https://doi.org/10.48047/AFJBS.1.02.2019.83-116

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Phytochemical Profiling and Therapeutic Significance of Plant Secondary Metabolites

Somesh Thapliyal[1*], Prashant Kumar Desai[2], Peeush Singhal[3]

1. Department of Pharmaceutical Sciences, Hemvati Nandan Bahuguna Garhwal University (A Central University), Srinagar Garhwal, Uttarakhand somesh.thapliyal@gmail.com
2. Department of Pharmacy, Lachoo Memorial College of Science and Technology, Jodhpur, Rajasthan prashant19481@gmail.com
3. Department of Pharmaceutical Sciences, Gurukul Kangri University, Haridwar, Uttarakhand peeushpharma@gmail.com

Volume 1 Issue 2 April 2019
Received 28 January 2019
Accepted 03 March 2019
Published 12 April 2019
doi:10.48047/AFJBS.1.2.2019.83-115

Abstract

Secondary metabolites (PSMs) of plants are large group of highly diversified organic compounds which do not directly participate in plant primary metabolism, growth, development or reproduction but serve several important functions for the plant, interact with other plants, and are increasingly being used in human therapeutics. In this detailed review, the chemical profile of the most important classes of PSMs (alkaloids, phenolics, terpenoids and sulfur compounds) is discussed, and questions concerning their biosynthetic pathways, regulatory mechanisms and their pharmacological relevance are raised. A systematic literature review was conducted according to PRISMA guidelines, including 94 peer-reviewed publications from 2001 to 2018. We found that the structural diversity of PSM could be extremely dependent on plant species, environmental conditions (UV radiation, temperature, pressure due to herbivores, etc) and peculiar biosynthetic route networks (shikimate, mevalonate and mevalonate-independent routes). Next, a critical comparison between the different extraction techniques was made based on the efficiency of the bioactive constituent(s) recovery, namely conventional Soxhlet extraction method and other emerging methods such as microwave-assisted extraction and supercritical fluid extraction. They have been used in therapeutic applications such as anticancer mechanisms), antimicrobial activity, modulation of antioxidant defence mechanisms, neuroprotection, metabolic regulation. The review concludes with a summary of the gaps in knowledge regarding metabolomics, synthetic biology and translation to clinical applications of PSM based therapeutics, and the recommendations for future research.

Keywords: Plant secondary metabolites; Phytochemistry; Alkaloids; Phenolics; Terpenoids; Extraction methods; Anticancer activity; Antioxidant; Biosynthesis; PRISMA review

1. Introduction

Studies have found the most abundant source of bioactive chemical compounds on plants. Sessile plants have developed an incredible array of biochemical armoury of secondary metabolites—these are not involved in primary growth and reproduction, but under ecological conditions they can confer significant survival value (Taiz and Zeiger, 2010; Dewick, 2009). The phytochemicals are chemical messengers involved in plant-environment interactions, and play role in defense against herbivores, pathogens, supplying herbivores, competing organisms, and also help them adapt to abiotic factors such as ultraviolet radiation, drought and extreme temperature (Freeman and Beattie, 2008; Ramakrishna and Ravishankar, 2011). Plant secondary metabolites (PSMs) are much more than ecological. Throughout the ages, the use of different plants has resulted in the production of compounds with medicinal, nutritional and industrial properties (Raskin et al., 2002; Cragg et al., 2014). However, in the present day, modern pharmacognosy has revealed that many of the clinically established drugs are either pure extracts of plants, or are semi-synthetic products or synthetics that are based on natural products from plants (Cragg and Newman, 2014; Katz and Baltz, 2016). According to the World Health Organization estimates, approximately 80% of the population in the world are using traditional medicine mainly plant based traditional medicine as their primary healthcare needs (Verde-Star and Garcia-Gonzalez, 2016). A huge number of PSMs exist having a wide variety of chemical structures. More than 200,000 different secondary metabolites are currently estimated with more than 20% of these classified as phenolics, terpenoids, alkaloids and sulfur containing compounds (Kroymann, 2011; Berini et al., 2018). They are products of relatively few biosynthetic pathways, the most important of which are the shikimate pathway, the mevalonate (MVA) pathway and the methylerythritol phosphate (MEP) pathway, but combinatorial enzymatic modifications and the species-specific regulatory mechanisms result in an extraordinary structural and functional diversity (Vranova et al., 2012; Avalos Garcia and Perez-Urria Carril, 2009). The functions served by PSMs are also very different. Bark beetles and other pests are structural and chemical enemies to which conifer bark contains as a structural and chemical defense, such as tannins and resin acids (Franceschi et al., 2005; Trapp and Croteau, 2001). Inducible defenses like phytoalexins and VOCs are triggered upon plant infection by a pathogen or herbivore feed on the plant, and often they also serve as aerial signals which “activate” neighboring plants to become more resistant to the pathogen (Ahuja et al., 2012; Loreto et al., 2014). PSMs are also involved in allelopathic interaction with growth and germination of other plant species that grow in the same neighbourhood (John and Sarada, 2012), and in mutualistic interaction with pollinators and seed dispersers, uses pigments and fragrance (Valenta et al., 2013; Caputi and Aprea, 2011).

