The Role of Plant Metabolites in Drug Discovery: Current Challenges and Future Perspectives

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Abstract

The search for new and novel drugs is never-ending. Despite advances in synthetic chemistry, nature nevertheless remains as an important resource for the drug discovery. For decades, a routine screening of ethnopharmacologically important plants, followed by chromatographic isolation has been the basis of bioprospecting. Recent

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advances in bioinformatics and in silico screening have further improved our rate and likelihood of finding medicinal metabolites. Multiple classes of naturally occurring plant secondary metabolites, such as polyphenols, terpenoids, and alkaloids have been proven to possess significant medicinal potentials including antioxidant, anti-allergic, anti-inflammatory, anticancer, antihypertensive, and antimicrobial activities. The compounds of interest are often used as a basis or inspiration for semisynthetic drugs with improved pharmacokinetic and pharmacodynamic parameters. However, when a new biologically active compound has been identified, further in vivo testing and clinical trials may not reflect results seen in vitro. As with any promising fields, pitfalls and drawbacks are inevitable; these include poor bioavailability and unknown pharmacodynamics/pharmacokinetics. The drug development is further complicated by other challenges, such as processing, formulation, scaling up, and intellectual property protection. This chapter aims to discuss on the prospects of plant metabolites leading to drug discovery, along with the process and outcomes of testing and the potential pitfalls or challenges faced by researchers and the pharmaceutical industry in this endeavour.

Keywords
Bio-active compounds · Biopiracy · HTS · Natural products · Secondary metabolites

2.1 Introduction

Natural products have provided mankind with the means to treat a myriad of diseases, illnesses, and ailments. This may be via herbal concoction designed by a traditional healer or by a robust pill produced by a large renowned pharmaceutical company. The nature has consistently provided humans with bio-active compounds that can be used directly as drugs or indirectly as drug leads (bio-active compounds that can be used as a template for the synthesis or semi-synthesis of new drugs). These natural products are compounds extracted from a variety of organisms including microorganisms, plants, aquatic life, and so on, although plants (the focus of this chapter) have by far received the most interest by researchers. Newman and Cragg (2012) comprehensively compiled and analysed the sources of new drugs over the past 30 years and observed that nearly 40% of the new drugs approved by the FDA (Food and Drug Administration) were of natural product origin or natural product-related origin, such as derivatives or synthetic mimetics of natural products. Recent findings show that there has been a surge in research regarding the use of plant metabolites as potential leads for drug discovery. Many of the plant metabolites of interest are actually secondary metabolites, which are non-proteinaceous compounds that are not directly involved in plant growth but are essential in plant-environment adaptation by providing defence mechanisms for survival such as the prevention of herbivory, antimicrobial activity, insecticidal activity, and UV

protection. Classes of secondary metabolites include alkaloids, steroids, phenolics, and terpenes (Gupta and Birdi 2017). This book chapter aims to collate and analyse the body of research regarding plant metabolites that have been or are currently being utilized as drugs or leads for the discovery of new drugs. Further, it discusses on the benefits of utilizing natural products, more specifically plant metabolites, as leads for drug discovery, a comprehensive compilation of plant metabolite-based drugs, a collation of prospective plant metabolite leads that are currently undergoing preclinical trials, a brief introduction of lead-based drug discovery and development, the intrinsic challenges involved in natural product drug discovery, and finally a commentary on the future perspectives of this field.

2.2 The Natural Product Approach to Lead-Based Drug Discovery

Natural product-based research has long been an attractive field of drug discovery. After all, nature has provided an abundant source of therapeutic bio-active compounds since the dawn of human civilization. Even today, this notion is evident by the prevention or risk reduction of certain diseases (such as metabolic disorders) by consumption of plant-based diets. Foods rich in beta carotene help maintain vision health and prevent cataract formation, whereas scurvy is prevented via the intake of citrus fruits high in vitamin C. These bio-active compounds may be present in any part of a plant, be it in the bark, root, bulb, wood, rhizome, tuber, leaf, aerial parts, and flowers. As such, scientists need to isolate the specific compounds of interest from carefully chosen plant species for the development of drugs to treat a myriad of diseases, infections, and ailments (Gurib-Fakim 2006). Classic examples of plant metabolites that were directly used as active compounds in drugs include (1) the opioid morphine that was derived from the *Papaver somniferum*, which is still in medical use today as an analgesic for both chronic and acute pain, and (2) the alkaloid quinine which was derived from the bark of the cinchona tree, previously the only known cure for malaria. Other examples include (3) the secondary metabolite tropane alkaloid to create atropine from *Atropa belladonna*, and (4) Taxol (paclitaxel), a complex terpene from the bark of *Taxus brevifolia* used to treat cancer (Niu et al. 2006; Khazir et al. 2014). The success of using plant metabolites is evident in our history and thus remains a promising field to further explore.

Researchers are also able to use plant metabolites as lead compounds for drug discovery rather than using the original active compound. There are a myriad of reasons for this, such as to reduce the expression of certain side effects or to reduce cost of production and or development of the drug. Lead compounds are molecules that can be used as a template for the design of a fully or semisynthetic drug derivative or analogue. For example, salicin was originally extracted from willow tree bark, leading to the eventual discovery of the acidic active form, salicylic acid. Salicylic acid itself is useful for pain, fever, and inflammation management but also exhibited undesirable side effects, most notably gastric irritation. To combat this issue, the pharmaceutical company Bayer set out to synthetically produce

acetylsalicylic acid, an analogue of salicin with reduced acidity, to ultimately circumvent the gastric irritation issue. Bayer's acetylsalicylic acid is best known as Aspirin®, the first synthetic drug to be commercialized (Miner and Hoffhines 2007).

The most compelling factor in using plants for lead-based drug discovery is the structural diversity and complexity provided by plant metabolites. They also are typically rich in chiral centres and with a wide range of pharmacophores, as well as a high degree of stereochemistry, which allows for initial modification targets. Another key advantage of utilizing natural products in drug discovery efforts is that natural product bio-active compounds can be associated with their innate biosynthetic molecular recognition (Newman and Cragg 2012). They are biologically validated structural entities that are often multi-targeted in its action, unlike many synthetically produced drugs that carry the motto "single compound, single target". This property of plant metabolites imparts a "metabolite-likeness" to the potential new drug entity, implying that the drug will not only be able to perform its designed therapeutic function but may also have the potential to be substrates for other systems (Newman et al. 2015). Compared to synthetic compounds, plant metabolites have a wider range of chemical space that resembles the chemical space of drugs. The combination of innate therapeutic properties and the various structural advantages of plant metabolites provide a solid basis for the use of plant metabolites as lead compounds.

