



Review

Ethnomedical research and review of Q'eqchi Maya women's reproductive health in the Lake Izabal region of Guatemala: Past, present and future prospects



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ABSTRACT

Ethnopharmacological relevance: In Central America, most Maya women use ethnomedicines for all aspects of their reproductive cycle including menstruation, pregnancy and menopause. However, very few of these plants have been documented, collected and tested in appropriate pharmacological assays to determine possible safety and efficacy. The aim of this work was to provide an overview of information on the ethnomedical uses, ethnopharmacology, chemistry and pharmacological research for medicinal plants used for women's reproductive health in Guatemala, with a special emphasis on the Q'eqchi Maya of the Lake Izabal region, to demonstrate therapeutic potential and support future research in the field. **Materials and methods:** Reviews of the ethnobotanical, ethnomedical and ethnopharmacological literature were performed for 30 plants collected in the Lake Izabal region of Guatemala and used by the Q'eqchi Maya for treatment of reproductive health issues were performed up to and including July 2015 using multiple databases, library searches for abstracts, books, dissertations, and websites.

Results and conclusions: Review of the published research confirms that many of the plants used by Q'eqchi Maya women for the management of reproductive health issues have pharmacological activities, including analgesic, anti-inflammatory, estrogenic, progestagenic and/or serotonergic effects, that support the use of these plants and provide plausible mechanisms of action for their traditional uses. Furthermore, a new serotonin agonist, 9, 10-methylenedioxy-5, 6-Z-fadyenolide was isolated, thereby demonstrating an untapped potential for drug discovery. However, to date much of the pharmacological assays have been in vitro only, and few in vivo studies have been performed. Considering the large percentage of the Maya population in Guatemala that use traditional medicines, there remains a significant lack of pharmacological and toxicological data for these plants. Future research should focus on the safety and efficacy of medicinal plants using in vivo preclinical studies and clinical trials, as well as chemical analysis. Since medicinal plants from the Piperaceae are most commonly used as traditional medicines by the Q'eqchi Maya women, and new bioactive compounds have been identified from Piper species, investigations of commonly used plants from this family would be an appropriate place to start. Data generated from such studies would contribute to Guatemala's national effort to promote a complementary relationship between traditional Maya medicine and public health services.

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Abbreviations: COX-2, Cyclooxygenase 2; E2, Estradiol; ER α , ER β , estrogen receptor alpha and beta; ERE, estrogen responsive element; GC, granulosa cells; 5-HT, Serotonin; 5-HT $_{1-7}$, Serotonin receptors 1-7; IC $_{50}$, median inhibitory concentration; Ki, Inhibition constant; MCF-7, human breast cancer cells; mRNA, messenger ribonucleic acid; nH, Hill coefficient; PMS, premenstrual syndrome; P4, progesterone; PR, progesterone receptor; qPCR, quantitative polymerase chain reaction; SEAP, secreted alkaline phosphatase assay; TRAMIL, Traditional Medicine for the Islands

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1. Introduction

1.1. Past research in women's reproductive health in Mexico and Central America

Although much of our understanding of attitudes, symptoms and treatments that are associated with the female reproductive cycle, including menstruation, pregnancy and menopause has been derived from studies performed primarily in the U.S. and Europe, among homogeneous groups of Caucasian, middle-class, well-educated women (Lock and Kaufert, 2001), a growing number of ethnobotanical studies have recently been published on plants used for reproductive health in the tropics (De Boer and Cotingting, 2014; De Giselle, 2014; Kamatenesi-Mugisha and Oryem-Origa, 2007; Michel et al., 2006, 2007, 2010, 2012; Ososki et al., 2002). Data from cross-cultural research studies indicates that the attitudes, symptoms and treatment choices of women vary considerably depending on their geographical location, environment, health status and specific cultural paradigms that impact women's health (Avis et al., 2001; Michel et al., 2006; Rasor and Adler, 1999). For example, research studies of menstrual health in Latin America suggest that women experience a lower incidence of menstrual symptoms as compared with their US female counterparts (Pawlowski, 2004; Severy et al., 1993). Another large cross-cultural investigation of menstruation conducted among 14 different cultural groups in 10 countries found a that 23–34% of women in developing countries, including Mexico, had reduced rates of menstrual symptoms (including dysmenorrhea, bloating, psychological changes) (Severy et al., 1993). In addition, a 2004 study among Maya women ($n=177$) in the Yucatan revealed a low (28%) prevalence of menstrual pain, and the only variable found to be significantly related to the dysmenorrhea was the age at which the women gave birth to her first child (Pawlowski, 2004). These data differ significantly from the high prevalence (up to 80%) of somatic symptoms and premenstrual mood changes among US women (Sundell et al., 1990; Smith and Schiff, 1993). Reasons for these differences are unknown but presumably diet, lifestyle and the use of ethnomedicines all appear to be involved and impact overall women's health in these countries.

In terms of menopause, the limited number of studies investigating the attitudes, symptoms and treatment choices in Latin America report more positive attitudes and less symptomatology as compared to their U.S. and European counterparts (Beyene, 1986;

Canto-de-Cetina et al., 1988; Leon et al., 2007; Martin et al., 1993; Malacara et al., 2002; Sievert and Espinosa-Hernandez, 2003), yet, overall, knowledge about menopause among Latin American women is lacking (Dulón-Pérez et al., 2013; Leon et al., 2007). One frequently cited study involved menopausal Maya women ($n=78$) from Chichimila, Mexico (Martin et al., 1993). The results of this study revealed that, although follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels were as high in menopausal Maya women as those in U.S. postmenopausal women, none of these women reported having vasomotor symptoms that are usually associated with such hormonal changes (Martin et al., 1993). Another study among 107 Maya women in the Yucatan also found no symptomatology related to menopause other than the cessation of menstruation (Beyene, 1986). Interestingly, bone mineral density, an important intermediate outcome and risk factor for fracture, was also found to be lower in women in both Mexican studies as compared with U.S. postmenopausal women, yet there was purportedly no observed osteoporosis in the Mexican women (Beyene, 1986; Martin et al., 1993). Again diet, lifestyle and the use of traditional plant based medicines have been suggested as reasons for these discrepancies.

Reviews of the Latin American literature over the past 25 years shows a rapid growth in scientific research on natural products, particularly medicinal plants (Calixto, 2005). However, very few of these ethnomedical studies have focused on women's reproductive health, while, those that have, have concentrated specifically on herbs used to enhance fertility or terminate pregnancy (abortifacients) (Arenas and Moreno Azorero, 1977; Browner, 1985; Conway and Slocumb, 1979; Southam et al., 1983). In general, the daily use of plant-based medicines (ethnomedicines) by women in Latin America continues to be based primarily on empirical knowledge and there is little or no scientific or medical information available for the safety and efficacy of these botanical medicines (Bodeker et al., 2005; Calixto, 2005; Michel et al., 2006; Van Andel et al., 2014).

1.2. Guatemala as a site for ethnomedical research in women's reproductive health

Guatemala is located at the northern end of the Central American (CA) isthmus and is inhabited by over 14 million people (Beyene and Martin, 2001). Guatemala is one of the regions with the greatest cultural and biological diversity in the world (Nations

et al., 1988). Unfortunately, the habitats that support such diversity are being lost at an alarming rate. In 1980, 41.9% of the country's land area was covered by forests, in 1990 that figure was reduced to 33.8% and then to 26.3% in 2000 (Nations et al., 1988). In addition to its environmental consequences, such losses will also have a serious impact on the diverse Guatemalan population, 60% of whom live in rural areas and rely on subsistence agricultural subsistence and the extraction of natural resources for their survival (Girón and Caceres, 1994).

Economically, Guatemala is one of the poorest countries in CA, yet is among one of the richest nations in terms of indigenous peoples and lifestyles, traditional medical knowledge and biological resources (Cáceres, 1996). In fact, Guatemala is the most ethnically diverse country in CA, with over 20 groups of ethnolinguistic Maya origin, making up almost 40% of the population (CIA, 2015). The majority of these populations live in extreme poverty, within remote rural areas that have little access to modern healthcare facilities or practitioners (PAHO: Pan American Health Organization, 2012). Being both a women and indigenous, Maya women are the most marginalized and oppressed populations in Guatemala. While some Maya women receive some formal prenatal care, it is estimated that ~70% of childbirths among Maya women are attended at home, of which ~75% are attended by midwives, and 5.5% get no medical assistance at all (PAHO: Pan American Health Organization, 2012). Furthermore, Guatemala's population growth is due to a very high individual expected birth rate. Less than 50% of all married women use contraception, and these women are expected to have at least five births during their lifetime, the highest number of the seven Central American countries (Hughes, 2004; Schooley et al., 2009). Higher birth rates are influenced by religious beliefs that do not support family planning, as well as sociopolitical concerns for the survival of the indigenous people (Schooley et al., 2009). Thus, most Maya births occur at home and attended are exclusively by a traditional birth midwife, and Maya women rely almost exclusively on herbal remedies for all stages of their pregnancy (Hughes, 2004; Orellana, 1987; Schooley et al., 2009).

In terms of menopause, the first report from Guatemala was a qualitative exploration of attitudes and symptoms associated with menopause among 27 Quiché, Tzutujil, and Cakchiquel Maya women in the Guatemalan highlands (Stewart, 2003). Similar to reports from Maya women in Mexico, these women had a very positive attitude about their entrance into menopause. However, these women reported experiencing menopausal symptoms similar to those of U.S. women including hot flashes, night sweats, moodiness and menstrual irregularities. Although the specifics of these therapies were not given, treatment for these symptoms included steam baths, lower abdominal massage by midwives and herbal medicines. No Western-style treatment, such as hormone therapy was sought to manage menopausal symptoms (Stewart, 2003). A large percentage of the Maya population in Guatemala continue to rely on traditional medical practices and medicinal plants as their primary source of healthcare (Cáceres, 1996).