Pharmacological activities of PSMs have been the subject of a great deal of scientific research from a therapeutic point of view. Flavonoids and phenolic acids are amongst the phenolic compounds that have been well characterized for their antioxidant, anti-inflammatory and enzyme inhibitory properties (Cheynier et al., 2013; Dai and Mumper, 2010; Nile et al., 2018). Some of the most widely used chemotherapeutic agents for clinical oncology are the alkaloids such as vincristine, vinblastine and camptothecin (Matsuura and Fett-Neto, 2017; Babbar, 2015). Asiatic acid, lupeol and ursolic acid are remarkable bioactive terpenoids that have potent anticancer, antimicrobial and hepatoprotective activities (Tian et al., 2017; Liu et al., 2015; Yeh et al., 2010). The therapeutic activity of these compounds is additionally enhanced, in their interaction with each other as well as with other medications (Mikulasova et al., 2016; Martelli and Giacomini, 2018). There are still some knowledge gaps on the connectivity of biosynthesis regulation, environmental conditions influencing the biosynthesis of metabolites and the translation of phytochemical knowledge into a formalized therapeutic use (Olivieto et al., 2017; Hall et al., 2008). Moreover, the optimization of extraction and analytical methods is still an important limitation in phytochemical studies as the choice of method plays a significant role in determining the composition, yield and bioactivity of extracts from plants (Ingle et al., 2017; Azwanida, 2015; Kaufmann and Christen, 2002).

This review aims to review systematically the classification of plant secondary metabolites, their biosynthetic origins, environmental control, extraction procedures and medicinal properties. Based on the PRISMA guidelines, we carefully reviewed 94 peer-reviewed papers published from 2001 to 2018 and summarised the current state of knowledge and identified key research gaps that need to be addressed in future studies.

2. Methodology

This systematic review was carried out according to those developed in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The literature survey included several electronic databases, such as PubMed, Scopus, Web of Science, and Google Scholar, from 2001 to 2018. The search strategy used was based on various combinations of the following keywords: “plant secondary metabolites,” “phytochemistry,” “alkaloids,” "phenolic compounds," "terpenoids," "extraction methods," "anticancer activity," "antioxidant," "biosynthesis," and "therapeutic applications."

The following papers were included in the study: (i) peer-reviewed original research and review papers; (ii) papers containing information about their classifications, biosynthesis, extraction, and/or therapeutic uses of plant secondary metabolites; (iii) published in English and/or Spanish language with full text available; and (iv) published between January 2001 and December 2018. The following abstracts were removed: (i) only conference proceedings abstracts; (ii) only primary metabolites studies; (iii) duplicate publications, abstracts, and/or inadequate methodological details. In total, 94 references were included and used in this review.

2.1 PRISMA Flow Diagram

The Systematic Literature Review (SLR) Process was adapted from available literature, as shown in Figure 1, PRISMA Flow Diagram of the Systematic Literature Review Process.

Figure 1. PRISMA Flow Diagram of the Systematic Literature Review Process

IDENTIFICATION *Records identified through database searching (n = 1,247)*
↓
SCREENING *Records after duplicates removed (n = 843) Screened by title and abstract (n = 843) Records excluded (n = 592)*
↓
ELIGIBILITY *Full-text articles assessed for eligibility (n = 251) Full-text articles excluded with reasons (n = 157): • Focus on primary metabolites (n = 62) • Insufficient methodological detail (n = 48) • Duplicate publications (n = 29) • Non-peer-reviewed sources (n = 18)*
↓

INCLUDED

Studies included in qualitative synthesis (n = 94) • Classification & biosynthesis: 28 studies • Extraction & analytical methods: 22 studies • Therapeutic applications: 26 studies • Ecological significance: 18 studies

3. Classification of Plant Secondary Metabolites

Plant secondary metabolites traditionally divided into four major groups, depending on their biosynthetic origin and chemical structure: phenolic metabolites, terpenoids (isoprenoids), nitrogen-containing metabolites (mainly alkaloids) and sulfur-containing metabolites (Tiwari and Rana, 2015; Kroymann, 2011). Within each class there is incredible variation in molecular structure and biological activity and/or ecological function (Dewick, 2009; Aniszewski, 2007). Information on the classification and representative compounds are summarized in Table 1.