2.3 Plant Metabolite-Based Drugs

Plant metabolites have played a major role in human medicine since the dawn of civilization; and often, it is easy to forget that many drugs we still use today can trace their roots back to plants. Since the time of the ancient Sumerians and Egyptians, willow bark (Salicaceae) was used to treat pains and fever. This analgesic activity is due to the presence of salicylates, a class of compounds of which aspirin (acetylsalicylic acid) is part of (Miner and Hoffhines 2007). In addition to aspirin, there are many examples of commonly heard compounds that are used as drugs. Caffeine and caffeine citrate, for example, are used to treat breathing issues in premature newborns. Morphine, codeine, and papaverine, amongst the most well-known of the naturally occurring opioids, all come from the opium poppy (*Papaver somniferum*, Papaveraceae) (Fabricant and Farnsworth 2001). Oseltamivir, under the tradename Tamiflu, is a well-known antiviral compound with plant origins. Although oseltamivir is now predominantly produced by recombinant *Escherichia coli*, star anise (*Illicium verum* Hook. f., Schisandraceae) was long used as the source of shikimic acid (required for oseltamivir production) (Wang et al. 2011). Quinine, an antimalarial drug that has been used for centuries, came from *Cinchona* (Rubiaceae) bark (Achan et al. 2011).

Quinine use has diminished following the discovery of the sesquiterpene lactone artemisinin, from *Artemisia annua* L. (Asteraceae). While perhaps not as ubiquitously known by the general populace, this compound has nevertheless had an impact on millions of people and earned its discoverer (Tu Youyou) half of the

Nobel Prize in Medicine (2015) (White et al. 2015). Since its discovery nearly five decades ago, artemisinin has been the subject of numerous studies and chemical derivatization due to artemisinin's poor bioavailability. These derivatives are currently the front-line treatment for malaria in 80 countries (WHO 2018). Coartem, the tradename for a drug based on an artemisinin derivative (artemether), obtained US FDA approval in 2009 (US Food & Drug Administration 2018), while numerous other derivatives are currently undergoing various stages of clinical trials worldwide (Koita et al. 2017; Anvikar et al. 2018; Daher et al. 2018). Artemether, artesunate (another artemisinin derivative), and dihydroartemisinin (the active metabolite of artemisinin compounds) formulations are on the 20th WHO Model List of Essential Medicines (a list of the world's most effective and relatively safe medications), along with aspirin, morphine, codeine, oseltamivir, quinine, and many other plant-based compounds and derivatives (summarized in Table 2.1) (WHO 2017).

Outside of this, there exist many plant-based drugs. A slightly dated but nevertheless extensive list of 122 plant-based drugs was previously compiled by Fabricant and Farnsworth (2001), although there are still many that were not included in that publication (either intentionally, erroneously, or due to comparatively recent discovery). In some cases, these drugs may be derivatives of other currently approved plant-based drugs. Examples include the aforementioned artemisinin derivatives (Koita et al. 2017; Anvikar et al. 2018; Daher et al. 2018); vinflunine, a vinblastine derivative (Bonfil et al. 2002); and exetecan, a topoisomerase I inhibitor for cancer treatment like topotecan and irinotecan, all three of which are derivatives of camptothecin (*Camptotheca acuminata* Decne.) (Cragg and Newman 2004).

Some, on the other hand, are not derivatives of existing drugs. For example, the alkaloid galantamine from the family Amaryllidaceae (notably *Galanthus woronowii* Losinsk.), where it was first isolated nearly seven decades ago, is an approved treatment for Alzheimer's disease and various other central nervous system-related dysfunctions. It enhances cholinergic function by inhibiting acetylcholinesterase and modulates the responsiveness of nicotinic acetylcholine receptors to acetylcholine (Heinrich and Teoh 2004). Calanolides A and B (and the derivative, 7,8-dihydrocalanolide B), non-nucleoside-specific reverse transcriptase inhibitors (NNRTI) undergoing clinical trials for the treatment of HIV, are dipyranocoumarins *from Calophyllum* spp. (Clusiaceae) (Kurapati et al. 2016). The β-triketone leptospermone from the family Myrtaceae (notably *Callistemon citrinus*) was the natural compound that ultimately resulted in the creation of nitisinone, a derivative used as the front-line treatment of hereditary tyrosinaemia type 1 (the inborn inability to metabolize tyrosine) for nearly three decades, and is currently undergoing trials as a treatment for alkaptonuria (Introne et al. 2011).

While plant metabolites are indeed used to treat a wide variety of ailments, their potential as anticancer drugs seems particularly noteworthy. There have been no less than 29 plant-derived anticancer compounds of clinical significance (Ali-Seyed et al. 2016). One such compound is betulinic acid, a pentacyclic lupane-type triterpenoid. It is found in many plants, most notably *Betula alba* (Betulaceae) (Müllauer et al. 2010). Its ability to induce apoptosis in a wide variety of cancers (Ali-Seyed et al. 2016) has led to the ongoing synthesis and testing of various derivatives as

Table 2.1 Compounds of plant origin on the 20th WHO Model List of Essential Medicines (WHO 2017)

Compound	Source	General use	References
Acetylsalicylic acid	Salicylic acid, from *Salix alba* (Salicaceae)	Pain and palliative care	Miner and Hoffhines (2007)
Caffeine and caffeine citrate	*Coffea* sp. (Rubiceae)	Neonatal care	Shrestha and Jawa (2017)
Morphine, codeine, and papaverine	*Papaver somniferum* (Papaveraceae)	Analgesic	Fabricant and Farnsworth (2001)
Oseltamivir	Shikimic acid, from *Illicium verum* (Schisandraceae)	Antiviral	Wang et al. (2011)
Quinine	*Cinchona officinalis* (Rubiaceae)	Antimalarial	Achan et al. (2011)
Artemisinin, artemether, artesunate, dihydroartemisinin	*Artemisia annua* (Asteraceae)	Antimalarial	Balunas and Kinghorn (2005)
Ephedrine	*Ephedra* sp. (Ephedraceae)	Spinal anaesthesia	Lee (2011)
Vecuronium	Malouetine, from *Malouetia bequaertiana* (Apocynaceae)	Muscle relaxant	McKenzie (2000)
Atropine	*Atropa belladonna* (Solanaceae)	Preoperative medication/ sedation	O'Brien (1974)
Metformin	Guanidine, from *Galega officinalis* (Fabaceae)	Type 2 diabetes treatment	Bailey (2017)
Warfarin	Dicoumarol, from the fungal metabolism of coumarin in *Melilotus officinalis* (Fabaceae)	Anticoagulant	Pirmohamed (2006)
Paclitaxel	*Taxus brevifolia* (Taxaceae)	Chemotherapy (various cancers)	Kampan et al. (2015)
Vincristine and vinblastine	*Catharanthus roseus* (Apocynaceae)	Chemotherapy (various cancers)	Noble (1990)
Irinotecan	Camptothecin, from *Camptotheca acuminata* (Nyssaceae)	Chemotherapy (metastatic colon cancer)	Xu and Villalona-Calero (2002)
Hyoscine/scopolamine	Nightshade (Solanaceae)	Palliative care and motion sickness	Müller (1998)
Amiodarone	Khellin, from *Ammi visnaga* (Apiaceae)	Antiarrhythmic	Fabricant and Farnsworth (2001)
Digoxin	*Digitalis lanata* (Plantaginaceae)	Antiarrhythmic	Hollman (1996)
Podophyllum resin	*Podophyllum peltatum* (Berberidaceae)	Genital and plantar warts	Fay and Ziegler (1985)
Benzyl benzoate	*Myroxylon balsamum* Harms and *M. pereira* (Fabaceae)	Lice and scabies	Popova et al. (2002), Seo et al. (2012)
Pilocarpine	*Pilocarpus* sp. (Rutaceae)	Antiglaucoma	Sawaya et al. (2015)

potential anticancer drugs (Chakraborty et al. 2015; Zhang et al. 2015; Khan et al. 2016; Huo et al. 2017).

