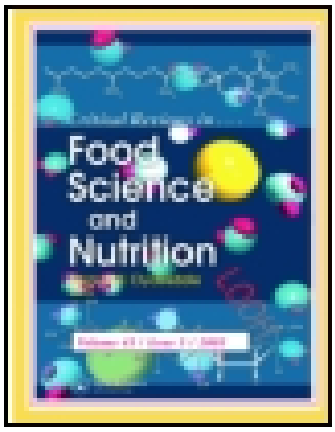

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Phytochemical-mediated Protein Expression Profiling and the Potential Applications in Therapeutic Drug Target Identifications

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ABSTRACT:

Many phytochemicals derived from edible medicinal plants have been investigated intensively for their various bioactivities. However, the detailed mechanism and their corresponding molecular targets frequently remain elusive. In this review, we present a summary of the research works done on phytochemical-mediated molecular targets, identified via proteomic approach. Concurrently, we also highlighted some pharmaceutical drugs which could be traced back to their origins in phytochemicals. For ease of presentation, these identified protein targets were categorized into two important healthcare-related fields, namely anti-bacterial and anti-cancer research. Through this review, we hope to highlight the usefulness of comparative proteomic as a powerful tool in phytochemical-mediated protein target identifications. Likewise, we wish to inspire further investigations on some of these protein targets identified over the last few years. With contributions from all researchers, the accumulative efforts could eventually lead to the discovery of some target-specific, low-toxicity therapeutic agents.

KEYWORDS:

Anti-bacterial, anti-cancer, enzyme, inhibition, medicinal plants, pharmaceutical

1. Introduction

Because of plants limited mobility, they are at a disadvantageous position when faced with threats from herbivorous animals, pathogenic microbial attacks and competition among members of the plant kingdom. To overcome these threats, plants resorted to a variety of physical (thorns, barks) and chemical defenses (Neilson et al., 2013). When faced with biotic and abiotic challenges, many plant species are known to synthesize a variety of phytoalexins, elicitors, antioxidants and assorted phytochemical molecules, to ensure their survival in the harsh environments. These plant-derived metabolites are of diverse chemical nature, including different derivatives of phenolic acids, flavonoids, alkaloids and terpenoids (Schnekenburger et al., 2014). Since the medieval age, these plant extracts, along with the phytochemicals in them, have been utilized for assorted health-promoting and diseasing-curing purposes by different ethnic groups all over the world.

Modern science also turns to medicinal plants and herbs for potential drug candidates, as scientists realize the vast library of phytochemicals beyond our imagination and turn to plants for inspirations of novel organic skeletons. The wide scope of the phytochemical library leads some opinions to believe that plants are much more skillful and efficient organic synthetic chemists than humans will ever be in the foreseeable future. In fact, it was estimated that more than half of the modern pharmaceutical drugs could be traced back to their origins in plants (Newman and Cragg, 2007). For examples, the classic anti-tumor drugs, vincristine and vinblastine, are based

on alkaloid skeletons derived from *Catharanthus roseus* (Madagascar periwinkle), a plant endemic to Madagascar Island in Africa (Facchini and De Luca, 2008), while irinotecan and topotecan, two camptothecin analogs with anti-cancer properties, were based on phytochemicals extracted from *Camptotheca acuminata* (Asian happy tree) (Sriram et al., 2005). The examples go on to include paclitaxel (a commercial anti-tumor drug) originated from diterpenoid extracted from the bark of *Taxus brevifolia* (pacific yew tree)(Kusari et al., 2014) and artemisinin (an anti-malarial bioactive compound) derived from the traditional medicinal plant *Artemisia annua* (Paddon and Keasling, 2014).

2. Scope of Review

Details about many of these phytochemical-mediated therapeutic effects remain elusive, and it is frequently unclear regarding the exact working mechanism. Among the reasons, minute sample quantities coupled with the analytical detection limits have thwarted the efforts to identify which signaling pathways, as well as which genes or protein components are being targeted by these bioactive phytochemicals. The recent advances in proteomic techniques (MALDI TOF-TOF, proteoChip, capillary gel electrophoresis) have overcome some of these challenges and eased the target identifications in these phytochemical-mediated actions (Kim et al., 2009; Lu et al., 2011). Through the combinatorial applications of comparative proteomic and other biotechnology techniques, researchers are able to investigate the cellular protein expression profiles, upon exposure to phytochemicals (Figure 1). By investigating the gene clusters with altered protein expression levels, researchers could start to deduce interlinks in the ever complex

cellular signaling networks. Detailed studies (protein sequencing and database search) on these phytochemical-mediated, differentially-expressed proteins could offer valuable insights of the mechanistic pathways involved. Further research in this direction could potentially lead to the design of target-specific and site-specific therapeutic agents, derived from phytochemicals. It is hoped that these high specificity inhibitors, accompanied by lower effective dosage, could help to evade the toxicity problems faced by many commercial drugs.