3.1 Phenolic Compounds

The phenolic compounds are the most prevalent and ubiquitous plant secondary metabolites, which contain one or more hydroxyl group(s) on an aromatic benzene ring structure (Seabra et al., 2006; Cheynier et al., 2013). The synthesis of these compounds occurs mainly through the shikimate pathway and the phenylpropanoid pathway, where the enzyme involved that regulates the process is phenylalanine ammonia-lyase (PAL) (Lattanzio et al., 2008). The phenolics can be simple, such as phenolic acids (gallic acid, caffeic acid) or more complex, polymeric phenolics such as condensed tannins and lignins (Dai and Mumper, 2010). Flavonols (quercetin, kaempferol), flavones (apigenin, luteolin), flavanones (naringenin, hesperetin), anthocyanins and isoflavonoids are the major subclass of phenolics found in these plants (Wang et al., 2018; Nile et al., 2018). In their review, Cheynier et al (2013) reported their biosynthesis, genetic regulation and ecophysiology, including their role in: The intestinal microflora does specific metabolic changes to flavonoids that leads to the formation of metabolites which are more easily absorbed and have unique biological properties (Surco-Laos et al., 2016; Velderrain-Rodriguez et al., 2014). UV-A radiation (Verdaguer et al., 2017), cold stress (Weidner et al., 2014), abiotic stress (Berini et al., 2018) all appear to have an impact on the phenolic content of plant tissues over the past few years.

3.2 Terpenoids (Isoprenoids)

Terpenoids are the biggest and most structurally diverse group of plant secondary metabolites with more than 55,000 known compounds (Singh and Sharma, 2015). Terpenoids are made up of isoprene units of five carbons and are classified as to the number of isoprene units per terpenoid e.g. monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), triterpenes (C30), tetraterpenes (C40). Terpenoids are synthesized through two distinct pathways: the cytosolic mevalonate (MVA) pathway that mainly leads to the production of sesquiterpenes and triterpenes; and the plastidial methylerythritol phosphate (MEP) pathway, in which mono- and diterpenes are produced (Vranova et al., 2012). Essential oil constituents which includes monoterpenes like limonene and geraniol are involved in composition of essential oil and are used as attractants or repellents for insects (Chen and Viljoen, 2010; Dubey and Luthra, 2001). Lupeol, Asiatic acid, ursolic acid and others terpenoids have been of great interest for their anticancer and anti-inflammatory effects (Liu et al., 2015; Tian et al., 2017; Yeh et al., 2010). Vanhercke et al. (2013) tried to metabolically engineer plant terpenoids for industrial feedstock applications, and Ghimire et al. (2016) reviewed the potential use of plant terpenoids such as squalene and its derivatives in the pharmaceutical sector. Volatile terpenes volatilized from the tissues of plants play important ecosystem roles in a variety of ways, such as in plant-plant communication, herbivore protection, and air chemistry (Loreto et al., 2014). In microalgal systems, it has been demonstrated that the terpenes that disrupt membranes (limonene) alter the structure of the lipid in the organisms (Concha et al., 2018).

3.3 Alkaloids

Alkaloids are a mixed class of nitrogen-containing secondary metabolites, which are generally formed from certain amino acid precursors, namely, tryptophan, tyrosine, lysine and ornithine (Aniszewski, 2007; Azimova and Yunusov, 2013). The compounds are distinguished by the presence of a basic nitrogen atom in the heterocycle, and can be useful in pharmacologic applications. These major structural classes include indole alkaloids (vincristine and vinblastine), isoquinoline alkaloids (morphine, codeine and berberine), tropane alkaloids (atropine and scopolamine), pyridine alkaloids (nicotine) and purine alkaloids (caffeine and theobromine) (Kumar, 2014; Kartsev, 2004). The alkaloids have important pharmaceutical applications, accounting for about 12% of the total number of plant natural products and they are some of the most prescribed drugs in the world (Babbar, 2015; Matsuura and Fett-Neto, 2017). In neurological disorders, plant alkaloids, like galantamine, in the treatment of

Alzheimer's disease have been developed as acetylcholinesterase inhibitors (Ng et al., 2015). The anticonvulsant activity of isovaleramida, isolated from Valeriana pavonii, is proof of the continuous possibility to discover new drugs with neuropharmacological activity based on alkaloid natural compounds, whereas the GABAergic activity of neroli essential oil illustrates the versatility of the neuropharmacological activity found in essential oils (Azanchi et al., 2014). Matsuura and Fett-Neto (2017) have reviewed the toxicity and mechanisms of action of these key plant alkaloids, emphasizing the potential toxicity and dual role of these alkaloids. Recent work in the field has suggested that alkaloids like umbelliprenin and auraptene have cytotoxic activity through the modulation of the expression of the gene myeloid cell leukemia type-1 (Mcl-1) (Motlagh and Gholami, 2017); indole-3-carbinol and its derivatives have a preferential effect on ERα+ breast cancer cells, exerting cytotoxic activity (Caruso et al., 2014).

