Chapter 1
Phytochemicals as Sources of Drugs

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1.1 Introduction

Plants have been used as medicines since ancient times, due to the presence of numerous phytocompounds that helps them to prevent and cure several diseases and disorders. They are utilized in different formulations such as herbal tea, extracts, decoctions, infusions, tincture or powder (Balick and Cox 1997; Thomas et al. 1999; Samuelsson 2004; Ujah 2019). Initially, humans started to utilize plants as food which was later segregated as medicinal plants with definite pharmacological action (Howes 2018). In the past, the methods for the application of a medicinal herb for certain ailment were mainly based on the history of the plant which was recorded in herbals. It has been reported from cave carvings and literatures that the medicinal plants are widely used in ancient health care systems, such as Ayurveda, traditional Chinese medications and several ancient medications from ancient civilizations

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including Mesopotamia, Persia, Sumeria, Egypt and Arab, as a source of medicinal drugs for the treatment of diseases. In Ayurvedic literatures such as Charaka Samhita and Sushruta Samhita of the first millennium BC, more than 700 medicinal plants are listed as a source of drugs (Shakya 2016) and about 7500 plants are estimated to be used in ancient Indian villages as local health traditions (Sarker et al. 2007). Likewise, traditional Chinese medical systems also utilized plants as a drug source for disease treatments, for more than a thousand years. It is noteworthy that China has 10% of the world's plant species which portrays their rich biodiversity. This diversity of plant species in China led to the publication of an ancient encyclopedia called Compendium of *Materia Medica* in 1593 which reports ~1000 species that are used in traditional Chinese medicinal practices (Yang et al. 2016a). Other olden civilizations, throughout the world, also have reported to have deep knowledge on using plants as medicinal source (Petrovska 2012).

Plants possess the ability to secrete secondary metabolites as a result of their metabolism, which is extracted as phytochemicals. This class of phytochemicals is required by plants for protection and maintenance but is not considered essential nutrients (Egbuna and Ifemeje 2015). These phytochemicals from medicinal plants are the source of a wide variety of modern natural drugs. Traditional medical systems possess enormous positive attributes to cure diseases; however, they utilize crude phytochemicals which may contain cytotoxic compounds (Thomas et al. 1999). Moreover, the developments in the extraction, purification and characterization of phytochemicals have opened doors for the production of several medicinal drugs which is better than traditional synthetic and conventional drugs. There are wide drug varieties of medicinal plants that are irreplaceable, even with the modern synthetic medicines. Furthermore, drugs from medicinal plants are highly bioactive, biocompatible, and bioavailable (Saraf 2010). The latest in-silico techniques as well as biomedical tools helps to bring out the maximum medicinal potential of plant extracts to make them as a significant drug source (Lagunin et al. 2014).

A plant is classified as a medicinal plant, when the whole plant, part of plants, their metabolites (crude extracts) or its purified and separated phytocompounds possess ability to cure diseases or reduce disease complications. There are numerous plants that are listed to contain medicinal properties with the help of traditional medical literatures and recent extensive researches in this field. The most important aspect of medicinal plant is their diverse availability for the large-scale production of drugs to match the growing population of patients with specific diseases (Naresh and Nagendraswamy 2016). It is noteworthy that various medicinal plants are obtained from wild forest and not from urban landscapes. Thus, medicinal gardens are gaining focus in recent times among botanists to make medically beneficial plants available for public usage in cities (Furlan et al. 2016). Pharmaceutical companies handle the need of large quantity of plants for scale-up production via systematic cultivation. High yielding medicinal plants are selected for a systematic cultivation process which serves as a source for natural drug products (Kumar et al. 2014). Organic farming was also recently introduced to avoid the interaction of fertilizers, pesticides or other chemicals while extracting the medicinal phytochemicals from the plants (Kala 2015). The phytochemicals with medicinal properties are extracted from the plant parts such as stem, root, bark, leaf, fruit, Heartwood and exudates. Literatures suggested that the

quantity and type of phytochemicals that are extracted from the medicinal plants depends on the part from which they are obtained. Common phytochemicals such as essential oils, phenols, terpenoids, carotenoids, xanthophyll and flavonoids are present in plants which are extracted as a drug source for several diseases (Xiao 2015). Apart from common phytochemicals, unique species related phytocompounds namely andrographolides, amaranthine, allicin and various other exclusive drug components (Susantiningsih et al. 2012; Zhang et al. 2015). All these phytochemicals were proved to process excessive biomedical applications which are formulated as medicinal drugs for long lasting effects and enhanced disease treatments.