Thus, because of the high indigenous populations and lifestyles, and the significant use of herbal medicines by the Maya population, as well as a lack of scientific studies of the safety and efficacy of these herbs, Guatemala is an excellent site for ethnomedical studies, and such studies are sorely needed. Moreover, due to the extensive use of herbal therapies by women, there is a critical need for studies that assess the safety and efficacy of plants used to treat reproductive health issues.

2. Review of the ethnobotanical and ethnomedical literature for Guatemala

The most comprehensive treatment of native Guatemalan

plants, as well as their medical uses during colonial times is recorded in the “*Recordacion Florida*”, which is a compilation of 60 medicinal plants, written by Francisco Antonio de Fuentes y Guzman in the late seventeenth century (Villatoro, 1996a,b,c). Other significant works on the subject include that of Fray Francisco Ximenez written in 1772, and entitled “*Historia Natural del Reino de Guatemala*”. In the 18th century, a scientific re-classification of New Spain, Mexican and Guatemalan flora was performed by Martin Sessé and Jose Mariano Mociño that was based on the system of Carl Linnaeus (Sprague, 1929).

Beginning in the 1930s, the interest in traditional Maya medicine slowly increased providing many publications on the subject of medical practice and medicinal plants, including the seminal work of Ralph Roys on Maya ethnobotany (Roys, 1931). Beginning in the 1960s and 1970s the Instituto Indigenista Nacional (National Indigenous Institute) also published a series of papers and books covering various aspects of traditional Maya medicine and medicinal plants in Mesoamerica (Adams, 1952; Carlson and Eachus, 1978). In addition, the historical review by Guerra (1964) provides an excellent account of the diverse medical techniques used by the Maya.

Since the 1970s there have been a number of studies conducted on the ethnobotany and ethnomedicine of the Maya, with a focus primarily on the Maya of Mexico, Belize and the western highlands of Guatemala (Arvigo and Balick, 1993, 1998; Berlin and Berlin, 1996; Heinrich et al., 1998; Orellana, 1987). In 1974 Mellen published “*El Uso de las Plantas Medicinales en Guatemala*” (Medicinal Plant Use in Guatemala) that describes the evolution of plants used as medicines in Guatemala, the role of traditional healers (curanderos) and midwives, and a list of over 100 medicinal plants commonly used in Guatemala (Mellen, 1974). In 1981 Morton described over 150 medicinal plant families in her “*Atlas of Medicinal Plants of Middle America*” (Morton, 1981). In 1984, Elfriede Pöhl a prominent botanist in Guatemala, published “*Plantas Comestibles y Toxicas de Guatemala*” (Edible and Toxic Plants of Guatemala), in which 63 plants are described giving their scientific name, botanical description, use and geographic distribution (Pöhl, 1984, 2006). A well cited book about Maya medicine in Guatemala was written in 1987 by Rebecca Orellana and entitled “*Indian Medicine in Highland Guatemala: the Pre-Hispanic and Colonial Periods*”. Orellana provides a thorough overview of Maya cosmology and beliefs about the origin of disease as well as the types of healers employed during pre-Hispanic and Colonial times followed by a description of 157 medicinal plant species, including their taxonomy, uses, chemical constituents, and known activities (Orellana, 1987). Others such as Villatoro, Comerford and Caceres produced a number of valuable publications that included not only the use of medicinal plants, but a very complete and culturally relevant description of the history of Maya medicine, Maya cosmology and methods to integrate traditional Maya medicine in the official system of healthcare of Guatemala (Cáceres, 1996; Comerford, 1996; Villatoro, 1996a–c). Over the past 20 years, the number of published articles in international journals of ethnobotany and ethnopharmacology has also increased, although many of these studies were conducted among Maya communities in Mexico or Belize (Amiguet et al., 2005; Ankli et al., 2000, 2002; Arnason et al., 1980; Cruz and Andrade-Cetto, 2015; Heinrich et al., 1992, 1998; Kufer et al., 2005).

2.1. Ethnobotanical literature of the Q'eqchi

The Q'eqchi are currently the third largest Maya population in Guatemala and occupy the largest geographic area of any other ethnolinguistic group in the country (Michel et al., 2012). Like all Maya communities, the Q'eqchi of the eastern lowlands (Izabal) maintain a rich tradition of medical beliefs and practices that

include the use of the native flora to treat a variety of illnesses. Compared with the numerous ethnographic and ethnobotanical studies on the Q'eqchi from the highlands (Cabarrus, 1974; Carlson and Eachus, 1978; Coe, 1996, 1999; Collins, 2001), few studies have focused on the lowland Q'eqchi (Amiguet et al., 2005; Arnason et al., 1980; Comerford, 1996) and, prior to our work (Cáceres et al., 2014; Michel et al., 2006, 2007, 2010, 2012) there were no other known ethnobotanical studies of the Q'eqchi from the eastern lowlands of Livingston, Izabal.

In 1977, Erwin Paul Dieseldorff published “*Las Plantas Medicinales del Departamento de Alta Verapaz*” (“Medicinal Plants of the Department of Alta Verapaz”), where he describes over 60 medicinal plants used in the Alta Verapaz region (Dieseldorff, 1977). He also includes the descriptions of three main types of healers used by the Q'eqchi people: the *ilonel* (the Q'eqchi term for healer), *aj tul* (the Q'eqchi word for *brujo* or “witch” who performs rituals to produce both good and bad outcomes) and the *aj ke* (the diviner who diagnoses and advises the ill). Other important works published on the Q'eqchi during this time include Carter's “New Lands Old Traditions” (1969), and “*El Mundo Kekchi de la Verapaz*” (“The Kekchi World of Alta Verapaz”), “*La Cosmovision K'eckchi en el Proceso de Cambio*” (“Q'eqchi Cosmovision in the Process of Change”) (Cabarrus, 1974), and the various publications of Richard Wilson (Wilson, 1972, 1993, 1994, 1995b, 1995a), with the most extensive being “Maya Resurgence in Guatemala: Q'eqchi Experiences” (Wilson, 1995a). Considering the considerable ethnographic attention the Q'eqchi have received, it is surprising that there are so few publications on the medical ethnobotany of the Q'eqchi, almost all of which are focused exclusively on the life and customs of the Q'eqchi of Alta Verapaz (Carlson and Eachus, 1978; Collins, 2001; Wilson, 1995b). Although ethnographic works on the Q'eqchi mention the intimate relationship between Q'eqchi identity and the environment and flora that surround them (Cabarrus, 1974; Wilson, 1993), only the work of Dieseldorff (1977) provides a list of medicinal plants used by the Q'eqchi, and Pesek et al. (2009) describes ethnobotanical surveys and spacial modeling of Q'eqchi medicinal plants in the Maya mountains of Belize. Additional published studies described the ethnobotany of the Q'eqchi of Southern Belize and documented the traditional medical beliefs and medicinal plants of the Q'eqchi in the same bioregion as the Q'eqchi of Livingston, Lake Izabal, Guatemala (Amiguet et al., 2005; de Gezelle, 2014; Heinrich et al., 1998). All other publications that provide detailed information on the medical ethnobotany of the Q'eqchi are limited exclusively to undergraduate thesis written within Guatemala. All of these are written in Spanish and none of them has been published outside of Guatemala. Furthermore, as with the majority of previous studies on the Q'eqchi, all of them focus on the Q'eqchi of Alta Verapaz. Consequently, our knowledge of the rich medical beliefs and practices of the Q'eqchi of the eastern lowlands (Lake Izabal), as well as the biological and chemical properties of the tropical medicinal plants that they use is extremely limited.

Other ethnobotanical explorations of medicinal plants in the same tropical bioregion as the Q'eqchi of Livingston, Izabal include the work of Rosita Arvigo and Michael Balick in Belize (Arvigo and Balick, 1998), a survey of medicinal plant use among the Garifuna of Livingston (Girón et al., 1991) and the extensive efforts of TRAMIL (Traditional Medicine for the Islands) to record the ethnopharmacology of the Caribbean region (Robineau, 1991, 2014; Robineau and Soejarto, 1996). Data from the ethnobotanical field interviews of TRAMIL are combined with a thorough review of the safety and efficacy of each plant. Results of this on-going endeavor have been disseminated in a variety of formats and include a widespread book entitled the *Vegetal Caribbean Pharmacopeia*, as well as pamphlets, videos, music and dance performances and community meetings.

2.2. Ethnomedical and women's health investigations of the Q'eqchi in Guatemala

The only known published work concerning women's reproductive health in Guatemala was the qualitative study by Stewart (2003) of attitudes and symptoms of menopause among Maya women in the Guatemalan highlands. While this study detailed the attitudes, symptoms and some of the treatments that the Maya women used for treatment of these symptoms, very little information on the actual treatments was included, and no herbal treatments were documented, collected or tested in pharmacological and toxicological assays. Thus, the important information relating to the safety and efficacy of herbal therapies used routinely by the Maya women was lacking.