3.4 Sulfur-Containing Compounds and Other Classes

Sulfur containing secondary metabolites like the glucosinolates and hydrolysis products (isothiocyanates), organosulfur compounds (OSCs) from Alliums (diallyl sulfide, allicin) and thiocyanates are important but relatively understudied class of PSMs. The compounds are obtained from sulphur-containing amino acids like methionine and cysteine, and have strong biological activity and spicy taste. Besides the sulfur compounds, other major classes of PSMs are phytoecdysteroids, which are chemical defense against plant-parasitic nematodes (Soriano et al., 2004), and other non-protein amino acids, which are antimetabolites against herbivores. Cyanogenic glycosides, which release hydrogen cyanide in the event of tissue damage do provide a good protection against generalist herbivores (Kroymann, 2011).

Table 1. Major Classes of Plant Secondary Metabolites: Classification, Biosynthetic Origin, Representative Compounds, and Biological Activities

Class	**Subclass**	**Representative Compounds**	**Biosynthetic Pathway**	**Key Biological Activities**
Phenolics	Flavonoids	Quercetin, kaempferol, apigenin, luteolin, catechin	Shikimate / Phenylpropanoid	Antioxidant, anti-inflammatory, anticancer (Nile et al., 2018; Dai and Mumper, 2010)

Phenolics	Phenolic acids	Gallic acid, caffeic acid, rosmarinic acid	Phenylpropanoid pathway	Antimicrobial, hepatoprotective (Cheynier et al., 2013; Martelli and Giacomini, 2018)
Phenolics	Tannins	Procyanidins, ellagitannins	Shikimate pathway	Astringent, antimicrobial, antioxidant (Lattanzio et al., 2008; Sumczynski et al., 2016)
Terpenoids	Monoterpenes	Limonene, geraniol, menthol	MEP pathway	Antimicrobial, insecticidal, aroma (Chen and Viljoen, 2010; Concha et al., 2018)
Terpenoids	Sesquiterpenes	Farnesol, artemisinin, umbelliprenin	MVA pathway	Antimalarial, cytotoxic (Motlagh and Gholami, 2017; Singh and Sharma, 2015)
Terpenoids	Triterpenoids	Lupeol, asiatic acid, ursolic acid	MVA pathway	Anticancer, anti-inflammatory (Liu et al., 2015; Tian et al., 2017; Yeh et al., 2010)
Terpenoids	Tetraterpenes	β-carotene, lycopene, lutein	MEP pathway	Antioxidant, provitamin A (Cutzu et al., 2013; Velderrain-Rodriguez et al., 2014)

Alkaloids	Indole alkaloids	Vincristine, vinblastine, reserpine	Tryptophan (Shikimate)	Anticancer, antihypertensive (Matsuura and Fett-Neto, 2017; Caruso et al., 2014)
Alkaloids	Isoquinoline alkaloids	Morphine, codeine, berberine	Tyrosine (Shikimate)	Analgesic, antimicrobial (Kartsev, 2004; Kumar, 2014)
Alkaloids	Tropane alkaloids	Atropine, scopolamine	Ornithine / Arginine	Anticholinergic, mydriatic (Aniszewski, 2007)
Alkaloids	Purine alkaloids	Caffeine, theobromine	Purine biosynthesis	Stimulant, antioxidant (Azimova and Yunusov, 2013)
Sulfur compounds	Glucosinolates	Glucoraphanin, sinigrin	Methionine / Cysteine	Anticancer, chemoprotective (Kroymann, 2011)
Other classes	Phytoecdysteroids	20-Hydroxyecdysone	MVA / MEP pathway	Anti-nematode defense (Soriano et al., 2004)
Other classes	Cyanogenic glycosides	Amygdalin, dhurrin	Amino acid precursors	Herbivore defense (Kroymann, 2011; Berini et al., 2018)

4. Biosynthesis and Regulatory Mechanisms

The biosynthesis of plant secondary metabolites is a very complex set of enzyme pathways, diverging from primary metabolic intermediates. Knowledge of such biosynthetic routes and their regulatory mechanisms is crucial to improve the production of pharmaceutically important compounds (Vranova et al., 2012; Avalos Garcia and Perez-Urria Carril, 2009) and for understanding evolutionary pressures that have given rise to the chemical diversity displayed by plants.

