NIS, NIS-LL, and NIS-LL muscular weakness subscores
Burekovic et al. (2008)	100 diabetic patients (type I and II) (a) ALA 600 mg/day, iv, followed by 3 weeks of 300–600 mg/day, *per os*	3 months	– Subjective and objective assessment of polyneuropathy symptoms	– ALA is effective in reducing the symptoms of diabetic polyneuropathy

(Continued)

Table 1 | Continued

References #	Patient groups and characteristics	Treatment duration	Measured parameters	Outcome
DIABETIC ANGIOPATHY				
Heinisch et al. (2010)	30 T2D patients (a) ALA (600 mg/day, iv) (b) Placebo	21 days	– Endothelium dependent and independent vasodilation, assessed by forearm blood flow.	– ALA improved endothelium dependent vasodilation.
Xiang et al. (2008)	– 42 subject with IGT test and 26 health controls (a) 300 mg ALA before GTT (b) Placebo		– Endothelium dependent FMD	– ALA improved endothelial dysfunction during acute hyperglycemia.
Vossler et al. (2007)	114 T2D patients (a) Tromethamine salt of R-ALA (dexlipotam; 960 mg/day) (b) Dexlipotam (1920 mg/day) (c) Placebo	4 weeks	– Percentage change in the FMD of brachial artery.	– No significant difference in FMD – Tendency toward a reduction of inflammatory markers and BP
DIABETIC RETINOPATHY				
Haritoglou et al. (2011)	467 T2D patients (a) ALA (600 mg/day, $n = 235$) (b) Placebo ($n = 232$)	2 years	– CSME	– 600 mg/day ALA did not prevent CSME
DIABETIC NEPHROPATHY				
Chang et al. (2007)	– 50 diabetic patients with ESRD who undergoing hemodialysis (3 times/week) (a) ALA (600 mg/day) (b) Control group	12 weeks	– ADMA – TC, hsCRP, ox-LDL, albumin, HbA1c	– ALA decreased ADMA levels significantly.
Morcos et al. (2001)	– 84 diabetic patients (T1D and T2D) (a) 35 patients (20 T1D, 15 T2D, ALA 600 mg/day) (b) 49 patients as controls	18 months	– Plasma thrombomodulin – Urinary albumin concentration (UAC)	– Plasma thrombomodulin decreased in treated group (increased in controls) – UAC unchanged in treated group (increased in controls)

ALA, alpha lipoic acid; ADMA, asymmetric dimethylarginine; BAP, biological antioxidant potential; BP, blood pressure; CRP, C reactive protein; CSME, clinically significant macular edema; DSPN, distal symmetric sensorimotor polyneuropathy; ESRD, end stage renal disease; FBS, fasting blood sugar; FMD, flow mediated dilatation; FFA, free fatty acid; GTT, glucose tolerance test; GH-Px, glutathione peroxidase; HDL, high density lipoproteins; hsCRP, high sensitivity CRP; HOMA index, homeostatic model assessment; IGT, impaired glucose tolerance; ISI, insulin sensitivity index; IL-6, interleukin-6; 8-iso-PG, 8-iso-prostaglandin; LDL, low density lipoprotein; MDA, malondialdehyde; NCV, nerve conduction velocity; NIS, neuropathy impairment score; NIS-LL, neuropathy impairment score in the lower limbs; NOS, nitric oxide synthase; ox-LDL, oxidized LDL; PL, polarized light; PPG, postprandial glucose; QSTs, quantitative sensory tests; TBARS, thiobarbituric acid reactive substances; TC, total cholesterol; TG, triglyceride; TNF-α, tumor necrosis factor alpha; TTS, total symptom score; VLDL, very low density lipoprotein.

of ADMA predicts cardiovascular events in different populations including T2D patients (Böger et al., 1997, 1998; Surdacki et al., 1999; Abbasi et al., 2001; Zoccali et al., 2001; Mittermayer et al., 2002; Stühlinger et al., 2002; Tarnow et al., 2004; Krzyzanowska et al., 2005, 2006, 2007). Indeed, ADMA may not only be a risk marker but also an etiologic factor in adverse cardiovascular events (Krzyzanowska et al., 2008). Reduced degradation of ADMA due to decreased DDAH activity has been proposed as an important mechanism for endothelial dependent vasodilator dysfunction in hypercholesterolemia (Ito et al., 1999) and diabetes (Lin et al., 2002). It has been reported that ALA decreases ADMA levels in the culture media of endothelial cells by increased expression and activity of DDAH and also of signal transducer and activator of transcription (STAT)3 phosphorylation. ALA also amplified STAT3 transfection-induced increase in DDAH II promoter activity (Lee et al., 2010). In two other studies, ALA was shown to reduce ADMA in diabetic patients with end stage renal disease (Chang et al., 2007) and with normal renal function (Mittermayer et al., 2010). These data provide a novel mechanism by which ALA regulates endothelial function.

Diabetic neuropathy is another potential clinical indication for ALA usage. At present, it is believed that several mechanisms contribute to the pathogenesis of this condition. These mechanisms for diabetic neuropathy are no longer being considered as being separate, but rather as a complex interplay giving rise to multiple interactions. The following mechanisms are thought to be involved in diabetic neuropathy: (a) increased flux through the polyol pathway with resulting sorbitol and fructose accumulation. Sorbitol production produces compensatory depletion of other organic osmolytes such as myo-inositol and taurine. The latter is an endogenous antioxidant, whose reduction leads to attenuation of oxidative defenses (Ziegler, 2006). Depletion of myo-inositol is associated with reduced Na^+–K^+-ATPase activity (Vallianou et al., 2009). (b) Endoneurial micro-vascular damage and hypoxia due to nitric oxide inactivation (Kishi et al., 1999). (c) Accumulation of AGEs and subsequent activation of NF-κB (Ziegler, 2006). (d) Homocysteinemia (Ambrosch et al., 2001). (e) Increased nerve lipid peroxidation (Low et al., 1997). (f) Modulation of MAPKs (Cavaletti et al., 2007). (g) Abnormal Ca^{2+} homeostasis and signaling (Hall et al., 2001) and (h) Decreased expression and level of neurotrophic factors, such as nerve growth factor, neurotrophin 3, and insulin like growth factor as well as alterations in axonal transport. LA has direct or indirect corrective effects on nearly all of these mechanisms. In a small study on a group of diabetic patients with neuropathy, ALA increased low pre-treatment level of plasma nitrates and nitrites, which are commonly, used markers of NO production. Increased NO production theoretically increases circulation to the neurons (Strokov et al., 2000). In another *in vitro* study, addition of ALA resulted in reduction of hyperglycemia-induced lipid peroxidation in both brain and sciatic tissues as measured by TBARS (Nickander et al., 1996). This effect on lipid peroxidation was confirmed in a study of 10 diabetic patients with peripheral neuropathy. A single daily dose of 600 mg LA for 70 days reduced serum lipid peroxidation (Androne et al., 2000). ALA also reduces protein glycosylation (measured by glycated hemoglobin), lower lipid peroxidation, and increase Na^+/K^+-ATPase activity in human red blood cells exposed to high glucose concentration (Jain and Lim, 2000).

The effects of LA on diabetic neuropathy have also been studied in streptozotocin-induced diabetic rats, where ALA promoted glucose uptake by nerve cells (Kishi et al., 1999), increased nerve myo-inositol (Kishi et al., 1999; Stevens et al., 2000), GSH levels (Böger et al., 1998; Stühlinger et al., 2002), Na/K-ATPase activity (Stevens et al., 2000), and nerve blood flow (Nagamatsu et al., 1995) while normalizing NAD/NADH ratios (Stevens et al., 2000).

CONCLUSION

The many unique properties of ALA and its interaction with other important antioxidant such as vitamin E, ascorbate, and GSH provide a fertile field for continued research. A number of experimental and clinical studies have shown the beneficial effect of ALA as a therapeutic agent for a diverse spectrum of diseases from heavy metal poisoning, radiation damage, ischemia-reperfusion injury, and neurodegenerative diseases to managing metabolic syndrome, diabetes, and its various complications. ALA/DHLA redox couple affects important biological processes including the regulation of several gene transcriptions and the activity of enzymes and receptors. Specifically in diabetes, it prevents beta cell destruction, enhances glucose uptake, and its antioxidant effects may be particularly useful in slowing the development of diabetic complications such as diabetic neuropathy. Given the growing evidence for increased oxidative stress and inflammatory pathways activated in diabetes, therapeutic paradigms may have to shift to target these processes through the use of compounds such as ALA. So far, the strongest evidence for the clinical use of ALA is from double blind, placebo controlled trials showing that LA administration significantly ameliorates polyneuropathies associated with diabetes. However, further work is necessary to assess the potential therapeutic roles for other diabetes-associated complications such as retinopathy and cataract. The lack of clear-cut benefits in some of the clinical trials to date does not disprove the benefits of ALA. It is believed that better designed experiments with appropriate doses, duration, and the selection of populations based on a specific pathophysiology may provide evidence for some of the hidden therapeutic potential of ALA.

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Ginsenosides as anticancer agents: *in vitro* and *in vivo* activities, structure–activity relationships, and molecular mechanisms of action

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Conventional chemotherapeutic agents are often toxic not only to tumor cells but also to normal cells, limiting their therapeutic use in the clinic. Novel natural product anticancer compounds present an attractive alternative to synthetic compounds, based on their favorable safety and efficacy profiles. Several pre-clinical and clinical studies have demonstrated the anticancer potential of *Panax ginseng*, a widely used traditional Chinese medicine. The anti-tumor efficacy of ginseng is attributed mainly to the presence of saponins, known as ginsenosides. In this review, we focus on how ginsenosides exert their anticancer effects by modulation of diverse signaling pathways, including regulation of cell proliferation mediators (CDKs and cyclins), growth factors (c-myc, EGFR, and vascular endothelial growth factor), tumor suppressors (p53 and p21), oncogenes (MDM2), cell death mediators (Bcl-2, Bcl-xL, XIAP, caspases, and death receptors), inflammatory response molecules (NF-κB and COX-2), and protein kinases (JNK, Akt, and AMP-activated protein kinase). We also discuss the structure–activity relationship of various ginsenosides and their potentials in the treatment of various human cancers. In summary, recent advances in the discovery and evaluation of ginsenosides as cancer therapeutic agents support further pre-clinical and clinical development of these agents for the treatment of primary and metastatic tumors.

Keywords: *Panax* genus, *Panax ginseng*, ginsenosides, anticancer activities, structure–activity relationship, molecular mechanism, pre-clinical pharmacology, clinical trials

INTRODUCTION

A BRIEF HISTORY OF GINSENG AND GINSENG PRODUCTS

Panax ginseng was discovered over 5000 years ago on the hills of Manchuria in China and since then held its place as a highly venerated medicinal plant in Traditional Chinese Medicine (Hemmerly, 1977). The ancient Chinese used it for various health benefits, ranging from overcoming fatigue to treating severe cardiac problems; ancient texts showed that ginseng was valued more than gold and was often reserved for treatment of royalty. The "*Shennong Bencao Jing*" written around 100 A.D. attributed longevity boosting properties to ginseng and stated that it was good for "enlightening the mind and increasing the wisdom" (Dharmananda, 2002).

The discovery of an American counterpart for this "miracle" plant happened in 1714, due to the efforts of Father Lafitau, a Jesuit priest, working with the Iroquois in Canada. Based on the theory that ginseng might grow wild in the Americas, due to a similarity in climactic conditions with China, Father Lafitau undertook a laborious search for the plant and eventually discovered that it was used as a medicinal herb by the native Indians too (Yun, 2001; Grafton, 2002). The British physicians F. Porter Smith and G. A. Stuart, working in China at the end of the nineteenth century, were among the first to introduce the medicinal qualities of this herb to a Western audience (Dharmananda, 2002). The popularity of ginseng in the western world grew as a "drug weary" population medicated heavily on increasingly toxic chemical compounds started to explore alternative and traditional forms of medicine. Ginseng began to be sold in the form of liquid extracts, capsules, chewing gum, teas, candy, and cigarettes. It was used for treating rheumatism, anemia, insomnia, and various other problems. Some even purchased ginseng products for their alleged aphrodisiac properties. Ginseng fragrances were incorporated in cosmetics and soaps. By 1980s an estimated five to six million Americans were routinely using ginseng products (Carlson, 1986). In fact, the sale of ginseng products in the United States alone was estimated to reach US$83 million in 2010.

In the early 1970s, reports, primarily from China and Korea, started claiming that the herb stimulated protein synthesis, lowered blood sugar and cholesterol levels, regulated rate of metabolism, protected against stress, and could therefore reduce mortality. Asiatic ginseng from eastern Siberia was reportedly used by Soviet cosmonauts and Olympic team trainees to reduce fatigue (Carlson, 1986). During the same period, the Fromm Operation based in Wisconsin funded research on the possibility of using compounds from American ginseng in cancer research (Carlson, 1986). Since then, ginseng's usefulness in cancer has been shown by extensive

pre-clinical and epidemiological studies (Attele et al., 1999; Court, 2000; Bespalov et al., 2001; Yoo et al., 2006). It is also demonstrated that prolonged oral administration of red ginseng extract (RGE) to rats dosed with a carcinogen such as DMBA, urethane, or aflatoxin B1, reduced the incidence and mass of tumors (Yun et al., 1983). Additionally, red ginseng has been shown to improve postoperative survival in patients with stage III gastric cancer (Suh et al., 2002). There is an increasing interest in developing ginseng products as anticancer agents, especially in combination therapy.

ETHNOBOTANY

The word *Panax*, first used by the Russian botanist Carl Anton Von Meyer, is derived from the Greek word meaning "all-healing" (Court, 2000). On the other hand, the English word ginseng is derived from the Chinese term "rénshen" which literally means "man root": a reference to the root's resemblance to the legs of a human. The *Panax* genus belongs to the family Araliaceae and consists of at least nine species, including *P. ginseng* (Asian ginseng), *P. quinquefolium* L. (Xiyangshen, American ginseng), *P. notoginseng* (Sanqi), and *P. japonicas* (Japanese ginseng; Lee and Zhao, 2009). Among them, *P. ginseng* is the most widely used ginseng and is indigenous to the Far East countries, most notably China and Korea. *P. quinquefolium* is indigenous to the United States and Canada (Lee and Zhao, 2009). Out of these, *P. ginseng* C. A. Meyer, *P. quinquefolium* L, and *P. notoginseng* have been extensively investigated for their physiological and pharmacological effects on human body.

Ginseng is a perennial herb, 60–80 cm tall. Its root, often 5–6 cm long, is fleshy, bifurcate, aromatic, and grayish white to amber yellow. The surface of root is wrinkled and furrowed, and its taste is sweetish at first, with a somewhat bitter after taste. Ginseng stem is simple, erect, and deep red; its flower is pink; and its fruit, a small berry, is red. The leaves are compound, digitate, oval, and thin. There are a total of five leaflets and the three terminal leaflets are larger than the two lateral ones (Yun, 2001; Coates et al., 2005).

CHEMICAL CONSTITUENTS OF PLANTS FROM THE GENUS *PANAX*

In general, the active or inactive chemical entities obtained from ginseng species can be classified into four categories as follows.

SAPONINS (OR SAPOGENIN GLYCOSIDES)

Saponins are glycosides consisting of a sapogenin moiety, which may be a steroid or a triterpene, attached to one or more sugar moieties. Ginseng saponins are generally called ginsenosides and are the main active principals of ginseng. They are often used as a marker for the quality control of ginseng drugs and commercial products. For example, the ginsenoside Rf is unique to Asian ginseng while F11 is found exclusively in American ginseng. Thus the Rf/F11 ratio is used as a phytochemical marker to distinguish American ginseng from Asian ginseng (Li et al., 2000; Assinwe et al., 2003).

The basic structures of ginsenosides are similar, and most ginsenosides consist of a dammarane steroid nucleus with 17 carbon atoms arranged in four rings. Ginsenoside structures are first elucidated by the Shibata group, and named as Rx according to their mobility on TLC plates, with polarity decreasing from index "a" to "h" (Sanada et al., 1974; Liu and Xiao, 1992). According to a Ginseng Evaluation Program led by the American Botanical Council of Austin, Texas, the ginsenosides Rb1, Rb2, Rc, Rd, Re, and Rg1 account for >90% of the total ginsenoside content of the *P. ginseng* root (Foster, 1996a,b; Chang et al., 2003). Whereas, ginsenosides Rb1, Re, Rd, Rc, Rg1, and Rb3, the six major saponins, make up more than 70% of total ginsenoside content in American ginseng (Wang et al., 2005). Ginsenosides are amphipathic in nature. The hydroxyl (OH) group attached to the steroid backbone in ginsenosides allows interactions between the polar head of the membrane phospholipids and the β-OH group of cholesterol, while the hydrophobic steroid backbone can interact with the hydrophobic side chains of fatty acids and cholesterol. These physicochemical interactions are mostly determined by the numbers and sites of polar hydroxyl groups on each ginsenoside. Based on such properties, ginsenosides with different numbers and sites of hydroxyl groups exhibit diverse activities. Till date, more than 100 ginsenosides have been isolated from *Panax* species and most of them exhibit four types of aglycone moieties (Liu and Xiao, 1992; Coates et al., 2005). Ginsenosides are classified into the following types based on the steroidal skeleton and number of hydroxyl groups/sugar moieties attached.

Protopanaxadiol

Protopanaxadiols (PPD; **Figure 1**) belong to the dammarane-type ginsenosides, such as ginsenosides Ra1, Ra2, Ra3, Rb1, Rb2, and Rb3, notoginsenoside R1, R2, Rs1, and Rs2, quinquenoside R1, and malonylginsenoside Rb1, Rb2, Rc, and Rd (Sanada et al., 1974; Kasai et al., 1983). More than 30 ginsenosides of the PPD type belong to the Rb series (Dharmananda, 2002). The metabolic pathway of the PPD-type ginsenosides has been extensively investigated, resulting in identification and characterization of several active metabolites, notably compound K (also referred to as compound M1 or IH-901; Hasegawa et al., 1996).

Protopanaxatriol

Protopanaxatriols (PPT; **Figure 2**) are also dammarane-type ginsenosides, including ginsenosides Re, Rf, Rg1, and notoginsenoside R1. The main structural difference between PPT and PPD is the presence of a hydroxyl group/sugar residue at C-6 in the PPT moiety (Sanada et al., 1974; Kasai et al., 1983).

Oleanolic acid (or aglycone oleanolic acid)

This type, including ginsenosides Ro, has a pentacyclic triterpene skeleton (**Figure 2**).

Ocotillol

This group has a five-membered epoxy ring at C-20 (**Figure 2**; Nakamura et al., 2007). Majonoside R2 from the Vietnamese ginseng is a prototype for this group (Konoshima et al., 1999).

POLYSACCHARIDES

Ginseng polysaccharides are water-soluble compounds containing various sugar moieties conjugated to uronic acid and include panaxanes A to U. They are acidic polysaccharides and have been shown to possess immunomodulating and anti-proliferative effects. Recent studies have identified an acidic polysaccharide,

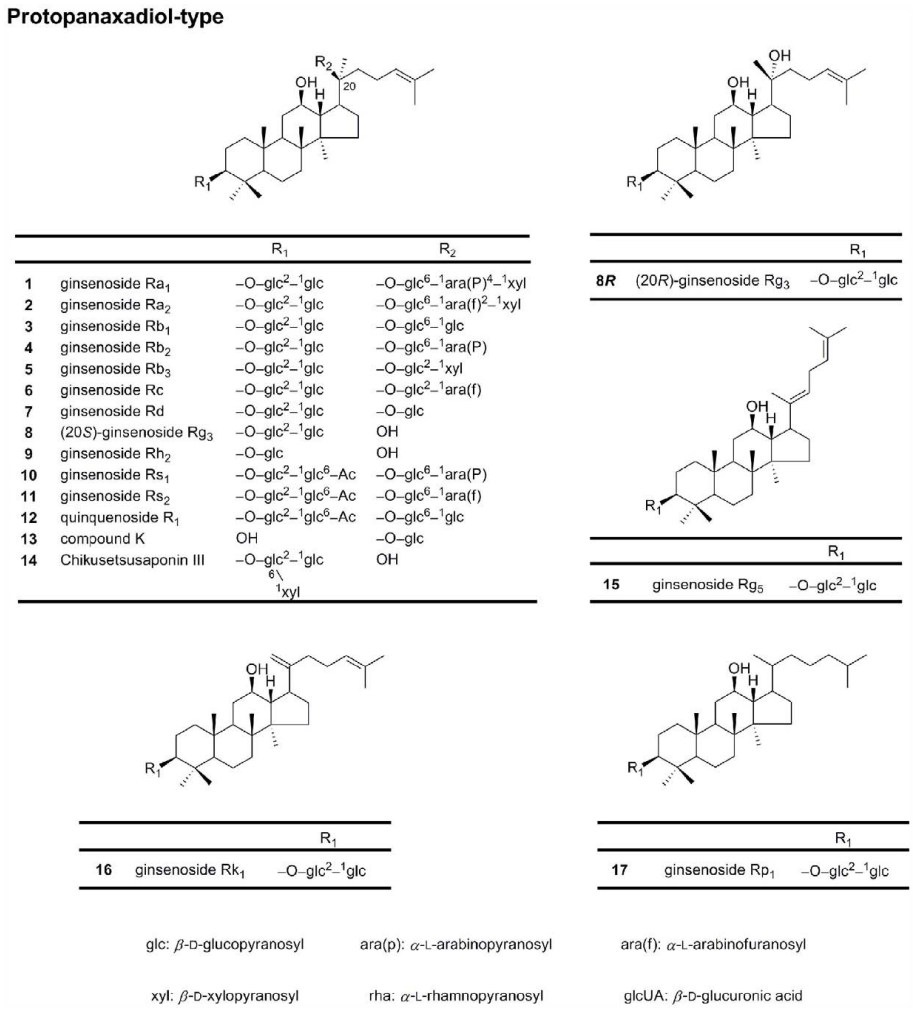

FIGURE 1 | Chemical structures of protopanaxadiol-type ginsenosides.

referred to as "ginsan," with immunostimulatory activity (Kim et al., 1998; Shim et al., 2007).

POLYYNES

The polyynes are a group of organic compounds with alternating single and triple bonds. In ginseng, these include falcrinol (panaxynol), falcarintriol (panaxytriol), acetic acid, or linolenic acid (Lee and Zhao, 2009).

FLAVONOIDS AND VOLATILE OILS

Besides the compounds mentioned above, some flavonoids and volatile oils also have been isolated and identified from *Panax* genus (Lee and Zhao, 2009).

STRUCTURE–ACTIVITY RELATIONSHIPS FOR GINSENOSIDE ANTICANCER ACTIVITY

Ginseng saponins (except oleanic acid-type ginsenosides) possess a four *trans*-ring rigid steroid skeleton, with a modified side chain at C-20 and sugar residues attached to the -OH of the aglycone. Structurally, most of the ginsenosides belong to PPD and PPT groups (Qi et al., 2010). For the PPD group, the sugar residues are attached to the β-OH at C-3 and another -OH at C-20 of the aglycone, e.g., ginsenosides Rb1, Rb2, Rc, Rd, Rg3, and Rh2. For the PPT group, sugar residues are attached to the α-OH at C-6 and another -OH at C-20 of the aglycone sapogenin, e.g., ginsenosides Re, Rg1, Rh1, and notoginsenoside R1 (Christensen, 2009). Structure–activity relationships elucidate the association between chemical structures and the pharmacological activities for a series of compounds. As summarized in **Table 1**, the anticancer activities of ginseng saponins are related to the following factors, including the number of sugar molecules, number and position of hydroxyl groups, and stereoselectivity (Qi et al., 2010).

NUMBER OF SUGAR MOLECULES

Anticancer activities increase with the decrease in the number of sugar moieties in a ginsenoside molecule. Ginsenosides with four or more sugar molecules, such as Rb1 and Rc, show no significant anti-proliferative effects (Yue et al., 2006; Li et al., 2009; Wang et al., 2009a); while Rd with three sugar residues weakly inhibits the growth of cancer cell (Yang et al., 2006; Li et al., 2009).

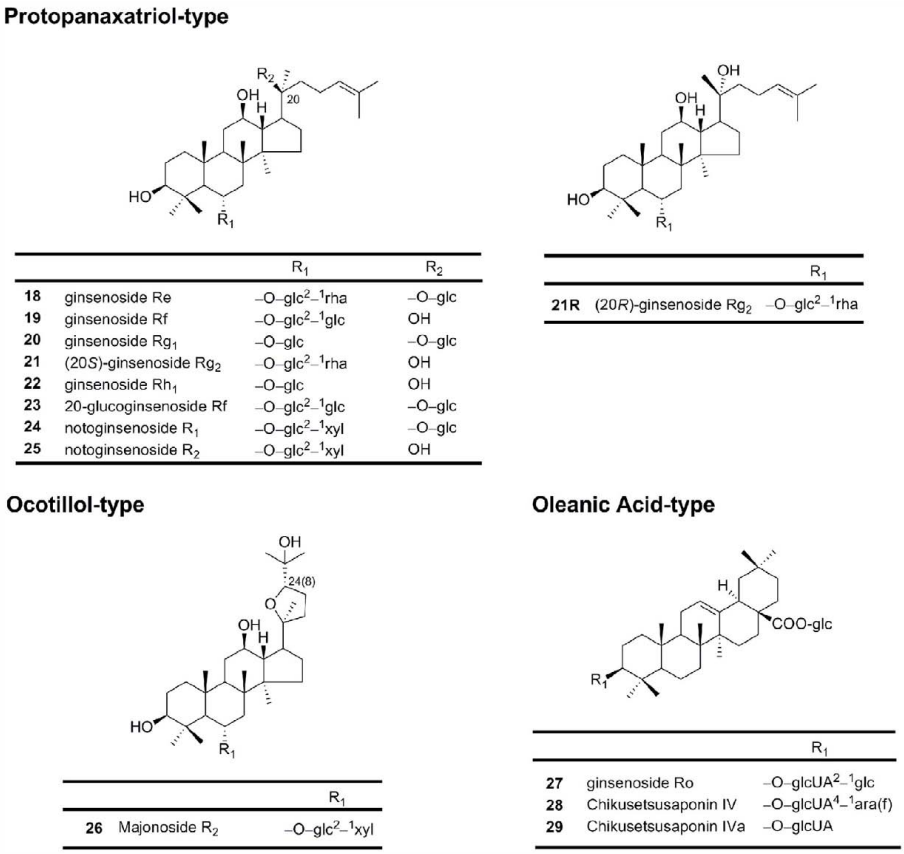

FIGURE 2 | Chemical structures of protopanaxatriol-type, ocotillol-type and oleanic acid-type ginsenosides.

Ginsenosides Rg3 (two sugar residues), Rh2 (one sugar residue), IH-901 (one sugar residue), PPT (no sugar residues), and PPD (no sugar residues) inhibit different types of cancer cells and also enhance the efficacy of conventional chemotherapy agents when given along with them (Hasegawa et al., 1995; Yue et al., 2006; Wang et al., 2007a; Yu et al., 2007). Rh2, 25-OH-PPD, and PPD show 5- to 15-fold relatively stronger anti-proliferative effects than Rg3 (Wang et al., 2007b). Among these compounds, PPD with no sugar residue shows the most potent anticancer activity. Qi et al. (2010) claim that introducing acetyl groups at selected positions increases the anticancer activity of PPD. It has also been suggested that neoglycorandomization might also increase anticancer properties (Langenhan et al., 2005). One might also expect to improve anticancer activity if heteroatoms like nitrogen and sulfur atoms are integrated into the steroid core structure or on the side chain.

Introduction of lipophilic groups in the side chains has been shown to increase anti-proliferative activity (Qi et al., 2010). For example, between the novel ginsenosides 25-OH-PPD and 25-OCH_3-PPD, both isolated from the fruits of *P. notoginseng*, the latter shows a lower IC_{50} value in pancreatic cancer cell line (Wang et al., 2009b,c). This type of modification may also improve the pharmacokinetic properties of the compound (Wang et al., 2008b, 2009a,b). A related linear relationship has been observed between Log Ps (also referred to as the lipophilicity) and Log IC_{50} of ginsenosides (Li et al., 2009). The presence of sugar moieties reduces the hydrophobic character of the compounds and decreases the permeability of the cell membrane. As interaction with the cell membrane decreases, so do the anticancer effects. Thus we see that in general, anticancer activity is inversely correlated to the number of sugar residues; a fact that may be attributed to the cellular uptake capability of the ginsenosides. The cellular uptake ratios of ginsenosides on cancer cells probably decrease with the increase of sugar numbers, due to progressive decrease in hydrophobicity (Ha et al., 2010).

SUGAR LINKAGE AT C-6

Differences in sugar linkage positions may also influence biological responses. The C-6 substituent differentiates the PPD and PPT groups of ginsenosides structurally. Ginsenoside Rh2 (PPD type) and Rh1 (PPT type), which possess a glucose linkage at C-3 and C-6, respectively, have similar chemical structures, but the anticancer effect of Rh2 is stronger than that of Rh1. F1 (20-*O*-glc-PPT) and Rh1 (6-*O*-glc-PPT) have the same number of sugar moieties and the same molecular weight, with different glucose attachment positions at C-20 and C-6, respectively. In a study on prostate cancer cell lines, F1 showed significantly higher cell inhibition than Rh1 (Li et al., 2009).

Table 1 | Possible structure–activity relationships of ginsenosides against cancer.

Ginsenoside	Nucleus	No. of sugars	Position of sugars/OH				Anticancer activity	Reference
			C-3	C-6	C-12	C-20		
Ra1	PPD	5	2	0	1-OH	3	None reported	
Ra2	PPD	5	2	0	1-OH	3	None reported	
Rb1	PPD	4	2	0	1-OH	2	Weakly anti-proliferative; anti-angiogenic	Leung et al. (2007)
Rb2	PPD	4	2	0	1-OH	2	Weakly anti-proliferative; anti-angiogenic	Mochizuki et al. (1995), Park and Cho (2009)
Rb3	PPD	4	2	0	1-OH	2	No anti-anti-proliferative activity	Xie et al. (2009)
Rc	PPD	4	2	0	1-OH	2	No anti-proliferative activity	Wang et al. (2009a), Li et al. (2009)
Rd	PPD	4	2	0	1-OH	2	No significant anti-proliferative activity	Wang et al. (2009a), Li et al. (2009)
Compound K	PPD	1	1	0	1-OH	0	Weakly inhibits cancer cell growth	Wang et al. (2007a)
Re	PPT	3	1-OH	2	1-OH	1	Not reported	
Rf	PPT	1	1-OH	1	1-OH	1-OH	Not reported	
Rg1	PPT	2	1-OH	1	1-OH	1	Anti-proliferative	Wang et al. (2007a)
Rg2	PPT	3	1	2	1-OH	1-OH	Anti-genotoxic	Wang et al. (2007a)
Rg3	PPD	2	2	0	1-OH	1-OH	Anti-proliferative, apoptotic	Wang et al. (2007a)
Rh1	PPT	1	1-OH	1	1-OH	1-OH	Strongly anti-proliferative	Jung et al. (2010a,b)
Rh2	PPD	1	1	0	1-OH	1-OH	Strongly apoptotic	Cheng et al. (2005)
25-OH-PPT	PPT	0	1-OH	1-OH	1-OH	1-OH	Weakly anti-proliferative	Wang et al. (2007b), Wang et al. (2008a)
25-OH-PPD	PPD	0	1-OH	0	1-OH	1-OH	Apoptosis; anti-proliferation	Wang et al. (2007b), Wang et al. (2008a), Wang et al. (2009b)
25-OCH_3-PPD	PPD	0	1-OH	0	1-OH	1-OH	Highly anti-proliferative and anti-apoptotic	Wang et al. (2008b), Wang et al. (2009a), Wang et al. (2009b)

PPD, protopanaxadiol-type; PPT, protopanaxatriol-type.

With a sugar substitute at C-6, the anticancer activity of ginsenosides is decreased compared to activity with linkages at C-3 or C-20. Molecular modeling and docking experiments confirm that the presence of a sugar moiety at C-6 increases the steric hindrance for these molecules to bind to their target proteins by blocking entrance into the extracellular binding pocket, and thus significantly reducing the anticancer activities of ginsenosides (Chen et al., 2009).

HYDROXYL GROUPS

It is known that polar substances interact with phospholipid head groups in the hydrophilic domain of the cell membrane. Ginsenosides interact with the β-OH of the cholesterol in the cell membrane and intercalate themselves into the plasma membrane. This leads to changes in membrane fluidity, and thus affects membrane function, eliciting a cellular response. This ability to insert and orient with the membrane is influenced by the number and site of polar hydroxyl groups. Differences in the number and position of hydroxyl groups influence pharmacological activity. Elimination of the double bond at C-24/25 and addition of hydroxyl or methoxyl at C-25 increases the anticancer potential of ginsenosides. The anticancer activities of 25-OCH_3-PPD, 25-OH-PPD, 25-OH-PPT, PPD, Rh2, and Rg3 have been systematically compared. 25-OCH_3-PPD and 25-OH-PPD show the most potent anti-proliferative, pro-apoptotic, cell cycle regulatory and inhibitory effects of tumor growth *in vivo* and appear to be promising candidates for further development as novel anticancer agents (Wang et al., 2007b, 2008a,b, 2009a,b).

Dehydration at C-20 increases the bioactivity of ginsenosides (Kang et al., 2006). Rg5 differs from Rg3 only by the presence of a hydroxyl group at C-20 in Rg3 and is approximately four times more effective than Rg3 at inhibiting cell proliferation (Lee et al., 1997; Shin et al., 2006). Rh3, however, is a dehydration product at C-20 of Rh2; both Rh2 and Rh3 induce differentiation of promyelocytic leukemia HL-60 cells, but the potency of Rh2 seems to be higher (Kim et al., 1998).

STEREOSELECTIVITY OF 20(*S*) AND 20(*R*)

20(*S*) and 20(*R*) forms of ginsenosides are stereoisomers of each other that depend on the orientation of the C-20 hydroxyl in ginsenosides. 20(*S*)-OH is spatially close to the C-12 hydroxyl of ginsenosides while 20(*R*)-OH is far. This difference in the stereochemistry produces different pharmacological effects. 20(*S*)-Rg3 scavenges hydroxyl radicals better than 20(*R*)-Rg3 (Lee et al., 2008). 20(*S*)-Rg3 is better aligned with the hydroxyl acceptor group in the ion channels than 20(*R*)-Rg3, making 20(*S*)-Rg3 an efficient regulator of voltage-dependent ion channels. Voltage-sensitive ion channels have been shown to play a significant role in the progression of cancer (Jeong et al., 2004; Fiske et al., 2006). In general, the 20(*S*) compounds have been shown to possess better anti-proliferative effects than their 20(*R*) counterparts whereas

20(*R*) compounds such as 20(*R*)-Rg3 have been shown to inhibit cancer cell invasion and metastasis.

Although a limited effect against cancer is observed, 20(*R*)-Rh2 inhibits osteoclast formation better than 20(*S*)-Rh2. It is possible that the anti-tumor properties of 20(*R*) ginsenosides are in part mediated through their angiosuppressive activity. These observations imply that the stereostructure of the C-20 hydroxyl may influence the biological and pharmacological effects of ginsenosides (Yue et al., 2006; Qi et al., 2010).

METABOLISM

When taken orally, PPD-type ginsenosides are mostly metabolized by anaerobic intestinal bacteria such as *Bacteroides*, *Fusobacterium*, *Eubacterium*, and *Provetella* to a PPD monoglucoside, compound K, via a step-wise cleavage of the sugar moieties (Lee et al., 2009a). In humans, compound K may be detected in the plasma from 7 h after the intake of PPD-type ginsenosides and in urine from 12 h after the intake. These findings suggest that compound K is the final metabolite of PPD-type ginsenosides (Lee et al., 2009a). Also, compound K exhibits moderate inhibition of the CYP2C9 activity, while PPD and PPT exhibit potent competitive inhibition against CYP3A4 activity (Hasegawa et al., 1996; Tawab et al., 2003).

MOLECULAR MECHANISMS OF ANTICANCER ACTIVITIES OF GINSENG AND GINSENG PRODUCTS

The initiation of tumorigenesis involves DNA alteration, which may be heritable in progeny cells. These alterations include mutations, oncogene activation rearrangements, amplifications, and methylation. The commencement of this transformation process is irreversible and is modified by agents that may act as promoters. Initiation and promotion give rise to an abnormal expression of cellular genes whose interaction hormones with cellular receptors trigger a cascade of intracellular biochemical signals resulting in the activation and repression of various subsets of genes. Hence, determining the molecular mechanisms in the development and progression of the neoplastic process is essential for understanding the activity of any compound. Although anticancer activities of ginseng products have been widely reported, the exact mechanisms of action are not fully understood. Based on the existing reports, we summarize the possible molecular mechanisms by which ginsenosides exert anticancer effects in the following text (**Table 2**; **Figure 3**).

MODULATION OF TUMOR CELL GROWTH, CELL CYCLE, AND CELL DEATH (FIGURES 4 AND 5)

Effects on DNA damage

An aqueous extract of ginseng root has been shown to exert a radioprotective effect against γ-ray induced DNA double stranded breaks and repair in cultured mouse lymphocytes; and reduce the relative strand scission factor (RSSF) by a factor of 2 (Kim et al., 1996). Several studies have shown that ginseng root extracts exhibit both lipid-soluble and water-soluble antioxidant activity (Lee et al., 2005b). This antioxidant action is mediated through free radical scavenging and through upregulation of antioxidant enzymes, thus attenuating DNA degradation. Both Cu/Zn-superoxide dismutase (Cu/Zn-SOD or SOD1) and catalase (CAT) are key enzymes involved in the metabolism of oxygen free radicals, and ginsenosides have been associated with the upregulation of these two enzymes at the level of gene expression and transcription (Rhee et al., 1991; Zhang et al., 1996; Kitts et al., 2000; Kim et al., 2002; Keum et al., 2003).

Effect on proliferation and differentiation

Studies show that, of all naturally occurring ginsenosides in the American ginseng plant Rg3, has the most potent effect on inhibition of cell proliferation. In fact, steam treatment increasing the concentration of ginsenoside Rg3 augments the anti-proliferative activities of American ginseng (Wang et al., 2007a). In the human prostate carcinoma LNCaP cell line, ginsenoside Rg3 displays good growth inhibitory activity. The expression of biomarker genes, including prostate specific antigen (PSA), androgen receptor (AR) and 5alpha-reductase (5alphaR), and that of the proliferating cell nuclear antigen (PCNA) are suppressed (Liu et al., 2000). Rh2 obtained from Asian ginseng also shows anti-proliferation, differentiation, and chemopreventive effects in various cancer cell types (Oh et al., 1999).

Studies show that p21 is an important target in mediating the anti-proliferative activities of ginseng. American ginseng extract (AGE) inhibits breast cancer cell proliferation by induction of this important cyclin inhibitor protein (King and Murphy, 2006). Rh2 has also been shown to inhibit cell growth in a concentration-dependent reversible manner, inducing a G1 arrest in cell cycle progression by upregulating the expression of p21 (Oh et al., 1999).

Ginseng is found to have the ability to induce transformation of neoplastic cells into normal cells (Lee et al., 2009b). Rh2 present in red ginseng inhibits the growth and colony forming ability of Morris hepatoma cells in soft agar suspension culture, and stimulates serum protein synthesis of these cells, thus transforming the neoplastic cells functionally and morphologically to those resembling original normal liver cells, a process known as "redifferentiation" (Odashima et al., 1989). Red ginseng (also known as Korean red ginseng) is a processed form of unpeeled ginseng that has been heated, either through steaming or sun-drying and contains predominantly the PPD ginsenosides (Lui and Staba, 1980).

Similarly, Rh1 and Rh2 cause differentiation of F9 teratocarcinoma stem cells via binding to a steroid receptor (Lee et al., 1993). Furthermore, Rh2 significantly induces B16 cell differentiation and increases melanin synthesis in B16 cells (Xia and Han, 1996). Cytotoxic effects of the PPD or PPT type of ginsenosides are inversely proportional both to the number of sugars attached to the aglycones and to the number of hydroxyl groups of the aglycones. Surprisingly, the type of side chain and the configuration of the hydroxyl group at C-3 in aglycones have not been found to play a significant role in determining cytotoxicity (Atopkina et al., 1999). Rg3 and Rh2 inhibit the proliferation of prostate cancer cell lines LNCaP and PC3, which may be associated with modulation of MAP kinases (MAPK; Kim et al., 2004). In addition, 20(*S*)-PPD and Rh2 reduce cell proliferation and increase sub-G1 cells in two cultured intestinal cell lines, Int-407 and Caco-2 (Popovich and Kitts, 2004).

Cell cycle regulation and regulation of p53/p21/MDM2 pathway

The cell cycle is a set of events, resulting in cell growth and division into two daughter cells. The stages are G1–S–G2–M. The G1–S transition in the cell cycle is controlled primarily via cyclin

Table 2 | Summary of the anticancer activities of major ginsenoside.

Ginsenoside	Source	Nucleus	Anticancer activity	Molecular mechanism	References
Ra1	*P. quinquefolium*	PPD	None reported	None reported	
Ra2	*P. quinquefolium*	PPD	None reported	None reported	
Ra3	*P. quinquefolium*	PPD	None reported	None reported	
Rb1	*P. quinquefolium*	PPD	Weakly anti-proliferative; anti-angiogenic	Inhibits capillary genesis; inhibits PEDF through estrogen receptor-β; inhibits TNF-α release; protects against oxidative stress.	Hwang and Hye (2010)
Rb2	*P. quinquefolium*	PPD	Weakly anti-proliferative; anti-angiogenic	Inhibits MMP-2; inhibits TNF-α release	Xu et al. (2008)
Rb3	*P. quinquefolium*	PPD	No anti-proliferative activity	Inhibits TNF-α release	Xie et al. (2009)
Rc	*P. quinquefolium*	PPD	No significant anti-proliferative activity	No particular mechanism reported	Wang et al. (2009a), Li et al. (2009)
Rd	*P. quinquefolium*	PPD	Weakly inhibits cancer cell growth	No particular mechanism reported	Wang et al. (2009a), Li et al. (2009)
Re	*P. quinquefolium*	PPT	Not reported	Not reported	
Rf	*P. ginseng*	PPT	Not reported	Not reported	
Rg1	*P. quinquefolium*	PPT	Anti-proliferative	Inhibits oncogenes c-myc, c-fos; downregulates nucleophosmin	Li et al. (2008)
Rg2	*P. ginseng*	PPT	Anti-genotoxic	Increases levels of p53 and p21	Wang et al. (2007a)
Rg3	*P. quinquefolium*	PPD	Anti-proliferative; apoptotic	Regulates mitochondrial cytochrome C, PARP, and C9; inhibits MMp-2 and 9; inhibits adhesion of metastatic cells to basement membrane.	Yue et al. (2006), Xu et al. (2008)
Rg5	*P. quinquefolium*	PPD	Strongly anti-proliferative	No particular mechanism reported	Lee et al. (1997), Shin et al. (2006)
Rh1	*P. quinquefolium*	PPT	Causes differentiation of teratocarcinoma cells; strongly apoptotic	Binds to steroid receptor; Inhibits TNF-α; Inhibited phosphorylation of JAK1, STAT1, STAT3, and ERK.	Jung et al. (2010a,b), Lee et al. (1993)
Rh2	*P. quinquefolium*	PPD	Induces differentiation of promyelocytic leukemia HL-60 cells; Inhibits osteoclast formation	Modulation of MAP kinases; increases sub-G1 cells; activates caspase-3 via Bcl-2 insensitive pathway; induce Fas receptor aggregation in a FasL-independent manner; regulates protein kinase A (PKA) pathway and the LPS/IFN-g-induced DNA-binding activity of AP-1; induced apoptosis via death receptor TRAIL-R1/DR4; inhibit the transport function of P-glycoprotein	Cheng et al. (2005), Choi and Choi (2009), Park et al. (1997), Kim et al. (2004), Li et al. (2011), Li et al. (2008), Odashima et al. (1989), Oh et al. (1999), Park et al. (1997), Popovich and Kitts (2004), Yi et al. (2009)
Rp1	*Semi-synthetic*	PPD		Inhibits IL-1 production; downregulation of the IGF-1R/Akt	Kang et al. (2011), Kim et al. (2009)
Notoginsenoside R1	*P. notoginseng*	PPT	Anti-proliferative	Inhibits TNF-α	Liu et al. (2010)
Notoginsenoside R2	*P. notoginseng*	PPT		No particular mechanism reported	
Majonoside R2	*P. vietnamensis*	OT	Anti-proliferative	No particular mechanism reported	
Ginsenoside Ro	*P. ginseng*	OA	None reported	No particular mechanism reported	
Chikusetsusaponin IV	*P. japonicus*	OA	None reported	No particular mechanism reported	
Compound K	metabolite	PPD	Apoptosis; Anti-proliferation	Inhibits COX-2 and production of PGE2; downregulates Akt phosphorylation; inhibits MMP-9	Jeong et al. (2010), Jung et al. (2006), Jung et al. (2010a,b), Kang et al. (2005), Kim et al. (2010a), Lee et al. (2010), Wong et al. (2010)

(Continued)

Table 2 | Continued

Ginsenoside	Source	Nucleus	Anticancer activity	Molecular mechanism	References
25-OH-PPT	*P. notoginseng*	PPT	Weakly anti-proliferative	No particular mechanism reported	Wang et al. (2007b), Wang et al. (2008a)
25-OH-PPD	*P. notoginseng*	PPD	Apoptosis; anti-proliferation	Downregulation of MDM2; activation of caspases; increase in p21	Wang et al. (2007b), Wang et al. (2008a), Wang et al. (2009b)
25-OCH_3-PPD	*P. notoginseng*	PPD	Apoptosis; anti-proliferation	Downregulation of MDM2, activation of caspases, increase in p21	Wang et al. (2008b), Wang et al. (2009a), Wang et al. (2009b)

PPD, protopanaxadiol-type; PPT, protopanaxatriol-type; OT, ocotillo-type; OA, oleanic acid-type.