Some previous reviews have focused on presenting and summarizing the advances in proteomic technologies, as well as elaborating on how proteomic approach could be used to identify therapeutic protein targets. For this review, we will focus instead on presenting the phytochemical-mediated therapeutic protein targets identified over the last few years, in two important healthcare-related fields: namely cancer therapy and pathogenic bacteria inhibition. It is hoped that this review would lead to increasing interests and further studies to elaborate on how these protein targets, may they be cancerous or bacterial proteins, are affected by phytochemical-derived bioactive compounds. With the extra knowledge gained, it is possible that some of these phytochemicals or their derivatives could further be developed into target-specific anti-tumor drugs or novel antibiotics.

3. Anti-bacterial Research Background

Among the different scientific research fields, one of the major concerns which continuously drawing the attentions of modern sciences is bacterial inhibitions. Members of the scientific research community are interested in bacterial inhibition research, as well as the

applications of anti-bacterial materials, because of their potent impacts on our life qualities and the promising commercial applications. The explorations of new antibacterial molecules, and their potential utilization as new classes of antibiotics, are of great interests to scientists in the pharmaceutical and medical fields (Cragg et al., 2014). This is especially so with the current emergence of multidrug-resistant bacterial strains which render many of the existing antibiotics ineffective (Gardete and Tomasz, 2014). The situation is grimmer if taking into account the decreasing antibiotic candidate in the pharmaceutical development pipeline. In view of this situation, increasing researches are aiming toward the identification of potentially useful antibacterial compounds or materials, especially those which specifically target critical enzymes in bacterial pathways (Machowski et al., 2014; Smith et al., 2013).

Currently, among the commercially available antibiotics, many function by targeting critical bacterial pathways such as bacterial cell wall biosynthesis, nucleic acid biosynthesis and protein translational machinery (Figure 2). Well known examples include penicillin, which is considered among the first available antibiotics and targets the bacterial cell wall biosynthesis. Representatives of other commercial antibiotics include but not limited to rifampin, sulfonamide, vancomycin and tetracycline which function by targeting the bacterial RNA elongation (Weiss et al., 2012), dihydropteroate synthase (Yun et al., 2012), cell wall biosynthesis (Gardete and Tomasz, 2014) and protein synthesis (Nguyen et al., 2014), respectively. Among these different anti-bacterial targets, protein translational machinery is a promising one, as the protein biosynthesis is critically essentially to the bacterial survival and highly conserved among different species of bacterial strains. Moreover, human and bacterial protein translational machinery are phylogenetically and structurally different among each other. Hence,

phytochemicals which target bacterial protein translational system would tend to be more selective with lower drug toxicity against mammalian system, while possessing broader inhibition spectrum against different species of bacterial strains.

3.1 Phytochemical-mediated Bacterial Protein Targets

Because of the diverse organic skeletons found in the phytochemical library, interests are mounting to screen and identified potential phytochemical structures with bacterial inhibition activities. Beside the traditional anti-bacterial targets mentioned above, other unconventional bacterial inhibition targets such as bacterial tyrosine kinases, are being explored for their applications as antibacterial targets, as tyrosine phosphorylation has been indicated with functional roles in bacterial physiology and virulence (Cozzone, 2009). In particular, bacterial tyrosine phosphorylation was reported to control the synthesis of surface polysaccharide, an important component during bacterial infection (Maeda et al., 2008). In addition to bacterial endogenous protein interaction networks, efforts are also directed toward the elucidation of the protein-protein interaction (PPI) networks between pathogen-human proteins, in the anticipation to identify potential new drug targets (Zoraghi and Reiner, 2013). Here, the authors highlighted the significance of utilizing assorted approaches (computational, pull-down, yeast two-hybrid) to construct global PPI networks which map out possible human proteins targeted by invading bacteria. It is hoped that these efforts could lead to the discovery of bioactive phytochemicals which target novel bacterial essential pathways, and render those multidrug-resistant bacterial strains vulnerable.

Previously, many of the phytochemicals are known for their bacterial inhibition activities, though the molecular mechanism frequently remains unknown. *Rhodomyrtus tomentosa* [Aiton] Hassk. (Downy rose myrtle) is a medicinal shrub originated from Southeast Asia. The leave extract from *R. tomentosa* had been reported with bacterial inhibitory activities (Limsuwan et al., 2009). Specifically, its rhodomyrtone (a xanthene derivative) was pinpointed as the potent phytochemical with anti-bacterial activity, parallel with inhibition potentials of commercial antibiotics. For instance, rhodomyrtone was reported with an MIC value of 0.39-0.78 µg/ml against MRSA strain, comparable to that of the commercial vancomycin (MIC: 1.25 µg/ml)(Limsuwan et al., 2009). Later, through the uses of proteomic approaches, differentially-expressed bacterial protein targets were identified, upon exposure to rhodomyrtone. Among these bacterial proteins identified, the groEL protein (60 KDa chaperonin), a protein involves in prevent misfolding and promotes refolding under stress condition, is especially noteworthy (Limsuwan et al., 2011). In addition, the reduced expression levels of exotoxin C and CAM factor, two bacterial virulence factors involved in pore-forming and early onset of infection symptoms, were reported in this rhodomyrtone-mediated bacterial protein profile study (Limsuwan et al., 2011). Based on this information, it is tempting to speculate that the rhodomyrtone may exert its antibacterial activity, partially by encouraging the aggregation of critical bacterial proteins, as well as reduce the virulence factor expressions.