Although much of this chapter is focused on specific purified compounds, it would be remiss to not at least briefly mention the rise of approved prescription drugs that are based on "cruder" preparations containing more than just one active compound. The first "botanical drug" (as these are termed) to be approved by the US FDA in 2006 was the green tea (*Camellia sinensis* (L.) Kuntze (Theaceae))-catechin based ointment Veregen™, for use on genital warts (Mishra and Tiwari 2011; Ahn 2017). In 2012, crofelemer, a proanthocyanin-based drug from the sap of *Croton lecheri* Müll.Arg., was also approved by the US FDA for treating diarrhoea caused by anti-HIV drugs (Ahn 2017). The herbal extract MF-101 (Menopause Formula 101, also known as Menerba) is currently undergoing phase 2 trials as relief for hot flashes in menopausal women (Froestl et al. 2014). Meanwhile, the neuropathic pain treatment nabiximols was the world's first cannabis-based prescription to obtain approval. Marketed as Sativex, this oromucosal spray contains Δ9-tetrahydrocannabinol (THC) and cannabidiol, two of *Cannabis sativa* main active ingredients (Flachenecker et al. 2014). It was originally launched in Canada (2005) (Mishra and Tiwari 2011) and is now readily available in many countries, including the UK, Germany, and New Zealand (Flachenecker et al. 2014). Ultimately, there is global demand for plant-based drugs, with a global market estimated at 1 trillion dollars, growing annually at a rate of 8–10% (Ahn 2017).

2.4 Prospective Plant Metabolites (Preclinical Trials)

While many plant metabolites have become accepted as drugs, there are easily thousands more potential drug leads that (for various reasons) have not undergone official clinical trials. Unfortunately, given the sheer number of compounds, any attempt to make an exhaustive list would be impossible within the confines of this book chapter. As such, this section of the chapter will discuss select examples, with emphasis on compounds that have been recently reported (2010 onwards) to exhibit in vivo activity. Needless to say, this section of the chapter will also not deal with crude extracts or fractions.

2.4.1 Anticancer Plant Metabolites

Cancer represents one of the leading causes of death worldwide. The ever-increasing number of cancer cases has brought adverse impact upon healthcare settings in terms of morbidity and mortality. As such, the search for therapeutic agents has become a major research field particularly in the past half century. More pertinently for this chapter, this increase in cancer research interest can be seen in the numerous natural anticancer drug-related publications and related clinical trials (Butler et al. 2014). Given the long-standing use of plants for treating various inflammations and tumours in ethnopharmacology, it is little wonder that researchers have successfully

identified numerous plant secondary metabolites as potential anticancer candidates (Greenwell and Rahman 2015). These include various alkaloids, terpenoids, glycosides, flavonoids, phenylpropanoids, etc. Many compounds from these classes have been shown to participate in the suppression of cancer-activating pathways, inhibition of oncogenes responsible for cancer formation, and activation of tumour apoptotic pathways.

For instance, several alkaloids such as vincristine isolated from *Catharanthus roseus* (L.) G. Don (Apocynaceae), paclitaxel from *Taxus brevifolia* Nutt. (Taxaceae), and omacetaxine mepesuccinate from *Cephalotaxus fortune* Hook. (Taxaceae) are currently used in anticancer therapy. Vincristine and paclitaxel affect tubulin, which eventually leads to metaphase arrest, triggering apoptosis and subsequent cessation of cancer proliferation (Horwitz 1994; Moudi et al. 2013). Omacetaxine mepesuccinate on the other hand is a ribosomal inhibitor that prevents protein translation (Gandhi et al. 2014). The successful examples of plant metabolite-based anticancer drugs have therefore garnered researchers' attention, thus making plant metabolites a focal point for potential anticancer drug development.

Alkaloids are nitrogen-containing biologically active secondary metabolites ubiquitously found in plants. As aforementioned, they have taken a major role in the drug discovery pipeline, demonstrating a wide array of structural diversity and remarkable pharmacological activity. Given the successful examples and the abundance, continuous efforts have been made upon testing alkaloids as new cancer drug candidates. Unfortunately, many alkaloids are also toxic, thereby requiring particular emphasis on dose to ensure the balance between their beneficial and toxic effects.

Harmine isolated from *Peganum harmala* L. (Zygophyllaceae), colchicine from *Colchicum autumnale* L. (Colchicaceae), and solamargine from *Solanum nigrum* L. (Solanaceae) have demonstrated remarkable in vivo tumour suppression in xenograft mouse models (Lin et al. 2016; Ruan et al. 2017; Tang et al. 2017). The intravenous administration of these compounds showed inhibitory effect via lowering tumour volume and size without affecting their body weight, thereby suggesting low toxicity to normal cells. Tang et al. (2017) further determined the action of solamargine in exhibiting its anti-lung cancer property via reducing the known tumour promoter (SP1) expression and activating tumour-suppressor proteins which eventually led to apoptosis.

Besides alkaloids, several phenolic compounds with anticancer activity have also been identified. The anticancer potential of phenolics has been recently explored via in vivo mouse xenograft models. The following examples are potential anticancer drug leads or candidates that have shown potential via in vivo testing (Table 2.2). In these studies, the route of administration used is injection via intravenous into tumour-transplanted nude mice unless otherwise stated. Unlike alkaloids, the dose of phenolics used was usually higher, yet with no toxic effect has been reported; this low toxicity may be a boon for their use as chemotherapy drugs. In general, all tumour-bearing mice administrated with these compounds showed reduction or inhibition in their tumour growth. Whenever possible, the sequential molecular events which lead to their anticancer property are presented.

Table 2.2 Examples of phenolic compounds with promising in vivo anticancer activity against a wide array of cancers

Phenolic compound	Sources	Potential anticancer target (in vivo)	References
Gallic acid	*Quercus lusitanica* (Fagaceae)	Bone cancer	Liang et al. (2012)
Silymarin	*Silybum marianum* (Compositae)	Oral cancer	Won et al. (2018)
Caffeic acid	*Cinchona succirubra* (Rubiaceae)	Lung cancer	Min et al. (2018)
Theabrownin	*Camellia sinensis* (Theaceae)	Lung cancer	Zhou et al. (2017)
Icariin	*Epimedium brevicornu* (Berberidaceae)	Oesophageal cancer	Fan et al. (2016)
Epigallocatechin gallate	*Camellia sinensis* (Theaceae)	Bile duct cancer	Kwak et al. (2017)
Silibinin	*Silybum marianum* (Compositae)	Pancreatic cancer	Nambiar et al. (2013)
Licochalcone A	*Glycyrrhiza glabra* (Fabaceae)	Gastric cancer	Hao et al. (2015)
Kaempferol	*Fragaria chiloensis* (Rosaceae)	Bladder cancer	Dang et al. (2015)
Eugenol	*Syzygium aromaticum* (Myrtaceae)	Breast cancer	Al-Sharif et al. (2013)
Ferulic acid	*Ferula assa-foetida* (Apiaceae)	Breast cancer	Zhang et al. (2016)
Gossypol	*Gossypium hirsutum* (Malvaceae)	Breast cancer	Xiong et al. (2017)
Luteolin	*Capsicum annuum* (Solanaceae)	Breast cancer	Sun et al. (2015)
Quercetin	*Ribes nigrum* (Grossulariaceae)	Breast cancer	Srivastava et al. (2016)
Chrysin	*Scutellaria discolor* (Lamiaceae)	Colon cancer	Bahadori et al. (2016)
Resveratrol	*Veratrum grandiflorum* (Melanthiaceae)	Colorectal cancer	Yang et al. (2015)
Ginkgetin	*Ginkgo biloba* (Ginkgoaceae)	Prostate cancer	Jeon et al. (2015)