4.1 Core Biosynthetic Pathways

The carbon skeletons of secondary metabolites in plants are formed by three main pathway networks: shikimate pathway, mevalonate (MVA) pathway and methylerythritol phosphate (MEP) pathway (Dewick, 2009; Vranova et al., 2012). Shikimate pathway is localized in the plastid; it transforms phosphoenolpyruvate (PEP) and erythrose-4-phosphate (E4P) into chorismate, which is used in the synthesis of aromatic amino acids, and in the phenylpropanoid pathway, to produce the vast spectrum of phenolic compounds (Lattanzio et al., 2008; Lin et al., 2010). The MVA pathway is located in cytosol and endoplasmic reticulum, and converts acetyl-CoA (start material) to isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), which are the sources of sesquiterpenes and triterpenes (Vranova et al., 2012). But the other metabolic pathway from pyruvate and glyceraldehyde 3-phosphate, called MEP pathway, is a localized pathway in plastids and is mainly important for the biosynthesis of mono- and diterpenes and is an alternative pathway to IPP and DMAPP pathway (Singh and Sharma, 2015). Such island-like parallel routes provide flexibility for plant metabolism, but make understanding of the allocation of flux and/or regulatory communication between the metabolic pathways more complicated (Vranova et al., 2012).

4.2 Pathway Regulation and Signal Transduction

Secondary metabolites are regulated by a number of mechanisms, including feedback inhibition, availability of the substrate, enzyme activity and gene expression. Transcriptional regulation of the phenylpropanoid pathway mainly occurs by the binding of MBW complexes of transcription factors to promoters, which include MYB, bHLH and WD40-repeat (Cheynier et al., 2013). The jasmonic acid (JA) and salicylic acid (SA) signaling pathways play important roles in the induction of secondary metabolism in response to biotic and abiotic stress (Freeman and Beattie, 2008; Ramakrishna and Ravishankar, 2011). Ramakrishna and Ravishankar (2011), reviewed recent advances on abiotic stresses like drought, UV, temperature, mechanical wounding, and heavy metal induced secondary metabolite biosynthesis by the generation of ROS and activation of the related signalling pathways in detail. The isoprenoid pathway network is a complex network with regulatory crosstalk between the MVA and MEP pathways as reported by Vranova et al. (2012), who were able to show retrograde plastidic signals coordinate isoprenoid biosynthesis between cellular compartments.

4.3 Environmental Modulation of Secondary Metabolism

Plant secondary metabolites are heavily influenced by environmental factors, both in regard to the amount of production and the quality of the production. The complexity of multi-factor interactions was shown by Berini et al. (2018) who found that abiotic factors (temperature, CO_2, soil moisture) affected the production of PSMs differently among five species of woody plants in the boreal-temperate transition zone. The phenolic content and antioxidant activity of germinating grapevine seeds has been found to be enhanced by cold stress (Weidner et al., 2014), and UV-A has been shown to stimulate accumulation of phenolics in several plant species by stimulating the phenylpropanoid pathway (Verdaguer et al., 2017). The study by Olivieto et al. (2017) gave a framework for the understanding of how environmental factors interplay to modulate production of PSM by means of interconnected regulatory network. Light quality and quantity is especially important for PSM biosynthesis; preliminary screening experiments conducted by Lin et al. (2010) revealed that plant clonal systems high in phenolics had a stronger capacity to remove nitrate from the system, suggesting a coordinated regulation of PSM phenolics biosynthesis and nutrient acquisition under cold latitudes. The effect of plant growth retardants in the production of PSM is also evident from the study on kharif pigeonpea (Cajanus cajan L.) by Rani and Sudhakar (2018) which showed that plant growth retardants significantly affected plant growth, yield and secondary metabolite production. Also, sample preparation techniques that involve drying can substantially change the metabolite content of the dried material; Abdullah et al., 2012 for instance, found that metabolite composition can vary in the dried leaves of the plant Misai Kucing (Orthosiphon stamineus), depending on the type of drying process used for sample preparation, and Mediani et al., 2013 have optimized the drying conditions which will give a maximum yield of metabolite(s) from Cosmos caudatus when used for optimal conditions.

4.4 Biotransformation and Metabolic Intermediates

The biosynthesis of complex secondary metabolites usually involves a series of enzymatic modifications and biotransformation reactions. Dubey and Luthra (2001) demonstrated that biosynthesis of monoterpenes can be regulated with the development of inflorescence of palmarosa as shown in the biotransformation of geranyl acetate to geraniol. Vanhercke et al. (2013) demonstrated that the re-direction of the metabolic flux towards a desired product in metabolic engineering tools requires very tight control of the expression levels and subcellular localization of the enzymes. One emerging idea is metabolic channeling, whereby metabolites in metabolic pathways are physically near the enzymes which catalyse their transformations, leading to their channeling and biosynthetic efficiency (Vranova et al., 2012). Human intestinal microflora biotransformation is a very important post ingestion metabolic process leading to the generation of bioactive metabolites with different pharmacological properties from the parent compound, which could have significant impacts on bioaccumulation, but also deserve attention for their possible therapeutic properties after biotransformation in the intestinal microflora (Surco-Laos et al., 2016; Velderrion-Rodriguez et al., 2014).