At the meantime, *Melastoma candidum* D Don (Melastomataceae family) is a medicinal plant distributed in the tropical and sub-tropical areas. The extracts from this plant were used

traditionally for toxic cleansing, treatments of traumatic injury and for eliminating stasis (Y. C. Wang et al., 2008). Furthermore, *M. candidum* extracts had also been tested with antibacterial activities (Y. C. Wang et al., 2008; Wong et al., 2013), though the bacterial inhibition mechanism remains unknown. Recently, the methanolic extract of *M. candidum* had been investigated for its effect on bacterial protein expression profiles. From the study, five differentially expressed bacterial proteins were discovered. Among them, the down-regulated elongation factor-Tu (EF-Tu), glutamate decarboxylase 1(Guzman et al.) and α-hemolysin were especially noteworthy (Wong et al., 2014). During bacterial ribosomal protein translation, EF-Tu interacts specifically with tRNAs in the elongation step. It is possible that the bioactive phytochemicals from *M. candidum* extract may exert their inhibitory actions via the bacterial protein translational pathway. Significantly, *M. candidum* extract was found to demonstrate inhibition activities against both Gram-positive and Gram-negative bacterial strains. This is of clinical importance, as it is generally more challenging to inhibit Gram-negative bacterial strains, due to the presence of outer membranes (Delcour, 2009) and efflux pumps (Nikaido, 1998). Hence, *M. candidum*-derived bioactive phytochemicals could potentially be developed into broad-range, target-specific antibacterial agents.

On the other hand, glutamic acid decarboxylase (GAD) has been implicated as the enzyme utilized by certain bacteria to counteract the influx of proteins from acidic environment (Gale and Epps, 1944). This property may help to enable the survival of enteric bacteria, such as *E. coli*, to evade the acidic environment of animal stomach. In this same study, the level of α-

hemolysin, a secreted bacterial toxin, was reduced in the culture medium, upon exposure to *M. candidum* extract. As α-hemolysin was reported as the bacterial toxin which involved in bacterial virulence and lysis of immunological-significant white blood cells. This observation has potential clinical importance. Previously, different research groups have reported on the observations that certain phytochemicals could synergistically enhance the inhibitory potentials of antibiotics (Hwang et al., 2013; Langeveld et al., 2014; S. Y. Wang et al., 2014). It is tempting to speculate that some of these synergistic phytochemicals may function in part by reducing the bacterial virulence, via the down-regulation of the α-hemolysin expression level. Further comparative proteomic works are needed to determine if this is the case or not.

Callicarpa formosana (beautyberry) is a flowering medicinal plant whose aqueous extract was previously reported with antibacterial activity (Wong et al., 2013). However, its bacterial inhibitory mechanism remains unclear. Recently, proteomic study has reported on the *C. formosana*-mediated down-regulated bacterial enzymes (triacylglycerol lipase, N-acetylmuramoyl-L-alanine amidase), upon exposure to *C. formosana* methanol extract (Yong et al., in press). The later amidase is especially noteworthy, as it was previously reported by others as a bacterial enzyme with functional role in the biosynthesis of peptidoglycans, an important building component in bacterial cell walls (Machowski et al., 2014). Nevertheless, further work is still needed to pinpoint which phytochemicals in the *C. formosana* extract are exactly responsible for this phenomenon. At the meantime, physiologically-stressed bacterial cells tend to elevate the expressions of those genes involved in oxidative stress defenses, to ensure the

survival of bacterial cells in the adverse environment. Plumbagin (a naphthoquinone derivative) is an anti-bacterial toxin originally isolated from the plant genus *Plumbago*. Exposure to plumbagin was previously reported to induce the expression levels of bacterial superoxide dismutase (SOD) and thiol peroxidase, two bacterial genes involved in oxidative stress defense (Chen et al., 2006). Investigation on phytochemicals which target bacterial defensive enzymes, such as amidase and SOD, could potentially be rewarding.