Amongst the various phenolics listed in Table 2.2 are flavonoids, one of the largest phenolic compound families present abundantly in our human diet (from plant-based food), with well-documented bioactivity. In fact, a meta-analysis conducted by Woo and Kim (2013) revealed that the risk of developing smoking-related cancer was inversely related to the dietary flavonoid intake. To further expound, quercetin improved the life span of treated breast tumour xenograft mice by five-fold as compared to the control group. It also limited tumour proliferation and expressed remarkable effect in promoting p53 apoptotic pathways (Srivastava et al. 2016). Meanwhile, kaempferol-treated mice (150 mg/kg of body weight) exhibited no noticeable change in their body weight, liver, lung, spleen, and kidney (indicating

minimal toxic effect) despite a large number of apoptotic cells (70%) being detected in the bladder tumour (Dang et al. 2015). Kaempferol was found to inhibit the cell cycle of cancer cells via its inhibitory action towards several markers such as c-Met, cyclin B1, and c-Fos. It also prevented tumour metastasis. Based on Sun et al. (2015), xenograft mice administrated with luteolin showed reduction in breast tumour development. Chrysin on the other hand when administered orally (8 mg/kg) to mice demonstrated suppression of colon tumour growth (Bahadori et al. 2016). Epigallocatechin gallate from *Camellia sinensis* (L.) Kuntze (Theaceae) downregulated the expression of carcinogenic proteins (Notch1, MMP-2/9 proteins) and declined the proliferation of colon cancer cells (Kwak et al. 2017).

Curcumin which was first isolated from *Curcuma longa* L. (Zingiberaceae) is deemed as a promising chemotherapy drug candidate due to its broad spectrum of anticancer properties. It has been shown to dysregulate the signalling pathway (STAT3 and NF-κB) for cancer cell growth and subsequently induce apoptosis (Vallianou et al. 2015). In a study by Jeon et al. (2015), ginkgetin from *Ginkgo biloba* L. (Ginkgoaceae) was found to reduce the expression of oncogenes in prostate tumour-bearing mice and inactivated the proliferating pathway of prostate cancer cells. Apart from that, licochalcone A from *Glycyrrhiza glabra* L. (Fabaceae) was able to inhibit tumour growth in mice transplanted with gastric tumour (Hao et al. 2015). Oesophageal tumour-bearing mice treated with icariin from *Epimedium brevicornu* Maxim. (Berberidaceae) was found to have high level of PUMA protein, a key regulator for mitochondria-dependent apoptosis (Fan et al. 2016). It also showed decline in Bcl-2 protein level which is responsible for apoptosis regulation. Silibinin and silymarin are both found in *Silybum marianum* (L.) Gaertn. (Compositae) and demonstrate potent anticancer activity. For instance, silibinin is currently undergoing clinical trials for patients with prostate cancer and had also been shown to reduce the number of CD31-positive cells (a marker for tumour angiogenesis) in pancreatic tumours (Nambiar et al. 2013). Aside from silibinin, xenograft mice which were intravenously injected with 200 mg/kg of silymarin had shown reduction in tumour volume and no histological change in their essential organs (Won et al. 2018). Resveratrol with an intravenous injection of 100 mg/kg upregulated the expression of miR-34c (a tumour-suppressor gene) in colorectal tumour-bearing mice but not in serum, indicating that its target is tissue-specific and not systemic. Besides, resveratrol-treated mice demonstrated decline in secretion of IL-6, a pro-inflammatory cytokine often associated with tumour development (Yang et al. 2015).

Studies that have covered other non-flavonoid phenolic compounds have successfully revealed several potential candidates, including eugenol from *Syzygium aromaticum* (L.) Merr. & L.M. Perry (Myrtaceae), gossypol from *Gossypium hirsutum* L. (Malvaceae), theabrownin from *Camellia sinensis* (L.) Kuntze (Theaceae), and phenylpropanoids (caffeic acid and ferulic acid) as well as gallic acid which are found abundantly in plant-derived foods and medicinal plants. Eugenol is a commonly used weak anaesthetic agent in the pharmaceutical field. Its potential use as an anticancer agent has been explored by Al-Sharif et al. (2013) who reported decrease in oncoprotein (NF-κB and cycline D1) and Cox-2 levels following administration of eugenol to breast tumour-bearing mice. Further gene profiling on the

breast tumour had revealed the upregulation of both $p21^{WAF1}$ (modulator of apoptosis) and apoptotic gene (Bax, active caspase-9) expression. The presence of eugenol also decreased Bcl-2 protein expression. Gossypol is a proven effective anticancer agent for prostate cancer which has recently completed phase 2 clinical trials. This compound suppressed oncoprotein (MDM2) production and exhibited anti-angiogenesis effect on breast tumour via inhibiting VEGF production (Xiong et al. 2017). Furthermore, gossypol reduced the micro-vessel density of breast tumours, which supported its anti-angiogenic action. The compound theabrownin, a major constituent in pu-erh tea, was reported by Zhou et al. (2017) to reduce lung tumour-developing incidence amongst tested mice.

Various small phenolic acids have also been reported to exhibit anticancer activity. Caffeic acid exerted anticancer activity when used singly and exhibited synergistic activity when co-administered with paclitaxel (Min et al. 2018). Ferulic acid on the other hand reduced breast cancer cell proliferation, inducing apoptosis and inhibiting tumour metastasis (Zhang et al. 2016). Gallic acid, delivered via drinking water to the bone tumour-bearing mice, inhibited angiogenesis. Histological examination of tumour cells further showed loosely arranged sarcoma cells, indicating destruction of the tumour. It also decreased bone tumour cell proliferation and promoted apoptosis (Liang et al. 2012).