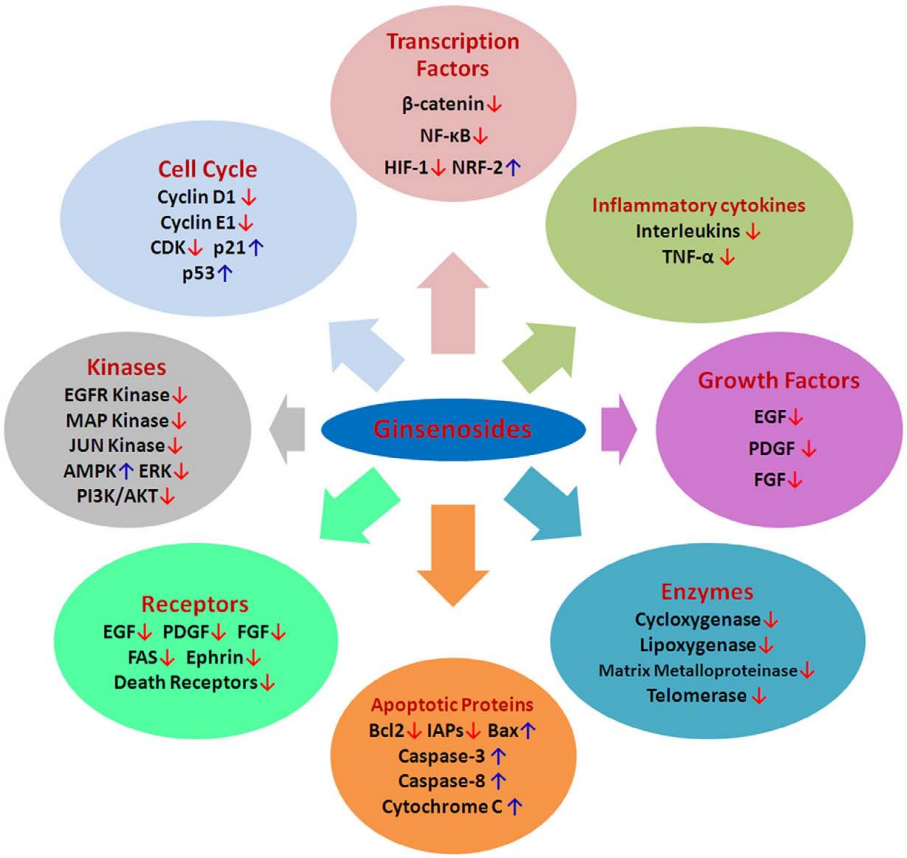

FIGURE 3 | Possible cellular and molecular mechanisms of ginsenosides aginst cancer. CDKs, cyclin-dependent kinases; MDM2, murine double minute-2; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; PDGF, platelet derived growth factor, MMP, matrix metalloproteinase; IAP, inhibitory apoptotic protein; TNF, tumor necrosis factor; NF-κB, nuclear factor κB; PI3K, Phosphatidylinositol 3-kinase; HIF-1, hypoxia-inducible factor-1; ERK, extracellular signal-regulated kinase; NRF2, nuclear factor (erythroid-derived 2)-like; AMPK, 5′ AMP-activated protein kinase; EGF, epithelial growth factor; ↑, upregulation; ↓, downregulation.

pathways, including cyclin D1 and cyclin cdks 2, 4, and 6. These proteins are, in turn, regulated by p21 and p27. At the very center of this cell cycle regulation is the tumor suppressor and transcription factor p53. The tumor suppressor p53 plays an important role in regulation of cell cycle and apoptosis. The MDM2 oncoprotein is an E3 ubiquitin ligase negatively regulates p53 function by conjugating ubiquitin to p53; thus altering its cellular localization and tagging it for proteasomal degradation.

Panaxydol, a polyacetylene compound obtained from *P ginseng*, induces cell cycle arrest at the G1 to S transition in HepG2 hepatoma cancer cells with concomitant increase in the protein levels of p21, pRb, and hypophosphorylated pRb in a

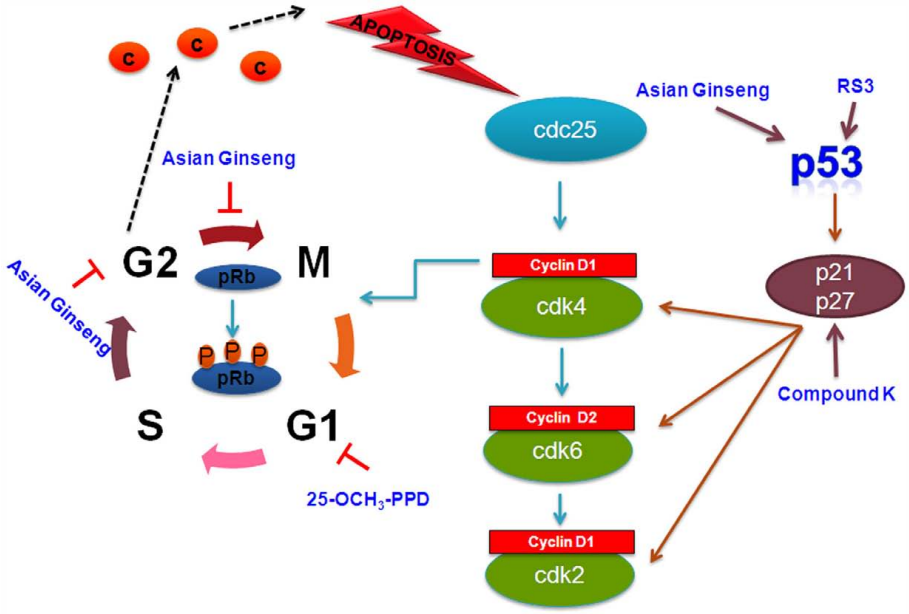

FIGURE 4 | Cell signaling pathways involved in cell cycle arrest targeted by selected ginsenosides. Rb, retinoblastoma protein.

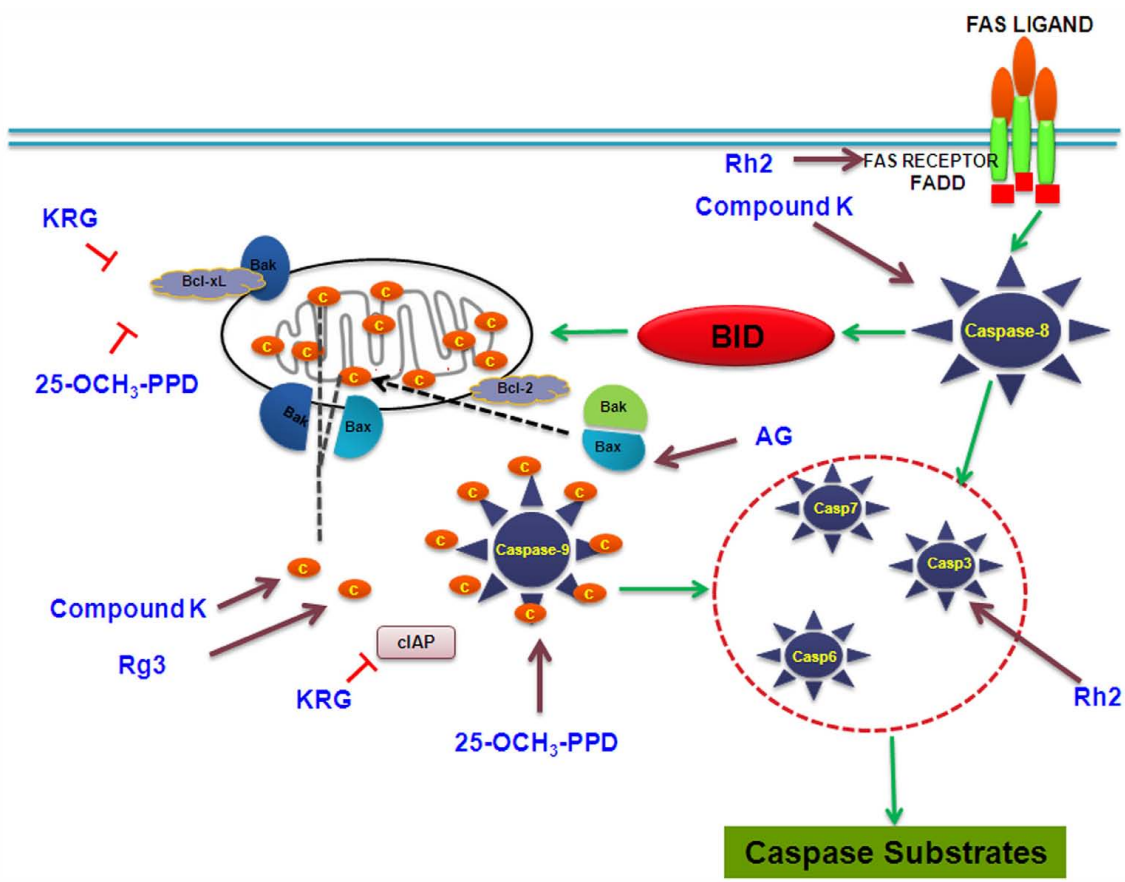

FIGURE 5 | Cell signaling pathways involved in apoptotic response targeted by selected ginsenosides. KRG, Korean red ginseng; AG: American ginseng.

dose-dependent manner (Guo et al., 2009). Rs3, a panaxadiol type ginsenoside isolated from the Korean red ginseng, upregulates p53 leading to induction of p21 which in turn initiates growth arrest and apoptosis. Studies show that ginsenosides selectively increase p53 expression at G2 phase of carcinoma cells and release cytochrome c from mitochondria, which is an essential requirement for apoptosis. An aqueous extract of Asian ginseng has been shown to induce MAPK and p53 signaling in LLC-1 cells, which suppresses cyclin B-cdc2 complex and in turn induces G2–M arrest and apoptosis. Compound K is known to induce the expression of p21, independently of p53 in U937 human monocytic leukemia cells (Kang et al., 2005; Jung et al., 2006; Wong et al., 2010). Wang et al. demonstrate that 25-OH-PPD inhibits cell growth and proliferation, induces apoptosis, and leads to arrest in the G1 phase of the cell cycle in prostate cancer. Further, in both PC3 and LNCaP prostate cancer cell lines, 25-OH-PPD reduces expression of MDM2, E2F1, Bcl-2, cdk2/4/6, and cyclin D1, which correlates with cell cycle arrest in G1 phase and anti-proliferative activity (Wang et al., 2007b).

Induction of cell death

There are two major pathways of cell death: apoptosis (death by suicide) and necrosis (death by injury). Apoptosis, also known as programmed cell death, involves a series of biochemical events, leading to characteristic cell morphology and death. It is typically accompanied by activation of specific proteases known as caspases (Nicholson and Thornberry, 1997). Necrosis is caused by external factors, such as infection, toxins, or trauma. Additional cell death mechanisms, such as autophagy (degradation of a cell's own components through the lysosomal action), entosis (internalization of a cell detached from the basement membrane into another cell; Overholtzer et al., 2007), paraptosis (non-apoptotic cell death mediated by MAPKs; Sperandio et al., 2004), and anoikis have been defined more recently.

Caspase activation. Caspases, or cysteine–aspartic acid proteases, are a family of cysteine proteases that play essential roles in apoptosis, necrosis, and inflammation. Numerous reports suggest the involvement of caspases in ginsenoside-induced apoptosis. Ginsenosides cause DNA damage, endoplasmic reticulum (ER) stress, and mitochondrial-dependent-induced apoptosis through the activation of caspase-3 (Cheng et al., 2005). Compound K has been shown to cause loss of the mitochondrial membrane potential followed by cytochrome c release from the mitochondria, resulting in the activation of caspase-9, -3, and concomitant poly ADP-ribosyl polymerase (PARP) cleavage, which are the indicators of caspase-dependent apoptosis (Lee et al., 2010). Caspase-8 plays a key role in compound K-stimulated apoptosis via the activation of caspase-3 directly or indirectly through Bid cleavage, cytochrome c release, and caspase-9 activation. Rg3 has been shown to facilitate the release of mitochondrial cytochrome c, PARP, caspase-9, and caspase-3 (Lee et al., 2010).

Aqueous extracts of American ginseng activate BAX and increase the levels of cleaved caspase-3 in p21-deficient cells causing cell death (King and Murphy, 2010). Korean red ginseng causes an increase in apoptosis, by downregulation of anti-apoptotic Bcl-2, Bcl-XL, and IAPs family members, and the activation of caspase-3 (Cheng et al., 2005). Rh2 activates caspase-3 via Bcl-2 insensitive pathway in the human hepatoma cell line SK-HEP1 (Park et al., 2010a). In structure and apoptosis relationship, the presence of sugars in PPD and PPT aglycone structures has been found to reduce the potency to induce apoptosis in the human leukemia (THP-1) cell line (Popovich and Kitts, 2002).

Induction of death receptors. Death receptors are cell surface receptors that transmit apoptotic signals initiated by specific ligands such as Fas ligand, tumor necrosis factor-α (TNF-α), and TRAIL (TNF related apoptosis-inducing ligand). These receptors play a crucial role in apoptosis and can activate a caspases-8 dependent cascade within seconds of ligand binding. Induction of apoptosis via this mechanism is therefore very rapid. Studies show that the ginsenosides such as Rh2 induce apoptosis via death receptor TRAIL-R1/DR4 in the human lung adenocarcinoma cell line A549. However, expression of Bcl-2 protein in A549 cells does not block Rh2 mediated apoptosis, suggesting that the DR4 pathway acts independently of the Bcl-2 family (Cheng et al., 2005; Choi and Choi, 2009). Intestinal bacterial metabolites of ginseng saponin such as compound K induce apoptosis in human hepatoblastoma cells by late activation of caspase-8 by Bid and DR5 (Lee et al., 2010). Contrastingly, studies with Korean RGE in U937 leukemia cell line show no change in levels of DR4, DR5, and TRAIL but the extract induces concomitant degradation of poly (ADP-ribose) polymerase, β-catenin and PLC-γ1 substrate proteins of caspase-3 (Park et al., 2009).

FAS receptor aggregation. Fas receptor is the ligand for Fas-activated apoptosis. Binding of the ligand promotes receptor clustering, DISC formation, and the activation of the caspase cascade. The ginsenoside Rh2 is shown to induce Fas receptor aggregation in a FasL-independent manner (Yi et al., 2009). Apoptosis in the HeLa cell line NCE induced by Rh2 upregulates the Fas receptor protein in a dose-dependent manner (Park et al., 1997). Ginsenosides are able to inhibit the proliferation of cancer cells and induce apoptosis by downregulating the expression of c-myc and Bcl-2 and upregulating the expression of Fas and p53. They also induce the cell cycle arrest and apoptosis in gastric/colon cancer cells and stimulate the activity of caspase-8, initiating Fas signaling pathway of apoptosis (Yi et al., 2009).

Autophagy. Autophagy is a type of programmed cell death occurring in response to cellular stress and starvation. Unlike apoptosis, in autophagy there is no cell enlargement, bleb formation, vacuole development, and enlarged/swollen nuclei (Elliott and Reiners, 2008). The ginsenoside Rk1 obtained from heat processed ginseng induces both G1 phase arrest and autophagy, but not apoptosis, at an earlier stage of treatment. Rk1-induced autophagy is documented by the conversion of microtubule associated protein light chain 3 (LC3)-I to LC3-II, an autophagosome marker, and monodansylcadaverine (MDC) incorporation into autolysosomes (Ko et al., 2009).

MODULATION OF PATHWAYS INVOLVING GROWTH SIGNALS

Growth factors play a critical role in the proliferation of tumor cells. Molecules like EGF, HER2, FGF, vascular endothelial growth

factor (VEGF), PDGF, and insulin growth factor (IGF)-1 have been associated with cell proliferation. The suppression of these growth factors or their receptors results in inhibition of tumor growth. For example, the inhibition of EGFR expression and decreased ERK1/2 activity attenuates cell survival and enhances induction of apoptosis in lung and pancreatic adenocarcinoma cells.

Inhibition of growth factors and their receptors

Several studies have shown that ginsenoside are capable of blocking VEGF. Rg3 is reported to downregulate the expressions of KDR (VEGF receptor-2) and VEGF protein and their mRNA in human lung squamous cancer SK-MES-1 cells (Wang et al., 2009d). It is reported that ginsenoside Rg_3 and cyclophosphamide (CTX) exhibit a synergistic effect on the inhibition of growth and angiogenesis in mice transplanted with ovarian cancer cells (SKOV-3; Xu et al., 2007). The treated mice show decreased PCNA labeling index and the VEGF expression is also significantly lower. Ginsenosides have also been shown to completely block EGF-induced DNA synthesis and cell growth in primary cultured rabbit renal proximal tubular cells (PTCs; Han et al., 2002). In contrast, the PPT and PPD fractions only cause partial blockade. In addition, the EGF-induced increase of c-*fos* and c-*jun* gene expression is completely blocked by total ginsenosides and partially by PPT and PPD saponins (Han et al., 2002). Compound K reduces the secretion of VEGF and increases the secretion of pigment epithelium-derived factor (PEDF) in HUVECs. In addition, Compound K effectively disrupts bFGF-induced neovascularization in the Matrigel plugs excised from mice *in vivo* (Jeong et al., 2010). A recent study (Dougherty et al., 2011) suggests that compound K inhibits the activation of EGFR signals, including pEGFR, pErbB2, pERK, and pAKT in an inflammation associated colon cancer model based on a high fat western diet.

Ephrin pathway

Ephrin receptors belong to the tyrosine kinase family of transmembrane proteins, capable of influencing cell–cell interaction and cell migration. When Ephrins bind to Eph receptors, the bidirectional signaling of the Eph/ephrin axis is activated; this results in tumorigenesis and is associated with angiogenesis and metastasis in many types of human cancer. Previously, the inhibition of Eph/ephrin signaling as a novel strategy for cancer treatment has been investigated. Using ChIP and microarray techniques, Luo et al. show that ginsenosides Rg3 and S_2H may exert effective anticancer activity through the Ephrin receptor pathway in human colorectal cancer cell line HCT116. The microarray analysis suggests that the activity of Rg3 is linked to upregulation of AKAPA8L (anchor protein) and downregulation of PITPNA (phosphatidylinositol transfer protein α) genes (Park et al., 2005; Luo et al., 2008).

INHIBITION OF ANGIOGENESIS

Angiogenesis is the process by which new blood vessels are formed from pre-existing structures. It is a crucial step in tumor growth and metastasis by supplying oxygen and nutrients. Ginseng derivatives have been shown to inhibit tumor growth by influencing neovascularization and the angiogenesis-related properties of endothelial cells (ECs; Leung et al., 2007). Ginsenoside Rb1 has been shown to inhibit capillary morphogenesis of ECs by regulating expression of PEDF through the estrogen receptor α (Leung et al., 2007). 20(*R*)-Rg3 inhibits proliferation, formation of capillary tube, and chemoinvasion of ECs induced by VEGF (Yue et al., 2006). 20(*R*)-Rg3 also reduces microvascular sprouting *in vitro* and inhibits neovascularization induced by basic fibroblast growth factor *in vivo* (Yue et al., 2006). The angiosuppressive effect of 20(*R*)-Rg3 may be related to the differential regulation of matrix metalloproteinases (MMP-2 and MMP-9) activities (Yue et al., 2006).

ANTI-METASTATIC EFFECTS

Metastases are multiple, widespread tumor colonies established by malignant cells that detach themselves from the original tumor and travel through the body often to distant sites. Some of these invading cells penetrate into a body cavity or the blood, lymph or spinal fluid and then are released forming tumors locally at the site of penetration. Enzymes capable of proteolytic degradation of extracellular matrix and basement membranes have been implicated in tumor progression and metastasis, especially the protease matrix metalloproteinase 9 (MMP-9). Matrix metalloproteinases, a family of zinc-dependent proteases, participate in several steps in tumor progression, including invasion, metastasis, and angiogenesis. Many studies implicate that the aberrant expression of MMP-9 plays an important role in tumor metastasis (Deryugina and Quigley, 2006).

20(*R*)- and 20(*S*)-Rg3 possess an ability to inhibit the lung metastasis of tumor cells such as B16-BL-6 melanoma and colon 26M3.1 by inhibition of the adhesion and invasion of tumor cells, and also by anti-angiogenesis activity (Mochizuki et al., 1995). Glucocorticoid receptor-induced downregulation of MMP-9 by panaxadiol (PD) and panaxatriol (PT) appear to be associated with the reduced invasive capacity of HT1080, a highly metastatic human fibrosarcoma cell line (Park et al., 1999).

Mochizuki et al. report that multiple administration of ginsenoside Rb2 after the intravenous inoculation of B16-BL6 melanoma cells results in a significant inhibition of tumor associated angiogenesis leading to suppression of lung metastases. In addition, the authors also show that Rg3 significantly inhibits the adhesion and invasion of B16-BL6 cells into reconstituted basement membranes (Matrigel; Mochizuki et al., 1995). Ginsenoside Rb2 has been reported to inhibit invasion to the basement membrane via MMP-2 suppression in some endometrial cancers such as HHUA and HEC-1-A cells, and can potentially be used as a medication for inhibition of secondary spreading of uterine endometrial cancers (Fujimoto et al., 2001). It is shown that ginsenoside Rg3 inhibits experimental pulmonary metastasis by highly metastatic mouse melanoma B16FE7 cells, by inhibition of the 1-oleoyl-lysophosphatidic acid (LPA)-triggered rise of intracellular Ca^{2+} (Shinkai et al., 1996). In addition, the invasive ability and MMP-9 expression of SKOV-3 cells decreases significantly after treatment with ginsenoside Rg3 (Xu et al., 2008). Compound K is effective in suppressing the expression of MMP-9 and thus inhibiting migration and invasion in metastatic murine colon adenocarcinoma cells (Choo et al., 2008). In another study, the aforementioned ginsenoside has also been shown to inhibit the secretion and protein expression of MMP-9 in astroglioma cells. The inhibitory effect of compound K on MMP-9 expression correlates with decreased MMP-9 mRNA levels and suppression

of MMP-9 promoter activity. The compound K-mediated inhibition of MMP-9 gene expression occurs via activator protein-1 (AP-1) because its DNA-binding and transcriptional activities are suppressed by the drug (Jung et al., 2006).

Recently, a study (Yoon et al., 2012) has shown that treatment with the Rd inhibits migration and invasion of the liver cancer cell line HepG2. This is accomplished by reducing the expression of MMP-1, MMP-2, and MMP-7, by blocking MAPK signaling, by inhibition of AP-1 activation, and by inducing focal adhesion formation and decreasing vinculin localization and expression. This leads to a significant anti-metastatic effect. Earlier studies have reported that Rd has negligible anti-proliferative activities (Wang et al., 2007a).

MODULATION OF KINASES (FIGURE 6)

Inhibition of PI3K-Akt activation

Akt (protein kinase B), a serine/threonine kinase, is a critical enzyme in signal transduction pathways involved in cell proliferation, apoptosis, and angiogenesis. Studies show that several ginsenosides such as compound K and PPDs inhibit the phosphorylation of Akt, mTOR, and their downstream substrates in a concentration- and time-dependent manner in human umbilical vein cells, and this inhibitory effect is seen downstream of phosphatidylinositol 3-kinase (PI3K). In fibroblast growth factor treated HUVEC cells, compound K downregulates the phosphorylation of Akt (Jeong et al., 2010). Additionally, in MCF-7 cells, 20(*S*)-PPD has been shown to synergistically enhance the cytotoxicity of tamoxifen by downregulating Akt, independently of estrogen receptor (Yu et al., 2007).

Activation of c-jun kinase

Studies show that activation of the JNK/c-Jun/AP-1 signal pathway induces the p21 activation, resulting in growth arrest. Compound K causes G1 phase arrest via the induction of p21 and activation of the JNK/AP-1 pathway. The apoptotic effect of compound K is exerted via the activation of c-Jun NH2-terminal kinase (JNK) and p38 MAPK (Kang et al., 2005; Jeong et al., 2010). Another study shows that panaxydol induces apoptosis through an increase in intracellular Ca^{2+} concentration, activation of JNK and p38 MAPK, and generation of reactive oxygen species (ROS) initially by NADPH oxidase and then by mitochondria. Apoptosis seen is caspase-dependent and occurs through a mitochondrial pathway utilizing generated ROS (Kim et al., 2011).

Modulation of AMPK pathways

AMP-activated protein kinase (AMPK) is a sensor of cellular energy states and is involved in apoptosis of cancer cells. Many cancers are characterized by increased expression and/or activity of enzymes (e.g., FAS and mTOR) that are inhibited by AMPK. Compound K is shown to exert cytotoxicity in MCF-7 human breast cancer cells through modulation of AMPK, followed by a decrease in cyclooxygenase-2 (COX-2) expression (Kim et al., 2010a). Compound K inhibits cell growth, induces apoptosis via generation of ROS, as well as decreasing COX-2 expression and prostaglandin E2 (PGE2) levels. These effects of compound K are induced via an AMPK-dependent pathway and are nullified by a specific AMPK inhibitor (Kim et al., 2010a).

ANTI-INFLAMMATORY PATHWAYS

Chronic inflammation is associated with increased cancer risk. The microenvironment provided by inflammatory cells is a crucial contributor in the neoplastic process, fostering proliferation, survival, and migration (Aggarwal and Shishodia, 2006). The inflammatory cells and regulators may facilitate angiogenesis and promote the growth, invasion, and metastasis of tumor cells.

Compound K suppresses the TNF-α-induced activation of nuclear factor kappa-B (NF-κB), expression of MMP-9, migration and invasion of cancerous cells, thus indicating that ginsenosides have the potential to suppress inflammation related metastasis (Choo et al., 2008). American ginseng has been shown to inhibit induced COX-2 and NF-κB activation in MDA-MB-231 and MCF-7 cell line (Peralta et al., 2009). Rg3 suppresses activation of NF-κB inhibiting cancer cell growth in colon cancer cell lines SW620 and HCT116 in a dose-dependent manner (Kim et al., 2010b). Blockage of ROS by NAC/catalase inhibits activation of NF-κB signaling increasing Rh2 induced cell death (Li et al., 2011).

The ginsenoside G-Rp1 dose-dependently inhibits interleukin-1 (IL-1) production (at both mRNA and protein levels) from LPS-treated RAW264.7 cells without altering cell viability. This compound also downregulates IκBα phosphorylation and NF-κB activation, but does not affect the upstream signaling enzymes such as MAPK and p85, a regulatory subunit of PI3K (Kim et al., 2009). PPD-type ginsenosides (G-Rb1 and G-Rb2) exhibit significant inhibition of TNF-α release with IC_{50} values of 48.0 and 27.9 mM, respectively (Park and Cho, 2009). Strong apoptosis-inducing saponins, Rh1 and Rh2, the main intestinal metabolites of Rg3 have been shown to be inhibitors of proinflammatory TNF-α and IL-1b in LPS/IFN-g-activated BV-2 cells, while increasing the expression of the anti-inflammatory cytokine IL-10 (Jung et al., 2010a,b). The prime targets of G-Rh2 are both the protein kinase A (PKA) pathway and the LPS/IFN-g-induced DNA-binding activity of AP-1, but not the cAMP responsive element binding protein or NF-κB. Both PPD and PPT type ginsenosides inhibit LPS-induced TNFα by blocking the phosphorylation of ERK 1/2, JNK, and c-Jun, as well as blocking NF-κB activation (Wu et al., 2007). RGE from steamed and dried *P. ginseng* is seen to inhibit *Helicobacter pylori*-induced MAPK signaling by repressing either nuclear factor NF-κB–DNA-binding activity or release of IL-8 and COX-2 in gastric epithelial cells (Park et al., 2007). RGE also inactivates c-Jun phosphorylation and represses redox-sensitive transcriptional activation, leading to reduced expression of IL-8 and 5-LOX mRNA in gastric mucosal cells (Park et al., 2007).

Rh1 significantly suppresses IFN-γ-induced iNOS promoter activity by inhibiting DNA-binding of several transcription factors, such as NF-κB, IRF-1, and STAT1 (Jung et al., 2010a). Furthermore, Rh1 inhibits the phosphorylation of JAK1, STAT1, STAT3, and ERK, which are upstream signaling molecules for IFN-γ-induced iNOS gene expression. This study also demonstrates that Rh1 inhibits IFN-γ-induced JAK/STAT and ERK signaling pathways and downstream transcription factors, and thereby iNOS gene expression.

Tumor promotion often accompanies an elevated ornithine decarboxylase (ODC) activity, acute inflammation and induction of COX-2 activity. Compound K, when applied topically onto shaven backs of female ICR mice leads to the inhibition of the

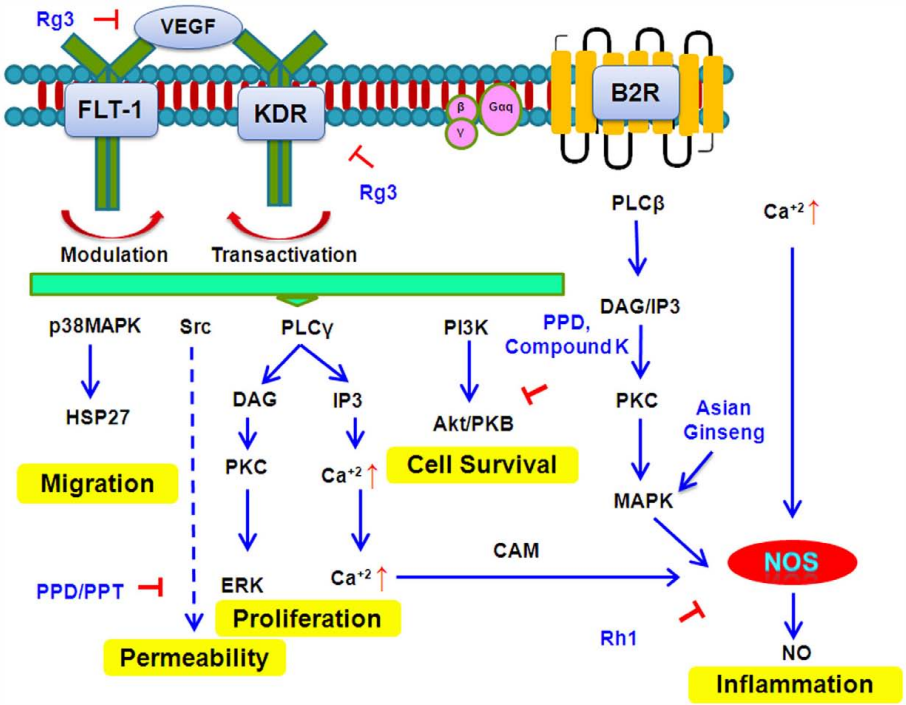

FIGURE 6 | Signal Transduction pathways targeted by selected ginsenosides.

12-*O*-tetradecanoylphorbol-13-acetate (TPA) induced expression of COX-2 and production of prostaglandin E2 (Lee et al., 2005a). The eukaryotic transcription factor NF-κB has been involved in intracellular signaling pathways associated with inflammation and carcinogenesis. Compound K has been seen to inhibit the activation of ERK1/2 and Akt signaling. When compound K is applied topically prior to TPA, expression and activities of ODC are inhibited dose-dependently (Lee et al., 2005a). In addition, compound K given prior to each topical dose of TPA markedly lowered the number of papillomas in mouse skin induced by 7,12-dimethylbenz(*a*)anthracene. Thus, COX-2 inhibition is an essential mechanistic pathway for tumor inhibition by compound K (Lee et al., 2005a). Rg3 is also shown to inhibit expression of COX-2 in TPA-stimulated mouse skin. Rg3 also exerts potent inhibitory effects on the activation of AP-1 responsible for c-*jun* and c-*fos* oncogenic transactivation (Keum et al., 2003). *P. notoginseng* extracts have been shown to reduce significantly the levels of serum TGF-β1, TNF-α, and IL-6 in a hepatic fibrosis model (induced by carbon tetrachloride) in Sprague-Dawley rats (Peng et al., 2009).

INHIBITION OF Wnt/β-CATENIN SIGNALING

Colorectal cancers are mostly initiated by aberrant activation of the Wnt/β-catenin signaling pathway (Kraus et al., 1994). The ginsenoside Rg3 has been shown to inhibit cell proliferation and viability of cancer cells *in vitro* by blocking nuclear translocation of the β-catenin protein and hence inhibiting β-catenin/Tcf transcriptional activity. Allelic deletion of the oncogenic β-catenin in HCT116 cells renders the cells more sensitive to Rg3-induced growth inhibition. In a xenograft colon cancer model, Rg3 effectively inhibits the growth of tumors derived from the colon cancer cell line HCT116. Histologic examination revealed that Rg3 inhibits cancer cell proliferation, decreases PNCA expression and diminishes nuclear staining intensity of β-catenin (He et al., 2011).

INHIBITION OF HYPOXIA-INDUCIBLE FACTOR-1

Hypoxia-inducible factor-1 (HIF-1) activates the transcription of genes that are involved in crucial aspects of cancer biology, including angiogenesis, cell survival, glucose metabolism and invasion. Intratumoral hypoxia and genetic alterations can lead to HIF-1α overexpression, which has been associated with increased patient mortality in several cancer types. In both Hep3B cancer and HEK293 immortalized normal cell lines, aqueous extract of Korean red ginseng (KRGW) attenuates the expression of hypoxia-induced genes without apparent cytotoxicity (Choi et al., 2011). Mechanistically, KRGW does not affect the synthesis, degradation, and translocation of HIF-1 in hypoxia. Interestingly, the authors found that KRGW represses the transcriptional activity of HIF-1 by interfering with the dimerization between HIF-1α and aryl hydrocarbon receptor nuclear translocator.

ACTIVATION OF Nrf2 PATHWAY

Nuclear erythroid 2 p45-related factor 2 (Nrf2) is a redox-sensitive basic leucine zipper transcription factor involved in the transcriptional regulation of many antioxidant genes. Nrf2 is inhibited in the cytoplasm by the anchor protein Kelch-like ECH-associated protein-1 (Keap1), a cytosolic protein that inhibits Nrf2 signaling by promoting proteasomal degradation of Nrf2. In the presence of

oxidative stress or chemical inducers, Nrf2 is released from Keap1 inhibition, translocates to the nucleus, and binds to antioxidant response element (ARE) consensus sequences. Activation of Nrf2 by chemopreventive agents influences expression of anti-oxidative phase-I and phase-II enzymes including heme oxygenase-1 (HO-1), NADPH quinone oxidoreductase-1 (NQO1), and glutathione (Lee and Johnson, 2004). An aqueous extract of wild ginseng has been shown to increase expression of GSTA2, GSTA3, and GSMT2 via an NRF-ARE pathway (Saw et al., 2010). Korean RGE has been shown induce HO-1 by NRF–ARE pathway in rat pheocromocytoma cells (Park et al., 2010b). Ginsenoside Rb1 exhibits protection against 6-hydroxydopamine induced oxidative stress in human neuroblastoma SH-SY5Y cells (Hwang and Hye, 2010).

INHIBITION OF hTERT

The human telomerase reverse transcriptase (hTERT), the active subunit of telomerase is activated in cancer cells and prevents telomere shortening, thus inhibiting of apoptotic processes. The inhibition of hTERT is an additional mechanism by which ginsenosides can induce cell death in cancer cells. Compound K is known to inhibit cell proliferation by inducing apoptosis and cell cycle arrest at the G1 phase (Kang et al., 2005). Compound K treatment reduces telomerase activity and downregulates the (hTERT) gene, resulting in the decreased expressions of its protein, and of the c-Myc and Sp1 proteins (transcription factors of hTERT) in U937 leukemia cells (Kang et al., 2006). Also, the hot water extract of Korean red ginseng causes significant inhibition on the growth of U937 leukemia cells which is associated with the inhibition of hTERT expression (Park et al., 2009).

INHIBITION OF MULTIDRUG RESISTANCE

A major difficulty in the treatment of human malignancies is the development of broad anticancer drug resistance by tumor cells. This phenomenon has been termed "multidrug resistance" (MDR). Among all ginsenosides, 20(*S*)-Rg3 is found to have the most potent inhibitory activity on MDR. Rg3 reverses MDR in drug-resistant human fibrocarcinoma KBV20C cells specifically and effectively in a dose-dependent manner (Park et al., 2005). Several ginsenosides, including 20(*S*)-Rh2, have been shown to inhibit the transport function of P-glycoprotein and increase sensitivity to cancer chemotherapeutics in resistant cells (Li et al., 2008).

CLINICAL STUDIES

Though many ginsenosides show a clear anticancer activity in cancer cell lines or xenograft tumor models, it is imperative to observe their effects in human subjects. Two Chinese studies have found that ginseng works synergistically with chemotherapy: One study examined 176 digestive tract cancer cases and found reduced toxic effects (Li, 1992). The other study involved a randomized trial of 63 stomach cancer cases (Lin et al., 1995). Both studies found improved immune function. Five studies examined the effectiveness of ginseng in relation to cancer mortality as an end point. Two were case–control studies (Yun and Choi, 1990; Rebbeck et al., 2007); two were prospective studies (Yun and Choi, 1998; Cui et al., 2006) while one was a randomized trial (Suh et al., 2002). The randomized study conducted in Korea found a 5-year disease free survival and significantly longer survival time among stage III gastric cancer patients during postoperative chemotherapy.

One of the case–control studies found no significant association between breast cancer risk and ginseng (Rebbeck et al., 2007). However, the case control study conducted in a Korean population reported a significant protective effect against total cancer risk (Yun and Choi, 1990). Even among subjects who consumed ginseng only one to three times a year, a protective effect was found. The protective effect was found among a variety of cancer types with both cohort studies reporting a significant protective effect.

Overall, fairly consistent protective effect of ginseng has been found against cancer risk and cancer mortality. Also, an improved immune function may be possible when ginseng is administered during chemotherapy. A protective effect might already be possible with minimal consumption of ginseng (one to three times a year; Yun and Choi, 1990). However, some of the above studies (Li, 1992; Lin et al., 1995) used compound Chinese herbal preparations containing ginseng extracts. It, therefore, becomes difficult to actually attribute the chemopreventive properties of the formulation specifically to a particular class of compounds, i.e., ginsenosides.

Also, sometimes conflicting and confusing results come to the fore. As an example, a large case–control study and a small cohort study have both suggested that ginseng use is associated with a 60–70% reduction in gastric cancer risk in Korean populations. However, when the study was attempted to be duplicated in a large prospective cohort study of Chinese women, no association between ginseng intake and gastric cancer risk was found (Kamangar et al., 2007).

A randomized, double-blind clinical study to investigate whether American ginseng might help cancer-related fatigue, suggests that American ginseng, at a dose of 750 mg/day, does not provide any benefit over that seen with a placebo However, the two highest doses of ginseng (1000 and 2000 mg/day) did appear to decrease fatigue more than a placebo, as measured by various scales of fatigue, vitality, and well-being. Data suggested that the higher doses of ginseng might be necessary for alleviating cancer-related fatigue (Barton et al., 2010).

In a randomized, double-blinded, placebo-controlled trial on 643 chronic atrophic gastritis patients in China, RGE powder was administered orally to each patient per week for 3 years and followed up for 8 years. The development of different types of cancers in the red ginseng subjects was compared to that of a placebo group and it was seen that administration of RGE powder for 3 years exerted significant preventive effects on the incidence of non-organ-specific human cancers in males (Yun et al., 2010).

CONCLUSION

Pre-clinical and clinical studies indicate that ginsenosides can be effective and safe anticancer agents. Ginsenoside saponins exhibit diverse molecular mechanisms of action, regulating most known modulators of carcinogenesis. Because of various mechanisms of cell death employed by ginsenosides, it may be difficult for cells to develop resistance to ginsenoside-induced cell death. Furthermore, the ability to kill tumor cells and relative non-toxicity to

normal cells make ginsenosides attractive candidates for drug development. However, the diversity in both chemical structure and biological activity leads to several challenges. Different species of ginseng have different saponin profiles. Also the mode of preparation of the plant extracts affects the quantity of its active anticancer principles. For example, heat treated red ginseng has a higher quantity of Rg3, Rh2, and PPD. As a result, it is often difficult to standardize studies and varying results have been obtained. What is needed today is a systematic study of a series of related compounds in established, well characterized cancer models to ascertain the relationship between the structure and anticancer activity of these compounds. These data should then be used to develop novel semi-synthetic ginsenosides with improved efficacy, pharmacokinetics, and bioavailability profiles. The use of modern combinatorial libraries and high throughput screening techniques coupled with an in-depth understanding of the molecular mechanisms underlying ginseng's biological activities is necessary to harness the full benefits of ginseng and ginseng products in treatment and prevention of cancer and other chronic diseases.

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REVIEW
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Mitigating the Impact of Admixtures in Thai Herbal Products

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Medicinal plants and their products are extensively used within indigenous healthcare systems in Thailand and several other nations. The international trade of herbal products has a noteworthy impact on the worldwide economy, and the interest in herbal products is expanding in both developing and developed countries. There has been rapid growth in the medicinal plant product market and a broadening consumer base interested in herbal products from Thailand. However, in herbal industries, ingredient substitution and admixture are typical issues wherein species of lower market value are admixed with those of a higher value. The adverse consequences of consuming adulterated drugs are invariably due to the presence of an unintended herb rather than the presence of an intended herb. It has also been argued that admixtures are intentional because of the lack of regulatory policies or centralized tests for product authentication. The consequences of species admixtures can extend from the reduced efficacy of a drug to decreased trade value. This study aims to clarify the nature and extent of species admixtures reported in the Thai herbal trade market and discuss the potential reasons for such adulteration. In the broader context of species admixtures, we strongly propose the establishment of multiple herbal crude drug repositories that can be developed to facilitate the use of comparative identity tests by industry, traders, and researchers to maintain authentic natural health product (NHP) standards and to certify the authenticity of NHPs. The proposition of the establishment of centralized testing (CT) could be a promising initiative in Thailand for the development of science and technology, and the herbal medicines produced as a result of CT could be dispensed as prescription drugs based on disease consideration instead of as health foods or nutraceuticals.

Keywords: admixtures, adulterations, medicinal plants, Thai herbal products, herbal database

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INTRODUCTION

Medicinal plants contribute significantly to both the indigenous and modern systems of medicine. Since ancient times, medicinal plants have been used in traditional home remedies and have formed a major component of the indigenous system of medicine practiced in many countries. It has been reported that 80% of the world's population depend on traditional herbal medicines, mainly plant-based herbal drugs, for their primary healthcare (Srirama et al., 2017). The international trade of medicinal plants and their herbal products contributes to the worldwide economy; thus, the demand for such herbal products is growing in both developing and developed countries. More than 28,187 species of plants are used for medicinal purposes (Medicinal Plant Names Services Portal, 2017).

These plants are used as either herbal medicines by the indigenous and rural communities or in the manufacture of pharmaceutical drugs. Currently, there are more than 1,000 herbal companies producing medicinal plant products, and their annual income is in excess of $60 billion (Newmaster et al., 2013). Due to commercialization and increased demand for traditional herbs, safety, quality, and assurance are major issues (Chen et al., 2017).

In recent years, an international resurgence of attention has occurred in the use of herbal products in human healthcare, which has led the herbal product market to thrive (Barry and Abratt, 2015). The increase in demand has far outpaced the availability of raw materials. Thailand's major exports of medicinal plants are used in pharmaceutical, perfumery, etc. Estimated exports of herbal products from Thailand indicate an increase from US$1.4 million in 2007 to US$11.5 million in 2011, recording a compounded annual growth rate of 12.67%. The major export markets of Thai herbal extracts are Myanmar (38.72%), the United States (13.15%), Cambodia (10.92%), Japan (9.50%), the United Kingdom (6.79%), and Hong Kong (3.31%), whereas the import markets include China, Brazil, the United States, Germany, Philippines, and India (https://report.nat.gov.tw). However, due to high demands, the substitution and adulteration of many medicinal plant species in medicinal plant products (Techen et al., 2004; Song et al., 2009; Suwanchaikasem et al., 2013; Urumarudappa et al., 2016; Srirama et al., 2017; Dechbumroong et al., 2018; Kumar et al., 2018), breweries such as teas (Stoeckle et al., 2011), and "nutraceuticals" (Bruni et al., 2010; Jaakola et al., 2010) have been documented.

Medicinal plants used in raw herbal trade are often marketed as powders; dry twigs including dry roots, rhizomes, leaves, etc.; and pills or tablets, and thus are usually difficult to identify morphologically. The major problems with the trade of raw herbal products include the adulterations and substitution of certain species with morphologically similar and geographically co-occurring species (Ved and Goraya, 2007; Urumarudappa et al., 2016; Srirama et al., 2017). In most of the Southeast Asian countries, including Thailand, over 80% of the medicinal plants that are in active trade are seldom cultivated. These are collected largely from the wild. The collection of these medicinal plants is largely performed by local people who often rely only on their knowledge or experience in identifying the species being collected (Menon, 2003; Seethapathy et al., 2015; Srirama et al., 2017). Thus, most often, there are admixtures of related species or morphologically similar species in the herbal trade samples. The possible reasons for adulteration are often attributed to the apparent confusion associated with local or vernacular names of plant species, lack of accurate raw plant material, and identical morphological characters of co-occurring species of plants (Mitra and Kannan, 2007; Kumar et al., 2018). Admixture is a common malpractice in raw material trade and a major problem in the herbal industry. Admixture is the practice of substituting an authentic crude drug partially or entirely with a different drug similar to the original drug but inferior in therapeutic and synthetic properties or the addition of low-efficiency drugs for the intentional or unintentional enrichment of profits (Mukherjee, 2002; Kokate et al., 2007). The adverse consequences of consuming adulterated drugs are invariably due to the presence of an unintended herb rather than the presence of an intended herb (Sunita, 1992; Uniyal and Joshi, 1993; Sarin, 1996). It has also been argued that admixture is deliberate because there is no standard monitoring tool in place or commercial test for product authentication. The consequences of species admixtures can extend from the reduced efficiency of a drug to the decreased trade value (Wieniawski, 2001), in addition to a threat to the safety of consumers (Song et al., 2009).

Since adulteration is very difficult to trace *in situ* and requires expertise, scientific methods have been developed to address medicinal plant adulteration in herbal trade, such as microscopic, macroscopic, organoleptic, and chemical analyses (Techen et al., 2014; Moraes et al., 2015; Mishra et al., 2016; Pawar et al., 2017; Raclariu et al., 2018). DNA technology has been heavily used to investigate the adulteration of species (Newmaster et al., 2013; Phoolcharoen and Sukrong, 2013; Kumar et al., 2015; Urumarudappa et al., 2016; Pawar et al., 2017; Dechbumroong et al., 2018; Liu et al., 2018; Raclariu et al., 2018; Seethapathy et al., 2018; Speranskaya et al., 2018; Kreuzer et al., 2019). Among the available tools for species identification and traceability, DNA barcoding is a low-cost and highly efficient approach that has the potential for automatization and rapid application (Galimberti et al., 2013; Mishra et al., 2016; Dechbumroong et al., 2018).

In this review, we attempt to elucidate the nature and extent of species adulteration reported in the Thai herbal trade market and discuss the possible underlying causes of adulteration. In the larger context of species admixtures, we believe and propose the need to certify the centralized system to develop an efficient mechanism to systematically evaluate traditional medicine and connect it with both national and international trade regulators. We strongly suggest the establishment of several crude herbal drug depositories to maintain authentic biological reference materials (BRMs) that can be developed to facilitate the use of comparative identity tests by industry, traders, and researchers and to certify herbal product authenticity. We strongly recommend the enforcement of existing laws and regulations concerning the quality of herbal products to govern herbal trade around the globe. The proposed establishment of centralized testing (CT) could be a promising initiative in Thailand for the development of science and technology. The herbal medicines produced as a result of CT could be dispensed as prescription drugs based on disease consideration instead of being designated health foods or nutraceuticals. CT could pave the way to increase the country's market size of phytomedicine and become a novel strategy in Thailand as an important contributor to the herbal product market and to maintain the safety and health of consumers.

MATERIALS AND METHODS

Literature Search on Medicinal Plants Used in Thai Herbal Pharmacopoeia (THP)

In this study, the list of plants in Thailand Herbal Pharmacopoeia (THP) was the main focus. A literature search was performed with various electronic databases [Google Scholar, Science Direct

(Scopus), and Web of Science] using specific search terms such as "Thai Herbal Pharmacopoeia mentioned plant names," "Thai medicinal plants," "Admixtures of Thai medicinal plants," and "Thai herbal medicine," which included peer-reviewed scientific studies and reports used to document traditional medicinal plants used in THP.

Origin of Thai Traditional Medicine (TTM)

Traditional medicines have been established after extensive experiences of over 100 years of practicing physicians in the indigenous systems of medicine (Kamboj, 2000). Traditional medicine of Thailand has an extensive history of use in various treatments and illnesses since the Sukhothai period (1238–1337) of Thai history. By the knowledge passed down through generations, a well-defined structure of traditional medicine has been developed, systematized, and is now recognized as Thai Traditional Medicine (TTM) by the Thailand Kingdom (SEARO, WHO, 2010). TTM is considered holistic medicine, as it involves not only the use and production of herbal medicine but also diagnosis, treatment, and pharmacy practice (Akarasereenont et al., 2015). The famous Thai massage (Nuad Thai) also originates from this system.