1.2 Drug Discovery Process

Drug discovery from natural products has brought about the isolation of valuable molecules. There are many examples of drugs isolated from plant sources, poppy seeds (seeds of *Papaver somniferum*), the source of morphine alkaloid which was isolated in the nineteenth century, avermectin the antiparasitics drug, quinine and artemisinin, which are used as antimalarials, lovastatin and its analogs that are used for lipid control, cyclosporine and rapamycins which are used as immunosuppressants in case of organ transplantation, paclitaxel and irinotecan the famous anticancer drugs (Harvey 2008). Nowadays, drug discovery is not only based on the isolation of active constituents, but also involves other techniques such as the high-performance liquid chromatography (HPLC) and standardization of herbal medicines using a marker compound (Newman et al. 2000; Butler 2004; Samuelsson 2004).

Drug discovery always begins with the collection and authentication of the selected plant (Fig. 1.1), and this should be done by a botanist, ethnobotanist, ethnopharmacologist, or plant ecologist. The plants of interest are either those species with known biological activity or used traditionally by man and thus their active compounds are required to be isolated. This approach depends on the attempt that the natural compounds purified from such herbs are probably more reliable than those gotten from plants having no history of human use (Katiyar et al. 2012). On the other hand, the collection may involve taxa that were not examined before and are randomly collected for a large screening program. In this respect, the intellectual property rights of the country from which the plants were collected must be protected (Baker et al. 1995). The second step involves the preparation of different plant extracts by the phytochemists and using the relevant pharmacological assays for the biological screening of these extracts. The third step is the purification and structure elucidation of the active compounds through bioassay-guided fractionation (Balunas and Kinghorn 2005). Recently, molecular biology has evolved as a fundamental technique in drug discovery through the usage of the proper screening assays directed towards physiologically relevant molecular targets.

However, according to Pan et al. (2013), drug discovery process from natural products can be categorized into three distinct phases, which are predrug stage, quasidrug stage, and full-drug stage:
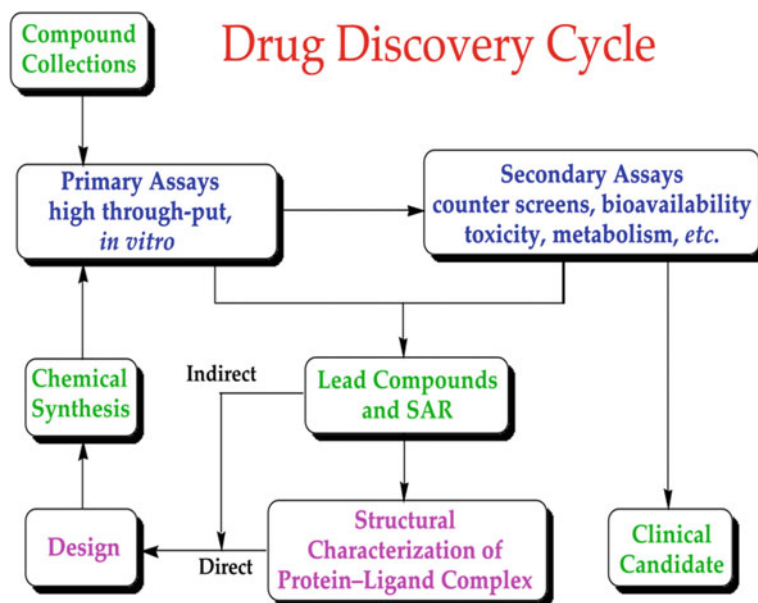
Fig. 1.1 Drug discovery cycle. (Source Boghog 2015. Licensed under Creative Commons Attribution-Share Alike 4.0 International)

1.2.1 Predrug Stage

Being the first stage, it involves the choice of the plants through one of three approaches, the edible plants, the traditionally used herbs, and scientific research (phytochemical analysis). The medical information about the therapeutic effects of herbs was mainly acquired by ancient people through many trials. Accordingly, Chinese herbal medicine and Indian herbal medicine, which were developed in ancient China, Japan, Korea, and India, are still influencing the modern healthcare system (Samy et al. 2008). The World Health Organization (WHO) stated that about 3.5–4 billion people in the whole world depend on herbal medicines for their health care, and about 85% of traditional medicine involves the use of plant extracts in what is named as the modern herbal medicine (Pan et al. 2013).