5. Extraction and Analytical Methodologies

The extraction and analytical techniques involved in isolating, identifying and quantifying bioactive compounds from plant matrices play a key role in phytochemical research. Different extraction methods influence the quantity of extracts that can be derived from them, the chemical composition and biological activity (Ingle et al., 2017; Sasidharan et al., 2011). Over the past two decades, the extraction process employed in this extraction process have changed from the conventional Soxhlet extraction and maceration method to other methods that are more advanced and have been introduced such as microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), pressurized liquid extraction (PLE), and supercritical fluid extraction (SFE) (Kaufmann and Christen, 2002; Azwanida, 2015).

5.1 Conventional Extraction Methods

Traditional extraction methods are still employed as they are simple, inexpensive and established. However, maceration is carried out by letting plant material extract in a suitable solvent at room temperature for a long period of time and provides gentle extraction conditions that can preserve thermolabile compounds (Sasidharan et al., 2011; Pandey et al., 2014). The Soxhlet extraction method involves continuous recycling of the solvent, and extracts the material from the thimble, which loaded with the plant material, is what is being processed to obtain the maximum amount of extracted compounds (Redfern et al., 2014; Tiwari et al., 2011). Tiwari et al. (2011) comprehensively reviewed all the screening and extraction techniques of phytochemicals and found that the selection of solvent is the most critical step to be taken in the extraction process because the solvent used would determine the efficiency of the extraction process depending on the polarity of the solvent. The study done by Duque et al. (2013) compared the quality of avocado oil from supercritical fluid extraction with the quality of avocado oil from conventional extraction method and found that extraction method would have significant effect on the quality parameters of avocado oil such as fatty acid composition, tocopherol content, and oxidative stability.

5.2 Advanced Extraction Technologies

This localization of heating will occur in the plant matrix when sunlight is absorbed by polar molecules and this localized heating will be enough to increase the amount of analytes released into the solvent, this process is called microwave-assisted extraction (MAE) (Kaufmann and Christen, 2002). Terigar et al. (2011) proved the scalability of MAE from laboratory to pilot scale for the extraction of soybean and rice bran; they obtained yields of almost the same amount as those of conventional methods but with much smaller extraction times. Ultrasound-assisted extraction (UAE) is a technique which involves generation of cavitation and creation of mechanical shear forces to facilitate the penetration of solvent into the plant tissues, which in turn can lead to an increase in the analyte solubilisation (Azwanida, 2015). The extraction method was found to influence the functional properties of the extract derived from Moringa oleifera leaves as observed by Vongsak et al. (2013) when optimizing the content of total phenolics, total flavonoids and antioxidant activity. In pressurized liquid extraction (PLE) or accelerated solvent extraction (ASE) the extraction is conducted at a higher pressure and higher temperature than usual, but with less solvent and at a higher extraction speed than normal, at high temperature (Methods optimization in ASE, 2013). Supercritical fluid extraction (SFE) can be used to extract thermolabile constituents at lower temperatures, and can be used to extract less polar to moderately polar constituents; particularly when using supercritical CO_2 (Duque et al., 2013). The extraction yield of bioactive compounds in Centella asiatica was found to be significantly influenced by the processing method employed (nano-powder form) by Borhan et al. (2013) and the extraction yield of different bioactive compounds of propolis was compared by Trusheva et al. (2007).

5.3 Analytical Techniques and Phytochemical Screening

Nowadays, there are many analytical methods that enable to characterize plant extracts at the molecular level. To date, the most appropriate methods for qualitative and quantitative analysis of phenolic compounds are high-performance liquid chromatography (HPLC) with mass spectrometry (MS) or diode-array detection (DAD) (Dai and Mumper, 2010; Tocci et al., 2018). Essential oil and the volatile terpenes found in a plant are most likely to be analyzed by GC-MS (Chen and Viljoen, 2010) Isolation of isolated compounds can be structurally identified uniquely with NMR spectroscopy, and it has played a key role in discovery of novel natural products (Katz and Baltz, 2016). A systematical analysis of metabolomics, both targeted and untargeted has revolutionized the way in which phytochemical profiling can be carried out, enabling the monitoring of hundreds of metabolites simultaneously (Hall et al, 2008). Natural product research still largely relies on the phytochemical screening methodology of various preliminary qualitative tests against their main compound classes (alkaloids, flavonoids, tannins, saponins and terpenoids) (Ingle et al., 2017; Pandey et al., 2014). Sasidharan et al (2011) provided comprehensive data about extraction, isolation and characterization of bioactive compounds, and it was recommended that there should be standardized process to obtain reproducible results. Standardization of the preparation of herbal drugs, such as optimizing processes of extraction, and pre-phytochemical screening strategies, have been analyzed in detail by Pandey et al. (2014) highlighting the importance of strict standardization for Herbals to be safe, effective, and of good quality.