TTM draws its traditional content from the Indian and Chinese systems (WHO, 2002), as indicated by the diversity of ethnic groups in Thailand's population (Trisonthi and Trisonthi, 1995), possibly due to historic migration from neighboring countries (Phumthum and Balslev, 2018). Maintenance of unique and distinctive knowledge of medicinal plants has been seen mainly in these communities. Additionally, a study on a typical mountain-residing indigenous community named "Karen" showed that having limited access to public healthcare systems encouraged them to accumulate a valuable method of preventing and treating diseases with herbal remedies (Tangjitman et al., 2015). However, TTM observed a decline in consumer acceptance from 1916 to 1977 due to the influence of Western medicine, although a subsequent revival in acceptance was observed after the WHO advised that traditional medicine and plant herbal medicine should be implemented in primary healthcare systems (Akarasereenont et al., 2015). Then, to accelerate the integration of traditional medicine into national healthcare system, a list of the national essential herbal drugs (NEHD) was established in 1999 according to the National Drug Committee (2006). Today, the Thai government also funds and strongly supports medicinal plant research and development.

To reduce the cost of imported drugs and improve access to healthcare, ASEAN members agreed to implement the WHO's traditional medicine strategy (WHO, 2002) in the year 2004, which in turn enhanced the use of traditional medicine, especially herbal medicine, in ASEAN healthcare systems. A recent investigation reports that Thai people spend several million bahts *per* year on unproven herbal products (Osathanunkul et al., 2016). Currently, alternative medicines are popular not only in developing countries, such as Thailand and India, but also in developed countries, such as the United States, Canada, and Europe. In support of this, an estimate shows that the United States alone spends over US$5 billion *per* year on herbal products (Osathanunkul et al., 2016). However, herbal products are considered supplements rather than medicines in the United States, which may be due to the absence of clinical data on their safety and efficacy.

Species Admixtures in Herbal Trade: Thai Herbal Pharmacopoeia

THP has listed 66 medicinal plants with a high therapeutic index as herbal medicine (**Table 1**). This study considered the listed medicinal plants to understand their status under the threat of adulteration in herbal trade (**Table 2**). A previous report has shown that 15.5% of the total number of plant species in Thailand is used as medicinal plants (Wakdikar, 2004). Recently, a number of studies have also shown that natural health product (NHP) market samples of raw herbal trade materials are often adulterated with other species (Kool et al., 2012; Newmaster et al., 2013; de Boer et al., 2015; Kumar et al., 2015; Urumarudappa et al., 2016; Mezzasalma et al., 2017; Pawar et al., 2017; Dechbumroong et al., 2018; Raclariu et al., 2018; Liu et al., 2018; Seethapathy et al., 2018; Speranskaya et al., 2018; Tungphatthong et al., 2018; Kreuzer et al., 2019).

A number of studies have reported species admixtures in herbal trade across countries. For example, DNA-based approaches have been used for the identification of species listed in Amazonian traditional medicine (Mezzasalma et al., 2017). *Trachelospermum jasminoides* is commonly used as traditional Chinese medicine and sold in markets in dried and sliced forms, which pose difficulties in traditional identification methods. Yu et al. (2017) used the nuclear region ITS2 to evaluate the 127 sequences representing *T. jasminoides via* the neighbor-joining tree method, which demonstrated the remarkable use of DNA barcoding to authenticate market samples. Using the ITS2 region, Chen et al. (2017) found that only 78% of the market samples contained the species listed on their product label. A few studies showed the use of both molecular and chemical markers and phylogenetic approaches for the identification herbal products, including dietary supplements. For example, a study using a phylogenomic approach analyzed the evolutionarily complex genus *Berberis* in order to develop DNA barcodes for the medicinally important species *Berberis aristata* for the regulatory purposes and quality control (Kreuzer et al., 2019). The combination of molecular and chemical markers ensures the quality of the Copalchi complex used in Mexican Herbal Pharmacopoeia and successfully differentiates between the species *Hintonia latiflora*, *H. standleyana*, and *Exostema caribaeum* (Cristians et al., 2018). Pawar et al. (2017) used traditional DNA barcoding techniques and chemical markers to identify frequently consumed botanical dietary supplements of ginkgo, soy, valerian, yohimbe, etc. The combination of DNA barcoding and nuclear magnetic resonance (NMR) was used for the identification of admixture in *Garciania* species (Seethapathy et al., 2018) and *Saraca acosa* (Urumarudappa et al., 2016). Wang et al. (2016) used DNA barcoding for the identification of processed *Angelicae sinensis* radix (Danggui) used in Chinese patent medicines (CPMs). *Lonicerae japonicae* Flos was used to produce hundred kinds of CPMs, single nucleotide polymorphisms (SNPs) were used to generate

TABLE 1 | Information on the 66 documented medicinal plants used in the Thailand Herbal Pharmacopoeia 2018.

Thai name	Scientific name	Family	Habit	Parts used	Treatment/application
Wannam (ว่านน้ำ)	*Acorus calamus* L.	Acoraceae	Aquatic perennial herb	Dried rhizome	Carminative
Matum (มะตูม)	*Aegle marmelos* (L.) Corrêa	Rutaceae	Tree	Fruits and bark	Antidiarrheal, stomachic
Hom (หอม)	*Allium ascalonicum* L.	Amaryllidaceae	Biennial herb	Dried bulb	Carminative, expectorant
Krathiam (กระเทียม)	*Allium sativum* L.	Amaryllidaceae	Herb	Bulb	Antimicrobial, antihyperlipidemic
Fa Thalai (ฟ้าทะลายโจร)	*Andrographis paniculata* (Burm. f.) Nees	Acanthaceae	Herb	Dried aerial part	Antidiarrheal, antipyretic, antiinflammatory
Thian Ta Takkatan (เทียนตาตักแตน)	*Anethum graveolens* L.	Apiaceae	Annual herb	Dried ripe fruit	Carminative, pharmaceutic aid
Kot (โกฐสอ)	*Angelica dahurica* (Hoffm.) Benth. & H	Apiaceae	Perennial herb	Dried root	Antipyretic, analgesic
Kot Chiang (โกฐเชียง)	*Angelica sinensis* (Oliv.) Diels	Apiaceae	Perennial herb	Roots	Blood tonic, treatment of mental disorders
Khamin Khruea (ขมิ้นเครือ)	*Arcangelisia flava* (L.) Merr.	Menispermaceae	Large climber	Stem	Stomachic, antidiarrheal, antibacterial
Maksong (หมากสง)	*Areca catechu* L.	Areceae	Small or medium sized tree	Ripe seed	Anthelmintic, antidiarrheal,
Kot Chula Lampha (โกฐจุฬาลัมพา)	*Artemisia annua* L.	Asteraceae	Annual herb	Dried aerial part	Antipyretic
Kot Khamao (โกฐเขมา)	*Atractylodes lancea* (Thunb.) DC.	Asteraceae	Perennial herb	Rhizome	Stomachic
Kot Kraduk (โกฐกระดูก)	*Aucklandia lappa* Decne	Asteraceae	Herb	Roots	Stomachic, carminative, antispasmodic
Sawat (สวาด)	*Caesalpinia bonduc* (L.) H. Roxb.	Fabaceae	Climber	Leaf	Laxative, antiflatulent
Phrik Khinu (พริกขี้หนู)	*Capsicum annuum* L.	Solanaceae	Annual or perennial herb	Dried ripe fruit	Gastro-intestinal stimulant, counterirritant
Thian Ta Kop (เทียนตากบ)	*Carum carvi* L.	Apiaceae	Perennial herb	Dried ripe fruit	Carminative, antiflatulent, pharmaceutic aid
Khun (คูน)	*Cassia fistula* L.	Fabaceae	Tree	Pulp	Laxative
Buabok (บัวบก)	*Centella asiatica* (L.) Urb.	Apiaceae	Herb	Aerial part	Mild diuretic, antiinflammatory, wound healing
Phet Sangkhat (เพชรสังฆาต)	*Cissus quadrangularis* L.	Vitaceae	Woody climber	Dried stem	Alleviation of hemorrhoidal symptoms
Makrut (มะกรูด)	*Citrus hystrix* DC.	Rutaceae	Shrub or small tree	Leaf	Pharmaceutics aid, carminative
Phaya Yo (พญายอ)	*Clinacanthus nutans* (Burm. f.) Lindau	Acanthaceae	Scandent shrub	Leaf	Antiinflammatory, antiviral
Thian Khao (เทียนขาว)	*Cuminum cyminum* L.	Apiaceae	Herb	Fruit	Carminative, expectorant, alterative
Khamin Chan (ขมิ้นชัน)	*Curcuma longa* L.	Zingiberaceae	Perennial herb	Dried rhizome	Stomachic, carminative, pharmaceutics aid, astringent
Khamin Oi (ขมิ้นอ้อย)	*Curcuma* sp.	Zingiberaceae	Perennial herb	Dried rhizome	Stomachic, antidiarrheal, emmenagogue
Lakkachan (ลักจัน)	*Dracaena cochinchinensis* (Lour.) S. C. Chen	Asparagaceae	Tree	Wood	Antipyretic, antiinflammatory
Pla Lai Phueak (ปลาไหลเผือก)	*Eurycoma longifolia* Jack	Simaroubaceae	Shrub	Roots	Antipyretic
Thian Khao Plueak (เทียนข้าวเปลือก)	*Foeniculum vulgare* Mill.	Apiaceae	Herb	Dried cremocarp and mericarp	Carminative, spasmolytic
Krachiap Daeng (กระเจี๊ยบแดง)	*Hibiscus sabdariffa* L.	Malvaceae	Annual herb or subshrub	Dried persistent calyx and epicalyx	Diuretic
Maenglak Kha (แมงลักคา)	*Hyptis suaveolens* (L.) Poit.	Lamiaceae	Shrub	Dried aerial part	Carminative, antimicrobial (topical)
Krachai Dam (กระชายดำ)	*Kaempferia parviflora* Wall. ex Baker.	Zingiberaceae	Herb	Rhizome	Tonic, carminative
Thian Daeng (เทียนแดง)	*Lepidium sativum* L.	Brassicaceae	Annual herb	Seeds	Expectorant, stomachic
Kot Hua Bua (โกฐหัวบัว)	*Ligusticum sinense* Oliv. cv. Chuanxiong	Apiaceae	Herb	Dried Rhizome	Carminative, blood tonic for menstrual disorder
Bunnak (บุนนาค)	*Mesua ferrea* L.	Calophyllaceae	Tree	Dried blooming flower	Cardiotonic, antipyretic

(Continued)

TABLE 1 | Continued

Thai name	Scientific name	Family	Habit	Parts used	Treatment/application
Phikun (พิกุล)	*Mimusops elengi* L.	Sapotaceae	Tree	Dried flower	Tonic, antipyretic
Mara Khi Nok (มะระขี้นก)	*Momordica charantia* L.	Cucurbitaceae	Annual or perennial climber	Fruit	Bitter tonic, internal heat alleviating
Mon (หม่อน)	*Morus alba* L.	Moraceae	Deciduous tree or shrub	Leaf	Mild antitussive
Kot Chada Mangsi (โกฐชฎามังสี)	*Nardostachys jatamansi* (D. Don) DC.	Caprifoliaceae	herb	Roots, Rhizome	Mild sedative, treatment of dysmenorrhea
Bua Luang (บัวหลวง)	*Nelumbo nucifera* Gaertn.	Nelumbonaceae	Perennial herb	Dried stamen	Cardiotonic, antipyretic
Kot Kan Phrao (โกฐก้านพร้าว)	*Neopicrorhiza scrophularia* Pennell	Plantaginaceae	Perennial herb	Rhizome	Antipyretic, stomachic
Thian Dam (เทียนดำ)	*Nigella sativa* L.	Ranunculaceae	Annual herb	Seed	Carminative, diuretic
Kaphrao Daeng (กะเพราแดง)	*Ocimum tenuiflorum* L.	Lamiaceae	Herb	Dried leaf	Pharmaceutics aid, carminative
Ya Nuat Maeo (หญ้าหนวดแมว)	*Orthosiphon aristatus* (Blume) Miq.	Lamiaceae	Perennial herb	Dried leaf and stem tip	Diuretic
Makham Pom (มะขามป้อม)	*Phyllanthus emblica* L.	Phyllanthaceae	Small or medium sized tree	Dried mature fruit	Expectorant, laxative, antiscorbutic
Thian Sattabut (เทียนสัตตบุษย์)	*Pimpinella anisum* L.	Apiaceae	Annual herb	Dried ripe fruit	Carminative, expectorant, pharmaceutic aid
Phlu (พลู)	*Piper betle* L.	Piperaceae	Woody climber	Leaf	Antifungal, antiallergic
Phrik Thai Dam (พริกไทยดำ)	*Piper nigrum* L.	Piperaceae	Climber	fruit	Aromatic, stomachic, carminative
Phrik Thai Lon (พริกไทยล่อน)	*Piper nigrum* L.	Piperaceae	Woody perennial climber	Dried unripe fruit	Aromatic, stomachic, carminative
Di Pli (ดีปลี)	*Piper retrofractum* Vahl	Piperaceae	Woody climber	Dried stem	Carminative, stomachic, antiinflammatory
Chaphlu (ชะพลู)	*Piper sarmentosum* Roxb.	Piperaceae	Herb	Leaf	Carminative
Sakhan (สะค้าน)	*Piper wallichii* (Miq.) Hand.-Mazz.	Piperaceae	Woody climber	Dried stem	Carminative, stomachic, antiinflammatory
Thian Klet Hoi (เทียนเกล็ดหอย)	*Plantago ovata* Forssk.	Plantaginaceae	Herb	Seed	Bulk-forming laxative
Chan Daeng (จันทน์แดง)	*Pterocarpus santalinus* L. f.	Fabaceae	Tree	Bark	Antipyretic, antiinflammatory, cardiotonic
Chan Khao (จันทน์ขาว)	*Santalum album* L.	Santalaceae	Tree	Dried heartwood	Cardiotonic, stomachic, nerve tonic
Chumhet Thet (ชุมเห็ดเทศ)	*Senna alata* (L.) Roxb.	Fabaceae	Herb or under shrub	Dried mature seed	Laxative, antifungal
Chumhet Thai (ชุมเห็ดไทย)	*Senna tora* (L.) Roxb.	Fabaceae	Herb or under shrub	Dried mature seed	Laxative, diuretic
Mawaeng Khruea (มะแว้งเครือ)	*Solanum trilobatum* L.	Solanaceae	Slender scrambling shrub	Fruit	Expectorant
Thaowan Priang (เถาวัลย์เปรียง)	*Solori scandens* (Roxb.) Sirich. & Ade	Fabaceae	Large woody Climber	Dried stem	Analgesic, antiinflammatory,
Tanmon (ตานหม่อน)	*Tarlmounia elliptica* (DC.) H. Rob., S. C.	Asteraceae	Scandent shrub	Leaf	Demulcent
Samo Phiphek (สมอพิเภก)	*Terminalia bellirica* (Gaertn.) Roxb.	Combretaceae	Large tree	Mature fruit	Laxative, carminative, astringent, expectorant
Samo Thai, Kot Phung Pla (สมอไทย, โกฐพุงปลา)	*Terminalia chebula* Retz.	Combretaceae	Large tree	Mature fruit	Laxative, carminative, astringent, expectorant
Rangchuet (รางจืด)	*Thunbergia laurifolia* Lindl.	Acanthaceae	Woody climber	Leaf	Detoxicant, antipyretic
Boraphet (บอระเพ็ด)	*Tinospora crispa* (L.) Hook. f. & Thom	Menispermaceae	Woody climber	Dried stem	Antipyretic, bitter tonic, stomachic
Thian Yaowaphani (เทียนเยาวพาณี)	*Trachyspermum ammi* (L.) Sprague	Apiaceae	Annual herb	Dried ripe fruit	Carminative, pharmaceutic aid
Phlai (ไพล)	*Zingiber montanum* (J. König) Link. ex A.	Zingiberaceae	Herb	Dried rhizome	Antiinflammatory, counterirritant, mosquito repellent
Khing (ขิง)	*Zingiber officinale* Roscoe	Zingiberaceae	Perennial herb	Dried rhizome	Carminative, antiflatulent
Krathue (กระทือ)	*Zingiber zerumbet* (L.) Sm.	Zingiberaceae	Herb	Dried rhizome	Antiflatulent, stomachic

TABLE 2 | Species admixtures in the herbal trade samples of medicinal plants listed in the Thai Herbal Pharmacopoeia and techniques employed for discrimination.

Thai name	Scientific name	Matrix type	Total number samples	Percentage of species admixture detected	Declared/ identified species	Discriminant technique employed	Reference
Maksong (หมากสง)	*Areca catechu* L.	Processed sample	45	38.09	Nil	Mini-DNA barcode	Song et al., 2017
Matum (มะตูม)	*Aegle maamelos,* (L.) Corrêa	Leaf, root, fruit	11	0	Nil	DNA barcode	Kumar et al., 2018
Fa Thalai (ฟ้าทะลาย)	*Andrographis paniculata* (Burm.f.) Nees	Dried sample, powder, capsule, tea	10	NQ	*Andrographis paniculata, Acanthus ebracteatus* and *Rhinacanthus nasutus*	DNA barcode	Osathanunkul et al., 2016
Thian Ta Takkatan (เทียนตาตั๊กแตน)	*Anethum graveolens* L.	N/A	N/A	NQ	*Trachyspermum ammi andFoeniculum vulgare*	DNA barcode	Schori and Showalter, 2001
Kot (โกฐสอ)	*Angelica dahurica* (Hoffm.) Benth. & Hook.f. ex Franch. & Sav.	Root	N/A	NQ	Nil	Metabarcoding and real-time PCR	Xin et al., 2018
Kot (โกฐสอ)	*Angelica dahurica* (Hoffm.) Benth. & Hook.f. ex Franch. & Sav.	Root	20	NQ	*A. anomala* and *A. japonica*	DNA barcode and SCAR assay	Noh et al., 2018
Kot Chiang (โกฐเชียง)	*Angelica* sinensis (Oliv.) Diels	N/A	N/A	NQ	*A. laxifoliata* and *A. nitida*	DNA barcode	Feng et al., 2010
Kot Chiang (โกฐเชียง)	*Angelica* sinensis (Oliv.) Diels	Root	13	NQ	Nil	HPLC fingerprints	Lu et al., 2005
Kot Chula Lampha (โกฐจุฬาลัมพา)	*Artemisia annua* Pall.	Dried herb, powder, tablet, tea	58	NQ	*A. atrovirens* and *A. indica*	High resolution melting (HRM) curve analysis and DNA barcode	Song et al., 2016
Phrik Khinu (พริกขี้หนู)	*Capsicum annuum* L.	Powder	5	NQ	*Beta vulgaris* and *Ziziphus nummularia*	RAPD-PCR and SCAR markers	Dhanya et al., 2011
Phrik Khinu (พริกขี้หนู)	*Capsicum annuum* L.	Powder	61	14.75	*Garlic, spring onion,* and/or *onion*	Real-time PCR and DNA sequencing	Kang, 2018
Khun (คูน)	*Cassia fistula* L.	Dried herb, powder,	12	0	Nil	DNA barcode	Seethapathy et al., 2015
Thian Khao, Yira (เทียนขาว/ยีหร่)	*Cuminum cyminum* L.	Powder	11	NQ	Nil	DNA barcode	Arunraj et al., 2016
Khamin Chan (ขมิ้นชัน)	*Curcuma longa* L.	Dried and fresh plant tissue	7	58.54	Nil	Bar-HRM technique	Osathanunkul et al., 2017
Pla Lai Phueak (ปลาไหลเผือก)	*Eurycoma longifolia* Jack	Root powder	46	50 or more	Nil	HPLC and two-dimensional electrophoresis (2DE)	Vejayan et al., 2018
Pla Lai Phueak (ปลาไหลเผือก)	*Eurycoma longifolia* Jack	Capsule, tablet, tea	11	27	*Holcoglossum* sp., *Nigella arvensis, Nigella sativa,* and *Ficus deltoidea*	DNA barcode and HPLC analysis	Abubakar et al., 2018
Pla Lai Phueak (ปลาไหลเผือก)	*Eurycoma longifolia* Jack	Capsule, beverage, instant coffee mix, tea	11	NQ	Nil	Bar-HRM analysis	Fadzil et al., 2018
Krachai Dam, Thai Ginseng (กระชายดำ)	*Kaempferia parviflora* Wall. ex Baker	Processed and packed commercial powder	7	58.54	Nil	Bar-HRM analysis	Osathanunkul et al., 2017
Bunnak (บุนนาค)	*Mesua ferrea* L.	Crude drug	6	33	Nil	DNA barcode	Kumar et al., 2018
Thian Dam (เทียนดำ)	*Nigella sativa* L.	Seed	10	NQ	*Allium cepa* and *Clitoria guianensis*	DNA barcode	Sudhir et al., 2016

(Continued)

TABLE 2 | Continued

Thai name	Scientific name	Matrix type	Total number samples	Percentage of species admixture detected	Declared/ identified species	Discriminant technique employed	Reference
Thian Dam (เทียนดำ)	*Nigella sativa* L.	Seed oil	N/A	NQ	Grape seed oil	Fourier transform infrared (FTIR) spectroscopy and gas chromatography	Nurrulhidayah et al., 2011
Phrik Thai (พริกไทย)	*Piper nigrum* L.	Fruit	N/A	NQ	*Carica papaya*	HPLC and antioxidative assay markers	Menghani et al., 2010
Phrik Thai (พริกไทย)	*Piper nigrum* L.	Powder	9	NQ	Chili	DNA barcode and HPLC	Parvathy et al., 2014
Phrik Thai (พริกไทย)	*Piper nigrum* L.	Seed	N/A	NQ	*Carica papaya*	RAPD markers	Khan et al., 2010
Chan Khao (จันทน์ขาว)	*Santalum album* L.	Oil	38	NQ	Nil	Gas chromatography–mass spectrometry	Howes et al., 2004
Chan Khao (จันทน์ขาว)	*Santalum album* L.	Oil	6	NQ	Nil	Multidimensional gas chromatography with simultaneous mass spectrometric and flame ionization detection	Sciarrone et al., 2011
Samo phiphek (สมอพิเภก)	*Terminalia billirica* (Gaertn.) Roxb.	Fruit	10	0	Nil	DNA barcode	Kumar et al., 2018
Samo phiphek (สมอพิเภก)	*Terminalia bellirica (Gaertn.) Roxb.*	Crude drug	12	NQ	Nil	PCR-RFLP and amplification refractory mutation system (ARMS)	Intharuksa et al., 2016
Samo Thai (สมอไทย)	*Terminalia chubula* Retz.	Fruit	13	0	Nil	DNA barcode	Kumar et al., 2018
Samo Thai (สมอไทย)	*Terminalia chebula* Retz.	Immature fruit	N/A	NQ	Nil	Chromatographic fingerprint analysis	Xie et al., 2006
Rangchuet (รางจืด)	*Thunbergia laurifolia* Lindl.	Leaf	8	NQ	Nil	PCR-RFLP	Suwanchaikasem et al., 2013
Rangchuet (รางจืด)	*Thunbergia laurifolia* Lindl.	Both fresh and dried sample, powder	10	NQ	Nil	Bar-HRM analysis	Singtonat and Osathanunkul, 2015

NQ, Not quantified; N/A, Not applicable.

mixtures of powdered CPMs for authentication, and other CPMs were generated through substitutions or as adulterants (Gao et al., 2017). Zhang et al. (2017) used SNPs for which traditional DNA barcoding has not been successful for the differentiation of *Echinacea* species.

Loop-mediated isothermal amplification (LAMP) is one of the approaches developed to identify herbal medicine species (Li et al., 2016). A study demonstrated that the recombinase polymerase amplification (RPA) assay can be developed into an efficient tool for the rapid on-site authentication of plant species in *Ginkgo biloba* herbal products to differentiate the two species *G. biloba* and *Sophora japonica* (as adulteration) (Liu et al., 2018). DNA barcoding and metabarcoding have potential for the quality control of herbal products (Raclariu et al., 2018). Recently, a study recounted the history of DNA-based methods for identification of botanicals, discussed some of the difficulties in defining a specific barcode or codes to use, and described how next generation sequencing technologies have enabled new techniques that can be used to identify these products with great authority and resolution (Moraes et al., 2015). High-throughput sequencing (HTS) methods were used effectively in the quality control and identification of food components. The HTS platforms, Illumina, and Ion Torrent, were used for the analysis of herbal teas, which yielded congruent results, both qualitatively and quantitatively (Speranskaya et al., 2018). These studies confirm that species admixtures may occur in the raw herbal trade. An intrinsic problem associated with the adulteration of herbal products is the effect it may have on user health and safety (Valiathan, 2006; Seethapathy et al., 2015; Srirama et al., 2017). Without regulation, such adulteration will decrease the efficacy and consumer confidence in herbal products, which will eventually cause economic damage to the raw herbal trade (Srirama et al., 2017).

Policies and Regulations Concerning Herbal Medicines

Recently, the large-scale international trade of herbal products has increased the concern for the safety and efficacy of herbal products (Walker and Applequist, 2012; Newmaster et al., 2013; Techen et al., 2014; Srirama et al., 2017). Since most herbal medicines are used in crude formulas in combinations of several herbs and often have extended usage, it is important that the species being used undergo strict validation, safety assessment, and quality and regulatory approval, similarly to modern medicinal drugs (Srirama et al., 2017). With continued development and improvements in DNA barcoding technology, especially with the combination of high-throughput techniques developed for DNA barcoding, a large number of samples can be assessed (Srirama et al., 2017). This technology could ensure the validation of raw herbal products and the identification of a large number of species *via* DNA-based methods (Srirama et al., 2017).

Although herbal medicines are widely used in healthcare systems for the treatment, diagnosis, and control of disease, quality control and proper regulation remain the foremost challenges worldwide. Every nation has its own official compendiums detailing the standardization and quality procedures for traditional medicine production. Although the WHO has passed stringent regulations related to traditional product production and formulation, very few countries have implemented regulations for herbal medicines, and most countries do not have proper guidelines for botanicals. Therefore, the quality of traded medicinal herbal products is not guaranteed (WHO, 2017).

Thailand became the 26th member of the WHO in 1984, which was a year after the Adverse Drug Reaction Monitoring Center (ADRMC) was associated under the authority of the Thai Food and Drug Administration (Thai FDA) (WHO, 2017). The Thai FDA collects reports from health product surveillance systems and programs through a database, Thai herbal database. The Thai herbal database is a potentially effective data source for identifying adverse events related to herbal products (Saokaew et al., 2011). This database provides specific data with respect to the adverse status of a particular plant species (http://thaihpvc.fda.moph.go.th/thaihvc/index.jsf#). However, there is an immediate need for a quality check portal before it reaches the consumer.

Quality Control: Centralized Testing Laboratory Proposition

Natural products are gaining popularity each day due to their safety and availability at an affordable price. The words "herbal," "natural," and "plant-derived" can be misleading at times, and it is important for the public to be made aware that herbal mixtures are medicines in their own right (Fan et al., 2012). Herbal medicinal preparations are formulations commonly consisting of 5 to 15 different herbs or a complex formulation consisting of several medicinal herbs and chemical drug constituents. A single herbal medicine may contain many natural constituents and/or a combination of numerous herbs that can give rise to interactions with hundreds of natural ingredients (Choi et al., 2002). This demands multiple stages of assessment of herbal extracts or products, such as pharmaceutical documentation, toxicology studies, and clinical studies for quality, safety, and efficacy, before entering the market.

A strong regulatory mechanism must be implemented to screen the safety, identity, and quality of herbal products (Srirama et al., 2017). Central to such a regulatory structure is the establishment of an effective entity to evaluate the credibility of species, assess the authenticity of the raw herbal products, and connect these data with trade regulators, both nationally and internationally. To safeguard the integrity of herbal trade, trade partners may need to access and utilize a biological reference guide developed through molecular diagnostic tools coupled with metabolite profiling. Ensuring herbal medicine quality also makes the products safer and more reliable, efficacious, sustainable, and marketable (Srirama et al., 2017). Imports and exports can be governed by such validations certified by nationally recognized government bodies/agencies (**Figure 1**). It is important that such a governing body also sets up a regulatory system wherein both traditional medicinal plants and their herbal products are placed. Furthermore, it is important that the molecular tools (DNA sequences) and chemical metabolite profiles are also available along with the herbal plant samples (Srirama et al., 2017). These tools would provide a simple reference for validation. However, Thai medicinal products containing mixtures of numerous herbs make it difficult, time consuming, and expensive to meet the requirements of identification (both by DNA-based and metabolite-based methodologies). However, with the emergence of new technologies, especially next-generation and high-throughput techniques, a large number of samples can be evaluated through meta-barcoding and chemical analysis. In addition to the potential for frequent authentication of herbal products, this setup can also identify a wide variety of medicinal plant species.

Safe and stable herbal extracts may be marketed if their therapeutic use is well documented and certified by a CT laboratory. There is an urgent need to develop a digital key that would enable easy identification of medicinal plants and their products, which could integrate floristic details, trade, drug databases, and DNA barcoding information (**Figure 2**). Electronic access to such database information can be made possible with internet access and international data sharing efforts, where a wealth of chemical and DNA sequence information can be made available and samples be compared. Users can easily search and compare by plant name or drug name to obtain complete details.

Conclusions

Medicinal plants and their products are extensively used in the indigenous and modern healthcare systems in Thailand and many countries. The international trade of herbal products has a major impact on the international economy, and the demand for herbal products is growing in both

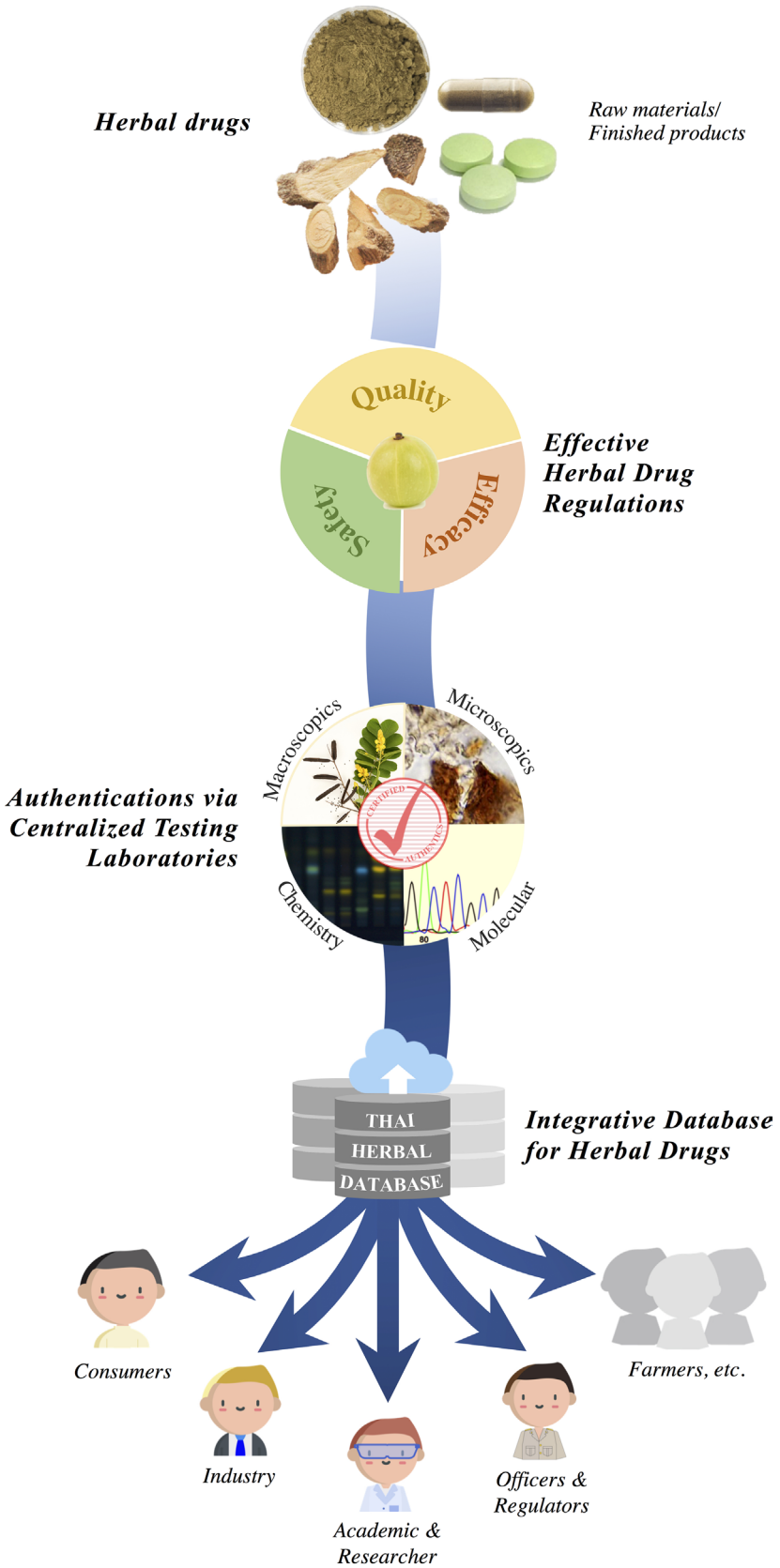

FIGURE 1 | Proposed regulatory framework of traded herbal drugs of Thailand.

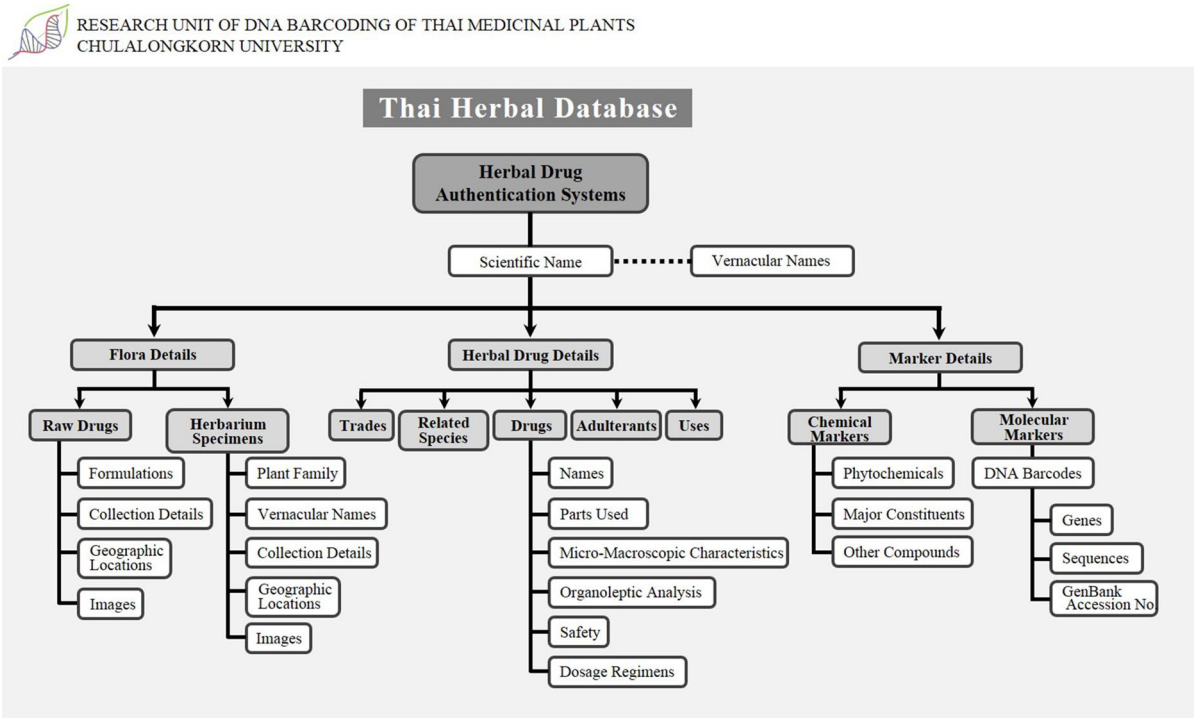

FIGURE 2 | Schematic representation of the Thai Herbal database.

developing and developed countries. This increase in demand has resulted in the substitution and adulteration of medicinal plants with other herbal products whose health benefits are unknown. The results of this study would potentially be useful in providing recommendations to address the increasing concerns of adulteration in raw drug trade and to propose mechanisms that can ensure quality standards in raw drug markets.

Our observations clearly support the claim that there is no established mechanism to connect and coordinate the herbal industry for the certification of herbal products in Thailand. Therefore, we propose the Thai herbal database that contains data on all medicinal plants, including floristic details such as taxonomic hierarchy, vernacular names in various languages, habitat, cultivation type, worldwide distribution maps, Thailand distribution maps (state and district), species images, herbarium images, general photographs, line diagrams, and synonyms. Genetic data include DNA marker information, gene sequences, GenBank accession number details, and chemical data include details of various kinds of tests such as TLC identity test, gas chromatography, gas liquid chromatography, and estimation of chemical compounds in drugs using HPLC and a complete list of major chemical constituents (marker compounds). It also contains a list of other chemical constituents and possibly important chemical compound structures as well as the status of import–export trade and the details of admixtures found in those particular plants. This database could play an important role in monitoring the medicinal plant trade and could be a promising initiative in Thailand for the development of science and technology and to provide consumers with access to all essential information. This herbal database concept could be a novel strategy in Thailand, generating transparency for all safety and quality measures and facilitating the prevention of admixtures in the herbal trade. This herbal database should be developed with utmost planning and made available to all researchers, academicians, people involved with regulatory policy and industry, and, most importantly, common people so that they may gain access to past and present studies. Additionally, in our opinion, accessibility of this herbal database to all researchers, traders, and consumers will allow the sensible development of drug safety measures. The use of this concept can allow governing bodies to improve the efficacy of herbal drugs at a considerable cost.

AUTHOR CONTRIBUTIONS

Conceptualization and design of the study: SS and SK. Data collection and formal analysis: SK. Validation and visualization: SK and CT. Conclusion: SS. Manuscript writing: SK. Review and editing: SS and SK.

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REVIEW
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Polygonum multiflorum-Induced Liver Injury: Clinical Characteristics, Risk Factors, Material Basis, Action Mechanism and Current Challenges

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Polygonum multiflorum Thunb. (PM), called *Heshouwu* in China, is a popular Chinese medicine in clinical practice. Several clinical studies have been conducted to evaluate the traditional therapeutic claims and to study the potential therapeutic activity of PM in dyslipidemia and neurodegenerative diseases, highlighting available clinical evidence. In recent years, reports on clinical adverse reactions of Raw Radix *P. multiflorum* (RPM) and *P. multiflorum* Praeparata (PMP) have been on the increase, especially with respect to liver injury. Most liver injury cases had been assessed for causality using RUCAM (Roussel Uclaf Causality Assessment Method) in this paper. However, the components of PM responsible for the reported hepatotoxic effects have not yet been identified. Moreover, many of the reports are contradictory, while studies on the mechanism involved in PM-induced liver damage are not comprehensive. This study was aimed at reviewing the status of research on liver injury due to PM, including clinical characteristics, risk factors, material basis research and mechanism of action, with a view to understanding PM-induced hepatotoxicity, and taking reasonable and effective measures to prevent it. In short, quality control is still one of the major safety problems in TCM drug safety concerns. The model of safety monitoring and risk management of PM drugs is not yet developed. Indeed, the characteristics and risk factors associated with PM require both proper understanding and control of the risk by strengthening standardization of clinical applications, basic science research, quality control in manufacturing, active monitoring methodology and enhancement of international communication and cooperation. Measures should also be encouraged and implemented to promote healthy development of the TCM industry.

Keywords: ***Polygonum multiflorum*** **Thunb., liver injury, safety evaluation, clinical characteristics, material basis, action mechanism, current challenges**

INTRODUCTION

Traditional Chinese Medicine (TCM), which plays a significant role in Chinese civilization, is widely used in western societies, Asia, Africa and the Middle East (Huang et al., 2014; Kim et al., 2016; Kasote et al., 2017; Lin et al., 2017; Sumei et al., 2018). TCM relies on natural products, mainly of herbal origin, used either as raw materials for decoction, as prepared herbal medicines, or as formulated traditional medicines. Herbal medicines and other botanicals are often used as complementary and alternative medicines which are believed safe, also has no strict safety and effective standards, should be paid great attention (Moreira et al., 2014; Lee et al., 2015; Wang et al., 2015; Alamgir, 2017; Cho et al., 2017; Cecilia and Orish, 2018; Jing and Teschke, 2018; Teschke et al., 2018). Herb-induced liver injury (HILI) refers to liver damage caused by TCMs, natural medicines and related preparations, resulting in adverse reactions such as dermatitis, nausea, vomiting, diarrhea, thrombocytopenia, coagulopathy, mental state changes, hepatotoxicity, nephrotoxicity, and electrolyte imbalance (Dag et al., 2014; Divya et al., 2016; Ming et al., 2017; Awortwe et al., 2018). However, the degree of HILI varies among different countries and regions (Zhou et al., 2013; Navarro et al., 2014). This variation may be related to the single-center retrospective investigations and different levels of HILI differential diagnosis in each center. Studies have shown that HILI is more common in people over 40 years old, and the prevalence in women is significantly higher than that in men (Takikawa et al., 2009; 2012; Chalasani et al., 2015). Most of the liver damage caused by Chinese herbal medicine is due to the components of herbal medicines. The first Chinese herbal medicine that produced liver damage is *Teucrium chamaedrys* L. Epidemiological investigations have shown 30 cases of patients in France with liver disease caused by *T. chamaedrys*, as a result of which it was removed from the French market in 1992. Since then, several cases have been reported in countries such as Canada and Spain (Larrey, 1992; Haslan et al., 2015). Liver injury caused by other Chinese herbal medicines in recent years has also been reported. These include *Polygonum multiflorum* Thunb. (*He Shouwu* in Chinese, hereinafter referred to as PM) (Lin et al., 2015; Wang et al., 2015; Guo et al., 2016); *Aloe barbadensis* Mill. (Yang et al., 2010; Jeonghun et al., 2014), *Atractylis gummifera* Salzm. ex L.(29), *Centella asiatica* (L.) Urb. (Jahan et al., 2012), *Larrea tridentata* (Sessé & Moc. ex DC.) Coville (Arteaga et al., 2005), *Mitragyna speciosa* (Korth.) Havil. (Harizal et al., 2010), *Morinda citrifolia* L. (Pawlus and Kinghorn, 2007; Yu et al., 2011), *Symphytum officinale* L. (Betz et al., 1994; Rode, 2002), and *Xanthium strumarium* L. (Kamboj and Saluja, 2010; Wang et al., 2011). Thus, the extensive use of these herbal medicines warrants safety measures. Indeed, TCM drug safety monitoring and risk management are becoming increasingly important tasks.

P. multiflorum Thunb. (PM), called *Heshouwu* in China, is a popular Chinese medicine in clinical practice. There are two forms of PM decoctions in the *Chinese Pharmacopoeia* (2015): Raw Radix *P. multiflorum* (RPM) and *P. multiflorum* Preparata (PMP). While RPM contributes to detoxification and bowel relaxation, PMP tonifies the liver and kidney, benefits *essence of blood* and *black beard*, and relieves hyperlipidemia, fatty liver, and osteoporosis (Xuan et al., 2007; Wang et al., 2014; Bounda and Feng, 2015). Several clinical studies have been conducted to evaluate the traditional therapeutic claims including anti-inflammatory bioactivity, dyslipidemia, sleep disorders, neurodegenerative disease. For example, PM was significantly tested for the treatment of the hyperlipidemia in a clinical study that enrolled 50 patients. The findings demonstrated that the lipid-lowering effect may be related to its regulating action of the genes involved in cholesterol synthesis and lipoprotein metabolism (Ke et al., 2000). Chen et al. have investigated the therapeutic activity of PM in Alzheimer's disease (AD) through a clinical trial (Chen L et al., 2010). The findings suggested that the scores for the Ability of Daily Living Scale and the Mini-Mental State Examination were significantly improved in the treatment group compared to the Chinese herb control group and the western medicine control group ($P < 0.01$). Moreover, in a randomized, Piracetam-controlled, single-center clinical trial, PM was evaluated as monotherapy for vascular dementia (VaD) (Li et al., 2008). The authors found that the total clinical effective rate was 71.25% and that the herbal medicinal had obvious therapeutic effect on VaD, with no relative adverse drug reactions. Therefore, the therapeutic effect of PM on neurodegenerative diseases is quite obvious and widely recognized. In addition, PM is also highly popular in TCM preparations, health foods and toiletries which are applied to *reinforce liver* and *kidney* and *black beard* (Guo et al., 2014; Hu et al., 2014; Lee et al., 2014). According to statistics, there are about 500 Chinese patent medicines and 200 health care products containing PM (State pharmacopoeia committee, 2015a).

In recent years, the incidence of drug-induced liver injury from PM and its related preparations have increased (But et al., 1996; Mazzanti et al., 2004; Jung et al., 2011). Incidents of PM-induced liver damage have led to extensive research interest in China and elsewhere (But et al., 1996; Park et al., 2001; Cho et al., 2009; Furukawa et al., 2010). In the present study, a comprehensive review of the relevant published literature was conducted in order to fully understand the clinical characteristics, pharmacovigilance practice risk and risk factors of PM-associated hepatitis so as to prevent future occurrences.

CASE REPORTS ON LIVER INJURY CAUSED BY *P. MULTIFLORUM* THUNB.

There is very scanty description of the toxicity of PM in ancient Chinese literature. Only 3 of the 42 ancient books which were published in Early China described the toxicity of PM, and highlighted that the incidence of PM-induced adverse reactions was low. In 1996, Hong

Kong reported the first case of liver injury caused by PM in China (Qi et al., 2013). Then, in 1966, British Medicines and Health Products Administration (MHRA) notified the public of a case of liver damage caused by PM preparation, and clearly stated that PM had safety problems; Canada, Australia, the United Kingdom and other countries made similar sensitizations (But et al., 1996). Multinational drug regulatory authorities have successively introduced policies to regulate and

even limit the use of PM and its preparations (Noda et al., 2009; Qi et al., 2013). In 2005, Adverse Drug Reaction Information Bulletin (No.9) of China Food and Drug Administration (CFDA) in a notice on the liver damage caused by *Polygonum* preparation *baishiwan*, recommended that patients should take it under the guidance of doctors, strictly observe symptoms and contraindications, and avoid overdose or long-term usage (CFDA, 2005). Patients were also advised to pay attention to liver function monitoring during the treatment period. The CFDA issued a circular prohibiting people with liver dysfunction from taking five kinds of drugs containing PM: *Yangxueshengfa* capsule, the first *Karasuma*, Radix piece, *shouwu* piece, and *shouwu* particles. These medicines were transferred to prescription drug management with revised specifications in 2013 (CFDA, 2013). In July, 2014, CFDA once again declared that strengthened supervision should be carried out on health foods containing PM, and made the following recommendations: (Kasote et al., 2017) Daily intake of raw products should not exceed 1.5 g, while the daily dose of preparations should not exceed 3.0 g; (Kim et al., 2016) the label of any health food containing PM should specify that the medicine is not suitable for people who have liver insufficiency or who have a family history of liver disease; (Sumei et al., 2018) there should be a precaution to the effect that the PM-containing product is not suitable for long-term excessive usage, and should not be taken along with drugs linked with risk of liver damage; and (Lin et al., 2017) patients must monitor liver function during the medication period (CFDA, 2014a). Moreover, CFDA issued a bulletin to draw attention to the risk of liver injury caused by oral administration of PM, requiring medical staff to fully understand the risk of drug use and patient status before using PM and its prescription preparations, while emphasizing the need to pay attention to the dose and course of treatment, and to avoid simultaneous use of PM and hepatotoxic drugs (CFDA, 2014b). Patients were advised to immediately discontinue the drug and seek medical attention if abnormal liver function was diagnosed. The National Adverse Reaction Monitoring team received more than 10,000 complaints about PM and its preparations, highlighting the issue of liver damage associated with the popularly used herbal medicine in China (National Adverse Monitoring System).