3.2 Perspectives

The coupling effects of dwindling antibiotic development in the pharmaceutical pipelines with the emergence of multi-drug resistant bacterial strains have steered the urgency of search for novel antibacterial agents. Many medicinal plants have previously been reported with bacterial inhibition activities, though frequently their mechanisms remain unclear. Illustrating using the aforementioned examples, we hope to highlight the plausibility of using comparative proteomic approach to identify bacterial proteins and signaling pathway targeted by antibacterial phytochemicals. However, the comparative proteomic approach alone is insufficient to lead to the discovery of novel antibacterial agents. For instance, in the observed down-regulated EF-Tu expression level, it is inconclusive to determine if bioactive phytochemicals from *M. candidum* is functioning similarly as kirromycin, a commercial antibiotic functions by targeting the bacterial EF-Tu.

Certain multidrug-resistant bacterial strains rely on drug efflux pumps, which transport the antibiotics out of the bacterial cells and render these bacterial cells resistant to antibiotic

treatments. Because of their clinical importance, much efforts have been focused on searching for novel phytochemical skeletons which could function as efflux pump inhibitors (plumbagin, shikonin)(Ohene-Agyei et al., 2014). Here, the expression level of bacterial efflux pumps may not be altered, upon exposure to these efflux pump inhibitors. In this or other similar situations, whereby the expression levels of the protein targets are not altered significantly, comparative proteomic approach may not be applied effectively for target identifications. In the aforementioned scenario, alternative biochemical assays, combined with activity-based assays, are necessary to shed some light on the phytochemical-mediated antibacterial mechanism.

4. Anti-cancer Research Background

Cancer represents a collective group of complicated diseases whereby the cells grow and divide in an unregulated manner. Despite decades of research and pharmaceutical efforts, the death incidence of cancer was still enormous. In the year of 2012 alone, cancer-related death was estimated to be 8.2 millions, with lung cancer (19%) leading the statistics (GLOBOCAN, 2012). The mechanism and causes of cancers are complex, and efforts are still on-going to shed light onto the possible links, causations, genetic predisposition, as well as the possible therapy (Figure 3). Among the many possible causes, accumulated mutations, as a result of oxidative damage by free radicals and adverse environment factors, have been popularly linked to the initiation of cancers. Likewise, defects in apoptosis intrinsic and extrinsic pathways are frequently implicated as the triggering events in cancer formation (Ghobrial et al., 2005; von Schwarzenberg and Vollmar, 2013).

Medicinal plants, rich with their secondary metabolites, are promising reservoir of diverse phytochemicals with potential anti-cancer activities. This scope is further encouraged by the fact that many of the currently available anti-cancer drugs introduced by the pharmaceutical industry could be traced back to their origin in phytochemicals. Classic examples of these phytochemical-derived commercial therapeutic agents include but not limited to paclitaxel, vinblastine and etoposide (Bhanot et al., 2011). Simultaneously, many other phytochemicals have been reported to inhibit proliferation and induce apoptosis in cancer cells (Chahar et al., 2011; Gescher et al., 1998). To illustrate the point, purified alkaloid extract derived from *Scutellaria barbata* has been shown to induce apoptosis of cultured human liver cancer cells (HepG-2 cells) (Wang et al., 2011), while aqueous extract of highland fern *Phymatopteris triloba* was reported with anti-proliferative activities against HeLa and K562 cancer cell lines (Chai et al., 2013). However, despite these apparent anti-cancer activities, the exact mechanism of these phytochemical-induced apoptosis remains unclear, and many fundamental questions remain to be answered. For instance, which cancer pathways or enzymes are being targeted by these phytochemicals, do these phytochemicals induced cancer cell death via stimulating of apoptosis intrinsic and extrinsic pathways, or via inhibiting of critical cancer cellular enzymes. It is hoped that by answering these questions, scientists could have a clearer picture on how these apoptotic phytochemicals exert their anti-proliferative roles. Undeniably, the advances in the mechanistic understanding could ease the potential commercial conversion and development of these phytochemicals into the next generation anti-cancer therapeutic agents.

4.1 Phytochemical-mediated Cancer Protein Targets

Currently, accumulating researches have been directed towards the study of how these apoptotic phytochemicals affect cancer cellular protein expression profiles. To illustrate the point, maslinic acid is a natural pentacyclic triterpene, frequently found in olive oil and medicinal plants such as *Aster yunnanensis* and *Eugenia gustavioides*. Maslinic acid and its derivatives had been reported by many research groups with apoptotic activities against different cancer cell lines (Juan et al., 2008; Lin et al., 2014; Parra et al., 2011; Bianka Siewert et al., 2013); however, the exact mechanism remains elusive. Through comparative proteomic study, it was reported that in maslinic acid-treated cancer cells, the expressions of dUTPase and stathmin (two cancer proteins involved in inducing early S and G2 cell cycle arrest) were down- regulated (Yap et al., 2012). Moreover, proteomic study by a different research group has further demonstrated that maslinic acid interfered with cytoskeleton protein expression (Rufino-Palomares et al., 2013). In this particular study, fourteen differentially-expressed cytoskeleton proteins were reported for the first time, upon exposure of colon cancer cells to maslinic acid. On the other hand, maslinic acid-induced apoptosis had been correlated with the activation of caspases and c-Jun N-terminal kinase (Reyes-Zurita et al., 2013). Furthermore, a recently reported study linked maslinic acid-induced apoptosis to the alterations and the eventual membrane damages on ovarian cancer cells (Bianka Siewert and Csuk, 2014). Efforts have also including study done with synthetic maslinic acid derivatives with higher selectivity and lower cytotoxicity (B. Siewert et al., 2014). Similarly, using human kerotinocytes as a model, the *Mucuna pruriens* (Fabaceae family) leaf extract was