Table 2. Comparative Analysis of Extraction Methods for Plant Secondary Metabolites

Method	**Principle**	**Advantages**	**Limitations**	**Target Compounds**	**Key References**
Maceration	Solvent soaking at ambient temperature	Simple; preserves thermolabile compounds; low equipment cost	Long extraction time; low efficiency; high solvent consumption	Phenolics, alkaloids, glycosides	Sasidharan et al. (2011); Pandey et al. (2014)
Soxhlet	Continuous solvent reflux	Exhaustive extraction; well-established; high reproducibility	High temperature exposure; long duration; thermolabile compound loss	Non-polar terpenes, lipids, waxes	Redfern et al. (2014); Tiwari et al. (2011)
Microwave-assisted (MAE)	Microwave heating of polar molecules	Rapid; reduced solvent use; high	Limited to polar solvents; potential thermal degradation	Phenolics, oils, polysaccharides	Terigar et al. (2011); Kaufmann

		efficiency; scalable			and Christen (2002)
Ultrasound-assisted (UAE)	Acoustic cavitation and cell disruption	Moderate temperature; enhanced mass transfer; energy-efficient	Equipment cost; scalability challenges; noise	Flavonoids, anthocyanins, phenolic acids	Vongsak et al. (2013); Azwanida (2015)
Pressurized liquid (PLE/ASE)	Elevated T and P above solvent boiling point	Fast; minimal solvent; automated; high throughput	High equipment cost; requires optimization of T/P parameters	Broad range: phenolics, alkaloids, terpenes	Methods optimization in ASE (2013); Ingle et al. (2017)
Supercritical CO_2 (SFE)	Extraction above critical T and P of CO_2	Mild conditions; selective; no solvent residue; green	High capital cost; limited to non-polar/moderately polar compounds	Essential oils, lipids, waxes, carotenoids	Duque et al. (2013); Vanhercke et al. (2013)

5.4 Optimization Strategies and Quality Considerations

The parameters like type and polarity of the solvent, extraction time, extraction temperature, solid to solvent ratio, particle size etc. should be optimized for the extraction of maximum amount of target bioactive compounds. An extensive use of systematic optimization of extraction conditions by response surface methodology (RSM) has been employed. The concentration of total phenolics and flavonoids content of Moringa oleifera leaves was optimized by Vongsak et al (2013) using RSM to obtain the optimal extraction conditions including extraction temperature, extraction time and concentration of the solvent. Likewise, Mediani et al. (2013) optimized the drying process conditions of Cosmos caudatus, maximizing the recovery of polyphenolic compounds, in an oven dryer. Abdullah et al. (2012) highlighted the effect of sample preparation on the ability to obtain metabolites on leaves of Orthosiphon stamineus through different drying methods. Borhan et al. (2013) also showed that using

Centella asiatica Nano powder processing resulted in a higher extraction rate compared to bulk powder. From the above observations, it is clear that extraction process not only has a technical role but also has a significant effect on the phytochemical composition and hence the biological potential of the extracts of plants.

6. Therapeutic Applications of Plant Secondary Metabolites

Plant secondary metabolites have almost all the major areas of pharmaceutical disease in human health. Until the present day, compounds isolated from plants have been used in ancient medicine systems of various parts of the world, as well as being used as lead compounds in the modern drug market (Raskin et al., 2002; Cragg et al., 2014; Katz and Baltz, 2016). The therapeutic activity of PSMs is discussed below in relation to the following: the prevention of cancer, pathogens, anti-inflammatory activity, neuroprotective activity, hepatoprotective activity, and regulation activity in metabolism.

6.1 Anticancer Activity

Plant derived anticancer agents are the most significant contribution of phytochemistry in modern medicine against cancer. The modes of action are diverse, such as induction of apoptosis, cell cycle arrest, inhibition of angiogenesis and metastasis and modulation of signaling pathways (Gali-Muhtasib et al., 2015). Beyond the classical apoptotic mechanism, plant-based anticancer drugs have been shown to induce other types of cell death such as the autophagy, mitotic catastrophe, necroptosis and parthanatos (Gali-Muhtasib et al., 2015).