1.2.2 Quasidrug Stage

This stage includes extraction of plants, isolation of pure compounds, structure/composition elucidation, and evaluation of the biological activities in which isolates are to be used as lead molecules for further drug development (Sasidharan et al. 2011).

1.2.3 Full-Drug Stage

The high potential of natural compounds bioactivities, makes them the major source of components used for constructing hybrid molecules in the development of anticancer, antioxidant, and antimalarial drugs (Decker 2011). Therefore, the multinational pharmaceutical organizations commonly spend a yearly measure of US$ 110 billion trying to find new medications from natural products.

As earlier stated, natural products have long been the source of new chemical entities (NCEs). In the period between 1981 and 2002, approximately 28% of NCEs are purely natural products or are inspired by the molecules derived from natural sources including semi-synthetic analogs (Newman et al. 2003). Since 1994, half of the approved drugs have been based mainly on natural products. Another 20% of NCEs during this period are like natural product analogs, meaning that the compound was synthesized according to a structure of a natural product (Newman et al. 2003). During the years 2005–2007, 13 natural product related drugs were also approved (Harvey 2008). Natural products can serve as a precursor for synthetic new compounds, with different structures and frequently with numerous stereocenters that can be defying in synthesis (Clardy and Walsh 2004; Koehn and Carter 2005).

Many structural features found in natural products poses challenges in the new drug discovery, such as a high number of chiral centers, great steric complexity, large number of oxygen atoms, low number of aromatic atoms relative to the heavy atoms, great number of solvated hydrogen bond donors and acceptors, high molecular rigidity, and diversity of ring systems (Feher and Schmidt 2003; Piggott and Karuso 2004; Clardy and Walsh 2004; Koehn and Carter 2005). Furthermore, since the heightening of enthusiasm for combinatorial chemistry, numerous synthetic and medicinal scientists is investigating the making of natural compounds and natural compounds-like libraries (Ganesan 2004; Tan 2004).

Medicinal plant-derived drugs either serve as new drugs by themselves or serve as drug leads that could be optimized by medicinal and synthetic chemists (See Chap. 2 for more information). Drug leads are not necessarily the newest chemical structures isolated during natural product drug discovery, but may also be the known compounds with new biological activity (Balunas and Kinghorn 2005).

Thousands of new molecular targets have been identified since the sequencing of the human genome (Kramer and Cohen 2004). Finding new screening assays aimed to act on these targets, known compounds isolated from traditionally used medicinal plants may be of essence on newly validated molecular targets. This could be observed on three examples, cucurbitacin I, which has been found to be highly selective in inhibiting the JAK/STAT3 pathway in tumors with activated STAT3 (Blaskovich et al. 2003), h-lapachone, which selectively kills tumor cells and don't affect normal cells by direct checkpoint activation during the cell cycle (Li et al. 2003), and betulinic acid that has selective cytotoxicity on melanoma via p38 activation (Pisha et al. 1995; Tan et al. 2003; Cichewicz and Kouzi 2004).

1.3   Efficacy of Medicinal Plants

The medicines from plants are better in bioactivity and are found to possess curative properties against various diseases, compared to chemical and synthetic medicines. Thus, the efficacy of drugs from medicinal plants would be higher than conventional drugs. Although, these predominantly depends on the type of plant parts and their processing, phytochemicals as well as other specific drug component extraction and purification procedures involved in the drug formulation (Briskin 2000). Initially, pre-extraction preparations of plant parts including a selection of fresh or dried, grinded or powdered samples and process of drying are significant in yielding bioactive compounds which eventually affects the effectiveness of plant-based medicines (Borhan et al. 2013; Vongsak et al. 2013). Conventional methods, namely maceration, infusion, percolation, decoction, steam and hydro-distillation are used for the extraction of phytochemicals that are later converted into drugs. However, drawbacks such as longer extraction time and smaller phytochemical yield exists as challenges in these traditional extraction methods (Azwanida 2015). Major modifications in these conventional methods and coupling of two or more techniques, subsequently increases their efficiency and yield of phytochemicals from these methods. Recently, extraction procedures such as accelerated solvent extraction (Nastić et al. 2018), Soxhlet, solid phase, sonication-assisted, micro-assisted and supercritical fluid extractions are used for the enhanced phytochemical extraction with high yield (Wang and Weller 2006). Similarly, methods to isolate and purify individual phytochemicals such as chromatography, hyphenated techniques, crystallization, ion exchange and solvent extraction using partition coefficient were used to segregate biologically active phytocompounds. The planar, low-pressure column, high-speed countercurrent, high-performance liquid and crystallization are the recent modifications in the conventional isolation and purification methods to enhance the specific phytocompound separation process. The separation of specific phytochemicals with the medicinal property via purification process is highly beneficial to enhance their efficacy and reduce cytotoxicity (Sarker et al. 2005).