investigated for its chemopreventive potential. Here, upon exposure to the *M. pruriens* extract, comparative proteomic approach pinpointed a collection of genes (T-complex protein 1, protein disulfide isomerase stress-induced phosphor protein 1) with reduced expressions. As these aforementioned proteins are frequently associated with stress response and protein oxidation, the research finding highlighted the promising role of *M. pruriens* extract as preventive agent of skin cancer diseases (Cortelazzo et al., 2014).

Meanwhile, proteomic study using fractionated extracts from *P. triloba*, a tropical fern species previously reported with cytotoxic activities (Chai et al., 2013), also lead the identification of differentially-expressed Heat Shock Protein-90 (HSP-90) in cancer cell culture (Wong and Chai, unpublished data). HSP-90 was previously reported as one of the four main classes of heat shock proteins, with functional role as 'molecular chaperone' and is critically essential for cancer cell survival (Albany and Hahn, 2014). Additionally, the importance of HDAC-HSP90 interplays in cancer cells has further highlighted the significance of HSP-90 inhibitors in chemotherapy (Krämer et al., 2014; Vahid et al., 2015). The attractiveness of HSP-90 as an anti-cancer target has lead to many studies focusing on identifying potential candidates of HSP90-specific inhibitors (Li et al., 2012). In two separately reported *in silico* docking and molecular dynamic simulation studies, three dietary phytochemicals (sesamin, matairesinol, resveratrol)(Singh and Konwar, 2014) and one natural phytochemical (taxifolin)(Verma et al., 2012) were suggested with inhibitory properties against HSP-90. Concurrently, a few synthetic and natural product-based small molecules (AT13387, 17-AAG, ganetespib) are under different

phases of clinical trial studies to evaluate their applications as HSP90-specific inhibitors in chemotherapy (Albany and Hahn, 2014). It is interesting in further study to determine if fractionated *P. triloba* extracts contain similar phytochemical skeletons as these aforementioned HSP90-specific inhibitors.

Traditional ethnomedicine has always been a promising and potentially rewarding reservoir to comb for useful phytochemicals. Previously, *Isodon rubescens*, a traditional medicinal herbs used for treating respiratory and gastrointestinal disorders, were found to demonstrate anti-neoplastic activities, and oridinin (a diterpenoid) has subsequently been identified as one of the main bioactive phytochemicals in *I. rubescens* (Sun et al., 2006). Comparative proteomic work has further identified eight differentially-expressed hepatocarcinoma proteins, upon exposure to oridinin. Excitingly, among these eight cancer proteins, chromobox protein homolog 1 (HP1 beta) and glycyl-tRNA synthetase (GlyRS) had further been pinpointed with functional roles in oridinin-mediated inhibition of telomerase and tyrosine kinase, respectively (H. Wang et al., 2011). These findings coincided with an earlier reported inhibition of cancerous tyrosine kinase by oridinin (D. Li et al., 2007). Similarly, in an anti-tumor study using *Rhizoma paridis*, a medicinal herb traditionally consumed for treatment of hepatopathy, the total saponins had been tested and lead to the identification of hepatocarcinoma proteins with altered expression levels (Cheng et al., 2008). Among the twelve differentially-expressed proteins identified in this study, the up-regulated DNase gamma and down-regulated heterogeneous nuclear ribonucleoprotein K (hnRNP K) were particularly

noteworthy, as the former protein is an enzyme implicated with functional role in DNA fragmentation of cancerous cell during apoptosis; while the latter protein was recently reported to be involved in cancer progression and tumorigenesis (Barboro et al., 2014). Co-incidentally, in a separate study using azactidine and decitabine (two DNA methyltransferase inhibitors used to treat leukemia), the expression of hnRNP was down-regulated by these two aforementioned methyltransferase inhibitors (Buchi et al., 2012). Currently, it remains unclear if the total saponins isolated from *Rhizoma paridis* contains phytochemicals with functional roles closely related to azactidine and decitabine, especially in view of the observation that hnRNP was down-regulated by these compounds. The significance of hnRNP as a promising anti-tumor target is further illustrated by a mechanistic study using camptothechin (an anti-tumor phytochemical isolated from *Camptotheca acuminata*)(Manita et al., 2011). In this study, the direct interaction between camptothechin and hnRNP A1 was demonstrated, using a T7 phage display work.