In our ethnomedical work with the Q'eqchi Maya women of Livingston, Guatemala, we investigated the symptoms and attitudes, as well as the herbal treatments associated with women's reproductive health and menopause (Cáceres et al., 2014; Michel et al., 2006, 2007, 2010, 2012). Data for these investigations were obtained through participant observation, semi-structured interviews, focus groups and plant walks with 50 Q'eqchi community members from the state of Izabal, municipality of Livingston, including five midwives, five traditional male healers and eight postmenopausal women (Michel et al., 2006). The results of these investigations demonstrated that the Q'eqchi Maya women in this region have their own cultural perceptions of women's health that impact their attitudes, symptoms and treatment choices during the menopausal transition. In focus group sessions with the Q'eqchi women, we found that women's reproductive health issues are considered cultural taboos by the Q'eqchi. Many women reported suffering from additional hardships when their spouse misinterpreted menopausal symptoms (vaginal dryness, sexual disinterest) as infidelity (Michel et al., 2006). Seven of the eight postmenopausal women interviewed had one or more symptoms during the menopausal transition, including headaches, anxiety, muscular pain, depression, and hot flashes. These results differ from previous studies in Mexico (Beyene, 1986; Martin et al., 1993), but are similar to the results of menopausal research conducted among other Maya groups from the highlands of Guatemala (Stewart, 2003). Although the Q'eqchi did not use a specific term for “hot flash”, three Q'eqchi women used the expression “baja presión” or a “lowering of blood pressure” to explain symptoms of profuse sweating followed by chills, heart palpitations, and emotional instability. The Q'eqchi Maya mentioned a number of herbal remedies to treat menopausal symptoms (Michel et al., 2006).

In terms of ethnomedical treatments for women's reproductive health, numerous plant species have been, and continue to be, used by Maya women and traditional healers in Guatemala (Michel et al., 2007). In our investigations, 48 medicinal plants used to treat conditions related to pregnancy, childbirth, menstruation, and menopause were documented under a Memorandum of Agreement between the University of Illinois at Chicago and the University of San Carlos, Guatemala City (Michel et al., 2007). The plants described by the Q'eqchi for their use in treating women's reproductive health ailments included 48 different plant species belonging to 26 plant families (Michel et al., 2007). Of these 48 plants, 31 were collected near Livingston, Guatemala by Dr. Joanna Michel, of which 30 were identified by botanists and taxonomists at the Herbarium in the University of San Carlos, Guatemala and then confirmed in Chicago in the herbarium at the Field Museum and included in Table 1.

Detailed documentations of collection sites, ethnomedical uses, preparation and administration of the ethnomedicine, as well as healing concepts were recorded for each of the plant species (Michel, 2006). Voucher specimens were collected were deposited

Table 1

List of 30 plant species used by Q'eqchi Maya women in the Lake Izabal region of Guatemala for treating reproductive health issues and collected by Dr. Joanna Michel (modified from Michel, 2006; Michel et al., 2007). Data on ethnomedical uses by other groups in Central America and other countries as they pertain to women's reproductive health are included. Biological and chemical information, and toxicity data are included where known.

Species/Voucher number	Q'eqchi name	Part used ^b	Medicinal use	Uses by other groups/countries	Biological and chemical data as it relates to uses for women's health	Toxicology
<i>Arachnothryx stachyoidea</i> (Donn. Sm.) Borhidi. [syn. <i>Rondeletia stachyoidea</i> Donn. Smith] (Rubiaceae); JM41	Kandel Che	Lf	Reposition womb, insomnia, "witchcraft"	None found.	Ethanol extracts of the leaves weakly bound to the serotonin receptors in vitro (Michel et al., 2007).	None found.
<i>Cecropia peltata</i> L. (Cecropiaceae); JM26	Guarumo	Lf	Expel placenta, lower womb, insomnia, nerves	Menstrual problems, to increase labor contractions (Cáceres, 1996; De Giselle, 2014).	Ethanol extracts bound to the serotonin receptors in vitro (Michel et al., 2007). Phytochemical analysis shows the presence of chlorogenic acid and isoorientin (Costa et al., 2011; Valdés et al., 2011).	Pre-clinical topical application showed no toxicity in Sprague Dawley rats (Shivananda Nayak, 2006).
<i>Cissampelos tropaeolifolia</i> DC. (Menispermaceae); JM39	Bejuco de ombligo, Xchup I el	Lf	Release of placenta	Used to induce labor and to speed up prolonged labor by the Q'eqchi in Belize (De Giselle, 2014). <i>C. pareira</i> L., a related species is used in other countries as an emmenagogue (Lewis and Elvin-Lewis, 2003).	Ethanol leaf extracts bound to the serotonin receptors (Michel et al., 2007); a 95% ethanol extract was antispasmodic and relaxed the uterus in rats, alkaloids such as warifiteine, methyl-warifiteine, berberine, hayatin and hayatidin found in other species of the genus (Semwal et al., 2014).	No experimental data found, but potentially an abortifacient (Michel, 2006).
<i>Citrus aurantium</i> L. (Rutaceae); JM27	Naranja agria	Lf	Nerves, insomnia, body aches, body sweats	Used throughout Central America to treat nervous conditions, insomnia, body aches, and body sweats (Cáceres, 1996).	Ethanol extracts bound to the estrogen receptors, reduce bone loss and decrease serum lipids in mice (Chiba et al., 2003). Flavonoid glycosides and flavones are present in the leaf extracts (Park et al., 1983).	None found.
<i>Clidemia setosa</i> (Triana) Gleason (Melastomataceae); JM50	Ixq Q'een	Lf	Fertility regulation Promote fertility in females, male contraceptive	Menstrual hemorrhages (Duke and Vásquez, 1994).	None found.	None found.
<i>Clidemia petiolaris</i> (Schltdl. & Cham.) Schltdl. ex Triana (Melastomataceae); JM63	Xa bol q'een	Lf	Postpartum hemorrhaging, fertility regulation ^c , blood clots	None found.	None found.	None found.
<i>Crossopetalum parviflorum</i> (Hemsl.) Lundell [syn. <i>Crossopetalum eucymosum</i> (Loes. & Pitt.) Lundell] (Celastraceae); JM13	Ra Mox	Lf	Cold body, body aches	None found.	None found.	None found.
<i>Dioscorea bartlettii</i> Morton (Dioscoreaceae); JM08	Cocolmea wild yam, xchup ixim qotz	Rh	Stagnant blood, anemia	Used to treat labor pains, dysmenorrhea, ovarian pain and vaginal cramping, and menopause (Rodriguez Martinez, 1987; Ososki et al., 2002). Used as a female contraceptive by the Q'eqchin in Belize (De Giselle, 2014).	Extracts of the rhizome contain diosgenin, and have estrogenic effects when taken orally (Higdon et al., 2001).	None found.
<i>Henriettea cuneata</i> (Standl.) Gleason (Melastomataceae); JM100	Ixq Q'een	Lf	Fertility regulation	None found.	Ethanol extracts of the leaves bound to the serotonin receptors 5HT1A and 5 (Michel et al., 2007).	Overuse may cause uterine hemorrhage (Michel, 2006).
<i>Hibiscus rosa-sinensis</i> L. (Malvaceae); JM06	Utz Uj'	Lf, Fl	Postpartum hemorrhaging, infertility, nerves	Used as an emmenagogue, abortifacient, labor inducement, dysmenorrhea, and for menstrual disorders (Cáceres, 1996; Lans, 2007). Used to treat excessive menstruation by Q'eqchi in Belize (De Giselle, 2014).	Extracts of the flowers had mild estrogenic effects in mice and rats (Murthy et al., 1997); Ethanolic leaf extracts bound to the 5-HT1A and 5 receptors in vitro (Michel et al., 2007). Antioxidant effects (Chen et al., 2003). Presence of anthocyanins, flavonoids, flavonoid glycosides and phenolics have been reported (Chen et al., 2003; Salib et al., 2011).	Methanol extracts of the leaves induced liver and kidney toxicity at a dose of 800 mg/kg administered to mice for 14 days, but 400 mg/kg showed no toxicity (Nath and Yadav, 2015).
<i>Hyptis verticillata</i> Jacq. (Lamiaceae); JM05	Verbena, chu pim	Lf	Release placenta, and the treatment of erratic menses that are associated with menopause	Oral contraceptive, pain reliever, anti-inflammatory (Domingez and Alcorn, 1985; Heinrich et al., 2004). Used to treat primary amenorrhea (De Giselle, 2014).	Antiinflammatory activities, hormone modulating effects (Picking et al., 2013). Leaf extracts bound to the 5HT7 receptor in vitro (Michel et al., 2007). Rosemarinic acid and sideroflavone (Heinrich et al., 2004). Lignans, terpenes, flavonoids, and alkaloids (Picking et al., 2013).	A crude extract showed no sub-chronic toxicity (Picking et al., 2013).

Table 1 (continued)