The medicinal drug entities extracted from phytocompounds possess medicinal properties against various microbial infections and diseases. The efficacy of a specific phytocompound is evaluated using in-vitro models or through in-silico methods which is further formulated and prescribed as medicine to be evaluated in in-vivo models, clinical trials and humans (Shobana and Vidhya 2016; Nivedha et al. 2017). It has been reported through several literatures that medicines formulated from plant-based compounds and chemicals possess antioxidant (Zhou et al. 2004), antibacterial (Khyade and Vaikos 2009), antifungal (da Silva et al. 2018), antiviral (Yang et al. 2016b), antialgal (Hussain et al. 2015), antimalarial (Chander et al. 2016), anti-diarrheal (Bellah et al. 2017), hypoglycemic (Loizzo et al. 2015), antitumor (Sahreen et al. 2015), anticancer (Clark and Lee 2016), anti-inflammatory (Van de Velde et al. 2016), anti-enteric (Kabir et al. 2017) and immuno-stimulant properties (Dügenci et al. 2003). The effectiveness of these properties in medicinal plants makes them to be a better drug candidate against diseases such as diabetes, neurodegenerative, cardiovascular, microbial infections and deficiencies

(Shankar et al. 2016). It also showed effective remedial potential against rare complications such as menstrual hemorrhage (Armand et al. 2018), menstrual disorders (Deoray and Page 2018), vaginal candidiasis (Sirilun et al. 2018), migraine headache (Nwidu et al. 2015) and Lafora (Wang et al. 2016). The dose and concentration-dependent efficacy of these plant extract-based medicines leads to their usage, despite the advent of numerous synthetic drug molecules (Elujoba et al. 2005). The latest computational methods, enhanced extraction, purification and characterization techniques in retrieving phytocompounds from plants and in formulating pharmaceutical drugs helps to improve the efficacy of medicines from plants (Atanasov et al. 2015).

The in-vitro analysis using bacteria, virus, algae and fungi helps to evaluate the antimicrobial properties of the phytocompounds. Disc diffusion assay, minimum inhibitory concentration (Discussed in subsequent chapters), calorimetric and spectroscopic analysis are used to analyze and optimize the antimicrobial activity of medicinal phytocompounds (Ahmad and Beg 2001). Similarly, egg, larvae and adult of mosquitos are reared in laboratory conditions to investigate the mosquitocidal properties of medicinal plant products (Chander et al. 2015). Normal and unhealthy cell lines of rabbit, mouse, monkey or human origin is used to establish the medicinal properties of phytocompounds in curing and reducing the complications of diseases (Dutra et al. 2016). The cell lines are utilized to evaluate the cytotoxicity of phytocompounds with remedial potential with (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (MTT), water-soluble tetrazolium salts (WST-1), Alamar blue, neutral red uptake, lactate dehydrogenase, propidium iodide, protein or deoxyribonucleic acid (DNA) content measurement, 3H-thymidine incorporation and bromodeoxyuridine incorporation assay (Omidi et al. 2017). In-silico methods such as computer simulations and statistical tools are also used to investigate cytotoxicity and efficacy of plant-based medicines towards rare diseases in which cells cannot be raised in in-vitro conditions (Zengin et al. 2018). After obtaining the highly efficient phytocompound from in-vitro models and in-silico studies, it is subjected to in-vivo analysis to estimate their toxic reactions and medicinal potentials in animal models (Gambini et al. 2015). It is also proved in recent times that isolated phytocompounds shows high bioactivity with less cytotoxicity, compared to crude extracts (Sharma et al. 2016). Thus, natural drugs from medicinal plants are gradually capturing the pharmaceutical market of synthetic drugs due to their effective disease curing or microbial growth inhibiting ability. However challenges in large-scale production, stability and isolation of individual compounds still exists as drawbacks for commercialization of natural drugs from medicinal plants.