Similarly, one step further, comparative proteomic works have also been performed using HeLa cytosolic and membrane fraction proteins, upon exposure to tubeimoside-1 (a triterpenoid saponin) isolated from *Bolbostemma paniculatum*, a medicinal herb previously reported with anti-tumor activity. Here, tubeimoside-1 was found to induced cell cycle arrest at G2/M phase, presumably by inhibiting the expression levels of selected cyclins, as well as interfering with the post-translational modifications (Xu et al., 2011). On the other hand, CIL-102 (an alkaloid derivative) was linked specifically to inhibit cancer cells proliferation by up-regulating the

cellular level of fumarate hydratase (a tumor suppressor), possible via modulating of the c-Jun N-terminal kinases (JNK1/2) and mTOR signaling pathways (Teng et al., 2013). The functional roles of JNK are complex and different among carcinomas (Bubici and Papa, 2014), with implication in multi-drug resistance during chemotherapy (Zhan et al., 2013). It is interesting for future works to test if CIL-102 function in a similar way as the known JNK inhibitor (SP600125 anthrapyrazolone) or via some other unknown pathways. The studies on phytochemicals with JNK-inhibition activities are of clinical importance, as JNK has been reported in the maintenance of stem-like tumor cells (Matsuda et al., 2012).

4.2 Perspectives

The cancer study is a complex field, with perplexed and interlinked mechanistic pathways. Frequently, scientific attempts to understand tumor developments are impeded by the vast information encoded in human genomes, the presence of allelic variants, and the sometime unchartered epigenetic frontiers. The situation is complicated further by the unalike cellular responses toward similar chemotherapeutic agents, among different types of carcinomas, as well as the multi-drug resistance phenomenon observed in chemotherapy. The occurrences of transient post-translational modifications, as frequently present on cancerous proteins, lead to additional challenges in cancer research. Frequently, these covalent modifications play critical roles in cancer development; however, their transient characteristics render it easier for them to evade the analytical detection. Nevertheless, with advances in proteomic methodology and instruments, these challenges could be overcome. Coupled with protocols such as phosphate

protein staining, flow cytometry analysis, and RNA interference (RNA_i) screening, comparative proteomic approach could continue to prove its worth in the efforts to identify phytochemical-mediated cancer proteins and their potential conversion into therapeutic drug targets.

5. Concluding Remarks

Currently, because of the therapeutic potentials and the diverse molecular structures of phytochemicals, many efforts have been diverted into the studies of various medicinal plants and their assorted bioactivities against different disease models. However, in many instances, no further effort is being reported in term of the molecular targets of these phytochemicals and the corresponding mechanistic studies. Through this review, we hope to highlight the potential usefulness of comparative proteomic as a powerful tool in phytochemical-mediated protein target identifications, may they be bacterial proteins or cancerous proteins. Furthermore, by reviewing the different phytochemical-mediated antibacterial and anti-cancer protein targets reported over the last few years, this review wishes to inspire further investigation on some of these protein targets identified via proteomic approach. On the other hand, comparative proteomic approach is not flawless. As mentioned previously, it is of no surprise for some low abundant proteins and some transient post-translational modifications to evade detection by proteomic approach alone. Likewise, certain membrane proteins with poor solubility could be challenging targets to detect using proteomic approach. With accumulative efforts from various research groups, it is hoped that comparative proteomic approach, combined with other techniques (X-ray crystallography, *in*

silico virtual screening, molecular docking study, RNA_i screening), could lead to the discovery and validation of target-specific, phytochemical-derived therapeutic agents.