The diagnosis of HILI represents a particular clinical and regulatory challenge with major pitfalls for the causality evaluation. RUCAM (Roussel Uclaf Causality Assessment Method) or its previous synonym CIOMS (Council for International Organizations of Medical Sciences) is a well-established tool in common use to quantitatively assess causality in cases of suspected drug induced liver injury (DILI) and herb induced liver injury (HILI). RUCAM represents a structured, standardized, validated, and hepatotoxicity specific diagnostic approach that attributes scores to individual key items, providing final quantitative gradings of causality for each suspect drug/herb in a case report (Benichou et al., 1993; Danan and Teschke, 2015). An *in situ* analysis of 169 cases of drug-induced liver injury caused by Chinese herbal medicine found that 14 kinds of Chinese herbal medicines exerted potential hepatotoxicity, and the number of cases caused by PM was the highest (Watanabe and Shibuya, 2004; Zhou et al., 2013; Zhu et al., 2015; Jing and Teschke, 2018). Literature on drug-induced liver injury published in Chinese Academic Journals Network Publishing Library revealed the screening of 194 Chinese patent medicines causing drug-induced liver injury, including 100 cases of PM which accounted for 51.6% (Chen and Cai, 1999; Zhi-Feng et al., 2013). In another study, Xiao et al. reported 193,714 cases of liver disease between 2007 and 2016 at The 302nd Hospital, out of which 5,710 cases had abnormal liver function history suspected to be related to drugs. It has been reported that 145 patients took PM or its preparations prior to being diagnosed with HILI (Wang et al., 2015; Wang et al., 2016). The clinical manifestations of liver injury induced by PM and its preparations include jaundice, sclera yellow staining, dark urine, nausea, vomiting, fatigue, weakness, stomach pain, abdominal pain, and loss of appetite (Dong et al., 2014; Xiang et al., 2015). The clinical manifestations of drug-induced hepatitis and viral hepatitis are basically similar. Most patients have a history of repeated medication and repeated onset (Banarova et al., 2012). Studies have revealed that that PM associated with liver damage can occur with no gender orientation, and at any age group (Xiang et al., 2015). In most cases, symptoms of liver damage occur about one month after taking the medicine, and they include fatigue, jaundice, anorexia, and yellow or tawny urine. The liver damage caused by PM in most patients is reversible after discontinuation of PM products, and active treatment can restore liver function, although some patients develop liver failure and die. It is hereby suggested that patients take PM products under the guidance of a physician or pharmacist, and avoid long-term usage or intake of high doses of the drugs. Basic characteristics of RUCAM have been provided in various publications (Danan and Teschke, 2018). The HILI signature should be determined according to the ratio R. In practice, two types of liver injury are considered for evaluation: hepatocellular injury ($R > 5$) and cholestatic/mixed liver injury ($R = < 5$) (Teschke, 2018). Furthermore, the HILI caused by PM between 2010 to 2019 referencing all publications that used RUCAM for causality assessment or positive reexposure tests are shown in **Table 1**.

PATHOGENIC ASPECTS OF LIVER INJURY CAUSED BY *P. MULTIFLORUM* THUNB.

Risk Factors of Liver Damage Caused by PM

Existence of Inherent Toxic Components

Multiple studies have shown that some chemical constituents of PM may be directly toxic to liver cells, but these constituents have not been fully identified. This will be summarized in detail in part IV.

Inappropriate Dosage and Medication Time

The Chinese Pharmacopoeia (2015 edition) stipulates that the dose of RPM is 3–6 g per day, while that of PMP is 3–12 g per day (State pharmacopoeia committee, 2015b). Although the toxicity of PM was rarely mentioned in medical literatures of past dynasties, several reports and information in recent years suggest that excessive and protracted usage of PM can cause toxic reactions in the body (Lin et al., 2015; Yang et al., 2018). In general, the toxicity induced by a medication is proportional to

TABLE 1 | Patient details recorded from published case reports and case series on liver injury caused by PM.

Reference	Reasons for medication	N. of case (M/F)	Age (Year)	HSW type	Assessment methods	Type of liver injury (number)	Outcome
Liu et al. (2013)	Grey hair	1(0/1)	20	PM	RUCAM	M(1)	Recover
Miao et al. (2013)	Health care, Hyperlipidemia	2(0/2)	52;61	RPM; PMP	NA	C(2)	Recover
Liu et al. (2013)	Vitiligo	1(1/0)	37	PMP	NA	NA	Recover
Gao et al. (2013)	Vitiligo	1(0/1)	14	PMP	NA	C(1)	Recover
Banarova et al. (2012)	NA	1(0/1)	33	Shou Wuwan	NA	NA	Recover
Shao and Li (2012)	Grey hair; Health care	2(1/1)	50;40	RPM; PMP	NA	H(2)	Recover
Hu et al. (2012)	Chronic nephritis	1(1/0)	36	PMP	NA	H(1)	Recover
Zhen and Zeng (2012)	Grey hair	1(0/1)	52	PMP	RUCAM	C(1)	Recover
(Li and Zhang (2012)	Grey hair; Hair loss	2(0/2)	50; 28	RPM	NA	NA	Recover
Chen S. X. et al. (2012)	Grey hair	1(1/0)	57	RPM	NA	C(1)	Recover
Liu et al. (2012)	Grey hair	1(0/1)	56	Yishen Wufa	NA	H(1)	Recover
Sun (2011)	Allergic rhinitis; Health care	2(0/2)	39; 72	RPM	NA	H(2)	Recover
Cao et al. (2011)	Grey hair	1(1/0)	28	PMP	NA	NA	Recover
Liu et al. (2011)	Hair loss	1(1/0)	26	Yangxue shengfa	NA	NA	Recover
Wu and Niu (2011)	Hair loss	1(0/1)	47	Yishen wufa	NA	NA	Recover
Liu et al. (2010)	Hair loss	1(0/1)	50	Shou Wuwan	Chinese Hepatitis Study	H(1)	Recover
Chen M. X. et al. (2010)	Health care	2(1/1)	46; 57	PM tea	NA	H(2)	Recover
Yun et al. (2010)	Grey hair	1(0/1)	51	Shou Wuwan	NA	NA	Recover
Furukawa et al. (2010)	NA	1(0/1)	53	Shou Wupian	NA	C(1)	Recover
Lei et al. (2015)	Hair loss	1(1/0)	26	Jingwu capsule	NA	H(1)	Recover
Xiao (2015)	Hair loss	1(1/0)	34	PM	NA	H(1)	Recover
Zhao (2013)	Grey hair	1(1/0)	39	Jingwu capsule	RUCAM	H(1)	Recover
Yan (2011)	Hair loss	1(1/0)	28	HSW tea	RUCAM	H(1)	Recover
Shen et al. (2010)	Leucoderma	1(0/1)	NA	PM preparation	RUCAM	H(1)	Recover
Li et al. (2015)	Hair loss	1(1/0)	26	Qibao Meiran Wan	NA	NA	Recover
Liu et al. (2015)	Hair loss	2(0/2)	47; 25	PM	NA	NA	Recover
Dong et al. (2014)	NA	18(13/5)	18-63	RPM; PMP	RUCAM	H(18)	Recover
Lian et al. (2013)	Grey hair; Hair loss; et al.	52(30/22)	22-69	PM	RUCAM	H (30); C (9); M (13)	Recover
Zhang et al. (2013)	NA	13(2/11)	35-66	PM	RUCAM	H (6); C (4); M (3)	12 Recoveries; 1 LT
Zhang et al. (2013)	Grey hair; Hair loss;et al.	36(23/13)	24-73	PM preparation	RUCAM	H (21); C (2); M (13)	33 Recoveries; 1 Cirrhosis; 2 Deaths
Ding (2012)	Grey hair; Hair loss;et al.	65(20/45)	34-71	PM preparation	RUCAM	NA	64 Recoveries; 1 Death
Guo (2012)	NA	15(7/8)	18-57	PM	RUCAM	H (8); C (3); M (4)	Recover
Xie et al. (2012)	NA	10(7/3)	36-56	PM	Maria	H (5); C (4); M (1)	9 Recoveries; 1 Death
Song (2011)	NA	26(14/12)	38-71	PM	The seventh edition of diagnostic criteria for internal medicine	NA	Recover
Wang (2011)	NA	20(13/7)	34-67	PM	NA	NA	Recover
Jung et al. (2011)	NA	25(18/7)	24-65	PM	RUCAM	H (18); M (7)	23 Recoveries; 1 LT; 1 Death
Chen S. P. et al. (2012)	Grey hair; Hair loss; et al.	12(7/5)	20-70	PM	RUCAM	H (4); C (4); M (4)	Recover
Liu and Li (2010)	NA	7(2/5)	31-64	PM	NA	NA	Recover

(Continued)

TABLE 1 | Continued

Reference	Reasons for medication	N. of case (M/F)	Age (Year)	HSW type	Assessment methods	Type of liver injury (number)	Outcome
Sheng and Shen (2016)	Health care; Hair loss;Grey hair	50(23/27)	30-70	PM preparation	FDA classification standard	H (30); C (3); M (17)	Recover
Zhu et al. (2015)	Hair loss; Grey hair; Health care	66(31/35)	8-63	PM preparation	RUCAM	H (61); C (1); M (4)	57 Recoveries; 4 Hepatic failure; 4 Cirrhosis;1 Deaths
Guo et al. (2015)	Vitiligo; Hair loss; Grey hair; et al.	15(6/9)	35-65	PM	NA	NA	Recover
Dong (2014)	NA	18(14:4)	19-63	PM	RUCAM)	H(18)	Recover
Wang and Li (2018)	Hair loss;Grey hair; High blood pressure	33(13/20)	17-86	PM preparation	RUCAM	NA	28 Recoveries; 1 invalid case; 1 aggravated case; 3 Deaths
Yang (2018)	Hair loss; Grey hair	30(17/13)	33-58	PM	NA	NA	Recover
Chang et al. (2018)	NA	39(16/23)	47.9 ± 14.3	PM preparation	RUCAM	H (36); C (1); M (2)	36 Recoveries;1 Chronic hepatitis case; 1 Cirrhosis
Li (2010)	Grey hair; Hair loss; et al.	11(6/5)	34-58	PM	NA	NA	Recover

the dose and the duration of exposure to the drug (Bárándi et al., 2010; Yue et al., 2018). People have little understanding of the side effects of PM, and even mistakenly believe that it is safe for a long time (Qi et al., 2013; Yun-Ho et al., 2016). Chen et al. explained the reasons why the doctors of previous generations ignored the toxicity of PM in *Shen Nong's Herbal Classics* (Chen, 1959; Liu, 1982; Jin et al., 2013). The medical practitioners of today regard PM as the top-up of tonics. In the past 20 years, people have long been biased towards the beneficial effects of PM, neglecting its toxicity (Qi et al., 2013). A long-term toxicity study on RPM revealed that continuous administration of RPM at a dose of 20g/kg for 13 weeks caused definite liver damage which was aggravated after 26 weeks, but was reversed after stopping the drug administration (Zhu et al., 2014). In a study on the degree of liver injury caused by different doses of RPM, rats were continuously administered daily doses equivalent to 20, 50, 100, 200 times of corresponding adult dose for 3 months (Hu et al., 2007). The results showed different degrees of liver damage such as inflammatory cell infiltration, hepatic sinus congestion, and kupffer cell proliferation (Hu et al., 2007; Yun et al., 2015). A comparison between the effects of RPM and its preparata on liver injury in rats found that the raw product produced liver damage when it was close to the clinical equivalent dose, while the preparata produced liver damage at a 4-fold dose of the clinical dose. Thus, long-term use of PM preparata also has certain degree of toxic side effects on the liver of rats (Jie et al., 2012; Wang et al., 2014). Continuously administration of different doses of aqueous extract of RPM showed that the activities and mRNA levels of CYP2E1, CYP1A2 were significantly inhibited in rat liver (Ma et al., 2014; Hao et al., 2015; Chun-Lian et al., 2017). A study of "quantity-time-toxic" relationship of hepatotoxicity induced by *Yangxueshengfa*, a PM preparation showed that serum ALT and AST activities peaked 12 h after administration of the drug, and returned to normal after 48 h (Li et al., 2011). It has been reported that *Yangxueshengfa* capsule at the dose of 2.50–12.00 g/kg significantly damaged liver tissue, while ALT, AST and traumatic brain injury (TBI) increased significantly with increase in dose (Xianfeng et al., 2016). Thus, although the occurrence of adverse reactions may be due to individual differences, medication overdose and long exposure to drug all of which are important risk factors for liver damage in PM or its preparations (Wang et al., 2012; He et al., 2016).

Immune Damage or Liver Metabolic Enzyme Genetic Polymorphism

Liver injury induced by PM in clinical application has obvious individual differences, indicating that several susceptibility factors exist in PM-induced liver injury. The traditional toxicological valuation model cannot effectively evaluate the specific liver damage at present. For this reason, Xiao's team constructed a low-dose LPS-induced drug immune-specific liver injury evaluation model for evaluation of the hepatotoxicity caused by RPM. The multi-component effect of RPM and the mechanism of hepatic injury induced by immune system have revealed the scientific nature of immuno-specific PM-induced liver injury (Dan et al., 2016). However, it is still not clear whether other immunologically active components are also involved in the PM-induced immune specificity.

Analysis of the clinical characteristics of patients with liver injury caused by PM and preparations showed that some patients taking normal or less than the normal doses of PM, and even some patients taking the drug only once in a relatively short period of time, developed liver damage. Several patients have a familial adverse reactions to liver organ damage (Liang et al., 2014; China, 2015; Cui et al., 2016; Cheng-Sheng and Sun, 2017). This is probably due to the presence of hereditary hepatic metabolic enzyme deficiency which in turn promotes the accumulation of toxic compounds in the process of biotransformation and metabolism, leading to PM-induced liver damage. The presence of hereditary deficiency of hepatic metabolic enzymes promotes

accumulation of several toxic compounds in the process of biotransformation and metabolism, leading to drug-induced liver damage. If the patient has an allergic constitution, this treats the metabolites and components of PM as haptens. After binding to their macromolecular carriers, the haptens form covalently-bound whole antigens which induce the production of antibodies and hypersensitivity. All of these factors can result in serious liver damage. The phase I and phase II metabolic enzyme genes are important in the development and progression of drug-induced liver injury. The genetic susceptibility of PM-related liver injury may be related to individual differences in phase I and phase II metabolic enzymes (Amacher, 2012; Ma et al., 2014; Feng et al., 2014). Genetic polymorphism in hepatic metabolism enzymes, or patient's hereditary hepatic metabolic enzyme deficiency can cause the accumulation of active constituents of PM during metabolism and biotransformation, leading to drug-induced liver damage (Yun et al., 2015). A study by Huang et al. found that the mRNA expressions of five subtypes of P450 enzymes in liver tissue decreased in a dose-dependent manner after treatment with aqueous extract of RPM, while high-dose treatment significantly inhibited the expressions of CYP1A2 and CYP2E1 mRNA in rat liver (Mei-Xi et al., 2016; Chun-Lian et al., 2017). In addition, other researchers have found that RPM inhibited the expression of CYP450 in rat liver (Zhang et al., 2015). Clinical studies have shown that genetic polymorphism of CYPlA2 differs between normal population and patients with RPM-induced liver injury, through significant differences in the proportion of CYP1A2*1C. This indicates that the activity of CYP1A2 enzyme is lower in patients with RPM-induced liver injury (Ma et al., 2014). Therefore, it is speculated that the mechanism of hepatotoxicity induced by RPM may be related to the genetic polymorphism of CYP450. Some scholars altered the metabolic capability of the liver using drug metabolizing enzyme inhibitors, so as to investigate the effect of trans-stilbene glucoside (the main component of PM) on the susceptibility models of liver injury. The results revealed that inhibition of drug metabolizing enzymes increased liver injury induced by stilbene glycoside, indicating that PM may exert liver damage in patients with different phase II metabolic enzyme gene polymorphisms or low function of these enzymes (Li et al., 2017). Based on the data of the National Center for Adverse Reactions and the clinical database of the drug-induced liver injury in the 302nd Hospital, Xiao et al. found that the incidence of liver damage induced by PM was low. There may be high-risk groups, suggesting that the liver damage of PM may be similar to heterogeneous liver injury. Endotoxin was used as inducer to make an animal model of specific liver injury and RPM was used as an evaluation drug to investigate the liver damage of rats. The results showed that a large dose of 72.5 g/kg caused no significant liver injury in normal rats, while the clinical dose 2 times equivalent (1.08 g/kg) of RPM caused liver injury in an idiosyncratic liver injury model induced by endotoxin, suggesting that there may be a group of people with idiosyncratic sensitivity to hepatotoxicity of RPM (Chun-Yu et al., 2015; Yue et al., 2018). Subsequently, the team also found that the occurrence of immunologically-specific liver injury in RPM was associated with abnormal inhibition of PPAR-γ pathway and overexpression of related inflammatory factors. Pioglitazone (a PPAR-γ agonist) significantly reverses liver damage. Therefore, it is possible to study Chinese medicines for the attenuation of RPM and the mechanism of attenuation (Lan-Zhi et al., 2017).

In summary, genetic polymorphisms of liver metabolic enzymes, metabolic enzyme defects, and immune stress status of different individuals are closely related to liver damage induced by PM.

Improper Processing Technology

Processing technology is important in the culture of TCM due to the advantages of improving the efficacy, modifying the property or reducing the side effects of medicines (Zhou and Zhang, 2006; Yang et al., 2016). There are great differences in the records of processing technologies for RPM, with up to twenty different processing methods. Inadequate degree of processing or inadequate processing methods may cause adverse effects on the liver. The toxicity of RPM is reduced by processing. However, *Yangshoushengfa* capsule, *Anshen Buxue* Liquid, *Runzao* Itching Capsule and *Qibao* Hairdressing Pills, all of which contain RPM, have been reported to cause liver damage, suggesting that inadequate processing may cause RPM toxicity. The use of stilbene glycoside and free anthraquinone contents as the only control indicators of processing is irrational (Yun et al., 2015). According to *Materia Medica* records, "processing of RPM is not nine times, do not sleep its poison. Non-black beans, do not kill its potential." Modern research has shown that RPM drug content profiles of rat blood were significantly different before and after processing, indicating differences in the liver metabolic pathways. Processing can reduce the hepatotoxicity of RPM, as reflected in serum total conjugated bilirubin (CBIL) and total bilirubin (TBIL) profiles (Ma and Wang, 1991; Yun et al., 2015). The constituents of RPM are dynamically changed during processing. After processing, the combined anthraquinone is hydrolyzed to free anthraquinone with diarrhea-reducing effects. The content of stilbene glycoside in RPM was significantly higher than that in PMP, while the contents of monosaccharides, disaccharides, tannins and phospholipids were gradually decreased with extension of processing time (Liu et al., 2008; Rong-Tsun, 2009; Han et al., 2015). A number of studies have shown that the processing of RPM is closely related to the occurrence of adverse reactions. Yang et al. studied the effects of the classic processing method named *nine-steamed and nine-sun*, and a modern processing method called *black bean juice steaming* recorded in Pharmacopoeia, on the chemical constituents of stilbene glycoside, free strontium and combined strontium. The results showed that the contents of these components resulting from the two methods were close, indicating that different processing methods may not affect changes in the components of RPM (Li and Gao, 2015). On the other hand, Pan et al. found that different processing methods caused changes in the contents or structures of the components of RPM, which in turn led to different degrees of liver toxicity (Liu et al., 2011). In summary, the differences in the processing of RPM, and the simplified processing techniques may be important factors for the adverse effects seen in clinical applications, especially hepatotoxicity. As long as the processing technology of RPM is

strictly controlled and standardized, the quality stability of products and the clinical efficacy can be guaranteed, thereby minimizing adverse reactions. It is worth noting that, due to the cumbersome, time-consuming, energy-consuming and long production cycle, many new technologies and methods have been introduced to selectively change the traditional processing technology of RPM. The classic method of processing has not been fully passed down. Peeling, processing taboos, steaming time, and processing accessories which affect the toxicity of RPM, have not received enough attention in the improvement of modern processing technology. This is also a key factor that affects the toxicity of RPM.

Medicinal Materials Issue

In many reports of adverse reactions, PM was purchased by patients themselves, and even some were derived from the farmer's market. Thus, it is difficult to ascertain the authenticity of the PM based only on the patient's oral account. There have been cases where the rhizome of Chinese monkshood was used as PM. The roots of the genus *Pteridophyte* and *Polygonum* are similar to the roots of PM. Consequently, it is difficult to rule out adverse reactions caused by counterfeit drugs and inferior drugs in many reports of liver damage induced by PM. In addition, ancient books have a detailed record of the origin and planting of PM (Yang, 2010). It is necessary to first of all ensure the quality of the medicine itself, and efforts should be made to use PM sold by regular medical units to ensure safety.

Improper Modern Preparation Process

Modern Extraction Process Can Cause Adverse Reactions

Herbal medicine is traditionally used in the form of water decoction which contains appreciable amounts of proteins, amino acids, polysaccharides and other components. In contrast, modern Chinese medicinal preparations generally use ethanol as a solvent in the extraction process. The chemical compositions of water decoction and ethanol extraction are different (Li et al., 2010; Lu et al., 2013; Wang et al., 2013). Compared with the former, ethanol can extract a lot of lipid-soluble constituents such as fatty acid components. These may appreciably alter the active ingredient spectrum, thereby increasing adverse drug reactions. The preparation process of RPM included in the 2015 edition of the Chinese Pharmacopoeia contains three methods: water decoction, ethanol extraction and direct infiltration after osmosis and pulverization. The types and contents of chemical components extracted by the different preparation processes are also different. Percolation process is used in the extraction of Ginseng *Shouwu* Capsule, *Baoxin* Tablet, and *Tongmai Yangxin* Oral Liquid. Although the percolation process is beneficial for extracting active components which are heat-sensitive, it will also change the chemical composition of the original prescription, resulting in an adverse reaction. Clinically, *Anshen Bunao* Liquid (water extract), Ginseng *Shouwu* Capsule (alcohol extract), and *Tianma Shouwu* Tablet have all been reported in liver injury, indicating that different preparation methods may cause RPM-linked adverse reactions.

Researchers have summarized the reasons for the adverse reactions associated with the modern preparation process. It is believed that the application of modern preparation technology increases the yield of active ingredients and also increases the yield of toxic components, which may lead to adverse reactions (Zhang et al., 2015). In some studies, Lu et al. compared the differences in hepatotoxicities of different PM extraction solvents, and found that 50% ethanol extract was the most toxic (Wang et al., 2015; Deng-Ke et al., 2017). In addition, Liang et al. compared the differences in hepatotoxicities of ethanol extracts and water extracts, and reported that the toxicity of alcohol extracts was much higher than those of water extracts and medicinal materials, suggesting that hepatotoxic substances of PM may be concentrated in the alcohol extract (Liang et al., 2010). In another study, Huang et al. used ultrasonic assisted method to extract anthraquinones from PM, and determined the optimal extraction conditions by orthogonal test, ignoring the fact that anthraquinones may be the toxic components of PM. This was in essence, a new extraction process in place of the traditional water extraction process which may cause adverse reactions in *Polygonum* preparations (Huang et al., 2015). In addition, stilbene glycosides, anthraquinone and polymerization proanthocyanidins were selected as indices of PM formula granules, which may produce adverse reactions in clinical usage of PM. These call for close attention.

Modern Pharmaceutical Technologies Can Cause Adverse Reactions

Modern preparation processes such as biosorption, use of macroporous resin columns, and membrane separation effectively improve the utilization of active components, but also inevitably increase the utilization of toxic components. At the same time, macromolecular components such as starch and polysaccharide are usually removed due to their relatively large molecular weights. They have the general effect of moderating the properties of medicines and their detoxification. Although their effects are generally minor, they may lead to increase in adverse reactions. In a study, Wei et al. used macroporous adsorption resin enriched with stilbene glycoside as the core of sustained-release microcapsules in PM (Rao et al., 2009). This is equivalent to increasing the content of the target component in the unit preparation and increasing the clinical dosage of the drug. This may increase the prevalence of adverse reactions in situations where the toxic component of the PM is unclear.

The causes of PM-induced liver damage are summarized in **Figure 1**.

Material Basis Research on Liver Damage Induced by PM

A total of 314 Chinese patent medicines containing PM were retrieved in the Chinese Pharmacopoeia and the Ministry of Standards, including 302 oral preparations and 12 external preparations. At present, among the proprietary Chinese medicines containing PM, only five varieties (*Yangxueshengf*a

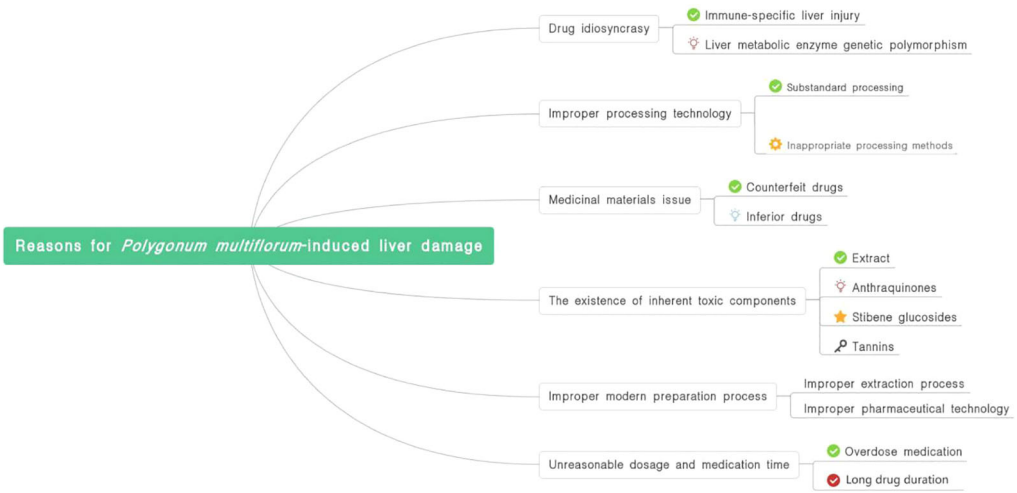

FIGURE 1 | Reasons for *P. multiflorum*-induced liver damage.

Capsule, *Shouwu* Pill, *Shouwu* Tablet, *Shouwu Yanshou* Tablet and *Shouwu Yanshou* Granule are managed as prescription drugs, while the rest are over-the-counter drugs (CFDA, 2013). The frequency of use of PMP is much higher than that of raw products among the oral preparations containing RPM. Analysis of adverse reactions have shown that thirteen types proprietary Chinese medicines contained PM, which accounted for 15.66% of varieties of RPM-containing preparations, while 37 types of proprietary Chinese medicines containing PMP accounted for 16.89% of varieties containing processed products (Lin et al., 2015).

The chemical components of PM are mainly stilbene glycosides, terpenoids, phospholipids, tannins and trace elements, but no toxic components have been identified to date. Stilbene glycosides, terpenoids and tannins are the major constituents of PM, and are the primary focus in the search for toxic components. In addition, PM is rich in phospholipids such as lecithin, inositol phospholipids, ethanolamine phospholipids, phosphatidic acid and cardiolipin. However, it is still unclear which component or components are involved in PM-induced liver injury.

Extracts

A comparison of acute toxicity in mouse has been made among the whole component, water extract and alcohol extract of RPM with respect to oral lethal dose (LD50), maximum tolerance dose (MTD), maximum lethal dose (MLD), cumulative death number and changes in mouse body weight (Wang et al., 2016). The results showed that the whole component was the most toxic, while the water extract was the least toxic. In a study on the effect of single administration of different components of RPM on "quantity–time–toxicity" in mice, it was found that the activities of ALT and AST peaked at 4 h in mice given higher doses of water extract for the duration of about 24 h (Huang et al., 2011). Serum ALT and AST activities reached peaked at 2 h and continued for 48 h after oral administration of higher doses. After 2 h of administration, the mice showed obvious hepatomegaly and increased liver index. Studies on the toxicity of RPM extract on L-02 human hepatocytes showed that the ethanol extracts of RPM and PMP were significantly more toxic to human hepatocytes than the aqueous extracts, suggesting that ethanol extract of RPM had a higher risk of liver damage (Lin et al., 2015). Ethanol extract of RPM was more hepatotoxic than water extract, due to its higher contents of emodin 8-O-D-glucopyranoside, emodin methyl ether-8-O-D-glucopyranoside, emodin and emodin methyl ether (possible hepatotoxic components) than in water extract (Lv et al., 2015). Some scholars have found that histological sections of mice showed signs of inflammatory response after 3 months of administration of RPM decoction. A study of the hepatotoxicity of different sources of RPM showed that the TCIDso of RPM ranged from 310 to 350 U·g-1, while that of PMP was 130 U ·g-1. Moreover, a comparison of the risk of liver injury caused 8 kinds of Chinese herbal medicines containing PM, using TCIDso as index, showed that *baishiwan* was relatively highly toxic. In a study, bilirubin was used as a substrate for UGT1A1 enzyme to investigate the inhibitory effect of bilirubin glucuronic acid binding on UGT1A1 enzyme activity *in vitro* and *in vivo* during bilirubin metabolism in rat liver. The extract had a strong inhibitory effect on UGT1A1, and the inhibition type was non-competitive inhibition (Qi et al., 2016). In a recent study, the hepatotoxic effects of long-term use of water extracts, alcohol extracts and formula particles of RPM were investigated in rats. The results showed that hepatocytes in different administration groups had different degrees of fatty degeneration and inflammatory cell infiltration. The order of toxicity of RPM was alcohol extract > formula particle group > water extract. It can be concluded that RPM is harmful to the liver, and that the toxicity of RPM is greater than that of PMP, which is consistent with clinical results. Although the hepatotoxicity of processed product is reduced, caution should be exercised in its clinical use.

Anthraquinones

The compounds present in PM are mainly emodin, rhein, emodin methyl ether, chrysophanol and aloe-emodin, and their pharmacological effects have been extensively studied. However, excessive use of these anthraquinones stimulate the gastrointestinal tract, resulting in symptoms of diarrhea, abdominal pain, bowel, nausea and vomiting. Severe cases have progressed quickly to paroxysmal tonic convulsions, convulsions, restlessness, and even respiratory paralysis. An analysis of the correlation between PM compounds and cytotoxicity revealed that 10 compounds may be related to the hepatotoxicity of PM, 7 of which are guanidine or glucoside compounds (Lin et al., 2015). Hepatotoxicity could be induced by emodin at high, medium and low doses, indicating that the anthraquinones may be the basis of potential hepatotoxic effects of PM (Can et al., 2015). Studies have demonstrated obvious hepatotoxicities of emodin and rhein. In a study on the correlation between different components of PM and hepatocyte apoptosis, Wei et al. showed that chrysophanol was likely to be the PM component responsible for liver injury. The results showed that emodin and rhein increased damage to L02 and BEL cells in a concentration- or time-dependent manner, while stilbene glycoside had no obvious cytotoxic effect. In addition, emodin, rhein and chrysophanol had no significant effects on the expression of liver CYP450. It has been shown that emodin accumulated in hepatocytes at high-dose repeated administration, with AUC and C_{max} showing upward trends, suggesting causal relationship between emodin and liver-induced damage (Ma et al., 2015). In another study, the liver injury and apoptosis induced by PM were attributed to chrysophanol and other substances. Eight kinds of toxic and controversial monomeric components have been identified in PM using high content analysis (HCA), and it was revealed that anthraquinones were responsible for its potential hepatotoxicity. Moreover, liver damage caused by PM was associated with mitochondrial abnormalities. The UGT1A1 enzyme in bilirubin metabolism has been used to determine the mechanism of hepatotoxicity caused by chemical constituents of PM. It was found that emodin-type anthraquinones selectively inhibited the activity of UGT1A1 enzyme in a structure-activity relationship. In addition, diterpene ketone and anthrone glucosides inhibited the activity of UGT1A1 enzyme. It was speculated that the inhibition may be the mechanism underlying the hepatotoxicity of anthraquinones in PM (Wang et al., 2016). Rhein, emodin and chrysophanol are the main components of anthraquinones in PM, which hepatotoxicity is related to total bilirubin and transaminase contents, inducing liver tissue inflammation, hepatocyte apoptosis and oxidative stress. Yang et al. used zebrafish as a model to study the hepatotoxicity of different extracts and different monomer components of PM (7 anthraquinones, 8 terpenoids, 7 anthrones, 3 cinnamic acid amides and 2 naphthol), and showed that the median lethal dose of emodin was the lowest (Yang et al., 2018). In conclusion, more studies are required to unravel the mechanism of toxicity of PM, and toxicity process in the body.

Stilbene Glycosides

At present, relevant research reports on stilbene glycoside-induced liver injury are rare. Liver damage has been reported in SD rats after oral administration of stilbene glycosides (Lv et al., 2011). Moreover, tannins present in PM have been shown to exert liver damage in rats in a synergistic effect with stilbene glycoside. Therefore, various chemical constituents of PM have been shown to be associated with hepatocyte injury. Other researchers used high, medium and low doses of stilbene glycoside for continuous gastric perfusion for 90 days to determine changes in liver enzymes and proteins, and found that the globulins, ALT and AST were increased significantly during the administration period (Zhe et al., 2016). After 15 days of withdrawal, except for the significant decrease of LDH, there were no significant changes in the other indices. This indicates that long-term use of stilbene glycosides may cause some damage to the liver, but liver function can be restored to normal levels after stopping administration of the drug. A comparison of the degree of liver damage by different extracts in a rat model showed that liver toxicity of the ethyl acetate extract was the most severe (Wang et al., 2016; Meng et al., 2017). On further analysis, it was found that cis-stilbene glycoside had the strongest cytotoxicity, suggesting that PM should be stored in the dark during the preservation of alcohol-derived or liquid medicines to avoid or reduce the formation of cis-diphenylethylene and reduce the occurrence of specificity in clinical applications.

Tannins

Tannins, which content is up to 15.7%, have been regarded as residue in the extraction process of PM, and have not been taken seriously, thereby ignoring their effect on efficacy and toxicity of PM. As the pharmacological effects of tannins become more prominent, their toxicological studies have begun to receive attention. Studies have found that tannins are important causes of cryptogenic liver damage associated with TCM (Blumenberg et al., 1960; Chandranayagam et al., 2013). Therefore, tannins have also become important aspects of the research on hepatotoxic components of PM. It has been reported that tannins in PM exert toxic effects on the liver. The combination of tannins and stilbene glycoside in different proportions can cause different degrees of liver damage in rats, through a mechanism be related to the decreased secretion of cholinesterase. The combination of tannins and stilbene glycoside damages the liver parenchyma cells through an irreversible synergistic effect. The hepatotoxicity of tannins extracted from RPM has been reported at medium doses, but there were no obvious liver damage at low doses. However, the liver damage was reversible. It has been shown that emodin, rhein, gallic acid, and resveratrol possess biological cytotoxicities, and it was speculated that gallic acid may be the main PM component responsible for liver injury (Yang et al., 2016). A study has found that 70% total ethanol extract of RPM and gallic acid were deleterious to hepatocytes (Chalasani et al., 2014).

Studies on the Mechanism of PM-Induced Liver Injury

The mechanism of liver injury caused by PM is still unclear. According to current literature reports, liver injury caused by PM comprises intrinsic liver injury and specific liver damage. Rat models were used in most studies, with metabolomics, proteomics and bioinformatics techniques. However, in-depth

mechanism of action remains unclear. A study in which serum metabolomics of mice treated with water extract of RPM were analyzed suggested that the liver damage by PM was related to lipid metabolism, amino acid metabolism and excretion of bile acid metabolites (Yun-Xia et al., 2017). Lin et al. demonstrated that the mechanism of liver injury induced by RPM has been attributed to oxidative phosphatization of mitochondrial function and abnormal conduction of TCA cycle signaling pathway, which can lead to hepatocyte apoptosis and abnormal metabolism of bilirubin (Longfei et al., 2017). In addition, extracts of RPM may cause differences in the expression of metabolic enzymes and alter the *in vivo* metabolism of PM components, which may further cause liver damage. A study has investigated liver injury in rats before and after preparation of PM, and screened the sensitive indicators of liver injury. The results showed that the liver injury potential of RPM was significantly lower than that of PMP. Serum DBIL and TBIL which reflect early liver damage can be used as sensitive indicators for liver toxicity monitoring. The mechanism of RPM-induced liver injury has been studied using *kidney yang* deficiency model rats. The study found that RPM administration upregulated TNF-α, while decreasing the activities of $Ca^{2+}Mg^{2+}$-ATPase and $Na^{+}K^{+}$-ATPase, suggesting that the increase in inflammatory factors caused disorder in mitochondrial function and liver damage. Epigallocatechn gallate (EGCG) is more cytotoxic than catechins and other enamel components, and it can selectively kill cells expressing OATP1B3. Thus, it can lead to an increase in the concentration of substrate for OATP1B3, which can induce hepatotoxicity (Zhang et al., 2013). In addition, mutation of CYP1A2*1C may be related to liver injury caused by RPM, but the specific mechanism involved in its induction of liver damage needs further studies (Benichou et al., 1993). Other possible mechanisms of PM-induced liver injury include long-term intake of PM leading to increased humoral immunity, increased CYP450 content in liver microsomes, suppressed expression of hepatic cytochrome P4502E1, and decreased CYP2E1 activity resulting in inability to clear substances in time. In addition, ethanol extract of RPM caused abnormal inhibition of PPAR-γ pathway and overexpression of related inflammatory factors (Zhou and Zhang, 2006). Liver tissues of rats treated with cis-stilbene glycosides were used to screen differentially expressed genes with gene chip, and it was shown that the TLR4-NFκB pathway may be an important gene pathway for specific liver damage, but the specific mechanism of action requires further research (Wang et al., 2016). In a study aimed at identifying the genetic basis of susceptibility to PM-induced liver injury, it was found that HLA-B*35:01 allele was a potential biomarker for predicting PM-induced damage in humans (Li et al., 2019). This is considered an innovative and progressive discovery

REASONABLE APPLICATION OF *P. MULTIFLORUM* THUNB.

At present, many classical prescriptions containing PM have been developed as Chinese patent medicine. Different preparation processes have changed the production process and traditional dosage forms, resulting in changes in the composition of the drugs, and also their efficacy and safety. The Chinese Pharmacopoeia represents the level of drug use, pharmaceuticals, and supervision in China. There is need to strengthen people's understanding of the safety and effectiveness of Chinese herbal medicines containing PM in line with the reality of modern applications. Post-marketing clinical research should be carried out for the 47 varieties of PM preparations; relevant safety information communication and feedback mechanisms should be established, and reasonable use guidelines should be drafted.

Clinically, liver damage due to PM and its preparations has a cumulative effect. In order to reduce the incidence of adverse reactions, it is necessary to take effective measures to ensure the safety of medication. The following suggestions are put forward: (Kasote et al., 2017) Before any patient takes the medicine, they should be fully consulted to understand their family history, allergy history and liver disease history. If the patient has a medical history as above, there is a greater risk of liver damage. Therefore, attention must be paid to rational use of drugs. (Kim et al., 2016) Reasonable control of the duration and dosage of administration should be established. Clinical applications of PM and its preparations should be started from small doses. The dosage should be gradually increased after careful observation of the patient's medication to prevent various adverse reactions caused by the accumulation of drugs. (Sumei et al., 2018) The monitoring of adverse reactions after drug administration should be strengthened. There is a certain incubation period for hepatotoxicity induced by PM under normal circumstances. Therefore, liver function should be carefully checked after medication. If abnormal liver function is diagnosed, the drug should be discontinued in time. (Lin et al., 2017) Systematic biology research should be fully applied by pharmacists, physicians and researchers to establish a comprehensive database of hepatic damage induced by PM, so as to provide a scientific and reasonable basis for the early diagnosis and prediction of hepatotoxicity. (Huang et al., 2014) Medical personnel should strengthen their understanding of the adverse reactions of TCM, and collect relevant information on the adverse reactions of PM and its preparations with respect to liver injury. Full attention should be paid to the clinical effects of drugs and possible related adverse reactions in order to provide better clinical services. In the current Pharmacopoeia of PM quality standard, the contents of the indicator components (stilbene glycoside, free anthraquinone, combined anthraquinone) which are related to hepatotoxicity indicate only the minimum limits, but are silent on the upper limits. Therefore, it is recommended that the Pharmacopoeia of the People's Republic of China and the processing regulations set limits on the toxicity indicators in the quality standard of PM. The Chinese medicine decoction production enterprises should strictly follow the regulations in formulating processing technology for PM so ass to ensure its quality.

The safety of TCM drugs is a concern throughout the life period of the drugs. Although pharmacovigilance is absolutely necessary to ensure public safety, measures should also be encouraged and implemented to promote the healthy development of TCM industry. Quality control is still one of the major safety problems in TCM safety concerns. The present study was carried out to review the research status of PM-induced liver injury with respect to

clinical characteristics, risk factors, material basis research, and action mechanism. The findings will be useful for further understanding of the hepatotoxicity induced by PM so as to take reasonable and effective measures to prevent it. The model of safety monitoring and risk management of PM drugs is still under investigation. Indeed, the characteristics and risk factors associated with PM require both proper understanding and control by strengthening standardization of clinical applications, basic science research, quality control in manufacturing, active monitoring methodology, and enhancement of international communication and cooperation.

AUTHOR CONTRIBUTIONS

YL, JN, and WW contributed to the conception of the study. YL, XY, MS, BM, and YD contributed significantly to analysis and manuscript preparation. YL, XY, XD, and LP performed the data analyses and wrote the manuscript.

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Herbal hepatotoxicity in traditional and modern medicine: actual key issues and new encouraging steps

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Plants are natural producers of chemical substances, providing potential treatment of human ailments since ancient times. Some herbal chemicals in medicinal plants of traditional and modern medicine carry the risk of herb induced liver injury (HILI) with a severe or potentially lethal clinical course, and the requirement of a liver transplant. Discontinuation of herbal use is mandatory in time when HILI is first suspected as diagnosis. Although, herbal hepatotoxicity is of utmost clinical and regulatory importance, lack of a stringent causality assessment remains a major issue for patients with suspected HILI, while this problem is best overcome by the use of the hepatotoxicity specific CIOMS (Council for International Organizations of Medical Sciences) scale and the evaluation of unintentional reexposure test results. Sixty five different commonly used herbs, herbal drugs, and herbal supplements and 111 different herbs or herbal mixtures of the traditional Chinese medicine (TCM) are reported causative for liver disease, with levels of causality proof that appear rarely conclusive. Encouraging steps in the field of herbal hepatotoxicity focus on introducing analytical methods that identify cases of intrinsic hepatotoxicity caused by pyrrolizidine alkaloids, and on omics technologies, including genomics, proteomics, metabolomics, and assessing circulating micro-RNA in the serum of some patients with intrinsic hepatotoxicity. It remains to be established whether these new technologies can identify idiosyncratic HILI cases. To enhance its globalization, herbal medicine should universally be marketed as herbal drugs under strict regulatory surveillance in analogy to regulatory approved chemical drugs, proving a positive risk/benefit profile by enforcing evidence based clinical trials and excellent herbal drug quality.

Keywords: herb induced liver injury, hepatotoxicity, herbal hepatotoxicity, herbs, evidence based trials, herbal traditional Chinese medicine, herbal modern medicine, herbal traditional medicine

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Introduction

Ancient Chinese and Egyptian papyruses describe medicinal use of plants for various ailments as early as 3.000 BC and thereby long before recorded history (Major, 1954; IARC Monographs, 2002). Starting at around that time, herbal traditional medicine originating from Mesopotamia, Egypt, and India influenced Byzantine, Greek, Latin, and Indian herbal medicine, thereby establishing principles of Ayurveda in India and developing traditional occidental herbal medicine, which subsequently became popular in numerous European and other Western countries (Major, 1954;

IARC Monographs, 2002; Raghavendra et al., 2009; NIH, 2013). Indigenous cultures such as African, North American, Middle and South American, Australian, and South Pacific islandic also used herbs in their healing rituals (IARC Monographs, 2002). In other parts of the world, herbal traditional Chinese medicine (TCM) emerged (IARC Monographs, 2002; Raghavendra et al., 2009) and influenced the traditional Oriental herbal medicine in Japan (IARC Monographs, 2002), called Kampo medicine (Nishimura et al., 2009), and other Asian countries such as Korea with its herbal traditional Korean medicine (Park et al., 2012). With TCM originating in China and Ayurveda in India, two most ancient yet living traditions of herbal medicine presently remain and experience some extension over the globe (Patwardhan et al., 2005). Although, most other ancient herbal medicine cultures vanished or are restricted to local use without potential globalization, many countries use herbal medicines (WHO, 2002, 2013).

The worldwide use of medicinal herbs has increased over the past years (WHO, 2002, 2013; Ekor, 2014), but their regulatory surveillance differ among countries due to lack of harmonization (WHO, 2005; Ekor, 2014). The world market for herbal medicines based on traditional knowledge is estimated at US$60,000 million annually (WHO, 2002), according to a UN report dating back to 2000 (UN, 2000). Alone in the United States, the total estimated herb retail sales in all channels rose from $4230 million in 2000 to $6032 million in 2013, corresponding to 42.6% overall and to 3.3% on an annual basis according to the data of the American Botanical Council (Lindstrom et al., 2014). These figures compare to the increased use of complementary and alternative Medicine (CAM), since an estimated $27 billion was spent by consumers of CAM in the United States in 1997 (Eisenberg et al., 1998) and $33.9 billion in 2007 (Nahin et al., 2009), equaling a rise of 25.5%. These figures considered all CAM related expenditures spent out of pocket on visits to CAM practitioners and purchases of CAM products, classes, and materials in the United States in 2007, with $14.5 billion spent on the purchase of nonvitamin, nonmineral, and natural products (Nahin et al., 2009); the widespread use of herbal medicine exerts a high economic power in our society with special financial benefits for herb producers, providers, and healers. Considering this enormous economic impact and the resulting expenditures, the question is whether these high costs as burden for the consumers and the society are warranted. In addition, herbal medicine is increasingly exposed to major pressure due to concerns of efficacy, safety (NIH, 2014a), and adverse reactions (Podsadzki et al., 2013; Ekor, 2014) such as liver injury (NIH, 2014a).

In this review article, we critically analyze actual key issues of herbal hepatotoxicity by herbal products of traditional and modern medicine and discuss future developments. The expressions of herbal hepatotoxicity and herb induced liver injury (HILI) are used synonymously. Some similarities of HILI exist with DILI (drug induced liver injury) (Teschke et al., 2013f).

Methods

Data Sources and Searches

We used the PubMed to identify publications on herbal hepatotoxicity and HILI which each provided hits of around 279.000 and 1.840.000. Our search was then further qualified and extended using additional keywords denoting herbal modern medicine and herbal TCM, providing additional hits. The first 100 hits of publications in each category were considered.

Study Selection

The focus of our search was on publications in English language, but relevant reports of other languages also were considered. The retrieved publications included case reports, case series and review articles and were analyzed whether they were appropriate and relevant for the topic of this article. Publications also were manually searched for additional publications not yet identified.

Data Extraction and Quality Assessment

Prior to our analysis, the publications were assessed regarding their scientific and clinical quality. Publications of relevance and good quality were preferred and considered for evaluation.

Key Issues

Herbal products in traditional and modern medicine are commonly perceived by the general population as well tolerated and devoid of major adverse reactions. One of the most important goals in clinical practice is to offer patients an efficient therapy for their ailment(s), without harming their health. However, efficacy and safety by the use of herbs in traditional and modern medicine are features that may apply to some herbs and patients but certainly not to others. Similar shortcomings are known from conventional chemical drugs, which also are not effective in all patients. The risk of rare adverse reactions occurring in various organs including the liver relates to both, herbs (NIH, 2014a) and synthetic drugs (NIH, 2014b).