1.4 Safety of Drugs from Medicinal Plants

The reports from the World Health Organization (WHO) revealed that more than 80% of the world population uses plant-based medicines for healthcare and 25% of drugs in pharmaceutical market are derived from plants (Li et al. 2009). Recently,

natural drugs from plants are tipped to be safer than the synthetic drugs. Despite these positive aspects, the lack of safety among natural phytomedicines is a significant drawback of concern. The common thoughts of safety and devoid is not true in all cases of phytomedicines as they lack quality control during production, disparities of active ingredients in distinct plant parts, imprecise identification of plant species and unclear nomenclature (Ekor 2014). The safety of extracted medicinal phytocompounds also depends on the geography, time and stages of plant harvest, soil, weather, nutrient levels and other conditions required for the growth of individual plants. The mixture of adulterants or contaminants in phyto-extracts and contamination via microbes as well as fungal toxins, heavy metals and pesticides also raises a question of safety towards phytomedicines (Brevoort 1998). Numerous literatures described that unsafe phytomedicine preparations, including lack of plant-drug interaction mechanism, adulteration and unsuitable formulation may lead to complications such as kidney disease, neurological, cardiovascular, dermatological effect, liver fibrosis, cellular and genotoxicity (Auerbach et al. 2012). Thus, cautious regulatory and safety monitoring procedure is advised as a prime importance to amplify the safety of phytomedicines (Saad et al. 2017).

In order to make sure the safety of natural phytomedicines is guaranteed, the international level standardization need be tightened for regulatory policies by healthcare professional and regulatory authorities. The route of administration, geographical origin, their compatibility and processing needs be evaluated to ensure the safety of phytomedicines by techniques mentioned in the previous section. Also, common binomial names for the medicinal plants needs be adopted to avoid challenges in identifying and collecting those plants. Therefore, a group of scientists from botany, phytochemistry, pharmacology and stakeholders are essential for the effective monitory of phytomedicinal safety. However, there exists weak regulation, high-profile safety concerns and lack of knowledge on the side-effects even in the developed countries. The side-effects can be caused due to over dosage, undeclared medicines, usage of different plant species, phytomedicines with synthetic drugs and contamination. Hence the need for regulation and safety measures to be implemented by each country which is quite difficult as a single plant species can be utilized either as medicine, functional diet or a diet supplement. It is advisable to form a global phytomedicine regulatory body in the future, which should draft safety rules for each continent to segregate medicinal plants, their formulation, dosage and ethical trials in humans.

The current regulatory policies have certain influence on the safety of the phytomedicines. In the UK, there are regulatory routes, including unlicensed phytomedicinal remedy until year 2011 that allowed unlicensed phytochemical to be available in the market. A 7-year transition period and simple licensing system was recently directed by the European Union to harmonize regulations on traditional phytomedicinal products. Also, the traditional phytomedicine registration scheme has been introduced in which a phytomedicine product is provided with a unique license depending on their efficacy, safety and quality. Similarly, Dietary Supplement Health and Education Act (DSHEA) in United States regulated natural drugs from plants that contain potential toxicity and side-effects towards humans (Ekor 2014).

Quality assessment of phytomedicines is highly essential in ensuring the safety of natural phytomedicines. The initial steps involved in quality assurance are authentication of plant species via genomic profiling, classical systematics, DNA Barcoding, utilization of good collection and agricultural practice, characterization of medicinal plant materials using micro and macroscopic techniques, identification and classification of medicinal plants via phytochemical profiling, better guidelines for documentation and authentication of medicinal plants and impurity profiling (Saharan 2011). Quality assurance of phytochemicals during extraction process can be achieved by Good Medical Practices (GMP) and Good Laboratory Practices (GLP) such as phytochemical profiling and standardization, harmonization via the analytical method in the entire supply chain, comprehensive quality measurement and transparency in the supply chain (Sharma 2015). These models of quality assurance are present to ensure safe plant formulations as medicines, functional foods, dietary supplements and nutraceuticals to correlate with international drug regulatory policies. However, the present models are only based on the efficacy and evidences of phytochemicals (Govindaraghavan and Sucher 2015). Thus, it is clear that strict quality control at each step of phytomedicine production with stringent regulatory measures will ensure the safety of natural drugs from plants, similar to synthetic drugs.