Apart from alkaloids and phenolic compounds, several other plant metabolites have also garnered attention due to their potency in hampering the proliferation of cancer cells. Tao et al. (2017) examined dioscin, a steroidal saponin found in *Dioscorea villosa* L. (Dioscoreaceae), for its activity against prostate tumour growth in xenograft mouse model. Dioscin demonstrated inhibitory effect against tumour growth via increasing production of ERβ. This protein is an oestrogen receptor and serves as a tumour marker where its reduction is associated with an increase in tumour cell proliferation. The inhibition of dioscin in tumour growth was further confirmed via reduction in tumour cell under microscopical analysis aided with haematoxylin-eosin stain. In addition, Tao et al. (2017) showed that ERβ-siRNA-modified mouse model nullified the action of dioscin, thereby proving that its mechanism of action was heavily reliant on ERβ regulation. Further confirmation of dioscin action was determined via molecular docking which revealed its strong binding affinity onto the ERβ activator. The subsequent dioscin-induced tumour cell apoptotic events were proposed to involve caspase-3 and Bax/Bcl-2 pathways. Another steroidal saponin, deltonin (found in *Dioscorea zingiberensis* C.H. Wright (Dioscoreaceae)), had demonstrated its anticancer activity via activating apoptotic pathways (Bax, activated caspase-3, caspase-9), inhibiting angiogenesis and declining the expression of pro-caspase-8/9 and Bcl-2 genes (Tong et al. 2011). Moreover, its administration improved the survival rate of colorectal tumour-transplanted mice. The triterpene glycoside actein isolated from *Cimicifuga foetida* L. (Ranunculaceae) on the other hand suppressed the development of breast tumour and metastasis (Yue et al. 2016). This compound reduced the level of angiogenic proteins such as CD34, suggesting its role as a tumour angiogenic inhibitor that resulted in its tumour growth-inhibitory action. Besides, it also decreased the expression of tumour metastasis-related genes (VEGFR1 and CXCR4).

2.4.2 Antidiabetic Plant Metabolites

Given the long-standing use of many plants in the treatment of diabetes, it is of little wonder that antidiabetic activity has been reported in numerous families across the plant kingdom. These compounds exert their hypoglycaemic activity through a myriad of mechanisms, including (but not limited to) insulinomimeticity, attenuating pancreatic beta cell function, increased hepatic glycogen synthesis, (Patel et al. 2012), pancreatic alpha amylase inhibition, and alpha glucosidase inhibition (Etxeberria et al. 2012).

For the most part, polyphenols are believed to contribute to the observed antidiabetic activity. This includes flavonoids like genistein, luteolin, daidzein, myricetin, quercetin, apigenin, and quercetin; hydrolysable tannins like pedunculagin, casuarictin, and strictinin (Etxeberria et al. 2012); and proanthocyanidins (Gonçalves et al. 2011). Unsurprisingly however, flavonoids may require high doses to exhibit in vivo activity. For example, a dose of 500 mg/kg of quercetin was required to reduce the blood glucose levels in Sprague-Dawley rats (Kim et al. 2011). There also exist many non-polyphenolic compounds with proven in vivo antidiabetic activity, including galegine (*Gallega officinalis* L., Papilionoideae), mycaminose (*Syzygium cumini* (L.) Skeels., Myrtaceae), bellidifolin, and swertiaperennin (*Swertia punicea* Hemsl., Gentianaceae) (Coman et al. 2012).

2.4.3 Antihypertensive Plant Metabolites

Like the trends observed in anticancer plant metabolites, alkaloids (Cong et al. 2014) and polyphenols (particularly flavonoids) (Ronchi et al. 2015) are often implicated in the antihypertensive activity of plants. Alkaloids from *Veratum nigrum* L. (Melanthiaceae) such as 12β-hydroxylveratroylzygadenine (VOG) are known antihypertensive agents but are also potentially neurotoxic and cytotoxic (Cong et al. 2014), unsurprising traits given the toxicity of many alkaloids.

Flavonoids are purported angiotensin-converting enzyme (ACE) inhibitors (Ronchi et al. 2015). However, as with most flavonoid-related bioactivities, they often require a reasonably high concentration to achieve a significant result in vivo and are not individually available in high concentrations in plant material to begin with. For example, a 160 mg/kg dose of the flavonone (±)-naringenin (isolated from the in vivo active methanolic extract of *Cochlospermum vitifolium*) was required to observe a significant decrease in rat systolic and diastolic blood pressure after 24 h. Curiously, naringenin had a much weaker effect on the systolic blood pressure and no significant effect on the diastolic blood pressure before 24 h. The slow action of naringenin implies that naringenin's metabolites, as opposed to naringenin itself, are likely involved in its antihypertensive activity (Sánchez-Salgado et al. 2010). Like with all other bioactivities, it is possible that cruder extracts (as opposed to pure compounds) may demonstrate antihypertensive activity due to synergism between the various compounds present (Ronchi et al. 2015).

Besides these, many plant-derived peptides are also ACE inhibitors. These come from a wide variety of commonly consumed plants and plant products: potatoes, rice, soybeans, yams, flaxseed, rapeseed, and canola to name a few (Pihlanto and Mäkinen 2013). They are very short sequences (often 2–8 amino acid long) with polar amino acid residues. Additionally, the presence of basic or aromatic amino acids may also improve their ACE-inhibiting activity (Pihlanto and Mäkinen 2013). A study by Nakahara et al. (2010) discovered nine ACE-inhibiting dipeptides from fermented soybean seasoning that successfully reduced blood pressure in both spontaneously hypertensive rats and Dahl salt-sensitive rats, with IC_{50} values ranging from 10 μg/mL to 1100 μg/mL. Nicotianamine, a common nonprotein amino acid, was also reported to exhibit antihypertensive activity in that same study. Sweet potato proteins also contain several 3–5 amino acid long sequences exhibiting antihypertensive activity in spontaneously hypertensive rats, with Ile-Thr-Pro exhibiting the best ACE-inhibiting activity (IC_{50} = 9.5 μM) (Ishiguro et al. 2012).

2.4.4 Antimicrobial Plant Metabolites

Protozoan-related diseases are often classified under neglected tropical diseases, despite affecting over a billion people worldwide and killing millions annually, often from underdeveloped and developing countries. While many of these diseases do have (typically decades old) treatments, their efficacies vary, and many are subject to issues such as poor bioavailability, high toxicity/multiple side effects, and unknown mechanism of action. Unfortunately, given that many of those affected come from poorer populations, further research into these treatments are often considered unlucrative (Schmidt et al. 2012a). Schmidt et al. (2012a, b) and Ogungbe and Setzer (2016) had previously published an extensive review of plant secondary metabolites with anti-protozoan potential, particularly against malaria (*Plasmodium*), trypanosomiasis (*Trypanosoma*, including the African sleeping sickness), and leishmaniasis (*Leishmania*).

To summarize their findings, unsurprisingly, multiple classes of plant secondary metabolites exhibited in vivo activity in rodents infected with protozoa. This includes the lignin (−)-hinokinin, the quinone isolapachol, a polyacetylenediol from *Bidens pilosa* (Asteraceae), the coumarins (−)-heliettin and (+)-3-(1′-dimethylallyl)-decursinol (Schmidt et al. 2012a), the germacranolides 11(13)-dehydroivaxillin from *Carpesium cernuum* and ineupatorolide A from *Carpesium rostulatum*, the guaianolide cynaropicrin from artichoke (*Cynara*), the clerodane diterpene 16α-hydroxycleroda-3,13(14)Z-dien-15,16-olide from *Polyalthia longifolia* var. *pendula* leaves (Annonaceae), and the quassinoids bruceolide, simalikalactone D from *Quassia amara* leaves, cedronin, and ailanthone from *Ailanthus altissima* (Schmidt et al. 2012a).