Hepatotoxicity Case Reports

General Aspects

Basic knowledge of hepatotoxicity by drugs and numerous other chemicals was summarized by Hyman Zimmerman in his pioneering book 25 years ago, briefly mentioning already some herbs as culprits and referencing a few case reports of herbal hepatotoxicity (Zimmerman, 1999). Since then, many more HILI cases emerged, which previously were reported (Pittler and Ernst, 2003b) and recently analyzed in publications on herbal TCM preparations (Teschke, 2014; Teschke et al., 2014c, 2015b), other commonly used herbal products (Teschke et al., 2013f), and herbal and dietary supplements (HDS) (Halegoua-De Marzio et al., 2013; Teschke et al., 2013d; Navarro et al., 2014; Robles-Diaz et al., 2015).

Epidemiology

Epidemiology data of hepatotoxicity cases in connection with herbal use are crucial to assess, both in traditional and modern medicine. Actually, the true prevalence of herbal hepatotoxicity is the total number of HILI cases in the population at a given time (Teschke et al., 2013f). It represents an estimate of how common herbal hepatotoxicity is within a population and at a fixed time. Conversely, the incidence of herbal hepatotoxicity is expressed as the total number of new HILI cases during a certain period

of time, divided by the number of individuals in the population initially at risk. Therefore, incidence differs from prevalence measuring new HILI cases; for chronic liver injury, these values may change.

Incidence commonly provides information about the risk of acquiring HILI, whereas prevalence signifies how widespread HILI is. The true prevalence and incidence of HILI (Navarro, 2009) and HDS (Navarro et al., 2014; Robles-Diaz et al., 2015) is unknown. Global epidemiology considerations of prevalence and incidence refer to all herbs contained in herbal drugs and herbal supplements, whereas specific epidemiology is restricted to one single herb. Global epidemiology data therefore may be used for health economy assessment whereas specific epidemiology data pertain to herbal product safety. For this purpose, true global prevalence and incidence of HILI still has to be determined through cohort studies or case control-studies, a difficult approach. For an appropriate assessment of the risks from a specific herbal product, there is lack of quantitative data for consumption of herbal products, number of HILI patients, and the population at risk. In addition, herbal product authentication is missing in most cases of suspected HILI and impedes causality assessment for the incriminated herb (Teschke et al., 2013f). Case underreporting and overdiagnosing also prevent determination of the true incidence; future studies will have to address these issues in order to provide firm data of prevalence and incidence in HILI.

For DILI by synthetic drugs, respective data are available: the estimated annual incidence rate of DILI at a coordinating center in Spain was 34.2 ± 10.7 cases per 10^6 inhabitants (Andrade et al., 2005), and in a French study it was 13.9 ± 9 per 10^6 inhabitants per year (Sgro et al., 2002).

Compilation of Hepatotoxicity Cases

For herbal TCM with potential liver injury, we identified 44 different TCM herbs and 21 herbal TCM mixtures, published in case reports and case series as provided by appropriate references (**Table 1**). These referenced reports present clinical case details, summarized in part also earlier (Teschke, 2014). The 12 most common Chinese herbal medicines with hepatotoxicity detailed in a recent review (Ma et al., 2014) are also included in the present compilation (**Table 1**).

Other herbs and herbal products unrelated to TCM showed reported potential hepatotoxicity for 111 items (**Table 2**), presented as an update of an earlier compilation (Teschke et al., 2012h). Most of the actual 111 items identified single herbs, rarely mixtures with HDS as examples with various ingredients (**Table 2**). Numerous other HDS with assumed potential hepatotoxicity are listed in compilations of other reports published just recently (Bunchorntavakul and Reddy, 2013; Navarro et al., 2014; Robles-Diaz et al., 2015) and hence were not included in the present compilation (**Table 2**).

In the past, some review articles focused exclusively on HILI by TCM herbs and herbal preparations (Ma et al., 2014; Teschke, 2014; Teschke et al., 2014c, 2015b) as a primarily neglected topic, which was otherwise considered as part of overall assessments on herbal hepatotoxicity in few publications (Zimmerman, 1999; Abdualmjid and Sergi, 2013; Bunchorntavakul and Reddy, 2013), including an official and well updated NIH statement (NIH, 2014a).

Symptomatology

Clinical symptoms of herbal hepatotoxicity in traditional and modern medicine are variable and described in published case reports, case series, and regulatory presented spontaneous reports as referenced (**Tables 1**, **2**). Symptoms are mostly unspecific and sometimes difficult to direct to the liver, which delays early recognition of the unfolding liver injury (Teschke et al., 2013f, 2014c; Ma et al., 2014). Clinical signs may emerge alone or in combination with other features, while jaundice is the symptom initially best recognized by the patient, facilitating the search for advice by the primary care physician. In detail, patients with herbal TCM hepatotoxicity experience fatigue (67.3%), jaundice (60.3%), anorexia (58.0%), nausea (35.9%), and fever (19.3%), but signs such as rash, pruritus, and pale stools have also been reported (Ma et al., 2014). In another study of 16 cases of Greater Celandine (GC) with established HILI, symptoms were present in 15 cases (Teschke et al., 2012a). Single or multiple symptoms were anorexia ($n = 3$), fatigue ($n = 5$), nausea ($n = 6$), vomiting ($n = 2$), dyspepsia ($n = 1$), bloating ($n = 1$), abdominal discomfort ($n = 1$), right upper quadrant pains ($n = 1$), epigastric pains ($n = 1$), unspecified abdominal pains ($n = 1$), fever ($n = 1$), dark urine ($n = 3$), pale stool ($n = 1$), pruritus ($n = 3$), and jaundice ($n = 15$) (Teschke et al., 2012a). For reasons of transparency, narrative case details and clinical data of patients with assumed HILI should be provided in tabular form, as done previously (Teschke et al., 2008a, 2011a, 2012a,b,d,e; Teschke, 2010a) and shown for GC hepatotoxicity as example (**Table 3**). Detailed information also allows characterization of HILI by a single herb such as GC (**Table 4**).

Although, clinical features are quite similar in HILI cases by traditional and modern medicine (Chau et al., 2011; Teschke et al., 2013f; Ma et al., 2014), there is one exception that relates to the hepatic sinusoidal obstruction syndrome (HSOS), formerly hepatic veno-occlusive disease (HVOD); this special liver injury is caused by pyrrolizidine alkaloids (PAs) contained in various TCM herbs, with its major diagnostic features of abdominal distension and pain, ascites, malaise, hepatomegaly, and body weight gain due to ascites and edema caused by fluid accumulation (Wang and Gao, 2014). Jaundice was most frequent with 84.8% in 100/118 cases of PA induced HSOS by Tu San Qi (*Gynura segetum*), ascites with 99.2% (121/122 cases, and hepatomegaly with 92.0% (104/113 cases) (Lin et al., 2011; Gao et al., 2012).

In a typical HILI case unrelated to PAs, the chronology of symptoms may follow a particular stepwise pattern, as described for HILI caused by Indian Ayurvedic herbs through an excellent observation by a patient under treatment for her vitiligo (Teschke and Bahre, 2009). Her symptoms started with pruritus, followed by loss of appetite, fatigue, nausea, vomiting, dark urine, light stool, until finally jaundice was recognized by her family physician; this sequence of symptoms stretched over almost 4 months under continued herbal medication.

Patients with HILI may be asymptomatic with increased values observed by chance, monosymptomatic, or polysymptomatic.

TABLE 1 | Compilation of reported cases with suspected hepatotoxicity by herbal traditional Chinese medicine (TCM).

Name with ingredients	Cases (*n*)	References
Ai Ye *Artemisia argyi*	na	Ma et al., 2014
An Shu Ling *Lycopodium serratum* or, rarely, *Corydalis* species, *Panax ginseng*, Pseudo ginseng, or two species of *Stephania*	1	Haller et al., 2002
Bai Fang *Angelica sinensis*, *Cyperus rotundus,* Ginseng, *Ligusticum wallichii*, *Paeonia alba*, *Rehmannia glutinosa*	1	Estes et al., 2003
Bai Shi Wan *Atractylis*, *Carthamus tinctorius*, *Dalbergia odorifera*, *Dioscorea bulbifera*, *Glycyrrhiza*, *Lithospermum erythrorhizon*, *Paeonia suffruticosa*, *Polygonum multiflorum*, *Psoralea corylifolia*, *Salvia miltiorrhiza*; Endoconcha sepiae, Ganoderma lucidum (mushroom)	1	Talari et al., 2010
Bai Xian Pi *Dictamnus dasycarpus*	1	Perharic-Walton and Murray, 1992
	1	Kane et al., 1995
	1	Vautier and Spiller, 1995
	2	Yuen et al., 2006
	4	Jang et al., 2008
	14	Kang et al., 2008
	2	Sohn et al., 2008
Bi Ma Zi *Rhicinus communis*	na	Ma et al., 2014
Ban Tu Wan *Angelica sinensis, Chaenomeles*, *Codonopsis pilosula*, *Notopterygium*, *Polygonum multiflorum*, *Rehmannia*, *Schisandra*	1	Cortez et al., 2012
Bo He *Mentha haplocalyx*		
Bo Ye Qing Niu Dan *Tinospora crispa*	2	Sangsuwan et al., 2004
Bofu Tsu Sho San *Angelica*, *Atractylis*, *Cnidium*, *Gardenia*, *Ephedra*, *Forsythia*, *Glycyrrhhiza*, *Gypsum fibrosum*, *Ledebouriella*, *Mentha*, *Paeonia*, *Platycodon*, *Rheum*, *Schizonepeta*, *Scutellaria*, *Zingiber;* Kadinum (talcum powder), sodium sulfuricum	1	Motoyama et al., 2008
Boh Gol Zhee *Psoralea corylifolia*	1	Hwang et al., 2001
	1	Nam et al., 2005
	3	Cheung et al., 2009
Cang Er Zi *Xanthium sibiricum*	na	Chau, 2008;
	na	Ma et al., 2014
Chang Shan *Dichora febrifuga Lour*	na	Ma et al., 2014
Chai Hu *Bupleurum falcatum*	28	Lee et al., 2011
Chaso *Camellia sinensis*, *Cassia tora* (syn. *Senna*), *Crataegus*, *Chrysanthenum morifolium Ramat.*, *Lotus*, *Lycium barbarum*; N-nitroso-fenfluramine	27	Adachi et al., 2003
Chi R Yun *Breynia officinalis*	2	Lin et al., 2002
	19	Lin et al., 2003
Chinese herbal mixtures (various) *Dictamnus dasycarpus*, *Gentiana scabra, Hedyotis diffusa*, *Paeonia suffructicosa*, *Paris polyphylla*, *Rehmannia glutinosa*, *Smilax glabra*, *Sophora subprostrata*;	1	Perharic-Walton and Murray, 1992
Angelica sinensis, *Bupleurum chinese*, *Dictamnus dasycarpus*, *Paeonia suffructiosa*, *Philodendron chinese*, *Saposhnikovia divaricata*, *Shisandra chinesis*, *Shizonepeta tenuifolia*, *Tribulus terrestris*;	2	Kane et al., 1995
Cocculus trilobus, *Dictamnus dasycarpus*, *Eurysolen gracilis*, *Glycyrrhiza*, *Lophatherum*, *Paeonia*, *Potentilla*, *Rehmannia glutinosa*;	1	Vautier and Spiller, 1995
Alisma plantago aquatica, *Artemisia capillaris*, *Bupleurum*, *Chrysanthemum morifolium*, *Circuma*, *Gardenia jasminoidis*, *Gentiana scabra*, *Glycyrrhiza*, *Magnolia*, *Paeonia*, *Plantago asiatica*, *Saussurea lappa*	1	Yoshida et al., 1996
Chuan Lian Zi *Melia toosendan*	1	Yuen et al., 2006
Ci Wu Jia *Acanthopanax senticosus*	2	Sohn et al., 2008
Da Chai Hu Tang *Bupleurum falcatum*, *Ginseng*, *Glycyrrhiza glabra*, *Pinellia*, *Scutellaria*, *Zingiber officinale*, *Zizyphus jujuba*	1	Kamiyama et al., 1997
Da Huang *Rheum palmatum*	1	Yuen et al., 2006
Du Huo *Angelica archangelica*	1	Björnsson et al., 2013
Fu Fang Qing Dai Wan Angelica dahurica, *Isatis indigotica* (Indigo naturalis), *Massa medicata fermentata* (yeast), *Salvia milthiorrhiza*, *Smilax glabra*	1	Verucchi et al., 2002
Gan Cao *Glycyrrhiza uralensis*, syn. Liquorice	1	Yuen et al., 2006
Ge Gen *Pueraria lobata*, syn. Arrowroot	2	Kim et al., 2009
He Huan Pi *Albizia julibrissin*	na	Ma et al., 2014
Ho Shou Wu *Polygonum multiflorum*, syn. He Shou Wu	1	Yuen et al., 2006

(Continued)

TABLE 1 | Continued

Name with ingredients	Cases (*n*)	References
	na	Ma et al., 2014
	1	Bae et al., 2010
Hu Bohe You *Mentha pulegium*, syn. Pennyroyal oil	na	Chau, 2008
Hu Zhang *Polygonum cuspidatum*	na	Chau, 2008
Huang Qin *Scutellaria baicalensis*	19	Gono et al., 2010
	2	Linnebur et al., 2010
	1	Yang et al., 2012a;
	1	Dhanasekaran et al., 2013
Huang Yao Zi *Dioscorea bulbifera*	na	Chau, 2008;
	na	Ma et al., 2014
Hwang Geun Cho *Corydalis speciosa*	1	Kang et al., 2009
Ji Gu Cao *Abrus cantoniensis*	1	Yuen et al., 2006
Ji Ji *Chloranthus serratus*	na	Chau, 2008
Ji Xue Cao *Centella asiatica*, syn. Gotu Kola	3	Jorge and Jorge, 2005
Jiguja *Hovenia dulcis*	1	Sohn et al., 2008
	1	Kang et al., 2008
	1	Kim et al., 2012
Jin Bu Huan *Lycopodium serratum* or, rarely, *Corydalis species*,	7	Woolf et al., 1994
Panax ginseng, Pseudo ginseng, or two species of *Stephania*	3	Horowitz et al., 1996
	1	Picciotti et al., 1998
	1	Divinsky, 2002;
	1	Haller et al., 2002
Jue Ming Zi *Cassia obtusifolia*, syn. *Senna obtusifolia*	1	Yuen et al., 2006
Kamishoyosan *Angelica sinensis*, *Atractylodes racea*, *Bupleurum falcatum*, *Gardenia*, *Glycyrrhiza glabra*, *Mentha haplocalyx*, *Moutan*, *Paeonia alba*, *Sclerotium Poriae Cocos*, *Zingiber officinale*	1	Inoue et al., 2011
Kudzu *Pueraria thunbergiana*	6	Kang et al., 2008
Ku Lian Zi Melia azedarach	na	Ma et al., 2014
Lei Gong Teng *Tripterygium wilfordii* Hook	na	Chau, 2008
	na	Ma et al., 2014
Long Dan Xie Gan Tang *Acebia*, *Alisma*, *Angelica sinensis*, *Bupleurum*, *Gardenia*, *Gentiana*, *Glycyrrhiza*, *Plantago*, *Rehmannia*, *Scutellaria*	17	Lee et al., 2011
Lu Cha *Camellia sinensis*, syn. Chinese green tea	1	Garcia-Moran et al., 2004
	1	Peyrin-Biroulet et al., 2004
	1	Gloro et al., 2005
	1	Bonkovsky, 2006
	1	Jimenez-Saenz and Martinez-Sanchez, 2006
	1	Bonkovsky, 2006
	1	Molinari et al., 2006
	5	Björnsson and Olsson, 2007
	3	García-Cortés et al., 2008
	34	Sarma et al., 2008
	36	Mazzanti et al., 2009
	1	Rohde et al., 2011
	47	Navarro et al., 2013
Ma Huang *Ephedra sinica*	1	Nadir et al., 1996

(Continued)

TABLE 1 | Continued

Name with ingredients	Cases (*n*)	References
	1	Borum, 2001
	3	Estes et al., 2003
	1	Skoulidis et al., 2005
	1	Reuben et al., 2010
Mao Guo Tian Jie Cai *Heliotropium lasiocarpum*	4	Culvenor et al., 1986
Onshido *Aloe*, *Camellia sinensis*, *Crataegus*, *Gynostemma pentaphyllum makino*, *Raphanus*; N-nitroso-fenfluramine	141	Adachi et al., 2003
Qian Li Guang *Senecio scandens*	na	Chau, 2008;
	na	Ma et al., 2014
Ren Shen *Panax ginseng*	6	Kang et al., 2008
Sairei To *Alisma*, *Atractylis*, *Bupleurum*, *Cinnamomum*, *Ginseng*, *Glycyrrhiza*,	1	Aiba et al., 2007
Pinellia, *Polyporus*, *Poria*, *Scutellaria*, *Zingiber*, *Zizyphus*	1	Tsuda et al., 2010
Shang Lu *Phytolacca acinosa*	na	Ma et al., 2014
Shen Min Black cohosh, Burdock, Cayenne pepper, *Ginkgo biloba*, Horse chestnut, *Piper nigrum, Polygonum multiflorum*, *uva ursi*; biotin, collagen (hydrolyzed), niacin, pantothenic acid, silica (from plant sources), soy isoflavones, vitamin A, vitamin B_6	1	Cárdenas et al., 2006
Shi Can *Teucrium chamaedrys,* syn. Germander	na	Chau, 2008
Shi Liu Pi *Pericarpium granati*	na	Chau, 2008
Shou Wu Pian *Achyranthes bidentata*, *Cuscuta chinensis*, *Eclipta prostrata*,	1	But et al., 1996
Ligustrum lucidum, *Lonicera japonica*, *Morus alba*, *Polygonum multiflorum*,	1	Park et al., 2001
Psoralea corylifolia, *Rehmannia glutinosa*, *Rosa laevigata*, *Sesemum indicum*,	1	Battinelli et al., 2004
Siegesbeckia orientalis	1	Panis et al., 2005
	3	Sohn et al., 2008
	1	Laird et al., 2008
	1	Furukawa et al., 2010
	1	Valente et al., 2010
	25	Jung et al., 2011
	1	Banarova et al., 2012
Tian Hua Fen *Trichosanthes kirilowii*	na	Chau, 2008
Tu San Qi *Gynura segetum*	2	Dai et al., 2006
	1	Chen et al., 2007
	1	Li et al., 2010
	52	Lin et al., 2011;
	116	Gao et al., 2012
White flood Qian Ceng Ta (*Huperzia serrata*), Wu Zhu Yu (*Evodia rutaecarpa*); beet root, caffein, cocoa bean, vinpocetine (from *Vinca* plant); acesulfame potassium, calcium silicate, carnitine tartrate, Carno-Syn® beta-alanine, citrulline, cryptoxanthin, folic acid, gamma-aminobutyric acid (GABA), glucuronolactone, selenium, L-norvaline, L-tyrosine, lutein, malic acid, ornithine, potassium gluconate, sucralose, sugar cane, watermelon flavor, zeaxanthin	1	Cohen et al., 2012
Wu Bei Zi *Galla chinensis*	na	Chau, 2008
Xi Shu *Camptotheca acuminata*	na	Chau, 2008
Xian Si Zi *Abrus Precatorius*	na	Ma et al., 2014
Xiao Chai Hu Tang *Bupleurum falcatum, Ginseng*, *Glycyrrhiza glabra*,	4	Itoh et al., 1995
Pinellia tuber, Scutellaria baicalensis, Zingiber officinale, *Zizyphus jujuba*	19	Lee et al., 2011
	1	Hsu et al., 2006
Yin Chen Hao *Artemisia capillaris*	7	Kang et al., 2008
	1	Sohn et al., 2008
Zexie *Alisma orientalis*	1	Yuen et al., 2006
Zhen Chu Cao *Phyllanthus urinaria*	1	Yuen et al., 2006

Data represent an update of cases retrieved from a selective literature search for published cases of herbal TCM associated with suspected hepatotoxicity (Teschke, 2014; Teschke et al., 2014c, 2015b) and of a recent compilation most common Chinese herbal medicines with hepatotoxicity (Ma et al., 2014). In some cases, causality for individual herbs and herbal mixtures was established using the CIOMS (Council for International Organizations of Medical Sciences) scale or its modifications, and by positive reexposure test results. For other cases, information was fragmentary and did not necessarily allow a firm causal attribution.

TABLE 2 | Compitalion of commonly used herbs and herbal products with reported hepatotoxicity.

Search items	Botanical names, ingredients, references
Acacia catechu	see Ayurvedic herb
Asterceae family	*Adenostyles alliariae* (Sperl et al., 1995)
Aloe	*Aloe perfoliata var. vera* (Rabe et al., 2005; Kanat et al., 2006; Bottenberg et al., 2007; Yang et al., 2010)
Amorphophallus Konjac	see Hydroxycut®
Arrowroot	*Maranta aruninacea* or *Tacca leontopetaloides* (Kim et al., 2009)
Atractylis gummifera	see Distaff thistle
Ayurvedic herbs	*Psoralea corylifolia, Acacia catechu, Eclipta alba* or *Bacopa monnieri, Vetivexia zizaniodis* (Teschke and Bahre, 2009)
Babchi	*Psoralea corylifolia,* see also Ayurvedic herbs (Nam et al., 2005)
Bacopa monnieri	see Ayurvedic herbs
Bajiaolian	*Dysosma pleianthum* (Kao et al., 1992)
Boronia Sm.	see Pro-Lean®
Buchu Tea	*Agathosma betulina*, *Agathosma crenulata* (Engels at al., 2013)
Bush tea	*Crotalaria species* (Smith and Culvenor, 1981)
Callile pis laureola	see Impila
Camellia sinensis	see green tea, Exolise®, and Hydroxycut® see X-elles®
Cascara sagrada	*Rhamnus purshianus* (Nadir et al., 2000)
Cassia angustifolia	see Senna
Centella asiatica	see Gotu Kola, see Pro-Lean®
Chamaerops humilis	see Saw Palmetto
Chaparral	*Larrea tridentata*, *Larrea divariatica*
syn. Creosot	(Katz and Saibil, 1990; Centers of Disease Control and Prevention, 1992; Smith and Desmond, 1993; Alderman et al., 1994; Batchelor et al., 1995; Gordon et al., 1995; Sheikh et al., 1997; Haller et al., 2002; Estes et al., 2003)
Chelidonium majus	see Greater Celandine, see Lycopodium similiaplex®
Chinese herbs	Unknown or up to 12 ingredients
Chlorophora species	see Kambala tea
Chrysanthemum leucanthemum	see Oxeye Daisy
Citrus aurantium	see X-elles®
Citrus paradisum	see X-elles®
Cyrana scolymus	see X-elles®
Cola nitida	see Pro-Lean®
Coltsfoot	*Tussilago farfara* (Roulet et al., 1988)
Comfrey	*Symphytum officinale*, *Symphytum asperum, Symphytum uplandicum* (Ridker et al., 1985; Weston et al., 1987; Bach et al., 1989; Ridker and McDermott, 1989; Miskelly and Goodyer, 1992)

(Continued)

TABLE 2 | Continued

Search items	Botanical names, ingredients, references
Compositae species	see Indian herbs
Creosot	see Chaparral
Crotalaria species	see Bush tea, see Rattlebox
Cyperus	see Pro-Lean®
Distaff thistle	*Atractylis gummifera* (Georgia, 1988)
Eclipta alba	see Ayurvedic herbs
Emblica officinalis	see Isabgol
Ephedra species	*Ephedra californica*, *Ephedra sinica* (Estes et al., 2003)
Exolise®	*Garcinia cambogia, Gymnema sylvestre,* White kidney bean, *Camellia sinensis*, L-Carnitine fumarate, Calcium, Magnesium chelate, Chromium chelate, Conjugated linoleic acid, Chitosan (McDonnell et al., 2009)
Fallopia multiflora	see Pro-Lean®
Foeniculum amare	see Herbalife®
Fucus vesiculosus	see Pro-Lean®
Garcinia cambogia	see Exilis®, see Herbalife®, see Hydroxycut®
Germander	*Teucrium chamaedrys, Teucrium polium* (Larrey et al., 1992; Mostefa-Kara et al., 1992; Dao et al., 1993; Mattéi et al., 1995; Laliberté and Villeneuve, 1996; Starakis et al., 2006)
Ginkgo biloba	see Pro-Lean®
Ginseng	see Bai Fang, see Dai Saiko To, see Pro-Lean®, see Xiao Chai Hu Tan
Glycyrrhiza glabra	see Dai Saiko To, see Xiao Chai Hu Tang
Gotu Kola	*Centella asiatica* (Jorge and Jorge, 2005)
Greater Celandine	*Chelidonium majus,* see also Lycopodium similiaplex® (Strahl et al., 1998; Greving et al., 1998; Benninger et al., 1999; Crijns et al., 2002; Hardeman et al., 2003; Stickel et al., 2003; BfArM, 2005; Rifai et al., 2006; Conti et al., 2008; Moro et al., 2009; Tarantino et al., 2009; EMA, 2010; Teschke et al., 2011a, 2012a, 2013b)
Green tea	*Camellia sinensis*, see also Lu Cha (**Table 1**) (Duenas Sadornil et al., 2004; Garcia-Moran et al., 2004; Abu el Wafa et al., 2005; Gloro et al., 2005; Bonkovsky, 2006; Javaid and Bonkovsky, 2006; Jimenez-Saenz and Martinez-Sanchez, 2006; Molinari et al., 2006; Martinez-Sierra et al., 2006; Björnsson and Olsson, 2007; Federico et al., 2007; Liss and Lewis, 2009; Sarma et al., 2008; Mazzanti et al., 2009; Verheist et al., 2009; Rohde et al., 2011; Teschke et al., 2011e, 2014e; Teschke and Schulze, 2012)
Groundsel	*Senecio longilobus, Senecio species*
syn. Senecio	Stillman et al., 1977; Fox et al., 1978
Guaraná	*Paullinia cupana* (Dara et al., 2008)
Gymnema sylvestre	see Exilis®, see Hydroxycut®
Hawthorn	see *Crataegus*
Hedeoma pulegoides	see Pennyroyal
Heliotropium	*Heliotropium eichwaldii, Heliotropium species*

(Continued)

TABLE 2 | Continued

Search items	Botanical names, ingredients, references
	(Mohabbat et al., 1976; Datta et al., 1978; Tandon et al., 2008; Kakar et al., 2010)
Herbalife®	*Solidaginis gigantea, Ilex paraguariensis, Petroselinum crispum, Garcinia cambogia, Spiraea, Matricaria chamomilla, Liquiritia, Foeniculum amare, Humulus lupulus,* Chromium, and various other ingredients (Hoffmann et al., 2005; Duque et al., 2007; Elinav et al., 2007; Schoepfer et al., 2007; Chao et al., 2008; Manso et al., 2008; Stickel et al., 2009; Chen et al., 2010; Jóhannsson et al., 2010; Appelhans et al., 2011; Manso et al., 2011; Appelhans et al., 2012; Manso, 2012; Halegoua-De Marzio et al., 2013; Teschke et al., 2013b)
Horse chestnut	see Venencapsan®, see Venoplant®
Hydroxycut®	*Camellia sinensis*, *Gymnema sylvestre, Amorphophallus Konjac, Paullinia cupana*, *Garcinia cambogia,* Caffeine, α-Lipoic acid, L-Carnitine, Calcium, Potassium, Chromium (Stevens et al., 2005; Jones and Andrews, 2007; Dara et al., 2008; Shim and Saab, 2009; Chen et al., 2010; Fong et al., 2010)
Humulus lupulus	see Herbalife®
Ilex paraguariensis	see Herbalife®, see Maté
Impila	*Callilepis laureola* (Wainwright et al., 1977; Wainwright and Schonland, 1977; Popat et al., 2001)
Indian herbs	*Compositae species* (Kumana et al., 1983)
Iroko	see Kambala tea
Isabgol	*Plantago ovata, Emblica officinalis* (Fraquelli et al., 2000)
Kambala tea	*Chlorophora excelsa, Chlorophora regia*
syn. Iroko	(Gunawan and Kaplowitz, 2004)
Kava	*Piper methysticum* (Strahl et al., 1998; Escher et al., 2001; BfArM, 2002; Bujanda et al., 2002; Denham et al., 2002; Weise et al., 2002; Estes et al., 2003; Gow et al., 2003; Humberston et al., 2003; Russmann et al., 2003; Schulze et al., 2003; Teschke et al., 2003, 2008a,b, 2011b, 2012c; Schmidt et al., 2005; WHO, 2007a; Christl et al., 2009; Teschke and Wolff, 2009, 2011; Teschke, 2010a,c; Teschke and Schulze, 2010; Teschke and Lebot, 2011; Schmidt, 2014)
Larrea divariatica	see Chaparral
Larrea tridentata	see Chaparral
Leucanthemum vulgare	see Oxeye Daisy
Liquiritia	see Herbalife®
Lycopodium serratum foot clubmass	see Lycopodium similiaplex®, see Wolf's
Lycopodium similiaplex®	*Lycopodium serratum, Chelidonium majus* (Conti et al., 2008)
Maranta aruninacea	see Arrowroot
Maté	*Ilex paraguariensis* (McGee et al., 1976)
Matricaria chamomilla	see Herbalife®
Mentha pulegium	see Pennyroyal

(Continued)

TABLE 2 | Continued

Search items	Botanical names, ingredients, references
Mistletoe	*Viscum album* (Harvey and Colin-Jones, 1981; Hyde, 1981; Colin-Jones and Harvey, 1982; Farnsworth and Loub, 1982; Stirpe, 1983)
Monascus purpureus	see Red Yeast Rice
Morinda citrifolium	see Noni
Nerium oleander	see Oleander
Noni	*Morinda citrifolium* (Millonig et al., 2005; Stadlbauer et al., 2005, 2008; Yüce et al., 2006; López-Cepero Andrada et al., 2007; Yu et al., 2011; Mrzljak et al., 2013)
Oleander	*Nerium oleander* (Altan et al., 2009)
Oxeye Daisy	*Leucanthemum vulgare, Chrysanthemum leucanthemum* (Mokhobo, 1976)
Paullinia cupana	see Guaraná, see Hydroxycut®, see Pro-Lean®
Pennyroyal	*Mentha pulegium*, *Hedeoma pulegoides* (Vallance, 1955; Sullivan et al., 1979; Anderson et al., 1996; Bakerink et al., 1996)
Petroselinum crispum	see Herbalife®
Petroselinum sativum	see X-elles®
Piper methysticum	see Kava
Phaseolus vulgaris	see Exilis ®
Plantago ovata	see Isabgol
Pro-Lean®	Ma Huang, *Paullinia cupana, Cola nitida*, *Centella asiatica, Salix alba*,*Ginkgo biloba, Fucus vesiculosus*, *Boronia Sm.*, *Ginseng*, *Fallopia multiflora*, *Cyperus*, Bee pollen, Caffeine, L-Tyrosine, Chromium, Vanadium, Magnesiumsalicylat, Folsäure, Vitamin B_{12}, and various other ingredients (Joshi et al., 2007)
Psoralea corylifolia	see Ayurvedic herbs
Pyrrolizidine alkaloids	see Bush tea, see Comfrey, see Groundsel, see *Heliotropium species*, see Indian herbs, see Maté, see Rattlebox
Rattlebox	*Crotalaria species*
syn. *Crotalaria*	(Tandon et al., 1976a,b)
Red Yeast Rice	*Monascus purpureus* (Roselle et al., 2008)
Rhamnus purshianus	see Cascara sagrada
Rooibos Tea	*Aspalathus linearis* (Engels et al., 2013)
Salix alba	see Pro-Lean®
Sassafras	*Sassafras albidum* (Larrey, 1997; Zimmerman, 1999)
Saw Palmetto	*Serenoa serpens, Chamaerops humilis* (Lapi et al., 2010)
Scullcap	*Scutellaria lateriflora, Scutellaria species* (MacGregor et al., 1989; Caldwell et al., 1994; Hullar et al., 1999; Estes et al., 2003; Yang et al., 2012a)
Scutellaria species	see Scullcap
Senecio	see Groundsel
Senna	*Cassia angustifolia* (Beuers et al., 1991; Seybold et al., 2004; Vanderperren et al., 2005)

(Continued)

TABLE 2 | Continued

Search items	Botanical names, ingredients, references
Serenoa serpens	see Saw Palmetto
Solidaginis gigantea	see Herbalife®
Spiraea	see Herbalife®
Symphytum	see Comfrey
Tacca leontopetaloides	see Arrowroot
Teucrium	see Germander
Tussilago farfara	see Coltsfoot
Valerian	*Valeriana officinalis* (MacGregor et al., 1989; Mennecier et al., 1999)
Valeriana officinalis	see Valerian
Venencapsan®	*Aesculus hippocastanum*, *Chelidonium majus*, *Melilotus officinalis*, Milfoil, *Silybum* Adans., *Taraxacum officinale* (De Smet et al., 1996)
Venoplant®	*Aesculus hippocastanum* (Takegoshi et al., 1986)
Vetivexia zizaniodis	see Ayurvedic herbs
Viscum album	see Mistletoe
Wolf's foot clubmass	*Lycopodium serratum* (Woolf et al., 1994; Horowitz et al., 1996; Conti et al., 2008)
X-elles®	*Petroselinum sativum, Citrus aurantium, Citrus paradisum, Cyrana scolymus, Camellia sinensis* (Mathieu et al., 2005)

Data are retrieved from a selective literature search for selective reports of herbs and herbal products with hepatotoxicity and actualized from a previous report (Teschke et al., 2012h). In numerous cases, causality was proposed, but not necessarily established and open for discussion.

Latency period describes the interval between initiation of herb use and time of onset, evidenced by emerging symptoms or increased liver values. Liver injury by herbal TCM develops slowly with clinical symptoms appearing between 1 week and 1 month (Ma et al., 2014), or up to 150 days (Chau et al., 2011); with a longer latency period of 5–260 weeks for green tea extracts (GTE) (Mazzanti et al., 2009; Teschke et al., 2014a); or 1 week–24 months for other herbs such as kava (Teschke et al., 2008a); and 28–134 days for Greater Celandine (GC) (Teschke et al., 2011a). Finally, published HILI symptoms (Chau et al., 2011; Teschke et al., 2013a; Ma et al., 2014) are similar to those of DILI (Andrade et al., 2004; Liss and Lewis, 2009).

Clinical Course

The clinical course of HILI is variable with details provided in most publications as referenced (**Tables 1**, **2**). For HILI cases, some details of treatment modalities by herbal products of traditional and modern medicine are provided, with focus on daily and cumulative dose, treatment duration, latency period, and reexposure duration (**Table 5**). With cessation of herbal use, clinical signs usually vanish along with improvements or normalization of initially increased liver values, as illustrated by few examples (Verucchi et al., 2002; Vanderperren et al., 2005; Teschke and Bahre, 2009; Furukawa et al., 2010; Valente et al., 2010; Yang et al., 2010). A well described dechallenge of liver values in suspected HILI is one of the key items to suspect causality for a particular herb. Patients with HILI caused by herbal TCM or modern herbal medicine commonly experience an acute type of liver injury, which is self-limited upon withdrawal of the offending herb with an overall good prognosis. Whether herbs may cause chronic forms of HILI has not yet been evaluated in detail (García-Cortés et al., 2008). However, persistence of increased liver values raises the question whether these are due to a preexisting liver disease present prior to herbal use rather than to HILI (Picciotti et al., 1998). The acute type of HILI rarely may progress to acute liver failure (Stadlbauer et al., 2005; Fong et al., 2010). This is a serious condition that may require a liver transplant and eventually leads to death (Perharic-Walton and Murray, 1992; Yoshida et al., 1996; Haller et al., 2002; Adachi et al., 2003; Estes et al., 2003; Yuen et al., 2006; Sohn et al., 2008; Fong et al., 2010). Between 1992 and 2008; in Seoul (Korea) alone, 24 patients underwent liver transplantation due to toxic hepatitis caused by herbal TCM (Sohn et al., 2008), causing concern in view of poorly documented efficacy of herbal TCM (Manheimer et al., 2009; Teschke, 2014).

Cessation of herbal use is the only therapeutic approach for HILI patients. Other options including evidence based therapy for treating patients with HILI are lacking, but on a case basis treatment was reported with glycyrrhizin (Inoue et al., 2011), ursodesoxycholic acid (Jorge and Jorge, 2005; Inoue et al., 2011), or corticosteroids (Weinstein et al, 2012).

Hepatotoxicity Criteria

HILI case assessment mandates clear hepatotoxicity criteria for disease characterization including causality assignment (Teschke et al., 2013f, 2014b). Laboratory-based criteria of HILI are best defined by alanine aminotransferase (ALT) and/or alkaline phosphatise (ALP) values, expressed as N in multiples of the upper limit of their normal range (**Figure 1**). For ALT, recommendations initially were at >2N (Bénichou et al., 1993; Danan and Bénichou, 1993) and currently are at >5N (Björnsson et al., 2012; Teschke et al., 2014c,d) or at 3N if total bilirubin values exceed 2N (Aithal et al., 2011); for ALP, values of >2N are considered diagnostic (Bénichou et al., 1993; Danan and Bénichou, 1993; Aithal et al., 2011). Restricting the ALT criteria to >5N will eliminate unspecific ALT increases and substantiate causality at a high level of probability (Björnsson et al., 2012). Considering patients with ALT values of >2N will initially also include numerous cases with nonspecific increases, which then require thorough assessment and stringent exclusion of causes unrelated to the used herb(s). For low threshold values, the rate of alternative diagnoses is high (Teschke et al., 2013g), findings that are plausible and not unexpected (Teschke et al., 2014e). Other values such as aspartate aminotransferase (AST) are not required, unless to be used as substitute for ALT if not available.

Concern emerges whenever hepatotoxicity is assumed even if liver values were only marginally increased, not reported, or not assessed. These problems are not uncommon for cases of assumed HILI, presented for instance by the US Pharmacopeia (USP) (Mahady et al., 2008) relating to both black cohosh (BC) (Teschke, 2010c; Teschke et al., 2011d,e; Teschke

TABLE 3 | Compilation of narrative case details and clinical data of patients with HILI by Greater Celandine (GC) and established causality.

Patient	Identification	Specific information for each individual patient
01	(Strahl et al., 1998), 42 years Female	GC extract of known brand name and manufacturer (3 capsules/day containing each 200 mg of probably dried herb for 9 months). Bloating as indication for treatment. Latency period of 2 months for first symptoms of itching and jaundice, at rechallenge 1 month. ALT 755 U/L, AST 350 U/L, ALP 221 U/L. Upon cessation of GC treatment, rapid decrease but not normalization of ALT values reported. Readministration of GC with positive result. Exclusion of virus hepatitis A-C and infections of other hepatotropic viruses reported, with lack of any details regarding hepatitis A, B, and C, CMV, EBV, HSV, or VZV. Exclusion of biliary obstruction by sonography. Exclusion of autoimmune hepatitis reported with lack of specified parameters. Normal values of iron and copper parameters. Liver histology: Acute hepatitis with confluent liver cell necroses and little inflammation. **Final diagnosis: highly probable GC hepatotoxicity.**
02	(Benninger et al., 1999), their case five 37 years Female	GC extract as drug of known brand name and manufacturer (unknown dose/day for 3 months). Atopic eczema as indication for GC treatment. Various herbal and homeopathic drugs as CD. Latency period of 3 months until symptoms of nausea and jaundice. ALT 813 U/L, AST 898 U/L, ALP 249 U/L. ALT course described. Positive reexposure test for GC. Five months after GC discontinuation, normalization of liver parameters reported. Exclusion of infections by HAV, HBV, HBC, HEV, CMV, and EBV. SMA 1:40, exclusion of AIH. Ultrasound examination with normal bile ducts. Liver histology not done. **Final diagnosis: highly probable GC hepatotoxicity.**
03	(Benninger et al., 1999), their case six 65 years Female	GC extract of unknown brand name and manufacturer (unknown dose/day for 3 months). Dyspepsia as indication for GC treatment. Latency period and symptoms not recorded. No CD. ALT 152 U/L, AST 89 U/L, ALP 451 U/L. After GC discontinuation, ALT course not sufficiently documented. Normalization of liver values 3 months after GC withdrawal. Exclusion of common causes for hepatitis reported, but lack of information regarding specific parameters. Lack of ultrasound data. Liver histology: Moderate drug induced hepatitis with low grade single cell necrosis. **Final diagnosis: probable GC hepatotoxicity.**
04	(Crijns et al., 2002), 42 years Female	Herbal mixture of GC and curcuma root (*Curcuma longa rhizoma*) of known brand name and manufacturer (unknown dose/day for 2 months). Not further described skin complaints as indication for treatment. Before admission, paracetamol (500 mg) tablet on one day. Latency period: 5 weeks until jaundice. Fever 40.5°C for 2 weeks, starting 2 weeks after initiation of GC treatment. ALT 1490 U/L, AST 838 U/L, ALP 265 U/L. Following cessation of the herbal mixture, ALT course described with normalization of liver values after 2 months. Exclusion of acute hepatitis A-C and infections by CMV, and EBV, but HSV and VZV not assessed. Normal titres of ANA, but no data of AMA, SMA, and LKM. Sonography with normal biliary tract. Liver histology: Severe acute hepatitis of viral or toxic drug cause. **Final diagnoses: probable GC hepatotoxicity and possible curcuma hepatotoxicity.**
05	(Stickel et al., 2003), their case two 69 years Male	GC extract as drug of known brand name and manufacturer (80 capsules within 5–6 weeks). Postprandial abdominal discomfort as indication for GC treatment. Latency period of 5–6 weeks until symptoms of weakness, abdominal pain in the right upper quadrant, nausea, jaundice, and dark brown urine. Medical history included cholecystectomy 4 years ago. Lack of regular comedication. Alcohol consumption below 20 g/day. ALT 881 U/L, AST 466 U/L, ALP 312 U/L. ALT course not recorded. Exclusion of acute viral hepatitis including HAV, HBV, HCV, CMV, EBV. Autoimmune parameters not assessed. By abdominal ultrasound and magnetic resonance tomography common and intrahepatic bile ducts inapparent. Liver histology: Cholestatic hepatitis compatible with drug toxicity. **Final diagnosis: probable GC hepatotoxicity.**
06	(Rifai et al., 2006), 58 years Male	GC extract as drug of known brand name and manufacturer (unknown amounts of tablets/day for 3 weeks). Biliary spasms as indication for GC treatment. Latency period: of 3 weeks until fatigue, dark urine, itching, jaundice, and pale stool. No CD. ALT 903 U/L, AST 380 U/L, ALP 516 U/L. After GC withdrawal well documented ALT course with ALT normalization within 4 weeks. Well documented exclusion of hepatitis A–C, and E, and of infections by CMV, EBV, HSV, and VZV reported. Well documented exclusion of infectious, autoimmune, metabolic, and genetic causes of acute hepatitis. Sonography with slightly thickening of the gall bladder and otherwise normal biliary tract. Liver histology: Lobular hepatitis with severe cholestasis and moderate inflammation that included also the bile ducts. **Final diagnosis: probable GC hepatotoxicity, but also possible causality for biliary disease.**
07	(Conti et al., 2008), 46 years Female	GC extract as solution of known brand name and manufacturer, containing also other herbs as are *Lycopodium serrata, Carduus marianus, Hamamelis, Ruta, Sepia, Pulsatilla, Collinsonia, and Hydrastis* (50 drops/day for 8 weeks). Insomnia and for sedation as indication for treatment. Latency period of 8 weeks until symptoms of nausea, anorexia, asthenia, and abdominal discomfort. Herbal mixture with various herbs and the potentially hepatotoxic *Lycopodium serrata* as CD. ALT 2,364 U/L, AST 737 U/L, ALP 255 U/L. Rapid decrease of ALT in the further course following treatment cessation with normalization after 2 months. Exclusion of HAV, HBV, HCV, CMV, EBV, and HSV. Specified serological tests for autoimmune diseases negative. Sonography without reported biliary tract abnormalities. Liver histology: Moderate mixed inflammatory infiltrate with eosinophils. **Final diagnoses: probable GC hepatotoxicity, probable *Lycopodium serratum* hepatotoxicity.**
08	(Moro et al., 2009), 65 years Male	GC extract as herbal tea derived from GC leaves (1 cup/day for 1 month). Pyrosis as indication for GC treatment. Lansoprazole 15 mg/day for 2 years as current CD. Latency period of 1 month. Asthenia, dyspepsia, dark urine, and jaundice as symptoms. ALT 4765 U/L, AST 3235 U/L, ALP not reported. ALT course not reported, but normalization of all liver parameters within 2 months. Three months before symptom onset, treatment with clarithromycin and amoxicillin for 1 week. All antibodies for not further

(Continued)

TABLE 3 | Continued

Patient	Identification	Specific information for each individual patient
		specified hepatic viruses resulted negative except for anti-HCV that was found positive despite negative HCV-PCR. Autoimmune parameters not reported. Hepatomegaly by ultrasound examination. Liver histology: Moderate drug induced hepatitis. **Final diagnosis: probable GC hepatotoxicity.**
09	BfArM, 2005, 95003848 32 years Male	GC extract as drug of known brand name and manufacturer (2 capsules/day for not clearly defined duration). Upper abdominal pains as indication for treatment. Latency period not report ed, jaundice as symptom. ALT 2196 U/L, AST 714 U/L, ALP 256 U/L. Upon cessation of GC treatment, decrease but not normalization of ALT and AST values, with lack of reported ALP value. Readministration of GC with pruritus and not further specified increases of liver values and lack of complete resolution upon dechallenge. Overall course of ALT not sufficiently documented, neither at first and second dechallenge, nor in the interval and after the second challenge. Undulating ALT values of unknown clinical significance. Exclusion of virus hepatitis reported, with lack of any details regarding hepatitis A, B, and C, CMV, EBV, HSV, or VZV. Exclusion of biliary obstruction by sonography and ERCP. Exclusion of autoimmune hepatitis with lack of reported parameters. Normal values of ceruloplasmin, α-1 Antitrypsin, and electrophoresis. Liver histology: Unspecific hepatitis with liver cell necroses. Poorly documented case including questionable rechallenge and lack of ALT normalization. **Final diagnosis: probable GC hepatotoxicity.**
10	BfArM, 2005, 96026841 55 years Female	GC extract as drug of known brand name and manufacturer (3 capsules/day for 6 weeks). Upper abdominal pains as indication for treatment. Latency period of 6 weeks with jaundice as symptom. Diltiazem 90 for several years and doxycycline for 10 days (start prior to jaundice) for treatment of erythema migrans as CD. ALT 2,016 U/L, AST 620 U/L, ALP 398 U/L. After cessation of GC treatment, normalization of ALT not reported and with 201 U/L on day 19 still increased. Overall ALT course poorly documented. Exclusion of hepatitis A, B, and C reported without details of assessed parameters. Lack of exclusion of virus infections by CMV, EBV, HSV, and VZV. Negative results for AMA, SMA, LKM, and actin. Exclusion of biliary obstruction by sonography and ERCP. Liver histology: compatible with drug induced liver injury **Final diagnosis: highly probable GC hepatotoxicity.**
11	BfArM, 2005, 98000501 65 years Male	GC extract as drug of known brand name and manufacturer (2–3 capsules/day for 42 days). To increase bile flow after cholecystectomy 20 years ago as indication for treatment. Latency period of 42 days with itching and jaundice as symptoms. ALT 461 U/L, AST 355 U/L, ALP 260 U/L, normalization not reported. After GC discontinuation, on day 12 ALT 235 U/L. Exclusion of hepatitis A–C and of infections by CMV, EBV, HSV, and VZV. AMA negative, exclusion of autoimmune hepatitis reported but individual parameters not described. Upon sonography and ERCP normal bile ducts after cholecystectomy. **Final diagnosis: highly probable GC hepatotoxicity.**
12	BfArM, 2005, 98001447 49 years Female	GC extract as drug of known brand name and manufacturer (3 tablets/day for 4 weeks). Upper abdominal pains as indication for treatment. Latency period of 3.5 weeks with reduced appetite, bloating, epigastric pain, nausea, vomiting, and jaundice as symptoms. ALT 2928 U/L, AST 1116 U/L, ALP 408 U/L. After GC discontinuation, on day 7 ALT was 1356 U/L, and on day 20 it was 426 U/L. Normalization of ALT has not been reported. Exclusion of hepatitis A-C, E and F, and of infections by CMV, EBV, and HSV, but not of VZV. Normal values of ANA, AMA, and SMA. Upon ultrasound and ERC, normal bile ducts and cholecystolithiasis or cholesterol polyps of the gallbladder, by ultrasound questionable cholecystitis. Liver histology: Severe portal hepatitis with beginning fibrosis. **Final diagnosis: probable GC hepatotoxicity.**
13	BfArM, 2005, 98001607 59 years Female	GC extract as drug of known brand name and manufacturer (3 tablets/day for 7 weeks). Vomiting, upper abdominal pains and gastro-esophageal reflux as indications for treatment. Latency period 20 days with tiredness, exhaustion, nausea, vomiting, and jaundice as symptoms. Asthma, treated with various sprays, and latent hyperthyroidism without treatment as comorbidities. Maximum values reported for ALT 960 U/L, AST 421 U/L, and ALP 425 U/L, with decrease but not normalization following GC discontinuation, but actual results have not been reported. Through histology, ERCP, and serology (HAV, HBV, HCV) other hepatobiliary diseases excluded, but details not reported. Missing exclusion of infections by CMV, EBV, HSV, and VZV. Poorly documented case. **Final diagnosis: probable GC hepatotoxicity.**
14	BfArM, 2005, 98008527 60 years Female	GC extract as drug of known name and manufacturer (3 capsules/day for several weeks). General discomfort as indication for treatment. Latency period of several weeks with abdominal pains, nausea, and jaundice as symptoms. Crataegus extract as CD. ALT 420 U/L, AST 451 U/L, ALP 288 U/L. At discharge after 4 weeks, ALT with 26 U/L still slightly elevated. Exclusion of acute hepatitis A–C and infections by CMV, and EBV, but HSV and VZV not assessed. Normal titres of ANA, AMA, SMA, and LKM. Sonography and ERCP with normal biliary tract. Liver histology: AIH or dug induced liver injury. **Final diagnosis: probable GC hepatotoxicity.**
15	(BfArM, 2005), 00000278 65 years Male	GC extract as drug of known brand name and manufacturer (3 capsules/day for 4 weeks). Bloating as indication for treatment. Latency period of 3.5 weeks with jaundice as symptom. Diclofenac (intermittent), sitosterols, butizide, raubasine, rescinnamine, and reserpine as CD. ALT 950 U/L, AST 570 U/L, normal ALP. Under treatment with cortisone and at discharge, ALT 193 U/L, but normalization of ALT with and without cortisone not reported. Exclusion of hepatitis A–C and of infections by CMV, EBV, and HSV reported. Normal titres of ANA, AMA, and SMA. Sonography and ERCP with normal biliary tract. Liver histology: Hepatitis with cholestasis. **Final diagnosis: probable GC hepatotoxicity.**

(Continued)

TABLE 3 | Continued

Patient	Identification	Specific information for each individual patient
16	(BfArM, 2005), 02001171 66 years Female	GC extract as drug of known brand name and manufacturer (0–2 capsules/day for 4.5 months). Dyspepsia as indication for treatment. Latency period of 4.5 months with reduced appetite and jaundice as symptoms. ALT 760 U/L, AST 408 U/L, ALP 337 U/L. On day 14 after GC cessation, ALT 379 U/L, and on day 24 ALT 207 U/L. Normalization of ALT not reported. Exclusion of hepatitis A–C reported, but details not presented. No exclusion of infections by CMV, EBV, HSV, and VZV. Autoimmune parameters not done. Sonography, MRCP and MRT with normal biliary tract. Insufficiency of the mitral valve. **Final diagnosis: probable GC hepatotoxicity.**

Narrative compilation with details of relevant clinical data of 16 patients with liver injury by the use of the herb Greater Celandine (GC), data are derived from a review article (Teschke et al., 2012a) and based on previous reports (Teschke et al., 2011a, 2012e). In these studies, highly probable and probable causality levels for GC were established in all patients presented as final diagnoses, using the updated CIOMS scale for the individual causality assessment. Half of the patients (cases 01–08) were derived from published case reports, the other half (cases 09–16) from spontaneous reports of the German regulatory agency (BfArM, 2005). Outcome was favorable in all cases. Abbreviations: AIH, autoimmune hepatitis; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMA, antimitochondrial antibodies; ANA, antinuclear antibodies; AST, aspartate aminotransferase; BfArM, Bundesinstitut für Arzneimittel und Medizinprodukte, Bonn; CD, comedicated drug(s); CIOMS, Council for International Organizations of Medical Sciences; CMV, cytomegalovirus; EBV, Epstein Barr virus; ERCP, endoscopic retrograde cholangiopancreaticography; GC, Greater Celandine; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HSV, herpes simplex virus; LKM, liver kidney microsomal antibodies; MRCP, magnetic resonance cholangiopancreaticography; MRT, magnetic resonance tomography; PCR, polymerase chain reaction; SMA, smooth muscle antibodies; VZV, varicella zoster virus.