Species/Voucher number	Q'eqchi name	Part used ^b	Medicinal use	Uses by other groups/countries	Biological and chemical data as it relates to uses for women's health	Toxicology
<i>Justicia breviflora</i> (Nees) Rusby (Acanthaceae); JM37 ^a	Rax Pim	Lf	Menorrhagia, postpartum hemorrhage ^c	None found	Leaf extracts bound strongly to the serotonin receptors in vitro (Michel et al., 2007). <i>J. pectoralis</i> had anti-inflammatory and estrogenic effects (Locklear et al., 2010; Telefo et al., 2002). Flavones and alkaloids found in other <i>Justicia</i> species.	None found.
<i>Justicia fimbriata</i> (Nees) V.A. W. Graham (Acanthaceae); JM16	Numay Pim	Lf	Insomnia, excess body heat, inflammation	None found.	None found.	None found.
<i>Mimosa pudica</i> L. (Mimosaceae); JM57	Dormilona Sleeping prickle	Lf	Insomnia, fertility	Used to treat anxiety, agitation and insomnia (Bum et al., 2011). The root used in childbirth and for infertility (Lans, 2007). Used to treat symptoms of menopause, uterine fibroids and insomnia by Q'eqchi of Belize (De Giséle, 2014).	Antiinflammatory effects in vitro and in animal models, effects due to the presence of flavanones (Patel et al., 2015). Anxiolytic effects in mice (Bum et al., 2011). Antifertility effects and prolongs the estrous cycle and disturbs the secretion of gonadotropin hormones in albino mice (Ganguly et al., 2007).	None found.
<i>Momordica charantia</i> L. (Cucurbitaceae); JM59	Sorosi	Lf	Dysmenorrhea, amenorrhea	Abortifacient, contraceptive, dysmenorrhea (Cáceres, 1996). Contains triterpenes, sterols, saponins (Robineau, 1991, 2014). Gynecological aid in Togo (Beloin et al., 2005). Used by Q'eqchi in Belize to treat symptoms of menopause, amenorrhea, and as a contraceptive (De Giséle, 2014). Also used for “suspended menses” in Dominican Republic (Ososki et al., 2002).	Several experimental studies demonstrated abortifacient properties (Grover and Yadav, 2004)	Two proteins isolated from the raw fruit demonstrated abortifacient properties (Basch et al., 2003).
<i>Neurolaena lobata</i> (L.) R. Br. Ex Cass (Asteraceae); JM31	Kamank, jackass bitters, gan mank	Lf	Dysmenorrhea, vaginal infection	Gonorrhea. Inflammations and nervousness, labor pains (Cáceres, 1996; Coe and Anderson 1996). Used as birth control and for delayed menstruation by Q'eqchi in Belize (De Giséle, 2014).	Leaf extracts had analgesic and anti-inflammatory effects in rats (Gracioso et al., 1998). Leaf extracts bound to the serotonin receptor 5HT7 (Michel et al., 2007). Anti-inflammatory effects attributed to sesquiterpene lactones (McKinnon et al., 2014; Walshe-Roussel et al., 2013).	Acute oral toxicity of an ethanol extract was in mice was > 5.0 g/kg; oral and intraperitoneal acute and sub-acute toxicity in mice was > 500 mg/kg (Cáceres et al., 1998)
<i>Ocimum campechianum</i> Mill. [syn. <i>Ocimum micranthum</i> Willd.] (Lamiaceae); JM04	Obej', albahaca benq	Lf	Dysmenorrhea, vaginal hemorrhaging	Emmenagogue (Gupta et al., 1979). Used to treat heavy menstruation, dysmenorrhea and postpartum hemorrhages by Q'eqchi in Belize (De Giséle, 2014).	Contains essential oils and alkaloids (Coe and Anderson, 1996; Pino Benitez et al., 2009; Sacchetti et al., 2004). Contains flavonoids and sesquiterpene lactones (Cáceres, 1996).	No cytotoxicity was found by the <i>Artemia salina</i> bioassay (Coe et al., 2010). An ethanol extract induced toxicity in brine shrimp (<i>Artemia salina</i>) and <i>Thamnocephalus platyurus</i> (Mayorga et al., 2010).
<i>Piper jacquemontianum</i> Kunth. [syn. <i>Piper aeruginosibaccum</i> Trelease] (Piperaceae); JM25	Ampom	Lf	Anemia, body aches	Nervous conditions (Barrett, 1994). Used to treat hot flashes in Belize (De Giséle, 2014).	Contains essential oils rich in linalool (Cruz et al., 2011). Ethanol extracts of the leaves bound to the serotonin receptors in vitro (Michel et al., 2007); the same extract bound to the estrogen and progesterone receptor and enhanced the expression of estrogen related genes (Cáceres et al., 2014). Dichloromethane extract exhibited antioxidant activity (Cáceres et al., 2012a).	None found.
<i>Piper auritum</i> HBK. (Piperaceae); JM03	Ob'el, cowfoot, pata de vaca	Lf	Dysmenorrhea, galactagogue	Rheumatism, ovarian pain, emmenagogue (Girón et al., 1991; Gupta et al., 1996). Used by Q'eqchi in Belize as a contraceptive, to treat postpartum hemorrhage, reduce infection and pain postpartum (De Giséle, 2014). <i>P. sanctum</i> used as an abortifacient (Lozoya, 1976).	Uterine stimulating effects in rats (Cáceres et al., 1995). Ethanol extracts of the leaves bound to the serotonin receptors in vitro (Michel et al., 2007); Contains essential oils, sesquiterpenes, flavone and alkaloids.	Tincture showed no cytotoxicity and LD ₅₀ in mice was 1802 mg/kg (Lagarto Parra et al., 2001).
<i>Piper sanctum</i> (Miq.) Schltdl. ex C.DC. [syn. <i>Piper andrum</i> C.DC] (Piperaceae); JM45	Nin qui ru chaq' q'een	Lf	Body aches, reposition womb		Essential oils, terpenes, piperolides present (Mata et al., 2004). Contains isoquinoline alkaloids, beta sitosterol (Mata et al., 2004)	May be abortifacient (Michel, 2006).
<i>Piper hispidum</i> Sw. (Piperaceae); JM32	Puchuq	Lf	Dysmenorrhea, amenorrhea, body	Used to treat pain and regulate menstruation (Coe and Anderson, 1996;	Ethanol extracts of the leaves bound to the serotonin receptors in vitro (Michel et al., 2007); Essential oils, benzoic acids, butenolides	None found.

			aches		Duke and Vásquez, 1994). Contains flavonones, chalcones, alkaloids (Coe and Anderson, 1996).	present (Michel et al., 2006; 2010; Pino Benitez et al., 2009).	
<i>Piper tuerckheimii</i> C. DC. (Piperaceae); JM14	Caite de Diablo	Lf	Inflammation, “loss of senses”, eclampsia, contraceptive		Used to treat heavy menstruation and menstrual cramps by Q'eqchi in Belize (De Giselle, 2014).	Extracts of the leaves had displacement activities in the GABA-T and GABA _A -BZD bioassays (Awad et al., 2009).	None found.
<i>Psychotria poeppigiana</i> Müll. Arg. [syn. <i>Cephaelis tomentosa</i> (Aubl.) Vahl.] (Rubiaceae) JM33	Ak Pere Tzo, perren pim	Lf	Postpartum hemorrhaging		Abortifacient (Coe and Anderson, 1996). Used to treat primary and secondary amenorrhea, heavy menstruation and hot flashes by Q'eqchi in Belize (De Giselle, 2014).	Ethanol extracts of the leaves bound to the serotonin receptors in vitro (Michel et al., 2007); Contains alkaloids, phenols and flavonoids (Coe and Anderson, 1996).	No experimental data found but potentially an abortifacient (Coe and Anderson, 1996; Michel, 2006).
<i>Scoparia dulcis</i> L. (Schrophulariaceae); JM58	Como Escobillo	Lf	Labor pains		Used for gynecological conditions (Barrett, 1994; Coe and Anderson, 1996).	Anti-inflammatory and anti-pain effects (Gupta et al., 2009). In vitro serotonin effects (Hasrat et al., 1997); Sedative effects in rats (Ahmed et al., 2001). Contains alkaloids, terpenes, phenolic compounds (Ahmed et al., 2001; Hayashi et al., 1988). Also betulinic acid, diterpenes, flavonoids (de Freitas et al., 2015; Fuentes et al., 2015).	None found.
<i>Senna reticulata</i> (Willd.) H.S. Irwin & Barneby. [syn. <i>Cassia reticulata</i> Willd.] (Fabaceae); JM30	Barajo	Lf	Dysmenorrhea		Treatment of menstrual pain (Coe and Anderson, 1996)	Phytochemical investigation (Messmer et al., 1968). The anthroquinones-chrysophanol, physcion, aloe-emodin, 1,3,8-trihydroxyanthraquinone, 3-methoxy-1,6,8-trihydroxyanthraquinone, emodin, chrysophanol-10,10'-bianthrone, and the triterpenes α and β -amirin, the steroids β -sitosterol and stigmasterol, as well as the flavonoid kaempferol anthraquinones were isolated from the wood (Dos Santos and Silva, 2008). An aqueous extract showed antioxidant activity and high polypheanol content (Lizcano et al., 2010)	None found
<i>Sida rhombifolia</i> L. (Malvaceae); JM94	Mesbel, mes'b'eel	Lf	Used to reduce labor pains, burning urine		Used to treat gonorrhea, as an emmenagogue, galactagogue, sedative and analgesic (Cáceres, 1996; Duke and Vásquez, 1994). Used to treat dysmenorrhea (Manfred, 1947). Used to speed labor and expel placenta by Q'eqchi in Belize (De Giselle, 2014).	Anti-implantation activities and stimulated rat uterus (Sattawongsakul, 1980). Antiinflammatory and antioxidant effects in vivo (Narendhirakannan and Limmy, 2012) Contains alkaloids, flavonols, sterols, tannins, polypheols and saponins (Assam et al., 2010).	No acute toxicity was demonstrated in mice (Assam et al., 2010).
<i>Smilax domingensis</i> Willd. (Smilacaceae); JM60	Chub Ixim, Cocolmeca	Rh	Night sweats associated with menopause (Michel et al., 2007).		Used to treat sexual impotence, rheumatism, physical weakness (Penna et al., 2003). Night sweats and hot flashes in Costa Rica (Doyle et al., 2009); leucorrhea and venereal diseases (Cáceres et al., 2012b).	Ethanol extract bound to the estrogen receptor and had in vitro estrogen agonist activity in MCF-7 cells (Doyle et al., 2009). Contains sarsasapogenin, smilasaponin, sarsaparilloside, sitosterol and others (Chen, 1999). Anthocyanins, flavonoids and saponins (Cáceres et al., 2012b). Antioxidant activity (Cáceres et al. 2012c)	No subchronic toxicity (2 g/kg, 90 days) was demonstrated (García-González et al., 2008)
<i>Solanum americanum</i> Miller (Solanaceae); JM72	Hierba Mora	Lf	Anemia, dysmenorrhea		Used to treat cervical cancer (Nellis, 1997).	Anti-inflammatory activity in cervix uteri (Nogueiras et al., 2010). Solasonione, solasodine, solamargine, tigotenine (Nogueiras et al., 2010).	Alpha-solamargine isolated from the fresh fruit showed a dose-mortality relationship with a median lethal dose of 42 mg/kg body weight intraperitoneally in rats (Al Chami et al., 2003).
<i>Zebrina pendula</i> Schnizl. (Commelinaceae); JM106	Cha cha	Lf	Dysmenorrhea		Used to treat Gonorrhea, amenorrhea (Cáceres, 1996; Asprey and Thornton, 1955).	Anti-inflammatory activity in vitro (Feng et al. 1962).	None found.
<i>Zingiber officinale</i> Roscoe (Zingiberaceae); JM01	Xinxibeer	Rh	Dysmenorrhea, night sweats		Dysmenorrhea, rheumatism, menstrual irregularities (Arvigo and Ballick, 1993).	Serotonergic effects (Marles et al., 1992; Penna et al., 2003). Contains essential oils, gingerols and other phenolics with antioxidant and anti-inflammatory activities (Park et al., 1998). Anti-inflammatory, and analgesic effects (Ali et al., 2008)	Generally considered as safe, no toxic or teratogenic effect were demonstrated (Ali et al., 2008)

^a Fl=Flower, Lf=Leaf, Rh=Rhizome.