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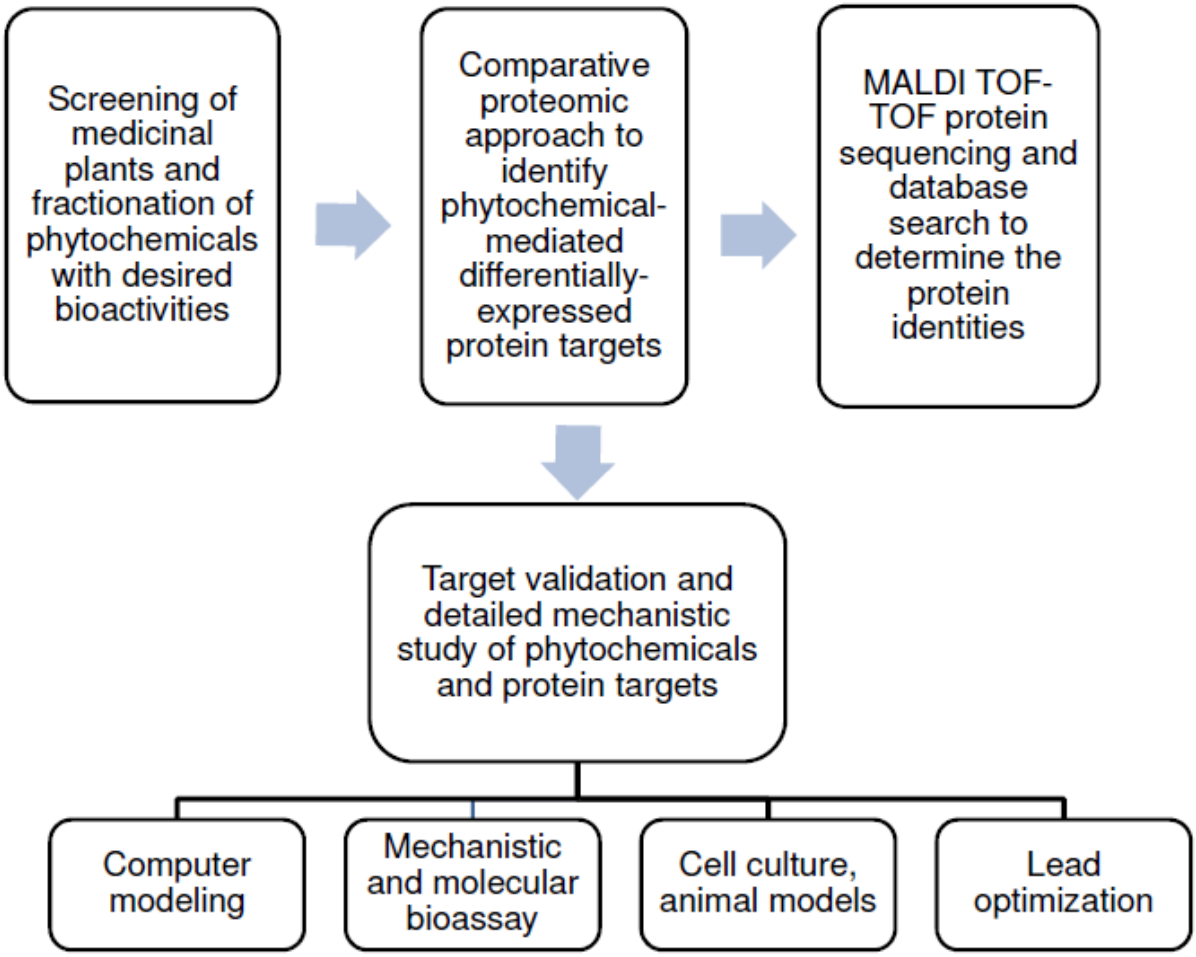

Figure 1. Flowchart for identification of phytochemical-mediated protein targets, along with the possible subsequent studies.