TABLE 4 | Preferred documentation as example: clinical characteristics of GC hepatotoxicity.

Characteristics of HILI by GC
Characterization of GC hepatotoxicity as a specific disease entity was feasible and based on high causality levels for GC in 16 patients with liver disease.
Causality for GC was graded highly probable and probable in 4 and 12 patients, respectively.
Among these 16 patients, there was an additional causality for comedicated curcuma graded as possible, for comedicated Lycopodium serratum graded as probable, and for biliary disease graded as possible.
The existence of GC hepatotoxicity has been verified by a positive reexposure test in two patients
Ages of the 16 patients ranged from 32 to 69 years with an average of 54.7 years, and the ratio of females: males was 10: 6.
Comedication with synthetic or herbal drugs and dietary supplements including herbal ones and herbal mixtures was used in the majority of assessable cases.
On average, the patients used 10 mg chelidonine daily with lack of daily overdose in any of the cases.
Treatment duration was 3 weeks to 9 months with an average of 2.4 months.
Latency period until first symptoms was 3 weeks to 4.5 months with an average of 1.7 months, which was considerably shorter than the treatment length.
Jaundice was the most frequently reported symptom, rarely also weakness, anorexia, nausea, vomiting, abdominal pains, dark urine, pale stools, and itching.
High serum activities are found for ALT but not for ALP, suggestive of a hepatocellular type of toxic liver injury in patients with GC hepatotoxicity.
Histology showed predominantly liver cell necrosis and hepatitis.
Outcome was favorable in all 16 patients, with lack of both acute liver failure and requirement of a liver transplant.
In one patient, good prognosis was sustained even after 7 months of continued GC Use despite presence of emerging GC hepatotoxicity.
GC hepatotoxicity usually represents the hepatocellular and idiosyncratic type of liver injury with its metabolic subgroup, characterized as acute clinical course.
The underlying mechanism(s) leading to GC hepatotoxicity as well as possible culprit(s) are still unknown.
In cases of liver disease, causality for GC was verified and creates concern regarding safety of patients and pharmacovigilance considerations.
Due to lack of epidemiologic data, the incidence of GC hepatotoxicity cannot accurately be calculated but appears to be low.

Preferred documentation: The data are based on the cases of 16 patients with GC hepatotoxicity and highly probable or probable causality levels for GC and derived from a previous report (Teschke et al., 2012a). Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; GC, Greater Celandine.

and Schulze, 2012) and green tea extracts (Sarma et al., 2008; Liss and Lewis, 2009); by the WHO, relating to kava (WHO, 2007a; Teschke and Wolff, 2009); the German regulatory agency BfArM (Bundesinstitut für Arzneimittel und Medizinprodukte) (BfArM, 2002, 2005) relating to kava (Schmidt et al., 2005; Teschke et al., 2008a); or the Drug Commission of the German Medical Association (DCGMA, 2011) relating to *Pelargonium sidoides* (PS) (Teschke et al., 2012b,e). In published case reports receiving the benefit of appropriate peer reviews, the presented HILI cases commonly provide high values of aminotransferases and/or ALP and basic data support of potential hepatotoxicity, as shown also for some cases with a positive reexposure test results (Teschke et al., 2014b). In other HILI case series, however, criteria were not or incompletely documented; neglecting these aspects in effect invalidates the causality assessment.

In spontaneous reports of regulatory agencies, a clear hepatotoxicity definition was provided by EMA (2007) but not by the U.S. Pharmacopeia (USP) (Mahady et al., 2008; Sarma et al., 2008), the German BfArM (2002), or the WHO (2007a). For instance, EMA mentions cases with assumed HILI by BC but clarifies that a causal attribution cannot be made with the required certainty in face of missing liver values (EMA, 2007). Consequently, missing regulatory hepatotoxicity definitions represent

TABLE 5 | Some chracteristics of daily and cumulative doses, treatment duration, latency period, and reexposure period of cases with hepatotoxicity by herbs of traditional and modern medicine.

Case	Sex Age	Herb Herbal mixture	Daily dose	Cumulative dose	Treatment duration	Latency period	Reexposure duration	References
1.	F/62y	Aloe	420 mg	37800 mg	3.0 month	2.75 month	1.0 month	Yang et al., 2010
2.	M/71y	Chaparral	1 tablet	90 tablets	3.0 month	3.5 month	1.0 month	Batchelor et al., 1995
3.	F/39y	Chinese herbal mixture	n.a.	n.a.	2.0 month	2.0 month	0.1 month	Kane et al., 1995
4.	F/ 9y	Chinese herbal mixture	n.a.	n.a.	6.0 month	5.25 month	1.0 month	Davies et al., 1990
5.	F/66y	Chinese Jin Bu Huan	0–2 tablets	60 tablets	3.0 month	2.75 month	0.5 month	Woolf et al., 1994
6.	M/46y	Chinese Jin Bu Huan	0–3 tablets	216 tablets	6.0 month	6.0 month	1.0 month	Woolf et al., 1994
7.	F/52y	Chinese Syo Saiko To	7.5 g	338 g	1.5 month	1.5 month	1.0 month	Itoh et al., 1995
8.	F/58y	Chinese Syo Saiko To	7.5 g	675 g	3.0 month	3.0 month	0.25 month	Itoh et al., 1995
9.	F/42y	Chinese Syo Saiko To	7.5 g	158 g	0.75 month	0.75 month	0.07 month	Itoh et al., 1995
10.	F/54y	Germander	600 mg	23,400 mg	1.3 month	1.3 month	1.0 month	Larrey et al., 1992
11.	F/25y	Germander	n.a.	n.a.	4.0 month	4.0month	0.75 month	Larrey et al., 1992
12.	M/48y	Germander	900 mg	81,000 mg	3.0 month	3.75 month	0.33 month	Larrey et al., 1992
13.	F/45y	Germander	260 mg	468,00 mg	6.0 month	6.0 month	0.25 month	Laliberté and Villeneuve, 1996
14.	F/42y	Greater Celandine	600 mg	162,000 mg	9.0 month	2.0 month	1.5 month	Strahl et al., 1998
15.	F/56y	Green tea	14 ml	210 ml	4.0 month	3.3 month	1.0 month	Jimenez-Saenz and Martinez-Sanchez, 2006
16.	F/37y	Green tea	n.a.	n.a.	4.0 month	4.0 month	1.0 month	Bonkovsky, 2006
17.	F/63y	Herbalife	n.a.	n.a.	4.0 month	3.5 month	n.a.	Hoffmann et al., 2005
18.	F/39y	Kava	60 mg	10,800 mg	6.0 month	6.0.month	0.5 month	Strahl et al., 1998
19.	F/49y	Mistletoe	50 mg	1500 mg	1.0 month	1.0 month	1.0 month(?)	Harvey and Colin-Jones, 1981
20.	M/61y	*Polygonum multiflorum*	n.a.	n.a.	0.033 month	0.033 month	0.033 month	Jung et al., 2011
21.	F/26y	Senna	100 mg	12,000 mg	4.0 month	3.0 month	n.a.	Beuers et al., 1991

In all 22 hepatotoxicity cases, causality for the respective herb or herbal mixture was ascertained by positive reexposure test results based on established criteria. Unclear: ?, Adapted data from a previous report (Teschke et al., 2014b).

confounding variables and result in false high signal cases due to regulatory case overreporting and overdiagnosing (Teschke et al., 2013f).

For reasons of transparency and assessment of case data quality, each HILI case series should provide tabulated information of available or missing case details, as done in various reports (Teschke et al., 2011a, 2012b,e) and shown as example (**Table 6**) (Teschke, 2010c).

HILI Case Characteristics

Hepatotoxicity classification is mandatory in cases of assumed HILI to facilitate further evaluation of reexposure results and CIOMS assessments (Teschke et al., 2013f). Based on specific laboratory constellations, differentiation of the hepatocellular, cholestatic or mixed form of hepatotoxicity is feasible by comparing serum activities of ALT and ALP at the time HILI diagnosis is first suspected (Bénichou et al., 1993; Danan and Bénichou, 1993; Teschke et al., 2014d). Enzyme activity is expressed as a multiple of the upper limit of the normal range (N), and the ratio (R) of ALT/ALP is calculated. Liver injury is classified as hepatocellular, if ALT > 2N alone or $R = 5$; cholestatic, when there is an increase of ALP > 2N alone or when $R = 2$; of the mixed type if ALT > 2N, ALP is increased, and $2 < R < 5$ (**Figure 1**). In a HILI case series of herbal TCM consisting of 27 patients, the pattern of liver injury was hepatocellular in 82% of the cases, cholestatic in 11%, and mixed in 7% (Chau et al., 2011).

Liver Histology

Liver biopsy in HILI and DILI cases requires special attention in any clinical hepatology setting, balancing benefits and risks for the patient (Teschke and Frenzel, 2014). Published and spontaneous HILI reports often contain detailed histological descriptions of liver biopsy findings, mostly associated with pictures obtained by microscopy. This erroneously implies that liver biopsy is an essential part of routine case assessments (BfArM, 2002; Teschke et al., 2008a, 2011a, 2012a,b,f,g; Teschke, 2010c). Histology data were also presented by narrative HILI case reports lacking even any causality for a particular herb (Teschke et al., 2012f,g). This raises the question whether a liver biopsy is justified, considering also that there were no histological findings recognized as specific for all hepatotoxicity cases (Ramachandran and Kakar, 2009). Liver biopsy in chronic hepatotoxicity cases to define prognosis in the absence of an expected specific therapy option remains debatable (Teschke and Frenzel, 2014).

To evaluate liver histology findings, a retrospective case analysis of pathological changes in HILI selectively caused by one single herb with established causality appears the best approach. For instance, HILI cases of kava and GC have such an established causality track. In 12 GC HILI patients with a probable or highly probable causality grading for GC, prevailing histological features included hepatitis, single or confluent liver cell necrosis, inflammation, rarely fibrosis, and cholestasis (Teschke et al., 2011a, 2012a,e). In eight HILI patients with a highly probable,

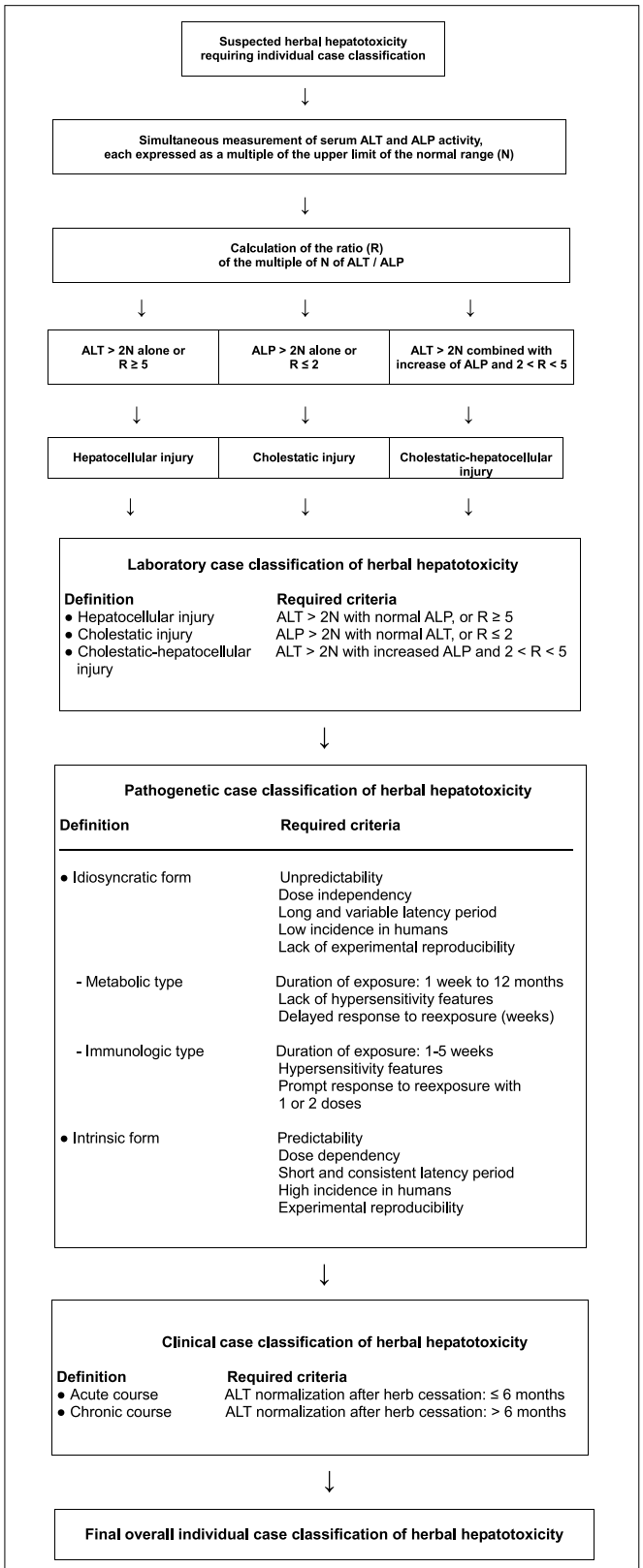

FIGURE 1 | Strategy for assessing HILI cases according to details presented previously (Teschke et al., 2013f). Abbreviations: ALP, alkaline phosphatase; ALT.

probable or possible causality for kava, liver histology showed hepatitis, liver cell necrosis, and rarely bile duct proliferation and intrahepatic cholestasis (Teschke et al., 2008a). Therefore, at least for the two herbs GC and kava, the histological features are quite uniform and restricted to two major features, hepatitis and liver cell necroses. These histological characteristics, however, are also found in most other liver diseases unrelated to herbs, obviating liver biopsy in suspected HILI cases due to unspecific results.

Additional insights are provided by the analysis of cases with positive reexposure tests, done unintentionally with the incriminated herb or herbal mixture, and available liver histology results. For instance, in HILI by a herbal mixture of TCM, total liver necrosis prevailed (Perharic-Walton and Murray, 1992); germander (*Teucrium chamaedrys*) caused hepatocyte necrosis with lobular inflammatory infiltration mainly by mononuclear cells, associated with slightly fibrous portal tracts containing inflammatory cells (Larrey et al., 1992); senna use resulted in liver cell necrosis around the central veins as well as portal and lobular infiltration by lymphocytes, histiocytes, and rare plasma cells (Beuers et al., 1991); chaparral intake was associated with hepatocellular necrosis combined with inflammation, portal tract expansion, mild cholestasis and fibrous septation (Batchelor et al., 1995); and the herbal TCM Chinese skullcap (*Scutellaria baicalensis*) was considered to cause acidophil bodies, ballooned hepatocytes, lobular inflammatory cell infiltrates including eosinophils, and portal tracts containing mononuclear cells and eosinophils (Yang et al., 2012a). Based on causality established by positive reexposure results, these few examples may provide some insight in morphological liver changes due to herbal use.

Histological features usually are not clinically relevant, but some clinicians still consider a liver biopsy an important part of the diagnostic work-up in suspected hepatotoxicity cases. The question is whether histological results changed the initial diagnosis or benefited the individual patient. In two cases of initially suspected HILI, however, histological findings of giant cell hepatitis were reported and completely ignored (Dunbar and Solga, 2007; Schoepfer et al., 2007), while the clinical course and this particular histological pattern best fitted with an existing severe virus infection with hepatic involvement rather than herbal hepatotoxicity (Teschke and Schwarzenboeck, 2009).

Clearly, the pathologist is not helpful offering diagnoses such as HILI or liver injury compatible with or suggestive for herbal use. Overall, liver histology as a supporting routine method for assessing HILI cases is not recommended, it commonly adds little new specific diagnostic clues as information to the case without benefit for the patient; as an invasive procedure, rare but potentially life threatening complications may occur (Teschke and Frenzel, 2014).

Alternative Causes

Unrecognized alternative diseases are a real clinical problem when caring for patients with initially assumed but later not confirmed HILI. Several hundred liver diseases have to be considered as diagnoses alternative to HILI, to be ruled out under clinical aspects and with specific diagnostic tools. As a reminder for clinicians, a checklist with details for these alternative diagnoses is available (**Table 7**) (Teschke et al., 2014d). Numerous missed

TABLE 6 | Preferred documentation as example: overview of known information regarding all 69 patients with primarily suspected but not established HILI by black cohosh (BC).

Presented information	Cases	Individual cases
Brand name	22/69	1,5,11,12,13,14,15,16,17,18,19,20,21,22,23,24,28,29,30,31,32,33
Manufacturer	12/69	1,5,11,17,18,20,28,29,30,31,32,33
Plant part	06/69	1,5,8,11,28,33
Solvent	02/69	1,11
Daily dose	11/69	1,3,5,6,7,11,12,13,16,17,18
BC drug	07/69	1,11,19,21,23,24,31
BC herbal supplement	01/69	5
BC polyherbal product	14/69	2,3,12,13,14,15,16,17,18,20,22,28,30,33
Date of BC start	20/69	1,3,4,5,6,7,8,10,11,12,13,15,18,20,21,22,27,28,29,33
Date of BC end	17/69	1,3,4,5,6,7,8,10,11,12,13,15,18,19,20,21,22
Date of symptoms	24/69	1,2,3,4,5,6,7,8,10,11,12,13,14,15,16,18,19,20,21,22,23,27,32,33
Temporal association	12/69	1,4,5,6,8,10,12,13,18,20,21,22
Time on BC	16/69	1,3,4,5,6,7,8,10,11,12,13,15,18,20,21,22
Time to onset	19/69	1,3,4,5,6,7,8,9,10,11,12,13,18,20,21,22,27,28,33
ALT value	15/69	1,2,3,4,5,6,7,8,10,11,12,13,15,23,32
ALP value	12/69	1,2,3,4,5,6,7,11,12,13,23,32
Hepatotoxicity criteria	14/69	1,2,3,4,5,6,7,8,10,11,12,13,23,32
ALT de-challenge	06/69	4,6,7,10,11,15
Biliary tract imaging	08/69	4,5,6,7,9,10,15,28
HAV	13/69	1,2,3,4,5,6,7,8,9,10,11,14,15
HBV	12/69	1,3,4,5,6,7,8,9,10,11,14,15
HCV	13/69	1,2,3,4,5,6,7,8,9,10,11,14,15
CMV	09/69	1,4,5,6,8,10,11,14,15
EBV	09/69	1,4,5,6,8,10,11,14,15
HSV	03/69	4,6,8
VZV	01/69	8
Co-medication/herbal mixture	23/69	2,3,4,5,6,7,11,12,13,14,15,16,17,18,20,22,23,27,28,29,30,32,33
BC undetermined product	13/69	4,6,7,8,9,10,25,26,27,29,32,34–69

PreferreThe group of the 69 cases consisted of 11 case reports (cases 1–11), 13 TGA cases from Australia (cases 12–24), 2 CADRMP cases from Canada (cases 25 and 26), 7 MedWatch/ FDA cases from the United States (cases 27–33), and 33 EMA cases from the European Union (cases 34–69). Details and references of the 69 cases were published earlier (Teschke et al., 2010). Abbreviations: ALP, alkaline phosphatase; ALT alanine aminotransferase; BC, black cohosh; CMV, cytomegalovirus; EBV, Epstein Barr virus; HAV, hepatitis A virus, HBV, hepatitis B virus, HCV, hepatitis C virus; HSV, herpes simplex virus; VZV, varicella zoster virus.

diagnoses were found upon reevaluation of initially assumed HILI cases, with similar problems for DILI (**Figure 2**) (Teschke et al., 2013g, 2014a). Exclusion of hepatitis E and infections by cytomegalovirus (CMV), Epstein Barr virus (EBV), herpes simplex virus (HSV), and varicella zoster virus (VZV) should be obligatory rather than facultative (**Table 7**).

Causality Evaluation

Reexposure

Establishing the diagnosis of HILI may be cumbersome in the usual clinical setting, with experts not available in place and in time. Since, convincing biomarkers for all HILI cases are lacking, the gold standard for the diagnosis of hepatotoxicity still is a positive unintentional reexposure test result, if available (Bénichou et al., 1993; Danan and Bénichou, 1993; García-Cortés et al., 2008; Chalasani and Björnsson, 2010). Details of essential criteria are based on the conclusions of an International Consensus Meeting, as referred previously (Bénichou et al., 1993). Accordingly, required data are baseline ALT levels before reexposure, designed ALTb, and reexposure ALT levels, designed ALTr. The reexposure test is positive, if ALTr = 2ALTb and ALTb is below 5N, with N as the upper limit of the normal value (**Table 8**). Other variations lead to uninterpretable results. Some HILI reports mentioned a positive reaction upon reexposure, and these cases were further analyzed. Not all reexposure tests could be confirmed, partially due to lack of any details; however, for numerous herbs and herbal preparations of TCM and modern medicine, valid reexposure test results confirmed causality in the assessed HILI cases (**Table 9**) (Teschke et al., 2014b,d); using these cases, some characteristic features of daily and cumulative doses, divergences between treatment duration and latency period, and reexposure duration are evident (**Table 5**).

CIOMS/RUCAM

Physicians at armlength from the patient with HILI are well advised to consider a pragmatic thorough clinical evaluation

TABLE 7 | Differential diagnoses of HILI.

Differential diagnosis	Diagnostic parameters	Diagnostic exclusion done for patient's assessment		
		Yes	NO	Partial
Hepatitis A	Anti-HAV-IgM	☐	☐	☐
Hepatitis B	Anti-HBc-IgM, HBV-DNA	☐	☐	☐
Hepatitis C	Anti-HCV-IgM, HCV-RNA	☐	☐	☐
Hepatitis E	Anti-HEV-IgM, Anti-HEV-IgG, HEV-RNA	☐	☐	☐
Cytomegalovirus (CMV)	CMV-PCR, titre change for Anti-CMV-IgM and Anti-CMV-IgG	☐	☐	☐
Epstein Barr virus (EBV)	EBV-PCR, titre change for Anti-EBV-IgM and Anti-EBV-IgG	☐	☐	☐
Herpes simplex virus (HSV)	HSV-PCR, titre change for Anti-HSV-IgM and Anti-HSV- IgG	☐	☐	☐
Varicella zoster virus (VZV)	VZV-PCR, titre change for Anti-VZV-IgM and Anti-VZV- IgG	☐	☐	☐
Other virus infections	Specific serology of Adenovirus, Coxsackie-B-Virus, Echovirus, Measles virus, Rubella virus, Flavivirus, Arenavirus, Filovirus, Parvovirus, HIV, and others	☐	☐	☐
Other infectious diseases	Specific assessment of bacteria, fungi, parasites, worms, and others	☐	☐	☐
Autoimmune hepatitis (AIH) type I	Gamma globulins, ANA, SMA, AAA, SLA/LP, Anti-LSP, Anti-ASGPR	☐	☐	☐
Autoimmune hepatitis (AIH) type II	Gamma globulins, Anti-LKM-1 (CYP 2D6), Anti-LKM-2 (CYP 2C9), Anti-LKM-3	☐	☐	☐
Primary biliary cholangitis (PBC)	AMA, Anti PDH-E2	☐	☐	☐
Primary sclerosing cholangitis (PSC)	p-ANCA, MRC	☐	☐	☐
Autoimmune cholangitis (AIC)	ANA, SMA	☐	☐	☐
Overlap syndromes	See AIH, PBC, PSC, and AIC	☐	☐	☐
Non alcoholic steatohepatitis (NASH)	BMI, insulin resistance, hepatomegaly, echogenicity of the liver	☐	☐	☐
Alcoholic liver disease (ALD)	Patient's history, clinical and laboratory assessment, sonography	☐	☐	☐
Drug induced liver injury (DILI)	Patient's history, clinical and laboratory assessment, sonography, use of the CIOMS scale	☐	☐	☐
Cocaine, ecstasy and other amphetamines	Toxin screening	☐	☐	☐
Rare intoxications	Toxin screening for household and occupational toxins	☐	☐	☐
Hereditary hemochromatosis	Serum ferritin, total iron-binding capacity, genotyping for C2824 and H63D mutation, hepatic iron content	☐	☐	☐
Wilson's disease	Copper excretion (24 h urine), ceruloplasmin in serum, free copper in serum, Coombs-negative hemolytic anemia, hepatic copper content, Kayser-Fleischer-Ring, neurologic-psychiatric work-up, genotyping	☐	☐	☐
Porphyria	Porphobilinogen in urine, total porphyrines in urine	☐	☐	☐
α_1—Antitrypsin deficiency	α_1—Antitrypsin in serum	☐	☐	☐
Biliary diseases	Clinical and laboratory assessment, hepatobiliary sonography, MRC	☐	☐	☐
Pancreatic diseases	Clinical and laboratory assessment, sonography, CT, MRT	☐	☐	☐
Celiac disease	TTG antibodies, endomysium antibodies, duodenal biopsy	☐	☐	☐
Anorexia nervosa	Clinical context	☐	☐	☐
Parenteral nutrition	Clinical context	☐	☐	☐
Cardiopulmonary diseases	Cardiopulmonary assessment of congestive heart disease, myocardial infarction, cardiomyopathy, cardiac valvular dysfunction, pulmonary embolism, pericardial diseases, arrhythmia, hemorrhagic shock, and various other conditions	☐	☐	☐
Addison's disease	Plasma cortisol	☐	☐	☐
Thyroid diseases	TSH basal, T4, T3	☐	☐	☐
Grand mal seizures	Clinical context of epileptic seizure (duration > 30 min)	☐	☐	☐
Heat stroke	Shock, hyperthermia	☐	☐	☐
Polytrauma	Shock, liver injury	☐	☐	☐
Systemic diseases	Specific assessment of M. Boeck, amyloidosis, lymphoma, other malignant tumors, sepsis, and others	☐	☐	☐
Other diseases	Clinical context	☐	☐	☐

This tabular compilation represents an update of a previous version (Teschke et al., 2013f). AAA, Anti-actin antibodies; AMA, antimitochondrial antibodies; ANA, antinuclear antibodies; ASGPR, Asialo-glycoprotein-receptor; BMI, body mass index; CIOMS, Council for International Organizations of Medical Sciences; CT, computer tomography; CYP, cytochrome P450; DPH, pyruvate dehydrogenase; HAV, hepatitis A virus; HBc, Hepatitis B core; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HILI, herb induced liver injury; HIV, human immunodeficiency virus; LKM, liver kidney microsomes; LP, liver-pancreas antigen; LSP, liver specific protein; MRC, magnetic resonance cholangiography; MRT, magnetic resonance tomography; p-ANCA, perinuclear antineutrophil cytoplasmatic antibodies; PCR, polymerase chain reaction; SLA, soluble liver antigen; SMA, smooth muscle antibodies; TSH, thyroid stimulating hormone; TTG, tissue transglutaminase.

Primarily suspected herbal and drug hepatotoxicity

↓ ↓

Missed diagnoses as alternatives to herbal hepatotoxicity	Missed diagnoses as alternatives to drug hepatotoxicity
Hepatitis E	Hepatitis B
Hepatitis by EBV	Hepatitis C
Hepatitis by HSV	Hepatitis E
Hepatitis by VZV	Hepaits by CMV
Giant cell hepatitis	Hepatitis by EBV
Infection with hepatic involvement	Virus hepatitis
Autoimmune hepatitis	Autoimmune hepatitis
LKM positive autoimmune hepatitis	Hemochromatosis
SMA positive autoimmune hepatitis	Wilson's disease
Primary biliary cirrhosis	Ischemic hepatitis
Overlap syndrome	Cardiac hepatopathy
Alcoholic liver disease/cirrhosis	Liver cirrhosis
Non-alcoholic liver cirrhosis	Fatty liver
Previous gastric bypass operation	Non alcoholic steatohepatitis
Cardiac hepatopathy	Alcoholic liver disease
Polytrauma (38)	Past liver transplantation
Preexisting liver diseases/cirrhosis	Unknown liver disease
Questionable liver disease	Gilbert's syndrome
Liver injury by co-medication	Benign recurrent intrahepatic cholestasis
Non-alcoholic steatohepatitis (	Bile duct diseases
Hyperthyroid hepatopathy	Tumors
Biliary diseases	Lymphoma
Pancreatitis	Systemic sepsis
Rhabdomyolysis by statin	Chlamydial infection
	Thyroid disease
	Postictal state
	Polymyositis

FIGURE 2 | Missed diagnoses in cases of initially suspected hepatotoxicity by herbs and synthetic drugs. Adapted from a previous report (Teschke et al., 2013f), which provides the respective references for each item presented above. Abbreviations: CMV, cytomegalovirus; EBV, Epstein Barr virus; HSV herpes simplex virus; LKM, liver kidney microsomes; SMA, smooth muscle antibodies; VZV, varicella zoster virus.

in connection with a prospective structured approach assessing causality, providing the diagnosis in time while the disease is unfolding and without delay due to waiting periods for expert rounds' conclusions months thereafter; this is a crucial issue worldwide. We clearly prefer the CIOMS scale (Council for International Organizations of Medical Sciences), also called RUCAM (Roussel Uclaf Causality Assessment Method), in its original form (Bénichou et al., 1993; Danan and Bénichou, 1993) or better its update (**Tables 10**, **11**) (Teschke et al., 2013a, 2014d). Discussions focused on strengths and weaknesses of CIOMS, a learning system and not immutable (Andrade et al., 2004; Rochon et al., 2008; Aithal et al., 2011; García-Cortés et al., 2011; Teschke and Wolff, 2011; Teschke and Schulze, 2012; Teschke et al., 2008c, 2013e, 2014d; Lewis, 2014; NIH, 2014b). Outlined suggestions for improvement and refinement are incorporated in the updated CIOMS scale (**Tables 10**, **11**) (Teschke et al., 2013e, 2014d). Included is now the search for additional competing causes such as sepsis; or autoimmune hepatitis, chronic hepatitis B and C, primary biliary cholangitis and sclerosing cholangitis, and genetic liver diseases. HBsAg and HBV-DNA quantification were added to distinguish HBV infection from immunization, as was HCV-RNA to correctly assess HCV infections. Specific diagnostic criteria now include PCR detection and titer changes of the respective antibodies (IgM, IgG) for CMV, EBV, HEV, HSV, and VZV infections. Hepatobiliary sonography was supplemented by color Doppler sonography including assessments of the liver vessels, endosonography, computed tomography (CT), and magnetic resonance cholangiography (MRC. Alcohol as risk factor is now specified by an intake of >2 drinks per day (>14 units/week) in woman and >3 drinks per day (21 units/week) in men, whereby one drink corresponds to 10 g ethanol. For comparison and method validation, causality has been evaluated in 101 hepatotoxicity cases by both the original and updated CIOMS scales, with identical causality results published in 6 studies (Teschke et al., 2014d).Therefore, the updated CIOMS scale was validated, and there is no need for further validation of the updated CIOMS scale vs. the original CIOMS scale.

A selective compilation shows that numerous international registries and regulatory agencies as well as associated groups

TABLE 8 | Conditions of unintentional reexposure tests in suspected HILI cases.

Reexposure test result	Hepatocellular type of liver injury		Cholestatic (±hepatocellular) type of liver injury	
	ALTb	ALTr	ALPb	ALPr
Positive	<5N	≥2ALTb	<2N	≥2ALPb
Negative	<5N	<2ALTb	<2N	<2ALPb
Negative	≥5N	≥2ALTb	≥2N	≥2ALPb
Negative	≥5N	<2ALTb	≥2N	<2ALPb
Uninterpretable	<5N	n.a.	<2N	n.a.
Uninterpretable	n.a.	≥2ALTb	n.a.	≥2ALPb
Uninterpretable	n.a.	n.a.	n.a.	n.a.

Conditions and criteria for an unintentional reexposure test, adapted from a previous report (Teschke et al., 2014b). Accordingly, required data for the hepatocellular type of liver injury are the ALT level, just before reexposure, designed as baseline ALT or ALTb, and the ALT levels during reexposure, designed as ALTr. Response to reexposure is positive, if both criteria are met: first, ALTb is below 5N with N as the upper limit of the normal value, and second ALTr ≥2ALTb. Other variations lead to negative or uninterpretable results. For the cholestatic (±hepatocellular) type of liver injury, corresponding values of ALP are to be used rather than of ALT. Abbreviations: ALP, Alkaline phosphatase; ALT, Alanine aminotransferase; n.a., not available.

actually apply CIOMS for HILI and DILI cases (**Table 12**), with its advantages over other causality assessing approaches (Teschke et al., 2013a) including the method of DILIN (Drug-Induced Liver Injury Network) (**Table 13**). As opposed to the DILIN method, CIOMS fascinates by its stringent scoring system (**Table 13**), with its generation of a quantitative assessment (score) to address probability, which is more precise than a simple yes or no (Gunawan and Kaplowitz, 2004). Support for CIOMS was actually provided by Navarro as DILIN member and senior author of a HDS case series by applying CIOMS for causality assessment (Halegoua-De Marzio et al., 2013), whereas CIOMS was not in favor of Lewis, another DILIN member (Lewis, 2014). Although, connected with an actual commentary on a single HILI case report that did not undergo any formal causality assessment, Navarro also correctly acknowledged that CIOMS/RUCAM is the most frequently referenced scoring system (Fenkel and Navarro, 2011). This assumption supports earlier systematic analyses of 2008 (Tajiri and Shimizu, 2008). Of 61 DILI reports that were reviewed in the PubMed database over the last decade, showing that CIOMS was the most used scale. In a recent confirmative study of 573 HILI cases (**Table 14**), CIOMS again was the most used method applied in 275 cases (48.0%) (Teschke et al., 2013g), in line with mainstream opinion (**Tables 12**, **14**) (Wai, 2006; Aithal et al., 2011; NIH, 2014a,b; Björnsson et al., 2012, 2013. The CIOMS scale was widely used for hepatotoxicity assessments in epidemiological studies, clinical trials, case reports, case series, regulatory analyses, and genotyping studies, as referenced in detail recently (Teschke et al., 2013f).

CIOMS is structured, quantitative, and specific and validated for hepatotoxicity, and considers all its core elements (**Tables 10**, **11**) (Teschke et al., 2014d). It was developed by an international expert panel and validated by cases with positive reexposure tests as gold standard, showing good sensitivity (86%), specificity (89%), positive predictive value (93%), and negative predictive value (78%) (Bénichou et al., 1993). Of note, the scales for the hepatocellular and the cholestatic (± hepatocellular) type of injury differ slightly (**Tables 10**, **11**).

The CIOMS scale was conceptualized and developed in consensus meetings organized at the request of the Council for International Organizations of Medial Sciences (CIOMS) (Bénichou et al., 1993; Danan and Bénichou, 1993), aiming to overcome experts' previous problems with unstructured and unquantified evaluations lacking defined and scored items,resulting in debated causality assignments. This CIOMS scale represented a breakthrough in DILI and HILI causality assessment methods and extended, specified, and quantified preceding versions (Danan, 1988; Bénichou, 1990). The basis for CIOMS was provided by eight experts in hepatology from 6 countries and included J. P. Benhamou (France), J. Bircher (Germany), G. Danan (France), W. C. Maddrey (USA), J. Neuberger (UK), F. Orlandi (Italy), N. Tygstrup (Denmark), and H. J. Zimmerman (USA) (Bénichou et al., 1993; Danan and Bénichou, 1993). These experts in the field evaluated DILI cases for case characteristics, hepatotoxicity criteria, liver injury pattern, and reexposure criteria; they standardized DILI case assessment with specific, quantitative items and validated their method with established positive reexposure DILI case results (Bénichou et al., 1993; Danan and Bénichou, 1993). CIOMS was developed for assessment of a single drug containing a synthetic product and may be used for a single herb containing multiple chemical constituents, but does not allow causality attribution to a single constituent.

CIOMS provides a differentiating range of causality grades for the responsible agent(s) and clearly delineates liver specific criteria for challenge, dechallenge, exclusion of unrelated diseases, and comedication (Bénichou et al., 1993; Danan and Bénichou, 1993). It even takes into account atypical chronology with +1 point for challenge periods of <5 days or > 90 days, whereas the period of 5–90 days renders +2 points (**Table 10**). It is well adapted for cases with missing data. Physicians suspecting herbal hepatotoxicity can easily use CIOMS, results are readily available within a few minutes.

To facilitate valid actual assessment and possible external reevaluation, CIOMS scale data should be provided individually point by point for each patient (**Tables 10**, **11**), along with the list of alternative diagnoses to be excluded (**Table 7**). In publications of HILI cases and for submission to regulatory agencies as spontaneous reports, the completed CIOMS scale with all items including individual and final scores should be supplied to ensure data transparency, as published before (Teschke et al., 2008a, 2011a,d,e, 2012b,d,h; Teschke and Bahre, 2009; Teschke, 2010a,c) and presented as example, using the CIOMS scale of the hepatocellular type of injury (**Table 15**).

Thus, we strongly recommend for HILI case assessment a sequential approach, starting with thorough clinical evaluations and concomitant prospective causality evaluation by the updated CIOMS scale (**Tables 10**, **11**), followed by optional expert opinion based on scored CIOMS items, if uncertainty remains, and finally for reasons of transparency, appropriate documentation of case details (**Tables 3**, **6**, **7**, **15**).

TABLE 9 | Analysis of reported positive reexposure test results in cases of suspected herbal Traditional Chinese Medicine (TCM) induced liver injury.

Case reexposure tests in cases of suspected herbal TCM induced liver injury
Chinese herbal mixtures
• 28-year old UK woman (Perharic-Walton and Murray, 1992): Chinese herbal mixture with 8 different herbs for 3–5 months. Jaundice. ALT value not available. Reexposure: episode of hepatitis reported without liver values, acute liver failure, died despite emergency liver transplantation. Both ALTb and ALTr not available → uninterpretable reexposure.
• 39-year old UK woman (Kane et al., 1995): Chinese herbal mixture with eight different herbs for 2 months. Short history of anorexia, nausea, fatigue, dark urine, yellow sclerae, jaundice. ALT 2440 U/L (normal 0–30) with R 68.3, ALT returned to normal after cessation. Reexposure after 6 weeks: ALT 1314 U/L. ALTb < 5N and ALTr = 2 ALTb → positive reexposure.
• 9-year old UK girl (Allen and Parkinson, 1990): Unclassified Chinese herbal medicine for 6 months. Nausea, anorexia, central abdominal pain, jaundice, pale stool for the past 4–21 days. ALT 1950 U/L (normal < 45) with R 13.1, ALT returned to 50 U/L after cessation. Intentional reexposure: ALT 315 U/L. ALTb < 5N and ALTr = 2ALTb → positive reexposure.
Ho Shou Wu
• 54-year old Korean woman (Bae et al., 2010): Unknown dose of Ho Shou Wu containing *Polygonum multiflorum* for one month. Diagnosis of toxic hepatitis. Cessation of Ho Shou Wu improved her condition. Reexposure started immediately after discharge with aggravation of liver values. English abstract and Korean article → uninterpretable reexposure.
Huan Qin
• 78-year old US woman (Yang et al., 2012a): Move Free Advanced® two tablets/day containing Huan Qin (*Scutellaria baicalensis*, Chinese skullcap), black catechu, glucosamine, and chondroitin for 3 weeks. Jaundice. ALT 1626 U/L (normal < 60) with R 10.2, ALT 678 U/L two weeks after cessation. Reexposure: ALT 1206 U/L. ALTb = 5N and ALTr < 2ALTb → negative reexposure.
Hwang Geun Cho
• 37-year old male patient from Korea (Kang et al., 2009): Hwang Geun Cho containing *Corydalis speciosa*. Jaundice. ALT 531 U/L with subsequent decline after cessation of the herb down to 146 U/ L. Unintentional reexposure two months after discharge: ALT 381 U/L. ALTb < 5N and ALTr = 2ALTb → positive reexposure.
Ji Xue Cao
• 61-year old Argentinian woman (Jorge and Jorge, 2005): Ji Xue Cao (*Centella asiatica*, syn.Gotu Kola) tablets for 30 days. Jaundice. ALT 1193 U/L and 2 months after cessation 18 U/L. Unintentional reexposure 7 months later: ALT 481 U/L. ALTb < 5N and ALTr = 2ALTb → positive reexposure.
• 52-year old female patient from Argentinia (Jorge and Jorge, 2005): Ji Xue Coa (*Centella asiatica*) for 6 months. Jaundice. Not further quantified elevated hepatic enzymes at beginning and after cessation. Unintentional reexposure 1 year later: ALT 1694 U/L. ALTb not availabe → uninterpretable reexposure.
Jin Bu Huan
• 66-year old US woman (Woolf et al., 1994): Jin Bu Huan 2 tablets at night two to three times a week for twelve weeks. Fever, nausea, fatigue for the past 5 weeks. ALT 782 U/L (normal < 35) with R 8.7, ALT declined to 47 U/L following cessation. Reexposure: ALT 941 U/L. ALTb < 5N and ALTr = 2 ALTb → positive reexposure.
• 46-year old US man (Woolf et al., 1994): Jin Bu Huan three tablets three times a week intermittently for 6 months. Fever, headaches, fatigue, tender hepatomegaly. ALT 394 U/L (normal < 35) 2 weeks after cessation with R 24.2, ALT subsequently 48 U/L. Reexposure: ALT 100 U/L. ALTb < 5 N and ALTr = 2 ALTb → positive reexposure.
• 50-year old US woman (Horowitz et al., 1996): Jin Bu Huan two to three tablets daily or intermittently for around 24 days. Fever. ALT 830 U/L and 330 U/L after cessation. Reexposure: ALT 540 U/L. ALTb = 5N and ALTr < 2ALTb → negative reexposure.
• 70-year old US woman (Horowitz et al., 1996): Jin Bu Huan three to four tablets at night three to five times a week for 31 days. Chills and fever 12 days after start of use, subsequently low-grade fever, malaise. ALT 408 U/L initially, 263 U/L after 2- week cessation, 67 U/L after 6-week cessation. Reexposure after 1 month: ALT 77 U/L. ALTb < 5N but ALTr < 2ALTb → negative reexposure.
Lu Cha
• 56-year old French woman (Peyrin-Biroulet et al., 2004): Mincifit® 14 ml/day containing green tea (*Camellia sinensis*, TCM Lu Cha) and *Cassia* sp. extracts for 15 days. Jaundice. ALT 54N with R 54.0, ALT normalization 2 months after cessation. Reexposure 5 years later with Dynasvelte forte® 8–12 g/day for 21 days (Green tea, *Coffea Arabica*, and chromium): ALT 99N. ALTb < 5N and ALTr = 2ALTb → positive reexposure.
• 45-year old Spanish man (Jimenez-Saenz and Martinez-Sanchez, 2006): Green tea infusion (6 cups/day) over 4 months. Asthenia and jaundice of ten days duration prior to cessation. ALT 1613 U/L (normal < 40) with R 4.3, ALT normalized within 2 months of cessation. Reexposure 6 weeks later: ALT 1460 U/L after 1 month of reuse. ALTb < 5N and ALTr = 2ALTb → positive reexposure.
• 37-year old Hispanic woman from the US (Bonkovsky, 2006): Green tea-containing product with various other herbal extracts for 4 months. Jaundice. ALT 1788 U/L (normal < 40) with R 21.7, ALT 92 U/L after withdrawal. Reexposure 1 year later for 1 month: ALT 1131 U/L. ALTb < 5N and ALTr = 2ALTb → positive reexposure.
• 23-year old Spanish woman García-Cortés et al., 2008: Green tea (*Camellia sinensis*) for 21 days. Jaundice after 19 days. ALT 56.9N with R 34.7, ALT 0.35N 3 months after withdrawal. Reexposure: ALT values not available. ALTb < 5N but ALTr not available → uninterpretable reexposure.
• 26-year old Spanish woman García-Cortés et al., 2008: Green tea for 121 days. Jaundice. ALT 32.1N with R 42.2, ALT dechallenge values not available. Reexposure: ALT values not available. Both ALTb and ALTr not available ? uninterpretable reexposure.
• 38-year old French woman (Sarma et al., 2008): Green tea (six caps Tealine®/day, containing also white and red tea) for 20 days. Symptoms not reported. ALT values not available. Reexposure: ALT value not available. Both ALTb and ALTr not available → uninterpretable reexposure.