Notably, multiple chalcones were reported to exhibit in vivo anti-leishmanial activity. This included licochalcone A (from Chinese licorice) and (−)-methyllinderatin from *Piper hostmannianum*, amongst many others. Many flavonoids (e.g. quercetin and quercitrin), xanthones (isolated from members of the Clusiaceae and

Gentianaceae families), and annonaceous acetogenins (from the family Annonaceae) also exhibited antiprotozoan activity. Unsurprisingly, alkaloids made a strong showing in this category as well, with in vivo antiprotozoan activity being exhibited by the quinolone alkaloid γ-figarine from *Helietta apiculata* bark (Rutaceae), the indole alkaloid isosungucine, the steroidal alkaloid α-chaconine, the quinazoline alkaloid peganine hydrochloride dehydrate from *Peganum harmala* L. (Zygophyllaceae), the alkaloid prosopilosidine from *Prosopis glandulosa var. glandulosa* leaves (Fabaceae), and the aromatic alkaloid cassiarin B from *Cassia siamea* (Fabaceae). Curiously, despite the commonly touted notion that plants are good sources of antibacterial compounds, it is nevertheless rare to find truly spectacular antibacterial compounds in plants and that most plant metabolite-based antibacterial activity tends to be relatively weak and/or only best demonstrated in vitro (Gupta and Birdi 2017). Unsurprisingly, bacteria and fungi remain the best sources of antibacterial and antifungal compounds.

2.5 Lead-Based Drug Discovery and Development Process

This section will primarily discuss (1) the various approaches to the selection of starting material, (2) the different high-throughput computational screening strategies, and (3) the selection of bioassay tests.

2.5.1 Approaches to the Selection of Starting Material as Leads for Drug Discovery

There are various methodologies to be considered in natural product-based drug development; and each can be specifically modified to complement the desired aim of the study. The general design classically begins with the identification and acquisition of plant biomass. The initial selection of starting material, i.e. the selection of plant to be studied, can be approached in a few ways. The standard set of tactics in selecting a potential starting material include (1) random screening, (2) ethnopharmacological investigation, (3) ecological approach, and (4) computational approach. The random screening approach involves the casual selection of starting material based on the availability and accessibility of the plant. While seemingly haphazard, this approach has the potential to identify unexpected compounds that could otherwise not be predicted by any currently available technology. The ethnopharmacological approach, on the other hand, is widely regarded as the classic approach to natural product drug discovery. It involves the transdisciplinary understanding of botany, chemistry, and pharmacology, along with history, anthropology, and the knowledge of people indigenous biomass source location. The ecological approach factors in the interactions between different communities in a specific environment. The hypothesis behind this approach is the notion that the metabolites produced by plants may be influenced by the interactions between the said plant with other organisms such as bacterial and fungal species, therefore

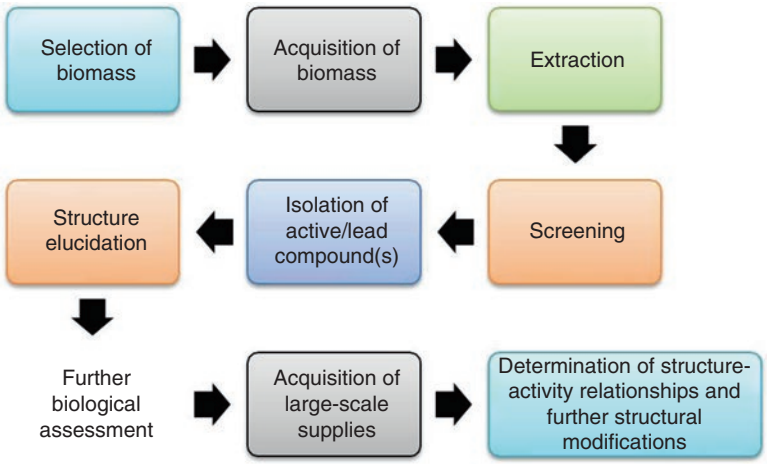

Fig. 2.1 The general design for natural product drug development

potentially producing a unique ecological function. Lastly, the computation approach of biomass selection is the in silico prediction of bioactivity based on chemical libraries. Examples of computation screening include in silico protein-ligand simulations, pharmacophore-based virtual screening, and molecular docking techniques. Once the biomass has been selected, researchers need to obtain a voucher specimen by the host country for their desired plant which states both genus and species before biomass acquisition can take place, to ensure accurate recording of botanical identification and nomenclature (Atanasov et al. 2015). The following steps are in accordance with Fig. 2.1.

2.5.2 Computational Screening Strategies of Lead-Based Drug Discovery: High-Throughput Screening (HTS)

Traditionally, screening for bioactivity involves the use of simple broad-based assays to check for desired bioactivities, such as antimicrobial activity or cytotoxicity. This is then followed up by the isolation of compounds, often using bioactivity-guided fractionation. While effective, this route is time-consuming, costly, and laborious. The need for more precise and specific screens that are rapid and cost-effective is in high demand. Progressively, screening methods are becoming more refined and systematic. Today, researchers often use various computational methods (ranging from specific biochemical-based screening to genetics-based screening) within the parameters of complementary high-throughput screening (HTS) strategies in hopes of identifying a potential bio-active lead compound with the desired chemical structure and biological properties (Cragg and Newman 2013). HTS is a process of drug discovery that assays biological or biochemical activity (e.g. profiling biochemical pathways of a large amount of potential lead compounds) using a

combination of various methods including robotics, data processing software, liquid handling devices, and sensitive detectors. The obtained results would then be analysed in hopes of identifying and understanding the interaction or role of a particular biochemical process.

One example of a computational-based HTS analysis is via in silico simulations to study binding properties of specific (yet hypothetical) protein-ligand combinations based on the compound's molecular structure and chemistry. These predictions offer researchers a higher chance of identifying a potential lead compound (also known as a "hit") for further experimental study. In order to analyse potential lead compounds, they must be compared to lead-like libraries. These libraries are a collection of chemically diverse compounds to test a wide range of biological interaction or vice versa in the efforts to focus on specific targets with a less diverse collection of chemical space. Typically, natural product-based approaches can be designed in a few ways: (1) target-oriented synthesis, (2) diversity-oriented synthesis, (3) biology-oriented synthesis, and (4) functional-oriented synthesis. With the appropriate HTS strategies and suitable library options, many descriptors of the compound can be measured for further analysis of drug potential. Examples of descriptors include molecular weight, logP, hydrogen bond donors, hydrogen bond acceptors, polar surface area, rotatable bonds, and aromatic ring count (Pascolutti and Quinn 2014). These descriptors are then analysed next to compound databases for correlation analysis as well as structure-activity relationship modelling. Another strategy is the use of pharmacophore-based in silico simulations in order to evaluate the physicochemical properties of target-ligand interactions based on the pharmacophore's 3-D model (Atanasov et al. 2015).

Unfortunately, the bioactivity of a "hit" generated by HTS screening is often underwhelming when tested in vitro. To address this issue, researchers may opt for phenotypic cell assays in a semi-HTS mode with natural product extracts (Newman and Cragg 2016). The phenotypic screen-based approach attempts to identify a lead prior to target identification and subsequent lead optimization. The molecular target screen-based approach, on the other hand, has a predetermined target identification before HTS screening for lead compounds and lead optimization (Zheng et al. 2013). The crude plant extracts must be free from contaminating materials such as tannins and pre-fractionated prior to cell-based or isolated protein HTS screening (Cragg and Newman 2013; Newman and Cragg 2016).