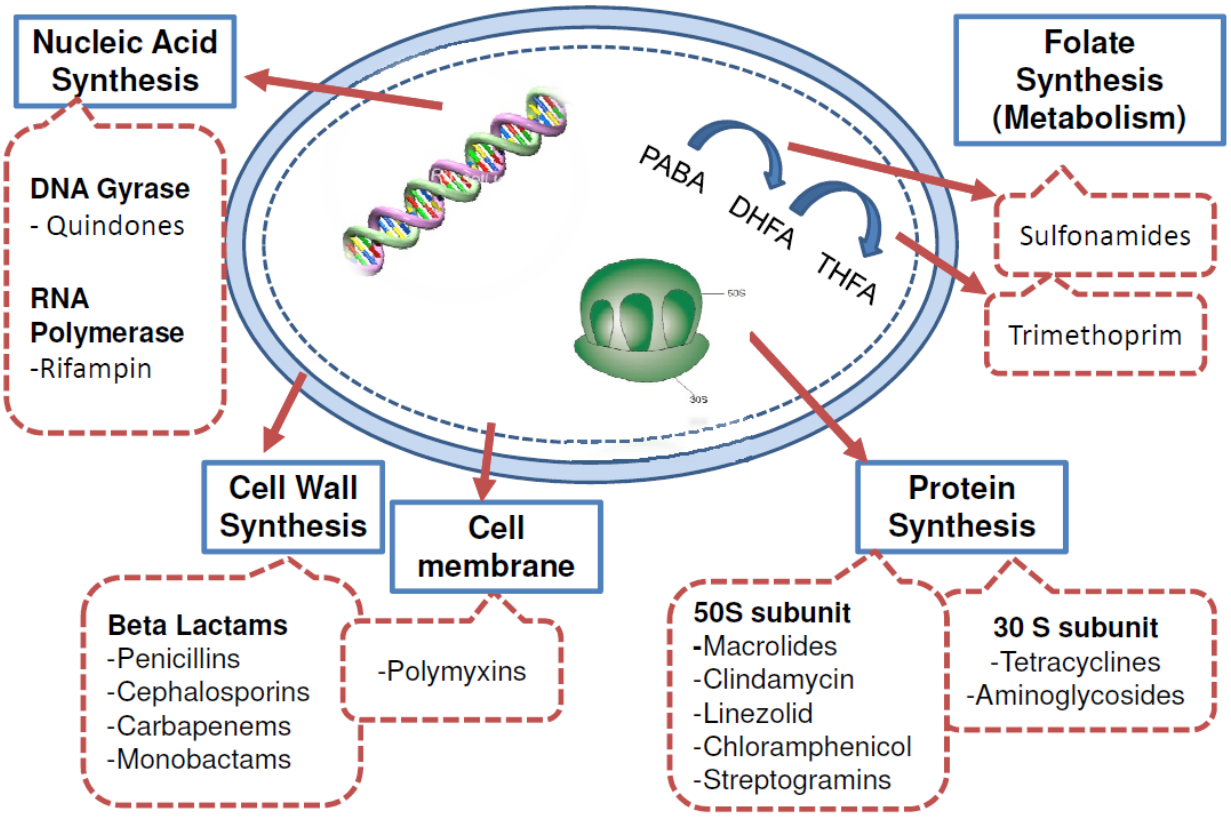

Figure 2. Critical bacterial pathways frequently targeted in antibiotics development. Examples of commercial antibiotics targeting each of these pathways are listed.

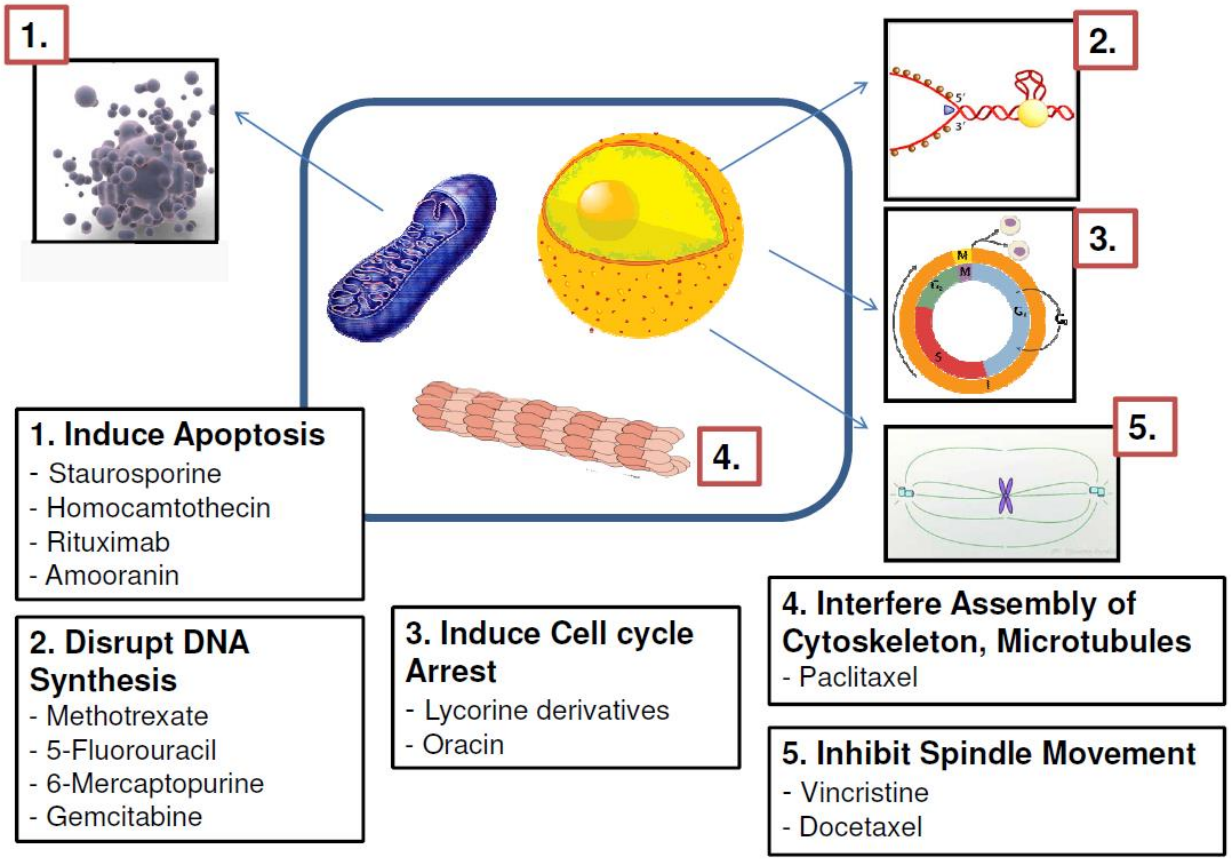

Figure 3. Anti-cancer strategies employed in chemotherapeutic drug development. Examples of chemotherapeutic agents corresponding with each of these strategies are listed.