(Continued)

TABLE 9 | Continued

Ma Huang

• 33-year old US woman (Nadir et al., 1996): Unknown daily dose of Ma Huang for around 4 weeks. Nausea, vomiting, abdominal discomfort after use for several days, jaundice under continuing Ma Huang use for another three weeks. ALT 832 U/L (normal < 65) with R 9.8. ALT dechallenge values not available. Intentional reexposure with a single dose 1 week after discharge: ALT 1586 U/L. Both ALTb and ALTr not available ? uninterpretable reexposure.

Polygonum multiflorum

• 61-year old Korean man (Jung et al., 2011): Unknown dose of *Polygonum multiflorum* Thunb for 1 day. Myalgia. ALT 818 U/L with R 21.6, 180 U/L after 9 days of cessation and ALTb < 5N as likely assumed. Reexposure after 11.5 months with a single dose of *P. multiflorum* Thunb: ALT 1520 U/L. ALTb < 5N and ALTr = 2ALTb → positive reexposure.

Shou Wu Pian

• 5-year old Netherland girl (Panis et al., 2005): Shou Wu Pian (three tablets daily) for four months. Jaundice. ALT 1543 U/L (normal < 39 U/L), 5 weeks after cessation 50 U/L. Reexposure with tablets Shou Wu Pian daily for 1 month: ALT 1277 U/L. ALTb < 5N and ALTr = 2ALTb → positive reexposure.

Xiao Chai Hu Tang

• 51-year old Japanese woman (Itoh et al., 1995): 7.5 g of Xia Chai Hu Tang daily for 7 weeks. Jaundice, with preexisting mild elevations of aminotransferases. ALT 855 U/L (normal < 35) with R 35.9, ALT decrease to 139 U/L upon cessation. Reexposure: ALT 186 U/L. ALTb < 5N but ALTr < 2 ALTb → uninterpretable reexposure.

• 52-year old Japanese woman (Itoh et al., 1995): Xia Chai Hu Tang 7.5 g daily for 6 weeks. Jaundice, preexistent ALT activity of 180 U/L (normal < 35). ALT 600 U/L, near normal 2.5 months after withdrawal. Reexposure: ALT 162 U/L. ALTb < 5N and ALTr = 2ALTb → positive reexposure.

• 58-year old Japanese woman (Itoh et al., 1995): Xia Chai Hu Tang 7.5 g daily for 3 months. Symptoms not reported. ALT 246 U/L (normal < 35) with R 5.0, ALT fell to near normal after 2 months of withdrawal. Intentional 7-day reexposure: ALT 265 U/L. ALTb < 5N and ALTr = 2ALT → positive reexposure.

• 42-year old Japanese woman (Itoh et al., 1995): Xia Chai Hu Tang 7.5 g daily for an unspecified time period to treat hepatitis A infection. Symptoms not specified, ALT 2 165 U/L (normal < 35) initially dropped with treatment to 42 U/L and increased 3 weeks after initiation of treatment. ALT 1335 U/L with normalization within 2 months after withdrawal. Intentional 2-day reexposure: ALT 195 U/L ALTb < 5N and ALTr = 2ALTb → positive reexposure.

*Compilation of some clinical details and laboratory values for assessment of reported positive reexposure test results in 25 cases with suspected herbal hepatotoxicity by TCM products. Data are derived from previous reports, which may provide additional details (Teschke, 2014; Teschke et al., 2014b). Unless otherwise stated, reexposure was commonly unintentional. Criteria for a positive reexposure test result were used as described in **Table 4**, restricted to criteria provided for the hepatocellular type of liver injury. Accordingly, essential data are the ALT levels at baseline before reexposure (ALTb), and the ALT levels during reexposure (ALTr). Response to reexposure is positive if ALTr = 2ALTb and ALTb < 5N, with N as the upper limit of the normal value. Other combinations lead to negative or uninterpretable results. Serum enzyme activities were provided in U/L or multiples of N. Details for calculation of the R value are presented before (**Figure 1**). Abbreviation: ALT, alanine aminotransferase; AST, aspartate aminotranferase; N, upper limit of normal; R, ratio; TCM, Traditional Chinese Medicine.*

DILIN method

With use restricted to its own country, the United States DILIN causality method (Chalasani et al., 2008) does not operate within the international HILI and DILI mainstream domains, as opposed to CIOMS (**Table 12**). The DILIN method may create problems even in its homeland when physicians are waiting for conclusions of expert circles at times HILI is unfolding. Representing a post-clinical, postponed evaluation rather than a rapid assessment of HILI as a critical disease, the DILIN method will not gain the same international popularity as its counterpart CIOMS (**Tables 9**, **10**, **12**), also due to other major shortcomings (**Table 13**).

Although, various CIOMS criteria (Danan and Bénichou, 1993) have been incorporated in the DILIN method (Rochon et al., 2008), the DILIN group missed the chance to establish the fundaments of a newly conceptualized causality method for DILI and HILI, considering the well known pros ad cons of CIOMS (García-Cortés et al., 2011; Teschke et al., 2013a, 2014d), rather than commenting on few shortcomings of CIOMS at the expense on its own DILIN method (Lewis, 2014). As opposed to the transparent CIOMS results shown by tables with individual scored items (**Tables 10**, **11**) and the applied scale with actual 15 assumed HILI cases (**Table 15**), the DILIN method lacks both transparency and individual item scorings (Teschke et al., 2013a, 2014d). Results presented as percentage ranges only and not given as clearly defined, individually scored items before are irrelevant. Proving a moderate reliability of their DILIN causality approach is far from concordance (Rochon et al., 2008; Teschke et al., 2014d); expert opinion validation therefore seems to be irrelevant. DILIN should close the present evaluating gap by recommending physicians in clinical practice to use CIOMS a priori to improve causality assessment already at origin of clinical data, while DILIN may later use these CIOMS-based itemized data for own assessment.

Naranjo scale

Causality assessment of hepatotoxicity cases by the Naranjo scale (Naranjo et al., 1981) with its known shortcomings is problematic (**Table 13**), although favored by the United States Pharmacopeia (USP) (Mahady et al., 2008) but rejected by the United States DILIN group (Lewis, 2014) and other groups (García-Cortés et al., 2011). This scale relates toxic drug reactions to general pharmacological drug actions rather than specifically to idiosyncratic reactions like hepatotoxicity; it contains drug concentrations and monitoring, dose relationship including decreasing dose, placebo response, cross-reactivity, and confirmation of the ADR using unidentified objective evidence, which are irrelevant for HILI (Naranjo et al., 1981; Teschke and Wolff, 2011; Teschke and Schulze, 2012). The hepatotoxicity unspecific feature of the Naranjo scale is unacceptable in suspected HILI cases, its results are heavily disputed (Liss and Lewis, 2009; Mahady et al., 2008; Sarma et al., 2008; Teschke, 2010c; Teschke and Wolff, 2011;

TABLE 10 | CIOMS scale for the hepatocellular type of injury in HILI cases.

Items for hepatocellular injury	Score	Result
1. TIME TO ONSET FROM THE BEGINNING OF THE HERB		
• 5–90 days (rechallenge: 1–15 days)	+2	
• <5 or >90 days (rechallenge: >15 days)	+1	
Alternative: Time to onset from cessation of the herb		
• ≤15 days (except for slowly metabolized chemicals: >15 days)	+1	
2. COURSE OF alt AFTER CESSATION OF THE HERB		
Percentage difference between ALT peak and N		
• Decrease ≥50% within 8 days	+3	
• Decrease ≥50% within 30 days	+2	
• No information or continued drug use	0	
• Decrease ≥50% after the 30th day	0	
• Decrease <50 % after the 30th day or recurrent increase	−2	
3. RISK FACTORS		
• Alcohol use (drinks/d: >2 for woman, >3 for men)	+1	
• Alcohol use (drinks/d: ≤2 for woman, ≤3 for men)	0	
• Age ≥55 years	+1	
• Age <55 years	0	
4. CONCOMITANT HERB(S)/DRUG(S)		
• None or no information	0	
• Concomitant herb/drug with incompatible time to onset	0	
• Concomitant herb/drug with compatible or suggestive		
• Time to onset	−1	
• Concomitant herb/drug known as hepatotoxin and with compatible or suggestive time to onset	−2	
• Concomitant herb/drug with evidence for its role in this case (positive rechallenge or validated test)	−3	
5. SEARCH FOR NON HERB/DRUG CAUSES		
Group I (6 causes)	Tick if negative	
• Anti-HAV-IgM	□	
• HBsAg, anti-HBc-IgM, HBV-DNA	□	
• Anti-HCV, HCV-RNA	□	
• Hepatobiliary sonography/color Doppler sonography of liver vessels/endosonography/CT/MRC	□	
• Alcoholism (AST/ ALT ≥ 2)	□	
• Acute recent hypotension history (particularly if underlying heart disease)	□	
Group II (6 causes)		
• Complications of underlying disease(s) such as sepsis; autoimmune hepatitis, chronic hepatitis B or C, primary biliary cholangitis or sclerosing cholangitis, genetic liver diseases	□	
• Infection suggested by PCR and titer change for		
• CMV (anti-CMV-IgM, anti-CMV-IgG)	□	
• EBV (anti-EBV-IgM, anti-EBV-IgG)	□	
• HEV (anti-HEV-IgM, anti-HEV-IgG)	□	
• HSV (anti-HSV-IgM, anti-HSV-IgG)	□	
• VZV (anti-VZV-IgM, anti-VZV-IgG)	□	
Evaluation of group I and II		
• All causes-groups I and II –- reasonably ruled out	+2	
• The 6 causes of group I ruled out	+1	
• 5 or 4 causes of group I ruled out	0	
• Less than 4 causes of group I ruled out	−2	
• Non drug or herb cause highly probable	−3	
6. PREVIOUS INFORMATION ON HEPATOTOXICITY OF THE HERB		
• Reaction labeled in the product characteristics	+2	
• Reaction published but unlabeled	+1	
• Reaction unknown	0	
7. RESPONSE TO READMINISTRATION		
• Doubling of ALT with the herb alone, provided ALT below 5N before reexposure	+3	
• Doubling of ALT with the herb(s) already given at the time of first reaction	+1	
• Increase of ALT but less than N in the same conditions as for the first administration	−2	
• Other situations	0	
Total score for patient		

*The updated CIOMS scale is derived and modified from a previous report (Teschke et al., 2014c).The above items specifically refer to the hepatocellular type of injury rather than to the cholestatic or mixed type (shown in **Table 11**). Abbreviations: ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; CIOMS, Council for International Organizations of Medical Sciences; CMV, Cytomegalovirus; CT, Computer tomography; DILI, Drug induced liver injury; EBV, Epstein Barr virus; HAV, Hepatitis A virus; HBc, Hepatitis B core; HBsAg, Hepatitis B antigen; HBV, Hepatitis B virus; HCV, Hepatitis C virus; HEV, Hepatitis E virus; HILI, Herb induced liver injury; HSV, Herpes simplex virus; MRC, Magnetic resonance cholangiography; N, upper limit of the normal range; VZV, Varicella zoster virus. Total score and resulting causality grading: = 0, excluded; 1–2, unlikely; 3–5, possible; 6–8, probable; ≥9, highly probable.*

Teschke et al., 2011d,e; Teschke and Schulze, 2012); this also pertains to a shortened version with only 5 of the original 10 items (Teschke and Schulze, 2012). Lack of hepatotoxicity specificity of the Naranjo algorithm was associated with missing definition of liver ADR, unclear time frame and latency period, undefined time frame for dechallenge, lacking risk factor definition, insufficient evaluation of alternative diagnoses, inappropriate assessment of comedication; and missing definition of a positive rechallenge test (Naranjo et al., 1981; Teschke and Schulze, 2012). This scale also was considered insensitive, allowing a possible causality even in the absence of essential data by virtue of the patient simply having taken the suspected agent (Liss and Lewis, 2009; Sarma et al., 2008). The Naranjo scale as modified by USP (Mahady et al., 2008) did not exclude alternative causes such as idiopathic autoimmune hepatitis, alcoholic or cardiac hepatopathy, other preexisting liver diseases, DILI, and drug-induced rhabdomyolysis (Sarma et al., 2008; Teschke, 2014; Teschke et al., 2011d,e). It

TABLE 11 | CIOMS scale for the cholestatic or mixed type of injury in HILI cases.

Items for cholestatic or mixed injury	Score	Result
1. TIME TO ONSET FROM THE BEGINNING OF THE HERB		
• 5–90 days (rechallenge: 1–90 days)	+2	
• < 5 or > 90 days (rechallenge: >90 days)	+1	
Alternative: Time to onset from cessation of the herb		
• ≤30 days (except for slowly metabolized chemicals: > 30 days)	+1	
2. COURSE OF ALP AFTER CESSATION OF THE HERB		
Percentage difference between ALP peak and N		
• Decrease ≥50% within 180 days	+2	
• Decrease <50% within 180 days	+1	
• No information, persistence, increase, or continued drug use	0	
3. RISK FACTORS		
• Alcohol use (drinks/d: >2 for woman, >3 for men) or pregnancy	+1	
• Alcohol use (drinks/d: ≤2 for woman, ≤3 for men)	0	
• Age ≥55 years	+1	
• Age <55 years	0	
4. CONCOMITANT HERB(S)/DRUG(S)		
• None or no information	0	
• Concomitant herb/drug with incompatible time to onset	0	
• Concomitant herb/drug with compatible or suggestive time to onset	−1	
• Concomitant herb/drug known as hepatotoxin and with compatible or suggestive time to onset	−2	
• Concomitant herb/drug with evidence for its role in this case (positive rechallenge or validated test)	−3	
5. SEARCH FOR NON HERB CAUSES		
Group I (6 causes)	tick if negative	
• Anti-HAV-IgM	□	
• HBsAg, anti-HBc-IgM, HBV-DNA	□	
• Anti-HCV, HCV-RNA	□	
• Hepatobiliary sonography / color Doppler sonography of liver vessels / endosonography / CT / MRC	□	
• Alcoholism (AST/ ALT ≥2)	□	
• Acute recent hypotension history (particularly if underlying heart disease)	□	
Group II (6 causes)		
• Complications of underlying disease(s) such as sepsis; autoimmune hepatitis, chronic hepatitis B or C, primary biliary cholangitis or sclerosing cholangitis, genetic liver diseases	□	
• Infection suggested by PCR and titer change for CMV (anti-CMV-IgM, anti-CMV-IgG)	□	
• EBV (anti-EBV-IgM, anti-EBV-IgG)	□	
• HEV (anti-HEV-IgM, anti-HEV-IgG)	□	
• HSV (anti-HSV-IgM, anti-HSV-IgG)	□	
• VZV (anti-VZV-IgM, anti-VZV-IgG)	□	
Evaluation of group I and II		
• All causes-groups I and II—reasonably ruled out	+2	
• The 6 causes of group I ruled out	+1	
• 5 or 4 causes of group I ruled out	0	
• Less than 4 causes of group I ruled out	−2	
• Non drug cause highly probable	−3	
6. PREVIOUS INFORMATION ON HEPATOTOXICITY OF THE HERB		
• Reaction labeled in the product characteristics	+2	
• Reaction published but unlabelled	+1	
• Reaction unknown	0	
7. RESPONSE TO READMINISTRATION		
• Doubling of ALP with the herb alone, provided ALP below 2N before reexposure	+3	
• Doubling of ALP with the herbs(s) already given at the time of first reaction	+1	
• Increase of ALP but less than N in the same conditions as for the first administration	−2	
• Other situations	0	
Total score for patient		

*For details see legend to **Table 10**.*

(Continued)

therefore appears that the USP approach (Mahady et al., 2008) is an invalid tool for causality assessment in suspected HILI, leading to the conclusion that quality of causality assessment is more important than quantity of counted cases, not vice versa (Teschke et al., 2012g). Use of this method has raised concern about judgment validity by the USP regarding cases of hepatotoxicty by green tea (Liss and Lewis, 2009; Teschke and Schulze, 2012).

WHO method

The WHO method in short consists of both the WHO scale and the global introspection by experts (WHO, 2000b) and was applied for assessing causality in cases of kava hepatotoxicity by the WHO (2007a) and of PS hepatotoxicity as erroneously assumed by the German regulatory agency BfArM and the Drug Commission of the German Medical Association (DCGMA, 2011), but the value of this hepatotoxicity unspecific method was heavily debated (Stammschulte and Gundert-Remy, 2012; Teschke et al., 2012b,c,d) and judged obsolete before (Teschke and Wolff, 2011), considering its known shortcomings (**Table 13**). In general, global introspection represents a strategy in evaluating the likelihood of drug causality for adverse reactions (Kramer, 1986). Surprisingly, this method also has never been validated for any ADRs (Teschke et al., 2012c); as early as 1986, global introspection by experts has been shown to be neither reproducible nor valid (Kramer, 1986). Both the questions and the answers are ambiguous (Teschke and Wolff, 2011). Specifically, the assessor considers factors that might causally link one or more drugs to an observed ADR, lists all factors, weighs their importance, and decides the probability of drug causation (Kramer, 1986). No specific check list or level of strength is given.

The WHO scale was not validated by a gold standard, is not quantitative, not specific for hepatotoxicity (WHO, 2000b; Teschke and Wolff, 2011; Teschke et al., 2012b,c,d, 2013b,e).

TABLE 12 | Selective compilation of international registries and regulatory agencies, and associated groups applying the CIOMS scale for causality evaluation in suspected HILI and DILI cases.

Cases	Products (*n*)	Country/Region	Group/Agency	References
DILI	Synthetic drugs	Spain Europe	Spanish Group for the Study of the Drug-Induced Liver Disease, Malaga	Andrade et al., 2004
DILI	Synthetic drugs (461)	Spain Europe	Spanish Group for the Study of the Drug-Induced Liver Disease, Malaga	Andrade et al., 2005
DILI	Synthetic drugs (28)	Spain Europe	Spain Hepatotoxicity Registry, Grupo de Estudio Para las Hepatopatías Asociadas a Medicamentos, Malaga	Andrade et al., 2004
HILIDILI	Various herbal TCM (15) Synthetic drugs (19)	Singapore Asia	National University of Singapore	Wai, 2006
HILI	Black cohosh (31)	Various countries Europe	European Medicines Agency	EMA, 2007
HILI	Herbs (13)	Spain Europe	Spanish Liver Toxicity Registry	García-Cortés et al., 2008
HILI	Various herbal TCM (159)	Korea Asia	Chungnam National University, Daejeon	Kang et al., 2008
DILI	Synthetic drugs (80)	Serbia Europe	Medicines and Medical Devices Agency of Serbia, Belgrade	Miljkovic et al., 2011
HILI	Herbal *Polygonum Multiflorum* (25)	Korea Asia	Gyeongsang National University School of Medicine, Jinju/Sungkyunkwan University School of Medicine, Changwon	Jung et al., 2011
HILI	Various herbal TCM (27)	Hong Kong	Hong Kong Herb-Induced Liver Injury Network (HK-HILIN), Hong Kong	Chau et al., 2011
DILI	Statins (73)	Iceland/Sweden Europe	National University Hospital Reykjvik/ University of Gothenburg/ Swedish Adverse Drug Reactions Advisory Committee (SADRAC)	Björnsson et al., 2012
DILI	Various synthetic Drugs	Spain Latin America	Spanish-Latin American Network on drug induced liver Injury, in progress	Bessone, 2012
HILI	Some Herbalife® products	Various countries	Various groups	Halegoua-De Marzio et al., 2013
HILI	Various herbal and dietary supplements (HDS)	Spain Europe	Spanish group for the Study of the Drug-Induced Liver Injury	Robles-Diaz et al., 2015

Reliability, sensitivity, specificity, positive and negative predictive values are unknown. Its scope is also limited since it cannot discriminate between a positive and a negative correlation, thereby stimulating overdiagnosing and overreporting (Teschke et al., 2013b). The WHO method ignores uncertainties in daily dose, temporal association, start, duration, and end of herbal use, time to onset of ADR, and course of liver values after herb discontinuation. Insufficiently considered or ignored are comedications, pre-existing liver diseases, numerous alternative explanations, and exclusion of virus infections by hepatitis A - C, CMV, EBV, HSV, and VZV (Teschke et al., 2012b,d). Similarly, case duplications and retracted cases remained undetected by the WHO method (Teschke et al., 2012a). Despite these flaws, the WHO method was used for causality assessment in herbal hepatotoxicity cases (Elinav et al., 2007; Schoepfer et al., 2007; DCGMA, 2011; Stammschulte and Gundert-Remy, 2012; Teschke et al., 2012b,d); claimed causality for PS was not confirmed after reevaluation in two studies (Teschke et al., 2012b,d).

Other approaches

Other attempts to evaluate causality in assumed HILI cases exist (Hung et al., 2011), also the ad-hoc assessment (Kaplowitz, 2001), which was preferentially used for kava cases by the German regulatory agency (BfArM, 2002) and in detail disputed subsequently (Teschke and Wolff, 2011), and the Karch & Lasagna method applied in some HILI cases of Herbalife® and considered obsolete recently (Teschke et al., 2013d), due to known shortcomings.

Questionable and Lacking Causality

Although causality was firmly established for various herbal TCM preparations as well as other herbs and herbal products in reported HILI cases (**Tables 1**, **2**) (Teschke et al., 2011a, 2012a,e, 2014a), causality problems emerged with a few herbs and herbal preparations as evidenced by some full length published reports with detailed analyses. Among these are black cohosh with a possible causality grading in one single HILI case (Teschke, 2010c) and lacking causality in another study (Naser et al., 2011), kava with a highly probable causality level in one HILI case confirmed by a positive reexposure test result (Teschke et al., 2008a), and a confirmed causality grading assessed by a positive reexposure test result for a Herbalife® product in a single HILI case (Teschke et al., 2013d); however, CIOMS/RUCAM based causality for some Herbalife® products was highly probable in one patient and probable in six patients, as preliminarily reported in abstract form without any case details including case data quality (Halegoua-De Marzio et al., 2013), which was described as poor and scattered before (Teschke et al., 2013c).

TABLE 13 | Core elements of the updated CIOMS scale vs. DILIN method.

Items	CIOMS scale	DILIN method
Accurate time frame of latency period (score)	+	0
Detailed time frame of challenge (score)	+	0
Clear time frame of dechallenge (score)	+	0
Recurrent ALT or ALP increase (score)	+	0
Definition of risk factors (score)	+	0
Details to exclude alternative diagnoses (score)	+	0
Assessment of HAV, HBV, HCV, HEV (score)	+	0
Assessment of CMV, EBV, HSV, VZV (score)	+	0
Liver and biliary tract imaging (score)	+	0
Color Doppler sonography of liver vessels (score)	+	0
Assessment of preexisting diseases (score)	+	0
Evaluation of cardiac hepatopathy (score)	+	0
Individual score of alternative diagnoses (score)	+	0
Qualified score of individual comedication (score)	+	0
Prior known hepatotoxicity of the herb (score)	+	0
Search for unintended reexposure (score)	+	0
Definition of unintended reexposure (score)	+	0
Qualified criteria of unintended reexposure (score)	+	0
Laboratory criteria for hepatotoxicity	+	+
Laboratory hepatotoxicity pattern	+	+
Hepatotoxicity specific method	+	+
Structured, liver related method	+	0
Quantitative, liver related method	+	0
Validated method for hepatotoxicity (gold standard)	+	0

Adapted and derived from a previous report (Teschke et al., 2013a). Latency period indicates time from herb start to symptoms, alternatively to abnormal liver tests. The symbol + shows that this item is present and the symbol 0 indicates lack of this item. Abbreviations: ALT, Alanine aminotransferase; ALP, Alkaline phosphatase; CIOMS, Council for International Organizations of Medical Sciences; CMV, cytomegalovirus; DILIN, Drug-Induced Liver Injury Network;EBV, Epstein Barr virus; HAV, Hepatitis A virus; HBV, Hepatitis B virus; HCV, Hepatitis C virus; HEV, Hepatitis E virus; HSV, Herpes simplex virus; VZV, Varicella zoster virus.

With Ba Jiao Lian (*Dysosma pleianthum*), this TCM herb was not further considered as hepatotoxic (Teschke, 2014; Teschke et al., 2014c, 2015b), since not all diagnostic criteria were fulfilled for cases of hepatotoxicity by this herb (NIH, 2014c; Teschke, 2014). In detail, after herbal use at recommended doses, the patients manifested abnormal liver function tests associated with nausea, vomiting, diarrhea, abdominal pain, thrombocytopenia, leucopenia, sensory ataxia, altered consciousness and persistent peripheral tingling or numbness. However, the increase of the aminotransferases was marginal, with preference of AST rather than ALT. The AST increase could reflect isolated damage of the mitochondria around the hepatic central vein or muscular damage, because of the associated increase of creatine phosphokinase, findings not in support for a clinically relevant toxic liver disease (Teschke, 2014). Evidence against a hepatotoxic potential was also provided for Jing Tian San Qi (*Sedum aizoon*) as another herbal TCM (Teschke, 2014), based on the results of recent studies showing that in patients with HSOS, the hepatotoxic PAs in the herbal TCM Tu San Qi (*Gynura segetum*) were responsible rather than the misidentified *Sedum aizoon* lacking these alkaloids (Dai et al., 2006; Gao et al., 2006, 2012; Wu et al., 2008; Lin et al., 2011; Wang and Gao, 2014).

Pathogenetic Aspects of HILI

Any HILI case report should describe details to ensure a pathogenetic case classification, using appropriate criteria that characterize two major forms of HILI (**Figure 1**). One of these is named idiosyncratic, the other one intrinsic (Zimmerman, 1999; Teschke et al., 2008a). The idiosyncratic form of injury is unpredictable and independent of the dose; its metabolic and immunologic subtypes require special attention in clinical practice (**Figure 1**). Conversely, the intrinsic form of liver injury is predictable and dose dependent (**Figure 1**). Although, valid data are lacking, it appears that most HILI cases are of the idiosyncratic rather than the intrinsic form.

Idiosyncratic Form

As an example, clinical assessment characterized kava hepatotoxicity as an idiosyncratic liver injury linked to a metabolic aberration in unusually susceptible humans, providing an overall low incidence of kava hepatotoxicity in the normal population (Teschke et al., 2008a). This rarity of kava hepatotoxicity was also considered in the recent kava trial and evaluated as a positive risk/benefit constellation (Court, 2014), opposing previous regulatory assumptions to the contrary (BfArM, 2002). In accordance with other HILI cases of the idiosyncratic form of injury, human kava hepatotoxicity is not reproducible in experimental animals. Therefore, results of preclinical assessments with kava in experimental studies showing lack of liver toxicity are not transferrable to humans with another susceptibility setting and are not suitable to ensure safe use in humans. Since experimental reproducibility is missing, the lack of an experimental model prevents analytical evaluations directed to a proposed molecular mechanism of kava hepatotoxicity. Regarding human kava hepatotoxicity, characteristics of the metabolic subtype of the idiosyncratic form of injury prevail, based on the variable duration of exposure of 1week up to 12 months, associated with a weak dose dependency (Teschke et al., 2008a). Overall, most plants are fairly well tolerated by humans, whether used as normal food, herbal drugs, or HDS.

The pathophysiology of idiosyncratic HILI in humans is difficult to assess due to lack of experimental reproducibility and hence missing existence of an experimental animal model of HILI. There are abundant studies related to effects of herbs on animals or in vitro cell systems, but uncertainty exists whether these experimental results are transferable to human idiosyncratic HILI conditions. However, pathogenetic aspects are well assessable for HILI cases of the intrinsic form, due to available animal models with experimental hepatotoxicity and the possibility of transferring their results to human conditions.

Intrinsic Form

Germander (*Teucrium chamaedrys*) hepatotoxicity is a typical liver injury of the intrinsic form, since it is dose dependent and reproducible in mice (Larrey and Faure, 2011). Due to its experimental reproducibility in animals, the molecular pathogenesis of Germander hepatotoxicity can easily be studied in experimental hepatotoxicity and transferred to human

TABLE 14 | Compilation of causality assessment methods used in suspected HILI cases.

Herbs Herbal products	*Ad hoc* (*n*)	WHO (*n*)	CIOMS (*n*)	Naranjo (*n*)	DILIN (*n*)	KL (*n*)	References
Kava	20						BfArM, 2002
Kava		30					Denham et al., 2002
Kava	20						Teschke et al., 2003
Kava			36				Stickel et al., 2003
Kava		80					Schmidt et al., 2005
Greater Celandine	23						BfArM, 2005
Black cohosh			31				EMA, 2007
Herbalife products		12					Elinav et al., 2007
Herbalife products		12					Schoepfer et al., 2007
Kava			26				Teschke et al., 2008a
Black cohosh				30			Mahady et al., 2008
Green tea				34			Sarma et al., 2008[
Black cohosh			4				Teschke and Schwarzenboeck, 2009
Black cohosh			9				Teschke et al., 2009
Kava			31				Teschke, 2010a
Hydroxycut					17		Fong et al., 2010
Black cohosh			22				Teschke et al., 2011e
Greater Celandine			22				Teschke et al., 2011a
Herbalife products						20	Manso et al., 2011
Various herbs			45				Chau et al., 2011
Greater Celandine			21				Teschke et al., 2012e
Pelargonium sidoides			15				Teschke et al., 2012c
Pelargonium sidoides			13				Teschke et al., 2012d
Sum (n)	63	134	275	64	17	20	
Sum (percent)	11.0%	23.4%	48.0%	11.2%	3.0%	3.4%	

The data are derived from a study evaluating alternative causes in suspected HILI cases (n = 573) (Teschke et al., 2013g). For the 275 CIOMS cases, causality assessment was performed with the updated CIOMS scale, the original CIOMS scale, or early CIOMS version. Abbreviations: Ad hoc, ad hoc approach; CIOMS, Council for International Organizations of Medical Sciences scale; DILIN, Drug Induced Liver Injury Network method; KL, Karch & Lasagna method; Naranjo, Naranjo scale; WHO, World Health Organization method.

Germander hepatotoxicity. Germander components are neoclerodane diterpenoids that are oxidized by the cytochrome P450 3A isoform into reactive metabolites. These deplete hepatic stores of glutathione and cytoskeleton associated protein thiols, form plasma membrane blebs, and cause apoptosis contributing to liver cell necrosis (Larrey et al., 1992; Larrey and Faure, 2011).

PAs are other good examples for the intrinsic form of liver injury, which again is clearly dose dependent, thereby predictable, and hence preventable. For herbs containing PAs, each consumer of these herbs is at a dose dependent risk developing HSOS as a specific entity of liver disease (Smith and Desmond, 1993; Sperl et al., 1995; Stillman et al., 1977; Fu et al., 2004). PA containing plants are probably the most common poisonous plants affecting not only humans but also livestock and wildlife, with more than 6.000 plants containing PAs and about 3% of the world's flowering plants containing PAs (Fu et al., 2004). Some of these plants have caused toxic liver disease, recognized as epidemics and sometimes primarily assigned to viral hepatitis and not necessarily to toxic plants (Tandon et al., 1976a,b, 2008). Human embryotoxicity caused by PAs has been described in a newborn whose mother drank one cup of a tea containing PAs per day throughout pregnancy (Roulet et al., 1988; Fu et al., 2004). Some PA containing plants such as *Crotalaria species* (Bush tea, Rattlebox), *Ilex paraguarensis* (Mate tea), *Symphytum species* (Comfrey), *Senecio species* (Groundsel), *Heliotropium species*, and *Compositae species* (Indian herbs) that caused HILI are tabulated (**Table 2**). These herbs also injure cattle and house animals (Fu et al., 2004) and cause experimental hepatotoxicity in animals (Lin et al., 2011). PAs can be quantified in the serum of patients with HSOS (Lin et al., 2011; Larrey and Faure, 2011). The pathogenesis of PA hepatotoxicity has been elucidated in experimental studies, which showed the involvement of hepatic microsomal cytochrome P450 in the activation of PAs (Larrey and Faure, 2011).

Finally, herbal TCM products containing more than 19 g dose of *Radix bupleuri* may increase the hepatotoxicity risk (Lee et al., 2011); this dose dependency was confirmed in experimental animals and provided insights into some pathogenetic processes (Liu et al., 2014).

Juristical Considerations

Black Cohosh

Legal aspects of HILI case assessment rarely provide particular juridical and clinical challenges. Two court decisions merit attention, one relates to BC and the other one to kava. In 2005, a report was published representing a case of a 50 year old woman

TABLE 15 | Preferred documentation as example: Tabulated causality assessment of 15 patients with primarily suspected HILI by *Pelargonium sidoides* (PS).

Items	Score	Patients 1–15														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. TIME TO ONSET FROM THE BEGINNING OF THE HERB																
• 5–90 days	+2	?	+2		+2		+2		+2	+2		+2	+2	?	?	
• <5 or >90 days	+1	?		+1		+1		+1			+1			?	?	+1
2. TIME TO ONSET FROM CESSATION OF THE HERB																
• ≥15 days	+1	?	+1	+1	+1	+1	+1	+1	+1	+1	?	+1	+1	?	?	+1
3. COURSE OF alt AFTER CESSATION OF THE HERB																
• Decrease ≥50% within 8 days	+3				+3								+3			
• Decrease ≥50% within 30 days	+2									+2						+2
• No information	0	0	0	0			0	0	0		0	0		0	0	
• Decrease ≥50% after the 30th day	0															
• Decrease <50% after the 30th day or recurrent increase	−2					?										
4. RISK FACTOR ETHANOL																
• Alcohol use (drinks/d: >2 for woman, >3 for men)	+1			?											?	
• No alcohol use (drinks/d: ≤2 for woman, ≤3 for men)	0	0	0		0	0	0		0	0	0	0	0	0	?	0
5. RISK FACTOR AGE																
• ≥55 years	+1	+1						+1							+1	
• <55 years	0		0	0	0	0	0		0	0	0	0	0	0		0
6. CONCOMITANT HERB(S)/DRUG(S)																
• None or no information	0	0	0			0	0	0		0		0	0	0		
• Concomitant herb/drug with incompatible time to onset	0										0					
• Concomitant herb/drug with compatible or suggestive time to onset	−1				−1											
• Concomitant herb/drug known as hepatotoxin and with compatible or suggestive time to onset	−2			−2					−2						?	−2
• Concomitant herb/drug with evidence for is role in this case (positive rechallenge or validated test)	−3															
7. SEARCH FOR NON HERB/DRUG CAUSES																
Group i (6 causes)																
• Anti-HAV-IgM							−			−	−					−
• Anti-HBc-IgM/HBV-DNA							−			−	−					−
• Anti-HCV-IgM/HCV-RNA							−			−	−					−
• Hepato-biliary sonography/color Doppler sonography of liver vessels							−			−						−
• Alcoholism (AST/ ALT ≥2)		−		?		−	−	−	−	−	−	−	−	−		−
• Acute recent hypotension history (particularly if underlying heart disease)		−			−	−	−	−	−	−	−	−	−			−
Group II																
• Complications of underlying disease(s) such as sepsis; or: autoimmune hepatitis, chronic hepatitis B and C, primary biliary cholangitis and sclerosing cholangitis, genetic liver diseases		?			−	?	+		−	+	+	+	−	+		−
• Infection suggested by PCR and titre change for																
CMV (Anti-CMV-IgM/IgG)										−						
EBV (Anti-EBV-IgM/IgG)										−						
HSV (Anti-HSV-IgM/IgG)																
VZV (Anti-VZV-IgM/IgG)																

(Continued)

TABLE 15 | Continued

Items	Score	Patients 1–15														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Evaluation of group I and II																
• All causes—group I and II—reasonably ruled out	+2															
• The 6 causes of group I ruled out	+1															
• 5 or 4 causes of group I ruled out	0															
• Less than 4 causes of group I ruled out	−2				−2				−2							
• Non drug cause highly probable	−3	−3	−3	−3		−3	−3	−3		−3	−3	−3	−3	−3	−3	−3
8. PREVIOUS INFORMATION ON HEPATOTOXICITY OF THE HERB																
• Reaction labeled in the product characteristics	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2	+2
• Reaction published but unlabelled	+1															
• Reaction unknown	0															
9. RESPONSE TO READMINISTRATION																
• Doubling of ALT with the herb alone	+3															
• Doubling of ALT with the herbs/drugs already given at the time of first reaction	+1															
• Increase of ALT but less than N in the same conditions as for the first administration	−2															
• Other situations	0															
• Total points for patient		0	+2	−1	+5	+1	+2	+2	+1	+4	0	+2	+5	−1	+1	+1

Preferred documentation of causality assessment using the scale of the updated CIOMS (Council for International Organizations of Medical Sciences), considering the items for the hepatocellular type of liver injury and the data of a previous report of 15 patients with primarily suspected HILI by Pelargonium sidoides (PS) (Teschke et al., 2012b). In the above section 6 of concomitant herb(s)/drug(s), the following products were considered: synthetic drugs, dietary supplements including herbal ones, and polyherbal products. In the section 7 (search for non-herb/drug causes), the symbol of—denotes that the obtained result was negative and that of + was positive, whereas lack of a symbol indicates that assessment was not performed. ALT denotes alanine aminotransferase, AST aspartate aminotransferase, HAV hepatitis A virus, HBc hepatitis B core, HBV hepatitis B virus, HCV hepatitis C virus, CMV cytomegalovirus, EBV Epstein Barr virus, HSV herpes simplex virus, PCR Polymerase Chain Reaction, VZV varicella zoster virus. Total points/causality: ≤0/excluded; 1–2/unlikely; 3–5/possible; 6–8/probable; ≥9/highly probable.

with fulminant liver failure and liver transplantation in assumed connection with the use of BC (Levitsky et al., 2005). A product liability action was filed by the patient after her recovery (Nebraska, 2006).

The decision of the judge answered the question whether in the specific case under discussion sufficient evidence establishes the herb as a generally or individually specific cause for the observed liver disease; for black cohosh, both aspects of causation were denied. General causation refers to the previously established hepatotoxicity by the same herb, but this was denied because of lack of convincing data. Specific causation refers to the case under discussion; this was also refuted on grounds of conflicting case data, poor case data quality, and numerous confounding variables (Nebraska, 2006). Our clinical diagnosis in this case was herpetic hepatitis and liver disease unrelated to BC or comedicated drugs, and CIOMS based assessment led to an excluded causality for both BC and comedicated drugs (Teschke and Schwarzenboeck, 2009): For this case of BC overdose, conclusions for an update of 22.12.2006 were provided (EMA, 2007): Worst case causality scoring would be possible, if comments of the expert would not be taken into account; because of the clinical experience of the expert and the requested obligatory causality in front of an American court, preference is given keeping the causality at a probable level (EMA, 2007). The conclusions of EMA are cloudy, difficult to reconcile, and ignore presented details, since the judge actually excluded both involved experts from expert testimony as to causation according to Daubert, rule 702 (Nebraska, 2006). As explained in detail, this rule requires that an expert be qualified to render a testimony on the subject, and that his testimony be reliable and relevant. None of the experts obviously met these and other required qualifications. Following the trial, USP reduced the causality for BC in this case from a probable to a possible level (Mahady et al., 2008). An erratum clarified the case conditions (Levitsky et al., 2008). Our clinical diagnosis in this case was herpetic hepatitis and liver disease unrelated to BC or comedicated drugs; CIOMS based assessment led to an excluded causality for both BC and comedicated drugs (Teschke and Schwarzenboeck, 2009). In retrospect, this court case calls for a thorough transparent documentation of HILI cases, associated with an unbiased expert opinion.

Kava

Kava was in the focus of another trial in connection with its marketing withdrawal by the German regulatory agency BfArM (Schmidt, 2014). Almost 12 years after the German regulatory agency BfArM issued an intermediate withdrawal of marketing authorization for products containing extracts of kava (*Piper methysticum*, Piperaceae) root and/or rhizoma (BfArM, 2002), the case has been reviewed by the German administrative court in Cologne (Court, 2014). According to the court's ruling on June

11, 2014, there was no justification for the ban of kava medicinal products issued by the German BfArM (Schmidt, 2014). The court ruled that based on available evidence, the benefit/risk ratio of kava medicinal products was confirmed as positive and must be considered as positive (Court, 2014), with credit given for previous reports (Schmidt, 2014) assessing causality in cases of assumed kava hepatotoxicity with the CIOMS scale (Teschke et al., 2008a, 2010; Teschke and Wolff, 2009; Teschke, 2010a). Credit was also given (Court, 2014) to the work of others (Sarris et al., 2013). Their double blind, randomized, placebo controlled trial was performed with a well defined noble kava drug that is on the market in Australia, showing both efficacy of kava in patients treated for their generalized anxiety disorders and lack of overt adverse reactions (Sarris et al., 2013), confirming kava efficacy based on a previous Cochrane study (Pittler and Ernst, 2003a). As a consequence of the court's ruling, German kava products have been formally restored to their market status on June 2002. As an update, BfArM appealed the court's ruling on June 30, 2014, but justification for the appeal has not yet been published (Schmidt, 2014). Clearly, the ruling is a major breakthrough, as it strengthens the legal certainty and predictability of regulatory decisions for herbal medicinal product manufacturers in general. It is also a call for the BfArM and other regulatory agencies to present transparent and appropriate clinical documentations of future HILI cases to be evaluated by clinically well trained regulatory assessors and external experts in the field, to be more self-critically, not to dismiss expert views to the contrary a priori, and providing rather than refuting original HILI case data in anonymous form to interested requesting scientists to assist in case evaluations.

TABLE 16 | Proposal for international harmonization: requirements for regulatory approved herbal drugs.

Specific international qualification required for regulatory approved herbal drugs
Good Agricultural Practices
Good Manufacturing Practices
Definition of plant family, subfamily, species, subspecies, and variety
Definition of plant part
Definition of solvents and solubilizers
Lack of impurities, adulterants, and misidentifications
Minimum of batch and product variability
Lack of variety to variety variability
Brand name with details of ingredients, plant parts, batch number, and expiration date
Manufacturer with address
Regulatory specification of indication of herbal drug use
Daily dose with details of the application form
Maximum duration of herbal drug use
Efficacy of the herbal drug proven by valid RCTs
Description of adverse reactions and their frequency
Information of risk/benefit profile
Internationally approved unified regulatory surveillance
Regulatory harmonization of updated CIOMS scale use to assess causality in suspected HILI
Placebo controlled randomized double blind clinical trials
Risk/benefit profiles

Data are derived and adapted from a previous report (Teschke et al., 2013c).

Essentials of Herbal Product Quality

Herb Authentication and Product Identification

Good quality of herbal drugs and other herbal products is prerequisite for safe human use (**Table 16**) (Teschke et al., 2013c). However, shortcomings of herbal products are well documented, both in herbal TCM and herbal modern medicine. Herbal authentication was an issue for BC (Sarma et al., 2008; Health Canada, 2010) and various TCM herbs (Haller et al., 2002; Teschke, 2014; Teschke et al., 2014b). There also was considerable debate whether kava products used by patients with kava hepatotoxicity might have been of poor quality including inappropriate herb authentication. This led to a comprehensive assessment and a proposal for a Kava Quality Standardization Code (Teschke and Lebot, 2011). Actually, several guidelines exist already for Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP), applicable to medicinal plants and herbal medicines to ensure their product quality (WHO, 2000a, 2003, 2006, 2007b). Despite these official precautionary recommendations for quality improvements, batch and product variability is not unusual (Lebot, 2006; Schmidt, 2007; Teschke and Lebot, 2011; Teschke et al., 2013c). Violation of GAP or GMP rules also will result in herbal products that lack efficacy, safety, or both.

When plants are considered for human use as ingredients of a herbal drug and dietary supplement, a clear definition and identification of plant family, subfamily, species, subspecies, and variety is mandatory, best done by a professional classical botanical description for any herb. Neglect may cause variation in plant family and species, contributing to the overall batch and product variability. Appropriate information should be provided by the manufacturers in the consumer's leaflet, thereby being available to the physician who suspects liver injury induced by a herbal product. The leaflet requires the name of the herbal product and the manufacturer's address who will provide additional information upon request. Therefore, all essential data of herb identification and the herbal product should be available before reporting HILI case details as spontaneous reports to the regulatory agencies or as case report publication. However, pitfalls are evident already at this stage of case evaluation.

In kava drug hepatotoxicity as an example, herb identification problems were evident. The manufacturers did not provide details on kava variety identification, so this specific information was missing in all spontaneous reports and case report publications (BfArM, 2002; Schmidt, 2007; WHO, 2007a; Teschke et al., 2008a; Teschke, 2010a,b, 2011). In the South Pacific region of origin, several hundred kava varieties exist—also called kava cultivars—and are grouped into noble, medicinal, and Two-Day varieties (Lebot, 2006; Schmidt, 2007; Teschke and Lebot, 2011; Teschke et al., 2011b). They differ in their kavalactone composition and their pleasant and unwanted, possibly toxic effects. In cases of suspected hepatotoxicity, it remained unclear which kava variety had to be incriminated. Interestingly, regulatory approval of kava drugs neither considered different kava varieties nor required respective labeling (BfArM, 2002). Thus, kava hepatotoxicity remains unexplained.

Other problems of herb or product identification have been described in detail in various cases of initially suspected herbal hepatotoxicity (EMA, 2007; WHO, 2007b; Mahady et al., 2008; Teschke et al., 2011a,b,d,e). Incomplete herb description complicates accurate association of herbs with liver injury and allows only general assumptions (Teschke, 2010c; Teschke et al., 2013e,f). Besides overall herb descriptions, the brand name of the herbal product has been provided in only a few case reports, and data for manufacturer, plant part, and extraction solvent normally was fragmentary (Teschke et al., 2011e, 2013c). For instance, the rate of undetermined herbal products was 10/16 cases (63%) among published case reports (Teschke et al., 2011e). This high rate questions the validity of any causality attribution. Additional problems arise from herbal mixtures, in which individual ingredients are not specified (Teschke, 2010c; Teschke et al., 2011d,e). Again, case reports have been published as HILI even if the patients were not sure whether they used a herbal product at all (Teschke et al., 2011e).

For GC, case reports assumed causality for hepatotoxicity in all 21 cases, but details on the GC product were fragmentary (Teschke et al., 2012e). Out of these 21 cases, seven patients used a GC monopreparation and four patients a GC polyherbal product, brand names and manufacturers were known in only nine patients. Fears of liability may contribute to the restriction of detailed product specifications by the authors. On the other hand, the regulatory agency did not hesitate to provide all relevant data of GC products from spontaneous cases of GC hepatotoxicity (Teschke et al., 2011a).

Therefore, unless complete data for herbal identification, ingredients, and name of the herbal product are provided in each HILI case, a valid causality assignment is not realistic. Identification problems are evident also in most HDS providing little specific information (Navarro et al., 2014; Robles-Diaz et al., 2015).