1.5 Current Uses of Natural Products as Drugs

Currently, several synthetic drugs and cosmetic consumers are gaining interest towards natural products due to the recent awareness about the toxic chemicals and reactions involved in those synthetic medicines. Among natural medicinal products from microbes, insects and animals, phytomedicines are accepted to possess high pharmaceutical significance due to their safety, ease production and wide availability. In recent years, plant based natural products are extensively employed in pharmaceutical, nutraceutical and dietary supplements. Nogueira et al. (2018) proposed molecular factories of plants for nutritional and industrial production of isoprenoids including tocopherols and carotenoids which is a major constituent of human diet. The plant-based natural products are widely used as medicines for the ailment of deadly diseases, including HIV and new diseases that are spreading in recent times (Vora et al. 2018). It is noteworthy that the natural phytoproducts are presently utilized in the treatment of neurodegenerative and cardiovascular diseases (Croft et al. 2018; Hussain et al. 2018) including Alzheimer's (Shal et al. 2018), diabetic neuropathy (Solanki et al. 2018) and cardiomyopathy (Uddandrao et al. 2018). The problem of multidrug-resistant and drug sensitive pathogens are tackled by natural plant-based drug products that are produced via modern safety measures and isolation methods (Shin et al. 2018; Vambe et al. 2018). The enhanced mosquitocidal property of natural phytomedicines helps to eradicate yellow fever (Nesterkina et al. 2018), Zika fever (Bajpai et al. 2018), tuberculosis (Gupta et al. 2018) and other protozoal diseases, namely leishmaniasis, schistosomiasis and

trypanosomiasis (Simoben et al. 2018). Phytochemical based drug delivery systems are widely explored by researchers in the treatment of diseases such as cancer (Karthikeyan and Laxmanappa 2015), diabetes (Ríos et al. 2015), menstruation disorders (Steenkamp 2003) and sexually transmitting diseases (Hossan et al. 2010). The recent and modern techniques in the fabrication and formulation of natural drug products make phytomedicines as a potential tool to cure these diseases. Extensive research in ethnopharmacology field will lead plant based natural drugs to be utilizable in the future to cure rare diseases such as hyperprolactinemia, hypogonadism syndromes (Besser et al. 1976), uterine contractility (Ekstrom et al. 1992), menorrhagia (Kingman et al. 2004), progeria (Jeyam et al. 2011), Morgellons (Kontos et al. 2017), micropsia (Sayin 2016) and porphyria (Smith and Foster 2018).

The awareness about natural products among common people leads to the transformation of medicinal advertisements to rely on commercials that explains the natural origin of the product. Thus, numerous natural plant-based products will be available in the pharmaceutical market. The improvement in the safety measures of phytomedicinal production and mass cultivation of plants to retrieve medicine from them will enhance the usage of plant-based medicines in the future. Nanoformulations of active phytocompounds for the targeted delivery of these compounds in target location and nanoparticles synthesized via phytochemicals are the future of phyto-therapies and phytomedicines. Liposomes, micelles, nanoencapsulations, dendrimers and polymers are some of the nanoformulations that are available for the targeted delivery of phytochemicals (Jeevanandam et al. 2016a). Phytochemicals such as flavonoids, phenols, terpenoids, essential oils, xanthophyll and several novel phytocompounds are used for the synthesis of less toxic nanoparticles with enhanced bioactivity, bioavailability and biocompatibility (Jeevanandam et al. 2016b). Recently, virus nanoparticles and virus-like nanoparticles are also employed for the formulation of active medicinal phytochemical to carry and deliver them at the target site of infection or disease (Aljabali 2018). Hence, the future of pharmaceutical industry lies in the emergence of novel methods to isolate, characterize, purify and formulate compounds and chemicals with medicinal properties from plants to cure and reduce complications of numerous diseases.