^b Voucher herbarium collection number (Joanna Michel collection #).

^c When combined with other herbs.

in the herbaria both in Guatemala and the USA. Portions of 100–500 g of the used plant part were collected and dried in a solar herb dryer. The dried plant materials were hand milled and approximately 100 g of each sample was extracted at the University of San Carlos laboratory with 500 ml of 95% ethanol. Aqueous extracts of some plant materials were prepared from dried plant materials in Chicago. One of the issues with ethnopharmacology and the testing of medicinal plants is the problem of solvents. Preparation of the ethnomedicines using fresh plant materials as an infusion or decoction tend to be common practice, however aqueous extracts contain high amounts of sugars, that may facilitate the growth of microbes. Thus the ideal situation would allow for collection, identification and pharmacological testing on site, to prevent contamination of aqueous plant extracts. However, since this is not feasible in the jungle, often alcohols such as ethanol or methanol are used for extraction to ensure that bacteria or fungi do not contaminate the plant extracts when they are sent out of the country for testing. The plant extracts from the Q'eqchi Maya were then sent to Chicago and tested in a wide range of bioassays pertinent to women's reproductive health.

3. Estrogenic and progestagenic effects of medicinal plants used in Guatemala

In female reproduction, estrogen (E2) and progesterone (P4) are the key ovarian hormones produced by the developing ovulatory follicle. These sex hormones are involved in all stages of female development and the reproductive cycle including ovulation, menstruation, pregnancy and finally the menopause. During ovulation, the serum levels of these hormones rise from the mid-follicular phase to facilitate the development of the dominant follicle (Berga and Naftolin, 2012). Androgens produced in the follicle theca cells are converted into estrogens by the granulosa cells and then the estrogens are secreted into the follicular fluid. Both E2 and P4 then act consecutively to regulate the concentrations of their respective receptors within the cell, ultimately initiating gene transcription. Numerous events prepare the endometrium for implantation, however in the absence of pregnancy, a decline in P4 levels leads to menstruation and cyclic repair (Critchley et al., 2001).

In terms of ovarian follicle ageing and reproductive senescence, a decline in specific functions of oocytes and granulosa cells (GC), as well as general cellular dysfunction, including a reduction in mitochondrial activity and energy failure induces GCs apoptosis that triggers follicular atresia (Wei et al., 2015; Matsuda et al., 2012). Since GCs are the main source of estradiol, as well as progesterone synthesis in the ovary, a reduction in their number and function leads to reduced levels of endogenous E2 and P4 and hence, menopausal symptoms (Wei et al., 2015). Thus, both E2 and P4 are essential to the female reproductive cycle and any medicinal herbs used for treatment of reproductive health should be screened for E2 or P4 activities.

E2 and P4 binding assays were performed as described in detail in our previously published reports (Doyle et al., 2009; Locklear et al., 2010; Michel, 2006; Michel et al., 2006, 2007). Competitive binding assays were performed to determine if Maya plant extracts displaced the binding activity of radiolabeled (^3H) estradiol or (^3H) progesterone to bind to the estrogen (ER) or progesterone (PR) receptor, respectively. Such activity would indicate the presence of chemical constituents within the plant that had binding affinity for either receptor, and further suggest that the plant may have E2 and/or P4 activities. Of 20 plant extracts tested, 12 bound to both the ER α and β , and 11 bound to the PR (Table 2). These results suggest that these extracts may act as both estrogen and progesterone agonists in vitro. Since premenstrual syndrome

(PMS), dysmenorrhea, fertility, perimenopause and the menopause are all associated with alterations circulating estrogen and progesterone levels, it is logical to hypothesize that traditional therapies used to treat these disorders may have estrogenic or progestagenic effects. Estrogens and progestins are used as hormonal therapy for the symptomatic management of dysmenorrhea, menopause and PMS symptoms (Nelson, 2008). Estrogens, either alone or in combination with progestins are clinically effective treatments for the relief of dysmenorrhea, vasomotor symptoms and have also been shown to relieve vaginal atrophy, prevent the loss of bone mineral density, and reduce fracture risk, including clinical fractures of the vertebrae and hip (Cirigliano, 2007; Gold et al., 2004). In cases of women with an intact uterus, it is necessary to give estrogens in combination with progesterone to avoid the development of endometrial hyperplasia and endometrial cancer (Gold et al., 2004). Thus, this suggests that the extracts used by the Q'eqchi for female reproductive issues have a plausible mechanism of action.

Of the plants tested, *Smilax domingensis*, *Piper hispidum* Sw. and *Cecropia peltata* L. were selected for further follow-up due to their active binding at ER α and ER β . The median inhibitory concentration (IC $_{50}$'s) of these extracts at ER α and ER β are presented in Tables 3 and 4 respectively.

3.1. ER reporter and endogenous gene assays

Of the 27 different plant families used by the Q'eqchi Maya women for reproductive health, some of the most prominent were the Piperaceae and Menispermaceae (Michel et al., 2007). Extracts from *Piper* and *Cissampelos* species bound to both the estrogen and progesterone receptors indicating potential agonist effects (Table 2, modified from Michel et al., 2006, 2007, 2010; Doyle et al., 2009). Active extracts from *Piper jacquemontianum*, *Piper auritum*, *Piper tuerckheimii* and *Cissampelos tropaeolifolia* were then tested in functionalized cell based bioassays including a SEAP reporter gene and by qPCR of ER-responsive gene expression in MCF-7 cells (Fig. 1, Cáceres et al., 2014; Cullen and Malim, 1995; Doyle et al., 2009). In the SEAP reporter gene assay, *P. jacquemontianum* (20 $\mu\text{g}/\text{ml}$) was estrogenic and enhanced E2 effects in MCF-7 cells (Cullen and Malim, 1995; Mahady et al., 2014). *P. tuerckheimii* was not estrogenic alone but significantly enhanced the effects of E2 on SEAP reporter gene expression (Fig. 1). In the endogenous gene assays, both *P. jacquemontianum* and *P. tuerckheimii* altered mRNA expression of E2 responsive genes in MCF-7 breast cancer cells (Fig. 2). *C. tropaeolifolia* (20 $\mu\text{g}/\text{ml}$) was also active (Fig. 2). In vitro data from cell-based assays indicates that these extracts not only bind to the ER, but that they also have a functional effect in that they act by either up-regulating the expression of ER-related genes (direct estrogenic effects) or by down-regulating the expression of ER-related genes (anti-estrogenic effects) in breast cancer cells. Thus, by regulating the hormone profile, the extracts could potentially treat PMS, dysmenorrhea, menorrhagia, and infertility. However, while these data support their use by the Q'eqchi Maya women in Guatemala for reproductive health disorders, is important to recognize that they have not been tested as yet in animal models or in clinical trials, and in vivo data in animal and human studies would be needed before any conclusions can be made (Cáceres et al., 2014). The other issues are the possible potentiation of breast cancer or estrogen-responsive cancers, but these issues would need to be resolved using appropriate animal models. Plant extracts that have an anti-estrogenic effect have a possible use as a chemotherapeutic agent for treating estrogen responsive cancers. Thus, these plants species would be good candidates for drug-discovery of novel anti-estrogens for cancer chemotherapy.

Table 2

Ethanol extracts of Q'eqchi medicinal plants that bound to both the estrogen and progesterone receptors (concentrations 50 µg/ml). Methods as described previously (Michel, 2006; Michel et al., 2006, 2007, 2010; Doyle et al., 2009). Table prepared and modified from Michel, 2006). Plant families, voucher number and species author citations are presented in Table 1.

Ethanol extract of Species	Plant part	% Binding ER α (mean \pm SD)	% Binding ER β (mean \pm SD)	% Binding to Progesterone receptor (mean \pm SD)
<i>A. stachyoidea</i>	Leaves	31 \pm 3.3	36 \pm 4.2	2 \pm 0.5
<i>C. peltata</i>	Leaves	61 \pm 9.8	57 \pm 6.1	34 \pm 4.0
<i>C. tropanolifolia</i>	Leaves	46 \pm 3.1	88 \pm 9.1	34 \pm 2.7
<i>C. tomentosa</i>	Leaves	48 \pm 5.5	52 \pm 4.9	15 \pm 2.0
<i>H. rosa-sinensis</i>	Leaves	43 \pm 4.9	38 \pm 4.0	38 \pm 6.2
<i>H. cuneata</i>	Leaves	56 \pm 4.4	55 \pm 5.6	43 \pm 3.3
<i>P. jacquemontianum</i>	Leaves	49 \pm 3.9	48 \pm 5.1	39 \pm 4.4
<i>P. auritum</i>	Leaves	49 \pm 4.3	42 \pm 3.2	29 \pm 1.1
<i>P. hispidum</i>	Leaves	49 \pm 2.6	76 \pm 4.5	49 \pm 4.3
<i>P. tuerckheimii</i>	Leaves	41 \pm 4.4	47 \pm 5.0	45 \pm 3.6
<i>S. domingensis</i>	Rhizome	67 \pm 6.1	69 \pm 3.4	32 \pm 2.8

*Lf=Leaf; extract 5:1 in 95% ethanol.