Plant Part Specification

Reports on HILI rarely provide details of the plant part used, ignoring specific toxic properties attributable to different parts of a plant. The regulatory recommendation for kava drugs was to use its peeled rhizome (Teschke and Lebot, 2011). In various assumed HILI cases by kava it remained unclear, whether also unpeeled rhizomes, peeled and unpeeled roots, and/or stem peelings were used, hampering evaluation of the causative agent of kava hepatotoxicity (WHO, 2007a; Teschke, 2011). For the U.S. FDA, peeled kava rhizomes were recommended for kava supplements (Teschke and Schulze, 2010), and according to the Australian Therapeutic Goods Administration, the commonly used medicinal kava products are derived from peeled rhizomes (Sarris et al., 2009). Plant part specification can be a major regulatory, agricultural, manufactural, pharmaceutical, and clinical issue (Teschke and Lebot, 2011).

Solvents and Solubilizers

Herbal drugs and supplements are specified as extracts that are either water based or prepared from organic solvents like ethanol or acetone, but regulatory advice is often lacking (Teschke and Lebot, 2011). Thus, herbal extracts will substantially differ in their composition depending on the solvent. In addition, numerous solubilizers like macrogol, craspovidon, mentha oil, methyl acrylic acid polymer and polysorbate polyols may be included in herbal products to facilitate gastrointestinal uptake (Teschke, 2010b). Therefore, solvents and solubilizers may influence the composition of chemicals in the herbal product and selectively affect the bioavailability for the liver as the target organ. These variations hamper causality attribution in suspected HILI cases, leading to the recommendation that kava drugs and supplements should be water based extracts lacking any solvents or solubilizers (WHO, 2007a; Teschke and Schulze, 2010; Teschke et al., 2011b).

Misidentifications, Impurities, and Adulterants

For herbal product quality, not only plant misidentification but also contaminants, impurities and adulterants still remain key problems (Kang-Yum and Oransky, 1992; Espinoza et al., 1995; Gertner et al., 1995; Huang et al., 1997; Ko, 1998; Ernst, 2002; Estes et al., 2003; Lebot, 2006; Schmidt, 2007; Seeff, 2007; WHO, 2007b; Mahady et al., 2008; Navarro, 2009; Teschke et al., 2009, 2011c,e; Health Canada, 2010; Larrey and Faure, 2011; Teschke and Lebot, 2011). Adulterants are not uncommon in herbal TCM mixtures; they usually consist of synthetic drugs to provide or fortify product efficacy. Although rarely addressed by analytical approaches in patients with actually reported HILI, Health Canada was the only regulatory agency with recently reported interest in the analytical assessment of herbal products to evaluate quality, providing evidence for misidentification of herbs in some products and presenting results that the accused herb was not present in the herbal products used by the affected patients (Health Canada, 2010).

It remains to be established to what extent misidentifications, impurities, and adulterants are responsible for individual HILI cases. For instance, possible causality for hepatotoxicity cases of the herbal TCM mixtures Chaso and Onshido was ascribed to N-nitroso-fenfluramine, found as adulterant in these slimming aid products that had been produced in China and sold in Japan (Adachi et al., 2003). However, there is only little clinical or experimental evidence for a potential hepatotoxicity by this adulterant (Kanda et al., 2003a,b; Lau et al., 2004). It rather appears that green tea as ingredient was the causative agent if supplied as extract (Teschke, 2014).

Misidentification may create major clinical challenges and harm dramatically the health of consumers, shown for the following cases (Teschke, 2014). Until 2008, overall 41 cases from China with HSOS, the former HVOD, were reported and causally attributed to the herbal TCM Jing Tian San Qi (*Sedum aizoon*, syn. Stonecrop) (Wu et al., 2008), but causal attribution to *Sedum aizoon* was obviously incorrect. *Sedum aizoon* lacks PAs, and when applied to experimental animals, HSOS did not eme.g., Lin et al., 2011, suggesting that a herb containing PAs likely is reponsible for the reported cases (Wu et al., 2008). In line with this is another hepatotoxicity case from Hong Kong with HSOS that initially also was ascribed to *Sedum aizoon*, but it turned out to have been caused by the herbal TCM Tu San Qi (*Gynura segetum*) (Lin et al., 2011). The name and appearance of *Sedum aizoon* is similar to the one of *Gynura segetum*, but botanical differentiation was considered possible for the eye of experts (Lin et al., 2011).

Comparative studies with both herbs provided clear supportive evidence for *Gynura segetum* as culprit for additional cases of HSOS as compared to *Sedum aizoon*. Respective studies in mice showed that *Gynura segetum* as the PA containing herb but not *Sedum aizoon* lacking PAs causes experimental HSOS as assessed by liver histology results (Lin et al., 2011). In an earlier experimental study, a model of the hepatic veno-occlusive disease was established by PAs derived from a herb described erronously as *Sedum aizoon* (Gao et al., 2006), which again does not contain PAs (Lin et al., 2011; Gao et al., 2012; Wang and Gao, 2014). This suggests that the described experimental model (Gao et al., 2006) was due to the action of a herb containing PAs, most likely *Gynura segetum* (Lin et al., 2011; Gao et al., 2012; Wang and Gao, 2014), rather than to *Sedum aizoon* lacking PAs (Gao et al., 2012). Based on these well founded considerations, evidence for a hepatotoxic potential of Jing Tian San Qi is lacking. The herbal TCM *Sedum aizoon* should therefore not be tabulated any more as hepatotoxic herb, as done until recently (Teschke et al., 2012h).

Gynura segetum was involved in other cases of herbal misidentification. In two Chinese women, HSOS emerged, which was induced by PAs of the herbal TCM *Gynura segetum* (syn. Ju Shan Qi, Ju Ye San Qi, Shan Chi, San Qi Cao, Shan Chi, Shan Chi) (Dai et al., 2006). Additional six cases were earlier suspected (Kumana et al., 1983, 1985); in at least four cases, the culprit was the PA containing herb *Heliotropium lasiocarpum* rather than *Gynura segetum* (Culvenor et al., 1986).

New Encouraging Steps

Modern medicine is well established on our globe but provides health facilities only to parts of the population with focus on patients who can afford the expenditures. Challenges of modern medicine include management of chronic disorders and orphan diseases, all at reasonable costs. To achieve this goal, support may come from herbal medicine, but this will require major efforts at various levels. Since abundant plants grow in all countries around the world and are ready to be used for treating human diseases, herbal medicine may have encouraging perspectives to become a global player, provided efficacy is proven and associated risks such as liver toxicity are limited and easily recognizable.

Progress in Developing Valid Diagnostic Biomarkers

Numerous valid clinical biomarkers exist and enable a firm diagnosis of most liver diseases unrelated to HILI and DILI, for instance by assessing specific antibodies of viral hepatitis (**Table 7**). New encouraging steps with the development of specific biomarkers for HILI are discussed (Larrey and Faure, 2011) in reference to a sensitive and specific assay enabling fthe detection of a reactive pyrrole-protein adduct in the serum of patients with HSOS. This disease was attributed to the Tusanqi preparation made erroneously with *Gynura segetum* containing PAs instead with *Segetum aizoon* lacking PAs (Lin et al., 2011). The results of this assay show that the patient actually consumed a herb containing PAs, which are metabolized in the liver to a reactive PA metabolite, reacting with a protein and forming an adduct (Larrey and Faure, 2011). However, this assay does not prove that PAs have caused the hepatotoxicity in this particular patient, needing supportive evidence in the clinical context. Measuring herbal toxins or their metabolites in the serum is useful in HILI cases in a setting of some intoxication, if high levels of the herbal toxin are expected in the serum due to large amounts of the consumed herb, high cumulative doses, or a prolonged degradation of the toxic herbal chemical. These conditions apply to HILI cases of the intrinsic form but not to those of the idiosyncratic form, which accounts for most HILI cases. For idiosyncratic HILI, similar restrictions apply regarding circulating micro-RNA (mRNA), presently investigated in intrinsic DILI and detectable in fluids including the serum (Zhou et al., 2013). Omics technologies, including genomics, proteomics, and metabolomics might well change but not revolutionize our understanding in the diagnosis of intrinsic hepatotoxicity (Yang et al., 2012b).

Interest in biomarkers to identify idiosyncratic hepatotoxicity risks in individuals who use drugs is continuing. For idiosyncratic DILI, numerous genetic and nongenetic risk factors have been described as possible biomarkers to predict DILI in some individuals (Chalasani and Björnsson, 2010), but whether these are useful to diagnose idiosyncratic HILI is unknown.

Conformed Diagnostic HILI Case Management

At an international level and to provide transparency and comparability, an overall accepted pragmatic stratification of HILI case assessment and data presentation should be adopted and more enforced. Sequential case management and presentation should focus on narrative case details for an overview of clinical features. This kind of information is best provided as table, easily also published even for a high number of cases, as illustrated for 16 HILI cases in a single report (**Table 3**); this facilitates HILI characterization caused by a single herb such as GC (**Table 4**). Detailed presentation of established criteria of hepatotoxicity definition, differentiation of hepatocellular, cholestatic, and mixed form of liver injury, and pathogenetic classification should be mandatory (**Figure 1**). Information of provided or missed case details and diagnostic parameters are valuable tools that signify case data quality and ensure transparency (**Table 6**). The concept of a sequential diagnostic approach in suspected HILI cases best starts with thorough clinical case assessments, subsequently combined with the use of the updated CIOMS scale (**Tables 10**, **11**) as the mainstream tool (**Tables 12**, **13**), and followed by expert opinion, if uncertainty remains. This worldwide applicable strategy allows transparency and provides a quick basis for final causality assignments of individual HILI cases by calculating individual and final scores of individual CIOMS items (**Tables 10**, **11**, **15**). This strategy of diagnostic harmonzation is pragmatic, time and cost saving, and facilitates potential reassessment by other clinicians, scientists, manufacturers, or regulatory agencies.

International Harmonization of Regulatory Efforts and Surveillance

Encouraging efforts are reported from the Chinese State Food and Drug Administration (SFDA), progress is underway to improve regulatory surveillance of TCM herbal products (Zhang et al., 2012). In 2012, the SFDA model of safety monitoring and risk management of TCM drugs was still under exploration,

with numerous regulatory and clinical issues. These include information on adulteration and counterfeit TCM drugs and clarification that except for SFDA approved Chinese and Western compound products, the addition of Western drugs into a TCM drug formula is illegal. SFDA has established examination methods and shelf sampling inspection of products in order to protect the safety of patients. It is not described whether SFDA proves causality in suspected HILI by TCM and CIOMS is used in analogy to other international registries and regulatory agencies (**Tables 12**, **14**).

The regulatory situation of herbal medicines has thoroughly been evaluated worldwide for most countries of all continents (WHO, 2005). For herbal supplements, regulatory control varies among countries and commonly is less stringent or missing, whereas regulatory approved herbal drugs in Europe are under strict regulatory surveillance, as are approved synthetic drugs (EMA, 2014; MHRA, 2014). Regulatory efforts regarding herbal medicine products should be advanced, aiming at an identical quality level in all countries. This harmonization is best achieved by regulatory lifting all HDS to the level of herbal drugs, provided new regulations are formulated and strictly followed, and preclinical and clinical safety as well as efficacy is proven. Consumers will benefit from worldwide pharmacovigilance harmonization and quality control standards of herbal drugs (**Table 16**), deviced as previously outlined for kava quality standards (Teschke and Lebot, 2011).

Sophisticated Evidence Based Trials

China is the country with an extremely high number of published randomized controlled clinical trials (RCTs) (Wang et al., 2007), evaluating herbal TCM, but their efficacy has rarely been established due to poor study quality (Manheimer et al., 2009; Teschke et al., 2015a,b). There is increasing awareness that valid evidence based clinical trials for any herbal treatment should be mandatory as shown for kava through a Cochrane analysis (Pittler and Ernst, 2003a), associated with a robust risk management and balanced risk/benefit profiles (Tang et al., 1999; Wang et al., 2007; Manheimer et al., 2009; NIH, 2014a,b; Teschke et al., 2015a,b). For Europe, these trials are commonly required by EMA and national regulatory agencies for herbal drug approvement; this established system should be adopted by the WHO for global harmonization.

Promising New Drug Research and Development

Plants are natural producers of chemical substances, enforcing great expectations that in the future more synthetic drugs are developed based on herbal ingredients being effective in human diseases. In fact, for most of history, herbal medicine was the only available medicine. It has also been estimated that one third to one half of currently used drugs were originally derived from plants (Bent, 2008). Encouraging developments are underway (Pelkonen et al., 2014), with focus on herbal TCM (Li et al., 2012; Zhao et al., 2012).

Globalization

With pragmatic modern drug medicine in competition, herbal medicine should contribute some of its items to a modern type of drug medicine as part of globalized health care systems at reasonable costs. On a long run, this approach appears feasible, provided traditional and modern herbal medicine conform to the expectations and needs of patients and consumers (Leonti and Casu, 2013), requiring new steps and improvements based on debated issues outlined above. Globalization of herbal medicine needs unrestricted exchange of herbal drugs among all countries. To ensure worldwide high quality, each herbal medicine should undergo strict regulatory surveillance and be classified as herbal drug according to internationally agreed criteria. Among these are strict adherence to GAP and GMP items, clear definitions of plants, plant parts, and solvents to be used, well defined indications and treatment modalities, proof of evidence based efficacy for the proposed indications, evaluation of adverse reactions, and positive risk/benefit profiles (**Table 16**). Presently, HDS fulfill these criteria only marginally and need a move to the herbal drug category; otherwise withdrawal from the market will be the alternative. Concern exists that presently limited scientific evidence exists to establish the safety and efficacy of most herbal products (Bent, 2008). Of the top 10 herbs in the United States, five herbs (ginkgo, garlic, St. John's wort, soy, and kava) have scientific evidence suggesting efficacy, but concerns over safety may temper the decision to use these products. Consequently, herbal products are not likely to become an important alternative to standard medical therapies or a global player, unless there are changes to the regulation and standardization of these products (Bent, 2008).

Concluding Remarks

Herbal use is common in traditional and modern medicine and requires more international harmonization to promote herbal medicine to a global player. In analogy to European regulatory settings, herbal medicinal products should be manufactured, marketed, and supervised as regulatory approved drugs similar to synthetic drugs. Certainly, this requires global consent and major efforts but it could open a global market for these drugs. International agreement should be reached on various issues, including best quality of herbal drugs, definition of indications, and proof of therapeutic efficacy by clinical trials, determination of therapy modalities such as posology, and assessment of adverse reactions in line with risk/benefit profiles. Present issues focus on poor herbal product quality, lack of proven efficacy, and rare adverse reactions including hepatotoxicity.These should be better recognized in the future by thorough clinical evaluation associated with the CIOMS scale as the best recognized causality assessing tool worldwide, possibly followed by expert opinion if uncertainty remains.

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The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety

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The use of herbal medicinal products and supplements has increased tremendously over the past three decades with not less than 80% of people worldwide relying on them for some part of primary healthcare. Although therapies involving these agents have shown promising potential with the efficacy of a good number of herbal products clearly established, many of them remain untested and their use are either poorly monitored or not even monitored at all. The consequence of this is an inadequate knowledge of their mode of action, potential adverse reactions, contraindications, and interactions with existing orthodox pharmaceuticals and functional foods to promote both safe and rational use of these agents. Since safety continues to be a major issue with the use of herbal remedies, it becomes imperative, therefore, that relevant regulatory authorities put in place appropriate measures to protect public health by ensuring that all herbal medicines are safe and of suitable quality. This review discusses toxicity-related issues and major safety concerns arising from the use of herbal medicinal products and also highlights some important challenges associated with effective monitoring of their safety.

Keywords: herbal medicines, adverse reactions, monitoring safety, challenges, public health

INTRODUCTION

The use of herbal medicines and phytonutrients or nutraceuticals continues to expand rapidly across the world with many people now resorting to these products for treatment of various health challenges in different national healthcare settings (WHO, 2004). This past decade has obviously witnessed a tremendous surge in acceptance and public interest in natural therapies both in developing and developed countries, with these herbal remedies being available not only in drug stores, but now also in food stores and supermarkets. It is estimated that up to four billion people (representing 80% of the world's population) living in the developing world rely on herbal medicinal products as a primary source of healthcare and traditional medical practice which involves the use of herbs is viewed as an integral part of the culture in those communities (Mukherjee, 2002; Bodeker et al., 2005; Bandaranayake, 2006).

The use of herbal remedies has also been widely embraced in many developed countries with complementary and alternative medicines (CAMs) now becoming mainstream in the UK and the rest of Europe, as well as in North America and Australia (Committee on the Use of Complementary, and Alternative Medicine by the American Public, Board on Health Promotion, and Disease Prevention, Institute of Medicine, 2005; Calapai, 2008; Braun et al., 2010; Anquez-Traxler, 2011). In fact, while places like the UK have a historical tradition of using herbal medicines (Nissen, 2010), the use is also widespread and well established in some other European countries (Calapai, 2008). In these developed countries, the most important among many other reasons for seeking herbal therapy is the belief that it will promote healthier living. Herbal medicines are, therefore, often viewed as a balanced and moderate approach to healing and individuals who use them as home remedies and over-the-counter drugs spend huge amount of money (in excess of billions of dollars) on herbal products. This explains in part the reason sales of herbal medicines are booming and represents a substantial proportion of the global drug market (Roberts and Tyler, 1997; Blumenthal et al., 1998; WHO, 2002a; Kong et al., 2003; Pal and Shukla, 2003; WHO, 2005a; Bandaranayake, 2006).

As the global use of herbal medicinal products continues to grow and many more new products are introduced into the market, public health issues, and concerns surrounding their safety are also increasingly recognized. Although some herbal medicines have promising potential and are widely used, many of them remain untested and their use also not monitored. This makes knowledge of their potential adverse effects very limited and identification of the safest and most effective therapies as well as the promotion of their rational use more difficult (WHO, 2002b). It is also common knowledge that the safety of most herbal products is further compromised by lack of suitable quality controls, inadequate labeling, and the absence of appropriate patient information (Raynor et al., 2011). It has become essential, therefore, to furnish the general public including healthcare professionals with adequate information to facilitate better understanding of the risks associated with the use of these products and to ensure that all medicines are safe and of suitable quality. Discussion in this review is limited to toxicity-related issues and major safety concerns arising from the use of herbal medicines as well as factors promoting them. Some important challenges associated with effective monitoring of safety of these herbal remedies are also highlighted with a view to helping refocus relevant regulatory agencies on the need for effectiveness and ensuring adequate protection of public health and promoting safety.

FACTORS RESPONSIBLE FOR INCREASED PATRONAGE AND SELF-MEDICATION WITH HERBAL MEDICINE

Essentially, herbal remedies consist of portions of plants or unpurified plant extracts containing several constituents which are often generally believed to work together synergistically. The recent resurgence of public interest in herbal remedies has been attributed to several factors some of which include (i) various claims on the efficacy or effectiveness of plant medicines, (ii) preference of consumers for natural therapies and a greater interest in alternative medicines, (iii) erroneous belief that herbal products are superior to manufactured products, (iv) dissatisfaction with the results from orthodox pharmaceuticals and the belief that herbal medicines might be effective in the treatment of certain diseases where conventional therapies and medicines have proven to be ineffective or inadequate, (v) high cost and side effects of most modern drugs, (vi) improvements in the quality, efficacy, and safety of herbal medicines with the development of science and technology, (vii) patients' belief that their physicians have not properly identified the problem; hence the feeling that herbal remedies are another option, and (viii) a movement toward self-medication (Bandaranayake, 2006).

The increasing utilization of herbs for self-medication by patients or individuals is also attributed to a number of other reasons such as (i) patients being uncomfortable about discussing their medical problems and fear lack of confidentiality in handling their health information, (ii) fear of possible misdiagnosis and wrong treatment by patients with non-specific symptoms or general malaise, and (iii) lack of time to see a physician; this is usually a reason where prior visit did not yield any positive experience (Studdert et al., 1998). Furthermore, patients' freedom of choice of a practitioner is also encouraging their utilization of alternative treatments and herbal remedies, although many select herbal medicines from a deductive approach based on anecdotal information, that is, "it worked for my friend or relative" (Parle and Bansal, 2006). So also, because of the influence of religion and greater level of spiritual consciousness, many individuals tend to be increasingly disposed to accepting therapeutic value of a treatment based on faith or intuition rather than scientific reasoning (Astin, 1998; Zeil, 1999). Herbal medicines, therefore, become particularly alluring when the body's natural capacity for self-repair, given appropriate conditions, is emphasized (Parle and Bansal, 2006).

In addition to all these above-mentioned factors, the marketing strategies and efforts by various manufacturers of herbal medicines and their sales representatives have seriously projected these products into greater limelight. Various advertisements in the mass media including television and radio programmes have significantly increased consumers' awareness and given the herbal products undue respectability and credibility (Brevort, 1998; Parle and Bansal, 2006). These advertisements are carefully presented to attract the different age groups of people that exist in the society. Children are encouraged to use herbs for their nutritional values to facilitate normal or healthy growth and development; young persons for their euphoric effects, supply essential ingredients to help them cope with daily stress and to prevent or slow the onset of aging; older persons for their anti-aging or rejuvenating effects and women for slimming and beauty enhancing effects (Parle and Bansal, 2006).

INFLUENCE OF REGULATORY POLICIES ON SAFETY OF HERBAL MEDICINES

It has been observed that most of the problems associated with the use of traditional and herbal medicines arise mainly from the classification of many of these products as foods or dietary supplements in some countries. As such, evidence of quality, efficacy, and safety of these herbal medicines is not required before marketing. In the same vein, quality tests and production standards tend to be less rigorous or controlled and in some cases, traditional health practitioners may not be certified or licensed. The safety of traditional and herbal medicines has therefore become a major concern to both national health authorities and the general public (Kasilo and Trapsida, 2011).

Until 2011, there were three possible regulatory routes by which an herbal product could reach a consumer in the UK. The unlicensed herbal remedy is the commonest route which does not have to meet specific standards of safety and quality neither is it required to be accompanied by safety information for the consumer (Raynor et al., 2011). Recently, the European Union (EU) implemented a directive after a 7-year transition period to harmonize the regulation of traditional herbal medicine products across the EU and establish a simplified licensing system in order to help the public make informed choices about the use of herbal products. This requires that all manufactured herbal products either gain a product license of the type needed to manufacture "conventional" products or become registered as a "traditional herbal medicinal product" (Routledge, 2008; Raynor et al., 2011).

Like conventional medicines, licensed herbal medicines hold a product license based on safety, quality, and efficacy. Hence, it is compulsory that they are accompanied by comprehensive information such as indications, precautions, how to use the product, side effects, how to store the product and regulatory information, for safe use. This information is usually provided on a leaflet inserted into the product package (Raynor et al., 2011). On the other hand, due to insufficient evidence of reproducible efficacy to meet regulatory standards, license cannot be obtained for some herbal medicines to sell these products. This led to the creation of a new category of traditional herbal registration (THR) with a transition period of seven years (European Union Herbal Medicines Directive, 2004). In line with this, the Traditional Herbal Medicines Registration Scheme, which is a "simplified registration scheme," was introduced in the UK. In this scheme, herbal medicinal products are required to meet specific standards of safety and quality, agree upon indications for use based on their traditional use and also provide information in a leaflet to promote safe use of the product by the purchaser (Raynor et al., 2011). However, this is not the case in many other parts of the world, especially in the developing countries where many unregistered and poorly regulated herbal products are sold freely on the market with little or no restraint. Furthermore, the common misconception that natural products are not toxic and are devoid of adverse effects often lead to improper use and unrestrained intake and this has also resulted in severe poisoning and acute health problems. This misconception is not limited to the developing countries. It also exists in

highly developed countries, where the general public often resorts to "natural" products without any proper awareness or information on the associated risks, particularly in the event of excessive or chronic use (UNESCO, 2013).

TOXICITY AND ADVERSE HEALTH EFFECTS OF SOME COMMON HERBAL MEDICINES

In most countries, herbal medicines and related products are introduced into the market without any mandatory safety or toxicological evaluation. Many of these countries also lack effective machinery to regulate manufacturing practices and quality standards. These herbal products are continuously made available to consumers without prescription in most cases and the potential hazards in an inferior product are hardly recognized (Bandaranayake, 2006).

It is important to reiterate the staggering rate at which interest and use of herbal medicines is expanding. Over the past decade, the use of herbal medicines represents approximately 40% of all healthcare services delivered in China while the percentage of the population which has used herbal medicines at least once in Australia, Canada, USA, Belgium, and France is estimated at 48%, 70%, 42%, 38%, and 75%, respectively (Foster et al., 2000; WHO, 2002b). In spite of the positive perception of patients on the use of herbal medicines and alleged satisfaction with therapeutic outcomes coupled with their disappointment with conventional allopathic or orthodox medicines in terms of effectiveness and/or safety (Huxtable, 1990; Abbot and Ernst, 1997), the problem of safety of herbal remedies continues to remain a major issue of concern.

The general perception that herbal remedies or drugs are very safe and devoid of adverse effects is not only untrue, but also misleading. Herbs have been shown to be capable of producing a wide range of undesirable or adverse reactions some of which are capable of causing serious injuries, life-threatening conditions, and even death. Numerous and irrefutable cases of poisoning have been reported in the literature (Vanherweghem and Degaute, 1998; Cosyns et al., 1999; Ernst, 2002). The toxicity evaluation of the polyherbal formula, Yoyo "Cleanser" Bitters®, conducted recently in our laboratory (Ekor et al., 2010), was prompted by an unpublished case report of a young male adult who had been on self-medication with this herbal product and was subsequently admitted to the hospital on account of liver failure. Yoyo "Cleanser" Bitters® is one of the herbal remedies that is widely advertised in the various Nigerian media and as such has gained so much public acceptance over time and continues to enjoy increased patronage among consumers, especially in the southwestern part of the country. Our study revealed that this herbal formula was capable of elevating plasma levels of liver enzymes and inducing hypokalemia following 30 days administration in rats. From our observation, the potassium loss (which is capable of predisposing to dangerous arrhythmias) was a greater risk associated with this herb during this sub-acute exposure or toxicity study. Prior to this study, we had evaluated the safety of "super B blood purifier" and "super B seven keys to power" mixtures in experimental model over a decade ago (John et al., 1997). These herbal mixtures were marketed by a registered Nigerian company which cultivated medicinal plants and manufactured medicinal herbal preparations. The herbal blood tonics were well patronized by common folks who claim their efficacy according to the manufacturer's stipulation that "they are safe, give strength and cleanse the blood and body of infection." We obtained the herbal constituents (*Entandrophragma utile* and *Anacardium occidentalis*) and investigated the individual plant extract as well as the herbal tonics made from them. Although, all the extracts and tonics proved safe during acute toxicity study, chronic toxicity testing revealed splenic enlargement in 10% of mice that received *E. utile* or either of the two tonics and one case of lung tumor (John et al., 1997). Recently, Auerbach et al. (2012) reported an association between traditional herbal medicine use and the development of liver fibrosis among study participants in Uganda. A number of Chinese herbal medicines and other herbal medicines from different parts of the world have also been implicated in cases of poisoning. Many of them have been shown to contain toxic compounds which are capable of reacting with cellular macromolecules including DNA, causing cellular toxicity, and/or genotoxicity (Rietjens et al., 2005). For the purpose of brevity and other obvious constraints, adverse reactions of only a few commonly used herbal medicines are described below.

ARISTOLOCHIC ACIDS AND *Aristolochia* SPECIES

Following the discovery of the nephrotoxic and carcinogenic potentials of aristolochic acids (Vanherweghem et al., 1993), several studies confirmed their genotoxic activity (Kohara et al., 2002; Fang et al., 2011; Hwang et al., 2012). Schmeiser et al. (1996) demonstrated the presence of aristolochic acids-related DNA adducts in renal tissues of patients. These mutagenic adducts when formed are usually poorly repaired (Sidorenko et al., 2012) and are capable of persisting for years in DNA (Nortier et al., 2000). Aristolochic acids I and II have been identified in different Asian medicinal plants and also reported to be present in slimming products. This has led to the banning of medicinal products containing these acids in Belgium, UK, Canada, Australia, and Germany (Hashimoto et al., 1999; Lee et al., 2002; Zhou et al., 2013).

Misidentification of medicinal plants and mislabeling herbal medicinal products are sometimes responsible for some of the observed adverse events or interaction and that is the reason it is important to assess herbal medicines for possible presence of adulterants. In the UK and many other countries *Aristolochia fangchi* was linked to the development of subacute interstitial fibrosis of the kidney referred to as "Chinese herbs nephropathy" (Cosyns et al., 1994; Tanaka et al., 1997a,b; Stengel and Jones, 1998; Lord et al., 1999). Also, recently a 75-year-old man was reported to have died in Australia from kidney failure associated with a toxic preparation containing the root of *Aristolochia fangchi* which he purchased over the internet for psoriasis (Chau et al., 2011). This case report suggested the chronic use of aristolochic acid-containing herbal product as the most likely cause of the patient's death. Similar cases had previously been reported in Taiwan and Japan (Yang et al., 2002; Hong et al., 2006). Consumption of aristolochic acid-containing Chinese herbal products has also been demonstrated in several studies to be associated with increased risk of urothelial cancer (Li et al., 2008; Yang et al., 2009; Lai et al., 2010; Chen et al., 2012).

A few years back, poisoning that was attributed to Fang-Ji (*Stephania tetrandra* S. Moore) in a weight-loss preparation (Cosyns et al., 1994; van Ypersele de Strihou, 1995; Reginster et al., 1997; Kessler, 2000) was actually caused by Guang-Fang-Ji (*Aristolochia fangchi*; Vanhaelen et al., 1994). The presence of aristolochic acids in the latter produced dramatic adverse reactions which led to nephrotoxic and carcinogenic events in more than 100 women using this weight-loss preparation (Zhou et al., 2004). In this instance, the similarity in the names of the two herbal products was responsible for the confusion and the unfortunate events.

Ephedra sinica

Ephedra is a very popular herb with long history of traditional use in respiratory conditions (Zhang et al., 1999; Bensky et al., 2004). This herb, whose efficacy has been demonstrated in a number of randomized double-blind clinical trials (Boozer et al., 2002; Haller et al., 2005; Kim et al., 2008), is currently included in the Chinese Pharmacopoeia for therapeutic use and classified as non-toxic. It is an ingredient in commonly used formulary preparations such as Xiaoqinglong Heji for common cold and Zhisou Dingchuan Koufuye for asthma (Chinese Pharmacopoeia Commission, 2010). *Ephedra* has been marketed in the US as a weight-loss dietary supplement and its use associated with a number of serious cardiovascular and central nervous systems (CNSs) adverse effects (Verduin and Labbate, 2002; Hackman et al., 2006; Hallas et al., 2008; Chen et al., 2010). Several case reports have also linked the use of *Ephedra sinica* and *Ephedra*-containing dietary supplements to adverse events such as hepatotoxicity (Skoulidis et al., 2005; Schoepfer et al., 2007), neurotoxicity (Varlibas et al., 2009), and transient blindness (Moawad et al., 2006).

Aconitum SPECIES

Aconitum carmichaeli and *Aconitum kusnezoffii* are used traditionally for pain relief (Chinese Pharmacopoeia Commission, 2010). The toxicity of the medicinal plants derives primarily from the presence of diester diterpene alkaloids such as aconitine, mesaconitine, and hypaconitine in them (Xu et al., 2005). These medicinal plants constitute important ingredients in some commonly used herbal preparations like Sini Tang, Fuzi Lizhong Wan, and Guifu Dihuang Wan for stroke and heart failure, diarrhea and diabetes, respectively (Chinese Pharmacopoeia Commission, 2010). Poisoning due to homemade medicated liquor containing aconite and traditional medicine containing *A. carmichaeli* were reported in China between 1999 and 2008 (Liu et al., 2011). Severe cases of cardiac toxicity from consumption of aconitine-containing herbal preparation manifesting as ventricular tachycardia and fibrillation and eventually leading to death have been reported (Tai et al., 1992; Fujita et al., 2007; Dhesi et al., 2010; Lin et al., 2011). In other studies bradycardia and hypotension were observed (Chan, 2009). Clinical cases of aconitine poisoning from *A. kusnezoffii* consumption have also been reported (Chan and Critchley, 1994; Chan, 2002). Furthermore, the toxic effect of the combination of *A. kusnezoffii* and *A. carmichaeli* overdose which manifested as bradycardia and hypotension was also reported by Chan (2009) in a case study.

Tussilago farfara

Traditionally, *Tussilago farfara* has been used effectively for thousands of years for the treatment of acute and chronic cough and it is generally regarded as non-toxic (Chinese Pharmacopoeia Commission, 2010). Total alkaloids and senkirkine isolated from this plant, on the other hand, have been demonstrated to be hepatotoxic (Zhang et al., 2008). Recently, the potential health effects of the pyrrolizidine alkaloids found in *T. farfara* was reviewed and hepatic veno-occlusive disease and cirrhosis were suggested as the major potential disease outcome in human (Edgar et al., 2011). However, restrictions in intake of pyrrolizidine-containing herbs and further investigations were recommended because of paucity of data on toxicity assessment in human (Kim et al., 2013).

GARLIC (*Allium sativum*)

Garlic has found relevance for management of hypertension and hypercholesterolemia besides its use as a food or food additive. It is known to contain alliin, which on crushing or chopping in the absence of heat or acid becomes activated by alliinase to allicin. Adverse effects associated with garlic extract including burning sensation in the gastrointestinal tract, nausea, diaphoresis, and lightheadedness have been reported. This extract may also cause contact dermatitis and morbid spontaneous spinal epidural hematoma has been attributed solely to excess garlic ingestion (Rose et al., 1990).

Ginkgo biloba AND *Ginseng*

Ginkgo biloba has found widespread use in a variety of conditions and several products such as elixirs, extracts, tea, as well as capsules and tablets that may differ in terms of content, have been made from the dried root (Sparreboom et al., 2004). The whole root which contains ginsenosides is usually used because these compounds possess specific pharmacologic effects that may oppose each other (Chong and Oberholzer, 1988). Over 30 ginsenosides have been identified and these compounds are being investigated for their ability to inhibit cell proliferation, tumor cell invasion, and/or metastasis (He et al., 2011; Kim et al., 2012). Recently, the ability of ginsenosides to modulate signaling pathways involving cell cycle, inflammatory, or growth factor pathways, was demonstrated (Nag et al., 2012; Dunnick and Nyska, 2013). The leaf extracts of ginkgo had also been demonstrated to contain active compounds that had found usefulness in improving circulation and cognition (Boullata and Nace, 2000).

The plant extracts appear to be relatively safe, although headache, dizziness, restlessness, nausea, vomiting, diarrhea, and dermal sensitivity are the most common side effects that have been observed. *Ginkgo* has been demonstrated to be capable of inhibiting platelet-activating factor and altering bleeding times. Therefore, cautious use had been advised in individuals or patients on anticoagulants therapy (Boullata and Nace, 2000). The ability of ginkgo to induce liver cancer in experimental model was reported recently and genotoxic mechanisms were suggested to play some role in the carcinogenic process (National Toxicology Program, 2012; Dunnick and Nyska, 2013). Similar observation was made in the thyroid gland and further studies are required to determine whether the mechanisms for the ginkgo-induced thyroid tumors are also found in humans (National Toxicology Program, 2012;

Dunnick and Nyska, 2013). In addition to the carcinogenic effects in the liver and thyroid, ginkgo has also been shown to be capable of inducing tumorigenesis in the nasal cavity. The pathogenesis of the nasal lesions was suggested to be related to toxic metabolites of ginkgo reaching the nasal cavity from the systemic circulation (Reed, 1993; Sells et al., 2007). Some other authors, however, have attributed the nasal lesions to gastric reflux/post gavage reflux through the nasopharyngeal duct (Damsch et al., 2011a,b).

Although, *Ginseng* products as well as the published descriptions rarely report or specify the species, *Ginseng* used as herbal remedies has different botanical sources generally obtained from several *Panax* species including *Panax quinquefolius, Panax Ginseng*, and *Panax pseudoginseng*. In large or excessive doses, *Ginseng* has been reported to cause agitation, insomnia, and elevation of blood pressure while mastalgia and vaginal bleeding have been observed at recommended doses (Baldwin et al., 1986; Dunnick and Nyska, 2013). Other adverse effects that have reported include transient nervousness, excitation, insomnia, inability to concentrate, headache, epistaxis, and allergies. A case of severe but non-fatal Stevens–Johnson syndrome was reported following a 3-day *Ginseng* administration (Dega et al., 1996). Also, spontaneous bleeding from the iris into the anterior chamber of the eye was linked to the use of *G. biloba* extract (Rosenblatt and Mindel, 1997).Separate case reports had also linked the use of *G. biloba* to the development of bilateral subdural hematomas and subarachnoid hemorrhage (Rowin and Lewis, 1996; Vale, 1998).

KAVA (*Piper methysticum*)

Kava is known for its CNS depressant activity and it is commonly used as an anxiolytic agent. Commonly reported side effects of this medicinal plant include headache, dizziness, gastrointestinal discomfort, and localized numbness after oral ingestion. Large dosages has been shown to be capable of giving rise to dry, scaly skin, and yellowish discoloration of the skin and nails, photosensitivity and redness of the eye. Excessive consumption of kava may also lead to photophobia and diplopia (Boullata and Nace, 2000). The muscle relaxant and anticonvulsant effects of this plant have been attributed to its active pyrones constituent. Significant interaction of kava with other CNS depressants which may lead to a comatose state has been reported and as such concurrent use with these agents is usually not advised (Almeida and Grimsley, 1996; Wong et al., 1998). In 2002, the US Food and Drug Administration received several reports of liver-related injuries alongside that of a previously healthy young female who eventually required liver transplantation following consumption of kava-containing products. This prompted the FDA to alert consumers on the potential risk of severe liver injury associated with the use of kava-containing dietary supplements. This liver-related risks also prompted regulatory agencies in other countries including Germany, Switzerland, France, Canada, and the UK to warn consumers about the potential risks of kava use and also went on to consider the withdrawal of kava-containing products from the market (U.S. Food and Drug Administration, 2002). In over 25 reports of adverse events outside the US, kava-containing products were associated with liver-related injuries like hepatitis, cirrhosis, and liver failure with some of the affected patients eventually requiring liver transplants (U.S. Food and Drug Administration, 2002).

ST. JOHN'S WORT

Hypericum perforatum, popularly known as St. John's wort, contains active compounds, such as hypericin, hyperforin, and melatonin. It was once used against viral infections but has gained increased use for mild depressive symptoms (Bennett et al., 1998; Rey and Walter, 1998). Adverse effects reportedly associated with its use include allergic reactions, headache, dizziness, restlessness, fatigue, dry mouth, nausea, vomiting, constipation, and photosensitivity. Hyperesthesia and a syndrome of dyspnea and hyperventilation with flushing headache, mydriasis, nausea, palpitations, and tremor, have been reported (Rey and Walter, 1998). Schneck (1998) reported a hypomanic episode in a patient with a history of panic disorder who took St. John's wort tincture for 10 days. The hypomanic episode, however, resolved within 2 days of discontinuing the herbal remedy. Interaction of St. John's wort with antidepressants and anticoagulants has been demonstrated and the herbal remedy is usually not recommended in pregnancy because of its uterotonic activity (Rey and Walter, 1998).

CHALLENGES ASSOCIATED WITH MONITORING SAFETY OF HERBAL MEDICINES

With the enormous global consumption of herbal products and medicines, it is high time they were included in pharmacovigilance systems. In terms of population exposure alone, it is essential to identify the risks associated with the use of herbal medicines, and in this regard, the safety of these products has become an issue of great public health importance (WHO, 2004, 2005b). There is no doubt that the increasing cases of poisoning associated with the use herbal medicines in many parts of the world in recent times, is necessitating the need to ensure thorough toxicity assessment alongside active pharmacovigilance on these products in order to promote their safe use and protect public health (Zhou et al., 2013).

The development as well as implementation of the regulation of traditional or herbal medicines in different parts of the world is often confronted with several challenges. Challenges often encountered and common to many countries are those related to regulatory status, assessment of safety and efficacy, quality control, safety monitoring and inadequate or poor knowledge about traditional, complementary/alternative, and herbal medicines within national drug regulatory authorities (WHO, 2005b).

CHALLENGES RELATED TO THE REGULATORY STATUS OF HERBAL MEDICINES

The definition and categorization of herbal medicines vary from one country to another. Depending on the regulations applying to foods and medicines, a single medicinal plant may be categorized as a food, a functional food, a dietary supplement, or a herbal medicine in different countries. This introduces serious difficulty in the definition of the concept of herbal medicines for the purposes of national drug regulation while at the same time also confusing patients and consumers (WHO, 2005b). In the United States, for example, natural products are regulated under the Dietary Supplement Health and Education Act (DSHEA) of

1994 (U.S. Food and Drug Administration, 2012). By definition, a dietary supplement is a product that is ingested and is intended to supplement the diet and contains a "dietary ingredient." The dietary ingredients in these products may include vitamins, minerals, herbs, or other botanicals (U.S. Food and Drug Administration, 2011). Under the DSHEA, additional toxicity studies are generally not required if the herb has been on the market prior to 1994 (National Institute of Health (NIH) Office of Dietary Supplements, 2011). In this regard, the FDA bears the burden to prove that a herbal medicinal product or "dietary ingredient" is toxic or not safe for use. Additional major challenge in many countries is the fact that regulatory information on herbal medicines is often not shared between regulatory authorities and safety monitoring or pharmacovigilance centers (WHO, 2004).

CHALLENGES RELATED TO THE ASSESSMENT OF SAFETY AND EFFICACY

There is no gainsaying the fact that the requirements as well as the research protocols, standards and methods needed for the evaluation of the safety and efficacy of herbal medicines are much more complex than those required for conventional or orthodox pharmaceuticals (WHO, 2005b; Zhou et al., 2013). A single herbal medicine or medicinal plant may contain hundreds of natural constituents, and a mixed herbal medicinal product may contain several times that number. Suppose every active ingredient were to be isolated from individual herb from which the herbal medicine is formulated or produced, the time and resources required would be tremendous. Such an analysis may practically be impossible especially where an herbal product is a mixture of two or more herbs (WHO, 2005b).

CHALLENGES RELATED TO QUALITY CONTROL OF HERBAL MEDICINES

The quality of the source materials used in the production of herbal medicines determines to a large extent the safety and efficacy of these herbal remedies. Generally, the quality of source materials is dependent not only on intrinsic (genetic) factors, but also on extrinsic factors like environmental conditions, good agricultural, and good collection practices (GACP) for medicinal plants, including plant selection and cultivation. It is the combination of these factors that makes it difficult to perform quality controls on the raw materials of herbal medicines (WHO, 2004, 2005b). According to good manufacturing practice (GMP), correct identification of species of medicinal plants, special storage, and special sanitation and cleaning methods for various materials are important requirements for quality control of starting materials.

One of the major challenges often encountered in the quality control of finished herbal medicinal products, especially mixture herbal products, is the difficulty in ascertaining the inclusion of all the plants or starting materials (WHO, 2005b). Thus, the general requirements and methods for quality control of finished herbal products remain far more complex than for other pharmaceuticals (WHO, 2003, 2004, 2005b). To ensure safety and efficacy of herbal medicines, therefore, WHO continues to recommend the institution of quality assurance and control measures such as national quality specification and standards for herbal materials, GMP for herbal medicines, labeling, and licensing schemes for manufacturing, import and marketing, in countries where herbal medicines are regulated (WHO, 2004).

CHALLENGES RELATED TO SAFETY MONITORING OF HERBAL MEDICINES

In recent years, issues relating to increasing use of herbal products in developed countries, dependence of many people living in developing countries on plants as a major source of medicines coupled with absence or weak regulation of herbal medicines in most countries and the occurrence of high-profile safety concerns, have increased awareness of the need to monitor safety and deepen understanding of possible harmful as well as potential benefits associated with the use of herbal medicines (Rodrigues and Barnes, 2013). Adverse events arising from consumption of herbal medicines are attributable to several factors among which include the use of the wrong species of plant by mistake, adulteration of herbal products with other, undeclared medicines, contamination with toxic or hazardous substances, overdosage, misuse of herbal medicines by either healthcare providers or consumers and use of herbal medicines concomitantly with other medicines.

Although, the assessment of the safety of herbal medicines has become an important issue for consumers, regulatory authorities, and healthcare professionals, analysis of adverse events related to the use of these products is much more complex than in the case of conventional pharmaceuticals (WHO, 2005b; Zhou et al., 2013). It is also recognized that evaluation of safety is complicated by factors such as the geographical origin of plant material, different processing techniques, route of administration, and compatibility with other medicines (Zhang et al., 2012). Furthermore, there is lack of the knowledge and/or poor emphasis on the importance of taxonomic botany and documentation by most manufacturers of herbal medicines and this poses peculiar challenges during identification and collection of medicinal plants used for herbal remedies (Farah et al., 2000). In order to eliminate the confusion created by the common names, it is necessary to adopt the most commonly used binomial names (including their binomial synonyms) for medicinal plants. For example, *Artemisia absinthium* L., which contains an active narcotic derivative and capable causing CNS disorders and generalized mental deterioration, has at least 11 different common names. Seven of the common names bear no resemblance to its botanical name. Because common names are mainly used, *Heliotropium europaeum* (heliotrope), which contains potent hepatotoxic pyrrolidine alkaloids, is often confused with *Valerian officinalis* (garden heliotrope), known to contain valepotriates with sedative and muscle relaxant properties. This explains why it is important to provide the exact scientific name of the plant, the plant part used, and the name of the manufacturer when reporting adverse drug reactions of herbal medicines. Therefore, effective monitoring of safety of herbal medicine will require effective collaboration between botanists, phytochemists, pharmacologists, and other major stake-holders.

CONCLUSION AND RECOMMENDATIONS

The global acceptance and use of herbal medicines and related products continue to assume exponential increase. Issues relating to adverse reactions in recent times are also becoming more vivid, increasing in prevalence and no longer debatable because

of previous misconception of regarding or categorizing herbal medicinal products as "safe" because they are derived from "natural" source. The reality is that "safety" and "natural" are not synonymous. Therefore, regulatory policies on herbal medicines need to be standardized and strengthened on a global scale. Relevant regulatory authorities in different countries of the world need to be proactive and continue to put in place appropriate measures to protect public health by ensuring that all herbal medicines approved for sale are safe and of suitable quality.

Providers of medicines, such as physicians, nurses, and pharmacists, often have little training in and understanding of how herbal medicines affect the health of their patients. Many of them are also poorly informed about these products and how they are being used. Adequate training is now very essential since most patients are almost often on other types of prescription or non-prescription medicines. In spite of the fact that the active involvement of orthodox healthcare professionals is continuously solicited and huge responsibility lies with them in terms of their valuable contributions to safety monitoring of medicinal products, it is also very important that all providers of herbal medicines are sufficiently empowered to play a role in monitoring safety of herbal medicines. This, however, should be in collaboration with the orthodox healthcare professionals. For this to be effective, it would be essential to create an atmosphere of trust to facilitate adequate sharing of knowledge about the use and safety of herbal medicines. In fact, the education of healthcare professionals, providers of herbal medicines, and patients/consumers is vital for the prevention of potentially serious risks from misuse of herbal medicines.

Of crucial importance also is an appropriate knowledge base relevant to diagnostic and treatment decision-making. Furthermore, individual healthcare provider should also show sufficient commitment toward understanding the use of herbal medicines. This can be by asking relevant questions about the use of these herbal remedies among others whenever they encounter patients who are taking these medications. Health professionals who work in poisons centers and health information services also need to be informed about herbal medicines. Finally, as with other medicines for human use, it has become mandatory that herbal medicines are covered in every country of the world by a drug regulatory framework to ensure that they conform with required standards of safety, quality, and efficacy.

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