1.6 Advances in Tools for the Screening of Medicinal Herbs

According to the most recent statistics for the time period from 1981 to 2014, about 42% of 1562 newly approved drugs are derived from natural medicinal plants (Newman and Cragg 2016). Meanwhile, in the period from 1981 to 2010, the proportion of natural bioactive compounds is more than one half of the approved 1073 new types of small molecule drugs (Atanasov et al. 2015). The complexity of the chemical compositions of natural compounds and great variability in their structures, coupled with nonspecific adsorptions, false positive results, the undetectable trace amounts of active components have been a challenging factor (Potterat and Hamburger 2013; Zhu et al. 2013; Wu et al. 2016a). These obstacles represent the actual challenges in the identification of bioactive components and prediction of their

possible mechanisms of action. The good choice of in-vitro and in-vivo assays for screening of the plant bioactivity is a very important step in drug discovery from plants. The chosen bioassays should be characterized by simplicity together with good sensitivity and reproducibility. Numerous in-vitro models using purified proteins, cell-based target-oriented or phenotypic assays can be utilized for evaluation of the biological activities of natural products, in addition to the isolated tissues or organ models, and in-vivo preclinical animal models can be used (Atanasov et al. 2015).

In the past, the screening of plant-derived extracts and compounds utilized the forward pharmacological approach which starts with in-vivo animal tests, organ, tissue models, or bacterial preparations, followed by in-vitro testing to determine the mechanism of action. The forward pharmacology begins with determination of functional activity through phenotypic change detection in complex biological systems and then characterizes the molecular target of the active compounds, so it is named the phenotypic drug discovery. This traditional way of drug discovery was applied mainly before the development of the modern molecular biology techniques and the Human Genome Project (Takenaka 2001; Lee et al. 2012; Schenone et al. 2013; Zheng et al. 2013).

Now when the screening starts by testing of the plant-derived compounds ("libraries") against pre-characterized disease-relevant protein targets, in order to identify "hits", the biologically active compounds which are then studied using in-vivo animal models with the target of their validation this is named as a reverse pharmacology approach. The forward and reverse pharmacology approaches differ only in the stage when the assays are applied. The reverse pharmacology begins by identifying the promising pharmacological target to obtain the promising hit compounds which are then validated in-vivo and thus considered as a target-directed drug discovery (Takenaka 2001; Lee et al. 2012; Schenone et al. 2013; Zheng et al. 2013).

In-silico simulations are computational methods which can be used to suggest a protein ligand binding characteristic of a molecular structure that may be a known plant-derived compound. Compounds that give good results in the in-silico predictions can be promising candidates for further experimental work. Applying virtual screening for activity predictions have shown increasing rates of success (Hein et al. 2010). In-silico simulations can also act as valuable filter tools to predict new activities for an already known natural product as well as detection of ADME/T properties (Kaserer et al. 2014). These computational methods can be employed also to discover new binding sites on protein with already known structures. Pocket finders are used to identify solvent-accessible cavities in the protein surface that represent important ligand binding sites that can then be computationally analyzed (Rollinger et al. 2008).

The active compounds may exert their biological activity through regulating the body's signal transduction and maintaining normal metabolism. Or they may exert their action through the interactions with the disease-related drug targets, such as enzymes and receptors (Mulabagal and Calderon 2010; Wu et al. 2016b). Screening of the biologically active natural compounds targeting these enzymes and receptors, could not only provide new approaches for new drug discovery, but also help to know the mechanistic action of bioactive small natural molecules (Chen et al. 2016a, b).

In this respect, affinity ultrafiltration mass spectrometry (UF-LC/MS) is one of the potential techniques as it combines ultrafiltration with liquid chromatography-mass spectrometry (LC/MS), which achieve the affinity capture of active small molecules against biomolecule targets through high-throughput screening and rapid identification of biologically active compounds in the complex mixtures (Li et al. 2015; Tao et al. 2015).

In UF-LC/MS, the bio-affinity ultrafiltration process begins with the separation of the ligand– enzyme complexes from free components, and then those ligands released from the complexes will be further identified and quantified by high performance liquid chromatography mass spectrometry (HPLC–MS) analysis (Tang et al. 2015). Compared to the extremely complicated traditional procedures of screening for bioactive plant constituents, the UF-LC/MS method is not just very easy, but also greatly reduces the time required for screening, the consumptions of samples and expensive reagents (Chen and Guo 2017a, b; Li et al. 2009).