4. Serotonergic effects of medicinal plants used for women's reproductive health in Guatemala

In addition to the estrogen receptor, distribution studies have indicated that there is a high abundance of serotonin receptors in the brain that have been associated with the symptoms of PMS and menopause (Medina et al., 2007). Serotonin (5-HT) is involved in various physiological and pathological processes via its interaction with 14 distinct receptor subtypes, grouped in seven classes of receptors (5-HT_{1–7}) on the basis of amino acid sequence, pharmacology, and signal transduction pathways. The 5-HT₇ receptor is a G-protein coupled receptor with seven trans-membrane domains (Medina et al., 2007; Michel et al., 2007). 5-HT₇ receptors are found particularly in the hippocampus, thalamus and hypothalamus, areas of the brain associated with the symptoms of PMS and menopause (Medina et al., 2007). The presence of 5-HT₇ in the hypothalamus also correlates with its involvement in thermoregulation and endocrine function (Medina et al., 2007). Therefore along with compounds that mimic E2 and P4, agonists of 5-HT₇ may be useful for the treatment of menopause and other women's health disorders such as PMS. Of the Q'eqchi Maya medicinal plants collected, 20 extracts were tested in several serotonin receptor-binding assays (Michel et al., 2007). Of the extracts tested, 15 bound to the various serotonin receptors (Table 5). Eleven species were found to bind to the 5-HT_{1A} receptor, with activity defined as greater than 50% binding at a concentration of 50 µg/mL (Michel et al., 2007). At 81% *Psychotria poeppigiana* showed the strongest binding affinity for 5-HT_{1A}. Five species displayed significant binding to the 5-HT_{5A} receptor with *C. tropanolifolia* (94%), *P. poeppigiana* (78%), *P. hispidum* (76%), and *Hibiscus rosa-sinensis* L. (70%) being the most active extracts. Fifteen species also bound to the 5-HT₇ receptor. *P. hispidum* (96%), *Neurolepta lobata* (86%), *P. auritum* (83%), and *Hydrilla verticillata* (89%) showed the greatest binding affinity.

It has further been suggested that 5-HT₇ receptor-selective ligands may prove to be therapeutically useful for the treatment of psychiatric disorders, as well as disorders of circadian rhythms and insomnia, such as seen many menopausal women (Medina et al., 2007). Thus, these extracts may therefore represent novel therapeutic alternatives for the treatment of those disorders in which disturbances in circadian rhythms and sleep architecture are thought to be contributory factors. Finally, there is evidence to suggest that the 5-HT₇ receptor may play a role in other CNS disorders including, anxiety, cognitive disturbances and also

migraine probably via both peripheral and central mechanisms (Medina et al., 2007). Considering the fact that many women suffer from anxiety, depression and sleep disorders during menopause, it is very possible that the Q'eqchi Maya women use these plants for these purposes.

In addition to serotonergic effects, many of the collected plant extracts also inhibited the activity of cyclooxygenase-2 (COX-2) (Table 5; Michel, 2006). COX-2 is a pro-inflammatory enzyme that produces acute inflammation via the eicosanoids and cytokines. The expression of COX-2 by neutrophils results in the synthesis and secretion of inflammatory prostaglandin E₂, which is associated with inflammation and pain (Locklear et al., 2010). In terms of women's health, COX-2 is up-regulated in PMS and is associated with the pain of dysmenorrhea, a problem that has a high prevalence in Latin America, and many Maya women report the use of many plants for the treatment of body aches, labor pains, and dysmenorrhea (Table 1). Using the methodology described previously (Locklear et al., 2010), 13 of the plant extracts tested inhibited the activity of COX-2 (Table 6; Michel, 2006), suggesting a potential use for the treatment of dysmenorrhea, and other inflammatory conditions. As can be seen from Table 1, many of these plants used by the Q'eqchi Maya women for these conditions inhibited COX2, thereby indicating that the plant extracts have in vitro anti-inflammatory effects.

5. ER and serotonin agonist compounds isolated from *Piper hispidum*

The genus *Piper* (Piperaceae) has over 1000 species of herbs, shrubs, small trees and hanging vines distributed throughout both hemispheres (Cáceres and Kato, 2014). Species belonging to this genus are included in the Indian Ayurvedic system of medicine, as well as Maya traditional medicine in Central America (Michel et al., 2010). Extracts of *P. hispidum* leaves were shown to bind to the ER and PR, as well as the serotonin receptors 5-HT_{1A}, 5-HT_{5A} and 5-HT₇. A bioassay-guided fractionation resulted in the isolation of active compounds that were identified as the butenolides 1–3 (Fig. 3). The 9,10-methylenedioxy-5, 6-Z-fadyenolide (1) was identified as a new compound, while 5,6-Z-fadyenolide (2) and piperolide (3) are known compounds that had previously not been reported in *P. hispidum* (Michel et al., 2010). Compounds 1–3 were tested in the 5-HT_{1A} and 5-HT₇ receptor binding assays. Only compound 1 had binding affinity for the two subtypes of receptors with an IC₅₀ of 16.1 and 8.3 µM to 5-HT_{1A} and 5-HT₇, respectively. Using GTP shift assays, compound 1 was found to be a partial agonist of the 5-HT₇ receptor (Michel et al., 2010). These activities are consistent with the Q'eqchi traditional use of this plant for the treatment of disorders associated with the female reproductive cycle. One of the intrinsic problems related to in vitro testing of plant extracts is that in some cases the in vitro results do not translate well into in vivo or human studies. Therefore, in vivo studies are needed to confirm these activities.

6. Recent studies in the field

In recent studies, eight medicinal plants used to treat menopause in Guatemala were collected or re-collected and extracted including *Plantago major* L. (Plantaginaceae), *Wigandia urens* (Ruiz & Pavón) Kunth. (Boraginaceae), *Hamelia patens* Jacq. (Rubiaceae), *S. domingensis*, *Pimenta guatemalensis* (Lundell) Lundell (Myrtaceae), *Phlebodium pseudoaureum* (Cav.) Lellinger (Polypodiaceae), *C. peltata* and *Euphorbia lancifolia* Schldt (Euphorbiaceae) (Cáceres et al., 2014). The plant extracts were tested in a wide range of

Table 3

In vitro binding of ethanol extracts from *S. domingensis*, *P. hispidum* and *C. peltata* to the ER α and ER β . IC₅₀ values were determined by a non-linear, least squares regression analysis. Inhibition constants (K_i) were calculated using the equation of Cheng and Prusoff (1973) using the observed median inhibitory concentration (IC₅₀) of the tested compound, the concentration of radio-ligand employed in the assay, and the historical values for the K_D of the ligand obtained experimentally. The Hill coefficient (nH), defining the slope of the competitive binding curve, was calculated using Data Analysis Toolbox. Hill coefficients significantly different than 1.0, may suggest that the binding displacement does not follow the laws of mass action with a single binding site. Tables prepared using modified data from Michel (2006) and Michel et al. (2007).

Species/Voucher number	Plant part	IC ₅₀ ER α (μ g/ml)	K_i	nH
<i>C. peltata</i>	Leaves	50.0	None	None
<i>P. hispidum</i>	Leaves	50.3	14.4	0.798
<i>S. domingensis</i>	Rhizomes	28.9	8.27	1.15

Table 4

In vitro binding of ethanol extracts from *S. domingensis*, *P. hispidum* and *C. peltata* to the ER α and ER β . IC₅₀ values were determined by a non-linear, least squares regression analysis. Inhibition constants (K_i) were calculated using the equation of Cheng and Prusoff (1973) using the observed median inhibitory concentration (IC₅₀) of the tested compound, the concentration of radio-ligand employed in the assay, and the historical values for the K_D of the ligand obtained experimentally. The Hill coefficient (nH), defining the slope of the competitive binding curve, was calculated using Data Analysis Toolbox. Hill coefficients significantly different than 1.0, may suggest that the binding displacement does not follow the laws of mass action with a single binding site. Tables prepared using modified data from Michel (2006) and Michel et al. (2007).