2.5.3 Bioassay Selection Strategies of Lead-Based Drug Discovery

Bioassay selection strategies are typically based on personalized study objectives and the accessibility or availability of relevant technologies, machinery, expertise, and funding. (1) In vitro assay with purified proteins is a benchmark high-throughput bioassay technique, which aims to study the compound-ligand interactions in terms of functional activity and/or physical interaction. This method is typically more cost-efficient as it does not require animal models and facilities. However, the

negative prospects of this method include non-specific or irrelevant "hits". This in turn may translate to the failure of the lead compound when subjected to further cell-based experimentation. This leads to the discussion of (2) in vitro cell-based and/or target-oriented assays which are a more robust adaptation of the classical purified protein in vitro assays. It differs by utilizing cell culture techniques therefore providing data of the molecular target. Another in vitro approach is using (3) phenotypic cell-based assays. This method is more comprehensive than the first two approaches as it can validate results of protein-based assays and even help elucidate potential molecular mechanisms (Hughes et al. 2011). However, none of the bioassay approaches discussed so far are able to assure in vivo activity. Thus, before proceeding to in vivo testing, researchers often resort to (4) in situ and/or ex vivo assays. These assays utilize isolated animal tissues or organs rather than the entire animal model. This technique is not only more cost-efficient than in vivo assays (and therefore able to accommodate a larger sample size allowing for a higher throughput), but it also provides analysis on pathophysiology. Lastly, one of the final analysis done prior to clinical testing would be (5) in vivo testing in animal models. This method incorporates the use of live animal models, typically rodents. It analyses the efficacy of the drug in a living model in terms of bioavailability, side effects, and toxicity due to its comparatively higher degree of pathophysiological relevance to humans (Honório et al. 2013). At times, the animal models are subjected to "humanization" by genetically engineering the models to possess relevant proteins and targets (Hughes et al. 2011; Atanasov et al. 2015).

2.6 Challenges Involving the Use of Plant Metabolites as Leads in Drug Development

2.6.1 Accessibility to Starting Compound

Once a lead structure is identified, large-scale extraction and development may not necessarily be feasible. Natural product-based drugs often have intrinsic challenges, such as low availability of the active compound (e.g. low concentration in plant tissues or plant material is not easily obtainable). To combat this issue, scientists may resort to total or semi- synthesis of the original natural product compound. However, this route may be too costly and time-consuming, thereby making the approach potentially impractical. As aforementioned, plant material is not always readily obtainable; and in some cases, the acquisition of biomass may be heavily regulated. To promote research and development in the field of drug discovery, mutually beneficial agreements between host countries (typically with rich biodiversity, such as the tropics) and pharmaceutical companies are reached. Often, this includes specifics on royalty distribution, technical training of local collaborators, and technology sharing. The Convention of Biodiversity (CBD), held in 1992, designated policies regarding biomass resource ownership. These policies state that the host country has an "exclusive property of their bioresources and have the freedom to trade them like

any other commodity". Therefore, the host country needs to be recompensed in some way by pharmaceutical companies who intend to collect biological samples for the purposes of drug development (Mishra and Tiwari 2011; Cragg and Newman 2013; Atanasov et al. 2015).

2.6.2 Intellectual Property (IP) and Biopiracy

Intellectual property (IP) is a particularly important component in the discussion of drugs developed from nature. IP, particularly when legally enforced (i.e. patented), allows pharmaceutical companies to be compensated for their discovery, research, and investment into developing a new drug. This acts as a fiscal incentive for pharmaceutical companies to invest capital into research and development. There are three main sectors that would allow for patent applications: (1) the discovery of new chemical components, (2) processes or methodologies involved in attaining specific chemical entities, and (3) trademarks. The patent in question must also suffice in areas of novelty, usefulness, and nonobviousness. It is unfortunately increasingly difficult (and typically downright impossible) in many countries to patent naturally occurring compounds, especially after the US Supreme Court's decision in Association for Molecular Pathology v. Myriad Genetics, 569 US 12 (2013) (Wong and Chan 2014). This may be somewhat disincentivizing to the pharmaceutical industry, especially given that drug development can typically take well over a decade and cost hundreds of millions of dollars (Jachak and Saklani 2007). Additionally, traditional knowledge of indigenous people must be taken into consideration. The exploitation of natural substances with historical relevance to indigenous people may lead to biopiracy (Jayaraman 1997; Atanasov et al. 2015; Mishra and Tiwari 2011).

Biopiracy, the improper authorization of IP patents and/or commercialization of traditional medicine or its original biological resource, is a rising issue. It is estimated that up to 95% of all patents involving natural products and its respective medical use are held in developing countries. In 1995, an instance of biopiracy involved University of Mississippi's patent of turmeric, *Curcuma longa* L. (Zingiberaceae), for the use of wound healing. India challenged the patent claiming turmeric does not fulfil the "novelty" criterion required by patent applications and argued that turmeric had been a household remedy for various applications (including wound healing) for centuries. This claim was heavily supported by Ayurvedic texts and other published literature regarding Indian systems of medicine. The patent was revoked over a year after its initial grant (Jayaraman 1997). This incident clearly proved that natural products with therapeutic properties, especially those found in developing countries, are exposed to potential exploitation. Laws and regulations regarding biopiracy, patent application, IP protection, and ecosystem conservation are crucial to the economic and environmental sustainability of the host country (Mishra and Tiwari 2011).

2.6.3 Poor Bioavailability

As is often the case, preliminary in vitro screening of plant metabolites may lead to the discovery of several compounds which exhibit desirable bioactivity in vitro. Sadly, most of these "hits" end up as drug leads rather than drugs, with most in vitro bio-active compounds being utterly ineffective in vivo (Anand et al. 2007). As a matter of fact, researchers should never extrapolate the importance of in vitro testing. Therefore, in vivo assessments should be made before these compounds can be classified as the new "leads" or drugs. This step serves as a checkpoint to validate their actual bioactivities either in animal models or subsequent clinical trials (if it ever reaches this stage).

The poor bioavailability exhibited by most plant secondary metabolites is deemed as a typical setback for these compounds (Thilakarathna and Rupasinghe 2013). In normal in vitro assays, these issues are often overlooked since the compounds are in direct contact with the cells or chemicals. This discounts that the uptake and distribution of these bio-active metabolites are the key to their advancement in drug development. Therefore, establishment of a potential drug candidate's pharmacokinetic profile (absorption, distribution, metabolism, and excretion) is essential to justify the bioactivities of these compounds in body (Fig. 2.2). While there are plenty of possible administration routes for plant metabolites, many studies focus on oral delivery. For the sake of briefly illustrating the many factors involved in drug delivery (and without discounting the viability of the numerous