Affinity UF-LC/MS is a powerful method that can rapidly screen and identify small drug molecules bound to target proteins, and also be used for the screening of the leading compounds. Moreover, this technique can also identify the mechanism by which bioactive compounds exert their actions. For this reason, it is an important approach for drug discovery from the natural medicinal plants. There are still many challenges facing affinity UF-LC/MS. For example, it is mainly used for screening of small active molecules from medicinal plants at one or two protein targets. Moreover, reduction of the non-specific bindings of small drug molecules to ultra-filtration membrane so as to reduce the false positive results is an urgent issue to be solved in the future.

1.7 Problems and Way Forward

Despite the great prospects of natural products drug discovery, future attempts face many challenges. The process of drug discovery usually takes a long time, estimated from 10 years or more (Reichert 2003), which costs more than 800 million dollars (Dickson and Gagnon 2004). Although, much of this time and money are spent, especially on the purification and structure elucidation of the leads, however, one in 5000 lead compounds usually pass the clinical trials and be approved for use.

The first challenge in natural products drug discovery is the precise identification and nomenclature of the plant of interest, the step on which all the following steps are dependent. For this reason, a combination of methods are used for authentication of medicinal plants such as genetic, morphological and anatomical characterization in addition to the chemical characterization (Bucar et al. 2013). Accordingly, the collection of plant material and accurate documentation, botanical identification, as well as preparation of the herbarium vouchers are tasks that cannot be automated (David et al. 2015) and requires specialists who are not available anymore (Bucar et al. 2013).

The collections of medicinal plants from wild species have many difficulties, as the plant habitats may quickly vanish due to human over-consumption of these plants (David et al. 2015). In cases of imported plant material, many factors such as local wars, natural crisis, or changing laws for travelling between countries and the exportation of plant material may affect its accessibility.

When a plant becomes utilized as an herbal medicine or when one of its constituents proved its bioactivity, the plant becomes threatened by extensive collection and unstable harvesting systems (Cordell 2011). A typical example of this problem is the "Taxol supply crisis" (Kingston 2011). When Taxol proved clinically effective against ovarian cancer, the demand for Taxol suddenly increased. The process for its isolation from the barks of *Taxus brevifolia* L. (The western yew tree) starts with the collection of the bark, drying, preparation of plant extract, and purification of the compound. This process is time consuming. At this time, worries on the environmental effect of excessive bark gathering appeared (Kingston 2011). Although Taxol is meanwhile accessible via semi-synthesis, the problem of sustainable supply of Taxol still frequently occurs.

Limited availability of a bioactive, plant-derived natural product is also problematic, especially when it proves a very promising bioactivity and becomes a pharmaceutical lead. Natural products are usually isolated in small amounts which are deficient for the development of lead compounds. To improve the rate for natural compound isolation, new technologies should be incorporated. New applications of NMR and MS should be employed to facilitate compound isolation (Glish and Vachet 2003). Also, the use of high-throughput X-ray crystallography can be used in natural product discovery (Blundell et al. 2002). Coordinated effort with medicinal and synthesis experts is important to decide whether synthesis or semi-synthesis may be conceivable (Lombardino and Lowe 2004). Another strategy to enhance natural product compounds advancement may include the formation of natural product and natural-product-like libraries that join the characteristics of natural products with combinatorial chemistry (Koehn and Carter 2005).

Further complications appear because natural compounds usually have complex chemical structures with numerous chiral centers, which make the pathway for their synthesis or even derivatization very difficult (Henrich and Beutler 2013).

The incompatibility of natural products with high-throughput screening is another challenge in drug discovery (Koehn and Carter 2005). High-throughput screening of plant extracts followed by the identification of its biologically active compounds is highly difficult as it needs sample preparation and assay designs. In general, high-throughput screening may depend on cell-free or cell-based assays which should be reproducible, accurate, and reliable. The tested compounds should not decompose, precipitate, or interfere with assay reagents, but plant-derived compounds most probably fail to fulfil these requirements. Accordingly, sophisticated sample preparation or fractionation of the crude extracts prior to testing is highly demanded (Johnson et al. 2011; Maes et al. 2012).

1.8 Conclusion

Despite the challenges that faces scientists in natural product drug discovery, natural products and their derivatives have been sources for numerous clinically useful medicines. Therefore, natural products remain a potential lead compound and precursors for production of new medicines. The next chapter details the various phytochemicals and synthetic analogs, their mechanisms of action and structure-activity relationships. A table summarizing 150 natural plant-derived drugs, their uses, and sources were presented.

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