Species/Voucher number	Plant part	IC ₅₀ ER β (μ g/ml)	K_i	nH
<i>C. peltata</i>	Leaves	31.5	6.49	0.89
<i>P. hispidum</i>	Leaves	51.5	10.6	1.16
<i>S. domingensis</i>	Rhizomes	38.5	7.94	1.33

SEAP Reporter Assay

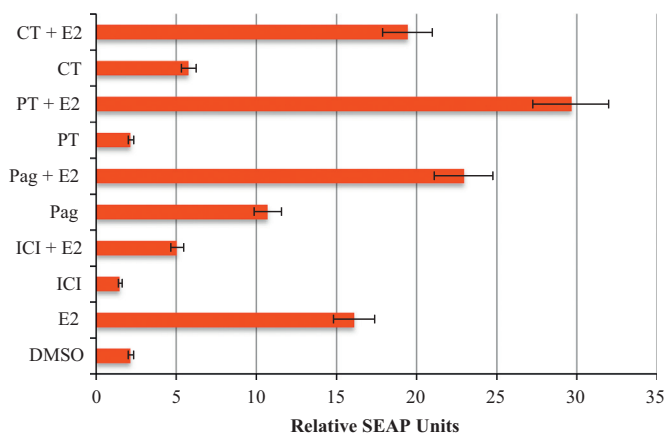


Fig. 1. SEAP reporter activity of *P. tuerckheimii* (PT), *P. jacquemontianum* (Pag) and *Cissampelos tropaeifolia* (CT). Methanol extracts of *P. aeruginosibaccum* (Pag) was estrogenic alone and also enhanced the SEAP activity of E2. *P. tuerckheimii* (PT) was not estrogenic alone, but interestingly, strongly enhance the activity of E2 in MCF-7 cells. *C. tropaeifolia* DC. (CT, 20 μ g/ml) was weakly estrogenic alone and also enhanced the SEAP activity of E2 in MCF-7 cells. Secreted embryonic alkaline phosphatase (SEAP) is a reporter widely used to study promoter activity or gene expression. It is a truncated form of human placental alkaline phosphatase (PLAP) by deletion of the GPI anchor. SEAP supplies and kits were obtained from InVivoGen (San Diego, CA) and performed as described by the manufacturer and methods were as previously described (Patel et al., 2014; Cullen and Malim, 1995).

bioassays including ER α and β and PR binding assays, and functional assays, serotonin (5-HT_{1A}) binding assays. Active extracts were further tested in cell-based SEAP reporter gene assays in transiently transfected MCF-7 cells and endogenous gene

pS2 and PR mRNA expression in MCF-7 cells

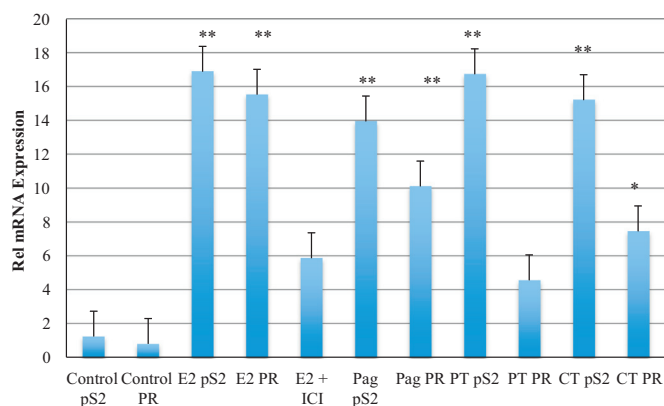


Fig. 2. In vitro expression of ER-responsive endogenous pS2 and PR genes in MCF-7 cells. Methanol extracts of *P. jacquemontianum* (Pag) and *P. tuerckheimii* (PT) altered mRNA expression of E2 responsive genes in MCF-7 cells. *C. tropaeifolia* DC. (CT, 20 μ g/ml) was also active. All three enhance the expression of pS2 and *P. aeruginosibaccum* significantly increased the expression of PR. Statistics were performed using one way ANOVA followed by Tukey's multiple comparison test. * $P < 0.05$; ** $P < 0.01$. Methods and data were as previously described (Doyle et al., 2009; Patel et al., 2014).

expression in MCF-7 cells (Cáceres et al., 2014). Ethanol extracts from five plants bound both to ER α and β (*C. peltata*, *P. major*, *W. urens*, *S. domingensis* and *P. guatemalensis* with an IC₅₀ range 28.9–51.5 μ g/ml); three plants bound to the PR receptor (*S. domingensis*, *P. major*, *P. guatemalensis*) and two bound to the 5HT_{1A} receptor (*H. patens* and *C. peltata*; Cáceres et al., 2014). Interestingly, in a recent study from Mexico it was reported that ethanol extracts from *H. patens* were not toxic in rat models, but had potent antinociceptive effects when administered to rats in doses of 100–200 mg (Alonso-Castro et al., 2015).

In vitro treatment of MCF-7 cells with the *S. domingensis* ethanol extracts (20 μ g/ml) induced SEAP reporter gene expression (using methods published in Cullen and Malim, 1995) in transiently transfected MCF-7 cells, indicating both estrogen agonist and antagonist effects. *S. domingensis* extracts (20 μ g/ml) enhanced the expression of an ERE reporter gene, and the expression of endogenous PR mRNA in MCF-7 cells (Cáceres et al., 2014; Doyle et al., 2009). *P. pseudoaureum* and *E. lancifolia* were not active in any of the test assays (Cáceres et al., 2014). Leaves and fruit of *Brosimum alicastrum* Swingle (Moraceae), a large evergreen canopy tree were collected in Guatemala (Patel et al., 2014). The fruit of the tree is also known as “Mayan bread fruit” or breadnut, and are used for the treatment of menopause and osteoporosis, and are thought to have hormone-like activities. Methanol extracts of the fruit displaced 3H-estradiol binding from the estrogen receptors (ER α and ER β , EC₅₀ 48 and 49 μ g/ml) (Patel et al., 2014). Methanol extracts of the fruit pulp including seeds, and an extract of the leaves (20 μ g/ml) induced a SEAP reporter gene in transiently transfected MCF-7 cells, which was inhibited by the ER antagonist ICI 182,780 indicating estrogenic effects were mediated through the ER (Patel et al., 2014). Conversely, a methanol extract of the fruit pulp alone was not estrogenic as compared with vehicle controls, and reduced SEAP reporter gene activity in a concentration dependent manner, suggesting potential anti-estrogenic effects (Fig. 4). Thus, in keeping with the reported traditional uses to treat menopause and osteoporosis by the Maya, *B. alicastrum* extracts have at least in vitro pharmacological activities that support these traditional uses.

Table 5

Ethanol extracts from Q'eqchi Maya plant species that bound to the serotonin receptors when tested at 50–100 µg/ml. Results and methods are described in Michel et al. (2006, 2007, 2010).

Species	Plant part	5HT _{1A} % Binding ^a	5HT _{5A} % Binding ^a	5HT ₇ % Binding ^b
<i>Arachnothryx stachyoidea</i>	Leaves	51	37	26
<i>Cecropia peltata</i>	Leaves	62	38	81
<i>Cissampelos tropaeolifolia</i>	Leaves	51	94	34
<i>Clidemia petiolaris</i>	Leaves	NT	NT	44
<i>Clidemia setosa</i>	Leaves	NT	NT	47
<i>Henriettea cuneata</i>	Leaves	55	43	NT
<i>Hibiscus rosa-sinensis</i>	Leaves	61	70	NT
<i>Hyptis verticillata</i>	Leaves	NT	NT	89
<i>Justicia fimbriata</i>	Leaves	48	28	17
<i>Neurolaena lobata</i>	Leaves	NT	NT	86
<i>Piper auritum</i>	Leaves	66	68	83
<i>Piper hispidum</i>	Leaves	63	76	96
<i>Piper Jacquemontianum</i>	Leaves	NT	NT	82
<i>Piper tuerckheimii</i>	Leaves	NT	NT	72
<i>Psychotria poeppigiana</i>	Leaves	81	78	67
<i>Senna reticulata</i>	Leaves	NT	NT	48
<i>Smilax domingensis</i>	Rhizome	27	2	16

NT = not tested.

^a Tested at a concentration of 50 µg/ml.

^b Tested at a concentration of 100 µg/ml.

7. Summary and future perspectives

7.1. Efficacy of medicinal plants used by the Q'eqchi Maya women

The purpose of this review was to provide an overview of the pharmacological support for the traditional uses of medicinal plants used by the Q'eqchi Maya from the Lake Izabal region of Guatemala for the treatment of women's reproductive health disorders. In general, Maya women in Guatemala regularly use medicinal plants to treat women's health concerns, yet pharmacological data on their safety and efficacy are largely absent. Review of the literature reveals that plants used by the Q'eqchi for reproductive health exhibited significant in vitro estrogen and progesterone effects, suggesting potential use for the treatment of PMS and menopause; serotonin agonist activities indicating potential use for the treatment of anxiety, depression, PMS, insomnia and menopause; COX2 inhibition indicating a potential use for treating inflammation and pain as seen in dysmenorrhea and labor pains. Thus, the experimental pharmacology supports many of the medicinal plants used by the Q'eqchi for women's reproductive health, and also provides a plausible mechanism of action.

7.2. Correlation of plant species used by Q'eqchi Maya and other groups

Literature reviews of many of the medicinal plants used for reproductive health by the Q'eqchi Maya in Livingston shows that many of these species are also used traditionally in other Central American countries and cultural groups. One example is *C. peltata* (Table 1), also known as Guarumo, or Trumpet Tree. The leaves of this plant are used by the Q'eqchi Maya to expel the placenta after childbirth, lower the womb, and to treat nervousness and insomnia. Other uses among the Maya of Belize and Guatemala and the Paya of Honduras include the use of an infusion of the leaves of this species as a bath for rheumatism, and as a tea to treat nervous conditions, menstrual problems, and to ease the pain of childbirth (Avis et al., 2001; Cáceres, 1996; Cosminsky, 2001; Comerford, 1996; Lentz, 1989). A recent study of traditional medicine for women's health issues among the Q'eqchi of the Toledo District of Belize documented the use of these leaves to quicken labor, and to

Table 6

Cyclooxygenase-2 is a pro-inflammatory enzyme responsible for inflammation and pain that has been associated with dysmenorrhea, PMS and menopause. The data presented in this table are a list of Q'eqchi medicinal plant extracts that inhibited the activity of COX2 in vitro (modified from Michel, 2006; methods Locklear et al., 2010).

Ethanol extract of plant species	Plant part	% Inhibition of COX-2 activity at 25 µg/ml
<i>A. stachyoidea</i>	Leaves	100
<i>C. peltata</i>	Leaves	103
<i>C. tropaeolifolia</i>	Leaves	101
<i>H. cuneata</i>	Leaves	102
<i>H. rosa-sinensis</i>	Leaves	94
<i>J. breviflora</i>	Leaves	100
<i>J. fimbriata</i>	Leaves	98
<i>P. auritum</i>	Leaves	101
<i>P. hispidum</i>	Leaves	99
<i>P. poeppigiana</i>	Leaves	102
<i>S. domingensis</i>	Rhizome	97

treat a difficult labor, when combined with other species (de Gezelle, 2014). In vitro data have shown that extracts of *C. peltata* leaves bound to both the estrogen receptors and the progesterone receptor, indicating potential hormonal activity that would be useful in the management of menstrual problems and for the management of menopause. In addition the extracts from *Cecropia* bound to the serotonin receptors 1A and 7, as well as inhibited the activity of COX-2 indicating its benefits in the treatment of nervousness, anxiety, insomnia and pain, all as cited by the Q'eqchi for its traditional use. However, the majority of this work was in vitro and the safety of the extracts was not assessed and no toxicological studies were performed.