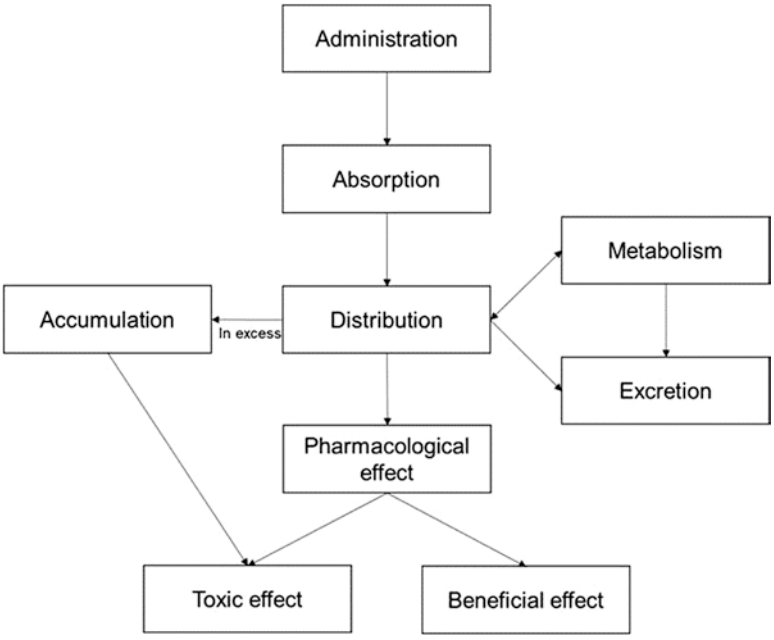

Fig. 2.2 An overview on the pharmacokinetic profile of plant metabolites in body

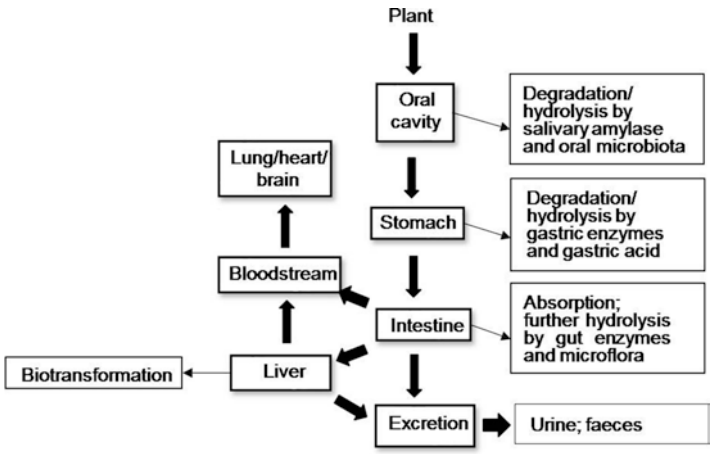

Fig. 2.3 Simplified illustration of pharmacokinetic pathways of plant metabolites via oral administration in body (thick arrow) and their serial bioavailability events (thin arrow)

other drug administration routes), Fig. 2.3 will use oral delivery as an example. The common complications faced by these "leads" in a complex biological body system via the oral administration include:

1. If the compounds have poor bioavailability, most of them will not be absorbed or successfully delivered to the site of action.
2. If the compounds are readily and rapidly being metabolized and/or excreted, this will decline the amount of the active form distributing or acting in the body.
3. If the route taken by the compound and/or site of action has unfavourable conditions for the maintenance of the active form (e.g. low pH), this may hinder their pharmacological effect as a result of the destruction or change in the compound's structure.
4. High-dose administration may seemingly help overcome the aforementioned issues but is not typically the best solution as it may cause excessive accumulation of the compounds or their derivatives in the body, thereby potentially leading to undesirable side effects or toxicity (Rein et al. 2013).

As an example, curcumin (as aforementioned) from the spice turmeric, *Curcuma longa* L. (Zingiberaceae), has been well-documented for its bioactivities including antioxidant, anticancer, anti-inflammatory, and antimicrobial activities. It was suggested that curcumin acts via multi-targets such as inhibition of cell proliferation and modulation of various signalling molecules.

Numerous clinical trials have been performed on curcumin after its discovery, making it a popular subject of study based on its pharmacological potential as new drug (Gupta et al. 2013). Unfortunately, its development as a new drug met a major obstacle: its poor bioavailability. The pharmacokinetic profile of curcumin showed that only trace amounts of curcumin were detected in human serum after oral

administration of 12 g of curcumin, while its concentration in plasma level was below detection limit (Lao et al. 2006). In addition to the poor absorption, whatever little curcumin that is absorbed is then subjected to metabolism either by the microflora or enzymes, via glucuronidation and sulfation (Anand et al. 2007). These metabolites may not share the same bioactivities of curcumin. As a result, the low availability of curcumin will greatly affect its distribution to its site of action, which in turn deteriorates its pharmacological effect and therapeutic value. This has limited the progression of curcumin research as a drug candidate. Hence, most recent studies have focused on finding an alternative approach to resolve this setback. These include rethinking the administration route, nanodrug formulation/design of transporter medium for drug delivery, protect curcumin from the metabolic pathways via a concomitant adjuvant, and structural modification for analogues with better bioavailability (Tian et al. 2017; Karade and Jadhav 2018; Peng et al. 2018).

Another promising candidate, epigallocatechin gallate (EGCG), suffers from a similar fate. EGCG is the main polyphenol found in green tea, and the wide-ranging beneficial properties of this compound have been studied intensively by many research groups worldwide. However, the poor bioavailability of EGCG has hampered its potential to be a new drug candidate: human subjects that ingested 2 mg EGCG/kg of their body weight had plasma EGCG concentrations below 80 ng/ml (Lee et al. 2002).

While the various routes of drug administration (above and beyond just oral delivery) lie outside the scope of this chapter, it is nevertheless crucial to correctly choose the route of administration. For example, an in vivo pharmacokinetic study using mouse model conducted by Banerjee et al. (2016) had revealed that the oral administration of andrographolide, isolated from *Andrographis paniculata* (Burm. f.) Wall. ex Nees (Acanthaceae), showed poorer bioavailability as compared to that of intravenous injection. Orally delivered andrographolide demonstrated a half-life which was two times faster than that of intravenous injection, suggesting its fast clearance in the body via oral route. However, even a more effective drug administration route does not guarantee "good" bioavailability per se: regardless of administration methods, it was found that andrographolide suffered from rapid metabolism and elimination from the body system of mice, thus ultimately still resulting in poor bioavailability. Given the complexity of mammalian biology, even positive results from in vitro studies do not necessarily grant plant metabolites a role as new drug candidate. The pharmacokinetic profile of these compounds is indeed one of the biggest pitfalls impeding attempts to translate them into a new drug. Nonetheless, these bio-active plant metabolites may still serve as lead compounds for clinically worthy derivatives.

2.7 Conclusions and Future Prospects

Drug discovery is a highly saturated field and can be approached in numerous ways: from the "traditional" ethnopharmacological approach of natural products to a completely synthetic approach. Although the search for drugs and drug leads from plant

metabolites is a relatively old field, research in this field has nevertheless continued to grow, incorporating new concepts, techniques, and technologies to improve the accuracy and rate of drug discovery. While the potential benefits of utilizing plant metabolites as leads in drug discovery are numerous (and indeed there are many success stories), there are nevertheless important components involved in natural product-based drug discovery that should be more explicitly addressed such as the discussion of intellectual property and biopiracy. Additionally, there is value in focusing on improving bioavailability and other pharmacokinetic and toxicity-related parameters that would otherwise impede the successful development of a plant-based drug. Nevertheless, the valuable pharmacological effect exhibited by these bio-active plant compounds as well as their diverse structural scaffolds has contributed numerous new leads and insights for future drug discovery.

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