7.3. Piperaceae is the most commonly used plant family by the Q'eqchi Maya

Of all of the plant families recorded, Piperaceae was the family most often mentioned by the Q'eqchi of Livingston, Guatemala for the treatment of women's health ailments (Michel et al., 2006). Interestingly, ethnopharmacological studies with the Q'eqchi of Belize also found Piperaceae to be the most common plant family used for the treatment of neurological and mental health conditions (Bourbonnais-Spear et al., 2005), and women's health conditions (de Gezelle, 2014). Traditional medicinal uses of *Piper* species relevant to this paper that have been supported by in vitro and/or in vivo activity include the use of *Piper betle* L. as an anti-fertility agent (Adhikary et al., 1989); *Piper methysticum* G. Forst. and *Piper chaba* Hunter to relieve depression and anxiety (Pittler and Ernst, 2000; Taufiq-Ur-Rahman et al., 2005); and *Piper kadsura* (Choisy) Ohwi and *P. chaba* Hunter for aches, pains and inflammation (Chiou et al., 2003; Taufiq-Ur-Rahman et al., 2005).

All of the *Piper* extracts tested in an earlier study were active in the ER and weakly active in the PR assays; had good activity in all serotonin bioassays, and strongly inhibited the activity of COX-2 in vitro. In the cellular reporter gene assays and endogenous gene assays, all *Piper* species tested either was directly estrogenic, or enhanced the activity of the positive control, estradiol, all suggesting estrogen agonist effects. Extracts of *P. hispidum* were found to act as agonists of both the ER and 5-HT₇ receptors, and a new natural product, 9,10-methylenedioxy-5,6-Z-fadyenolide, was found to be a 5-HT₇ agonist (Michel et al., 2010). *P. hispidum*, known as puchuk by the Q'eqchi, is used as a tea to treat dysmenorrhea, amenorrhea, menopause and body aches, but is also commonly known as cordoncillo in Nicaragua where it is used to treat aches and pains (Coe and Anderson, 1996; Michel et al., 2010), and in Peru to regulate menstruation (Coe and Anderson,

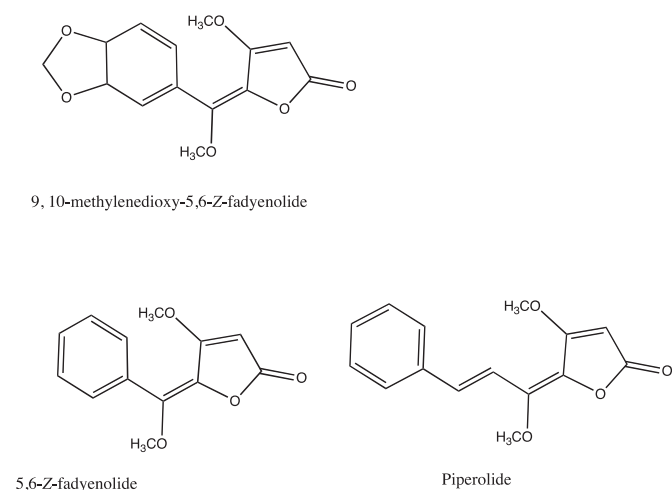


Fig. 3. Structures of new and known butenolides isolated from the leaves of *P. hispidum* (Michel et al., 2006, 2010).

1996; Michel, 2006; Michel et al., 2007). Thus, the in vitro pharmacology fully supported these traditional uses of this plant, and the identification of novel chemical constituents indicates that the use of ethnomedicine and ethnopharmacology can facilitate drug discovery programs.

7.4. Lack of in vivo, human and toxicological studies

Some criticisms of the reviewed work include the fact that most of the bioassays were performed in vitro, and animal studies need to be performed to confirm these works. Also, no direct cytotoxicity studies were performed, however there was no cytotoxicity observed in the MCF cells treated with the plant extracts. While the absence or presence of activity observed from in vitro bioassays does not necessarily correspond directly to in vivo efficacy, or suggest they are safe for human consumption, in vitro assays are a good starting point for most pharmacological studies and can often suggest mechanisms of action (Farnsworth, 1998). Other concerns include the reports that some plants are used to treat amenorrhea (maybe acting as an abortifacient), fertility and labor pains, suggesting a direct effect on the uterus or ovaries, or maybe substances that are toxic to the women or fetus (Farnsworth et al., 1975). Thus, in vivo studies to assess safety (toxicity) and efficacy are essential before plants can be recommended for use. Nevertheless these data do suggest that plants used traditionally to treat women's health complaints by the Q'eqchi Maya of Livingston Guatemala have a plausible mechanism of action and therefore do warrant further animal and human studies.

7.5. Issues of extraction methods for pharmacological studies and drug discovery

Other issues with the studies include the extraction methods that use organic solvents versus traditional infusing or boiling plants in water. Also, investigating individual plants versus the common Q'eqchi practice of using herbal combinations, somewhat similar to traditional Chinese medicine formulas. In terms of extraction procedures it is extremely difficult to use aqueous extractions (boiling or otherwise) due to the lack of facilities for large-scale lyophilization to prevent the extracts from being contaminated with microbes. Extractions with ethanol or methanol are usually a low cost method that are polar enough to extract out compounds that would be seen in aqueous extracts, without the excess of sugars that would be present in aqueous extracts and that would promote microbial contamination of the extracts.

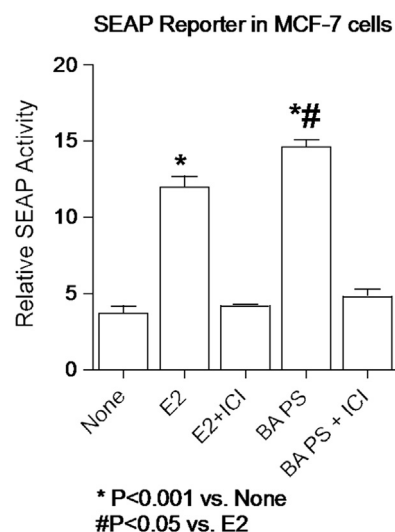


Fig. 4. In vitro SEAP reporter gene activity of *Brosimum alicastrum*. Methanol extracts of the fruit pulp and seeds (BAPS, 20 µg/ml) were estrogenic alone and enhanced the SEAP activity in MCF-7 breast cancer cells. The activity of BAPS was shown to be ER dependent as when MCF-7 cells were treated with BAPS and ICI 182780 (an ER antagonist that promotes degradation of the ER), the activity of the extract was significantly reduced demonstrating that the ER was necessary for this activity. Results and methods were previously reported (Cullen and Malim, 1995; Patel et al., 2014).

Another consideration that is especially important for the isolation and characterization of the chemical constituents, and subsequent pharmacological testing of the extract is to be able to use a solvent that allows for evaporation at low temperatures to prevent the degradation of potential active compounds. Solvent selection is critical if such studies are to be further pursued for drug discovery, as it has been shown that extraction of plant materials with aqueous-organic solvents (such as 80–95% ethanol or methanol) is more effective and efficient means of obtaining high compound yielding extracts (Sultana et al., 2009).

7.6. Ethnomedicine is an important part of overall healthcare

For most indigenous peoples in Guatemala and other countries, healers and ethnomedicines still represent the frontline of primary healthcare for these populations. In Guatemala up to 65% of the population is indigenous, and up to 80% rely on medicinal plants as their primary source of medical treatment (Cáceres, 1996; Villatoro, 1996a). Thus, pharmacological and chemical investigations to determine safety and efficacy of ethnomedicines and traditional healing methods are critically important for the health of not only indigenous women, but also the public health of indigenous communities as a whole. Furthermore, traditional medical knowledge coupled with ethnomedical and pharmacological investigations should be used in public health efforts to determine factors that may contribute to poor health, as well as to identify ways to incorporate safe and effective traditional medical practices into the health care of these communities. This approach can also supply new leads for drug discovery as many of the medicinal plants used by the Maya have never been tested scientifically, nor has much of the knowledge of traditional Maya medicines been documented or published. Thus, documentation, as well as chemical and pharmacological studies could contribute significantly to the overall health of the Q'eqchi Maya, but also may impact other cultures where new therapies are developed.

7.7. Conclusion and future priorities

The results of the reviewed works suggest that there is a basic scientific basis supporting the traditional use of these plants by the Q'eqchi Maya women from the Lake Izabal region of Guatemala. However, the work does not cover all of the Q'eqchi Maya, nor does it address the traditional medicines of the other Maya groups within Guatemala. Thus, some future research priorities should include a collection and compilation of these data, along with investigations of the quality, safety and efficacy of these medicinal products, particularly where they impact pregnancy. Since the Piperaceae is the family most often utilized by the Q'eqchi, it would be a good place to start, and where possible, priority should be given to animal and human studies. Since the Ministry of Health in Guatemala has been proactive in recognizing traditional Maya medicines, data from such studies would significantly contribute to Guatemala's national effort to promote a complementary relationship between traditional Maya medicine and public health services. Implementing such an approach as part of national public health efforts would not only be more effective and less costly than trying to provide everyone with Western medicine, but would likely be much more culturally acceptable for all involved.

Contribution of the authors

All authors contributed to the writing of this review.

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