In fact, indole alkaloids compounds like vincristine and vinblastine, extracted from Catharanthus roseus continue to be one of the most prescribed chemotherapeutic drugs as they are microtubule-disrupting drugs that inhibit the formation of the mitotic spindle (Matsuura and Fett-Neto, 2017; Kumar, 2014). Indole-3-carbinol and its n-alkoxy derivatives, for their ability to selectively target ER-positive breast cancer cells, as demonstrated by Caruso et al. (2014), represent an example of selective anticancer agents that are based on the receptor-expression profile. The NF-κB signaling pathway has proved to be one major pathway of interest: Pharmacologic inhibitors of IκB kinase were shown to inhibit the growth and migration of mammary carcinosarcoma cells in vitro and osteolytic bone metastasis in vivo by Idris et al. (2009). The compounds of triterpenoid type show a special promise: Liu et al. (2015) reported the ability of lupeol to induce apoptosis in human pancreatic cancer cells through the inhibition of the AKT/ERK pathway; Nyaboke et al. (2018) reported that lupeol from Zanthoxylum gilletii is cytotoxic towards multidrug resistant cancer cell lines. Asiatic acid inhibited the growth of lung cancer cells in vitro by altering the morphology of mitochondria, activating apoptotic pathways, consisting of caspase-3, -8 and -9, and activating ERK/p38 MAPK pathways; in vivo, Asiatic acid inhibited lung cancer cell proliferation (Tian et al., 2017; Wu et al., 2017; Hsu et al., 2005). Dichamanetin is a natural compound, a flavonoid, which can inhibit the growth of cancer cells by inhibiting the components involved in the signal transduction of ROS binding to mitochondria via apoptotic induced cell death (Yong et al., 2013). Extracts of Hypericum with a higher phenolics content have been shown to have anti-fungal activity, suggesting other therapeutic potential of phenolics-rich Hypericum extracts (Tocci et al., 2018).

6.2 Antimicrobial Activity

This interest in the search for alternative or complementary substances to the conventional antimicrobials, particularly the plant-based antimicrobial substances is due to the growing problem of antimicrobial resistance (Martelli and Giacomini, 2018; Vasconcelos et al., 2018). Cinnamon and its active components such as cinnamaldehyde, eugenol, and cinnamic acid, have antibacterial properties that include cell membrane disruption, enzymatic activity inhibition and quorum sensing (Vasconcelos et al., 2018). Mikulasova et al. (2016) demonstrated synergism of PSMs with antibiotics and plant extracts/essential oils against multidrug resistant (MDR) staphylococcus, pointing to the possibility of using PSMs as additives to antibiotics and to enhance the efficacy of existing antibiotics. A dual-active compound comprised on the combination of two active substances with antibacterial and antioxidant properties, as described by Martelli and Giacomini (2018), is an innovative strategy to address infections with oxidative stress. Hypericum species exhibit antifungal properties which have been linked with their phenolic composition (Tocci et al., 2018) and terpenoids and essential oils have been reported as having a wide range of antimicrobial activity (Redfern et al., 2014). Trusheva et al. (2007) reviewed the use of propolis extracts for their antimicrobial activity, and that this bioactivity is influenced by the method used to extract propolis.

6.3 Antioxidant and Anti-inflammatory Activity

The imbalance between production of reactive oxygen species (ROS) and antioxidant activities is called oxidative stress and is linked to pathogenesis of a variety of chronic conditions such as cardiovascular disorders, neurodegeneration, diabetes, and cancer (Martins et al., 2016). Phenolic compounds are one of the most potent natural antioxidants in plants and they act in a number of different ways, including acting as direct antioxidants and chelators of metals and activation of endogenous antioxidants such as the flavonoids mentioned above (Nile et al., 2018; Dai and Mumper, 2010). A comprehensive review on the antioxidant, anti-inflammatory and enzyme inhibitory effects of natural and synthetic flavonoids of plant origin was conducted by Nile et al. (2018) and they found that the hydroxyl number and position in the structure led to antioxidant activity. The antioxidant activity of the metabolite formed by the human gut flora has been demonstrated to be different from the parent compounds: in some cases the activity of the metabolites is superior to the parent molecule, in terms of both bioactivity and absorption (Surco-Laos et al., 2016). In commercial varieties of black/red rice, both free and bound phenolic were observed to be contributing to overall phenolic antioxidant activity (Sumczynski et al., 2016).

The in vivo antioxidant effects of phenolic compounds were reviewed by Martins et al. (2016) and numerous gaps in knowledge gained from in vitro experiments were identified that required bioavailability and metabolism to understand their in vivo antioxidant effects. Vattem et al. (2005) demonstrated that the antioxidant enzyme response in oxidatively stressed porcine muscle is an example of how the antioxidant properties of cranberry phenolics can be used as a natural preservative. Wang et al. (2018) gave a comprehensive review of the bioactive flavonoids of medicinal plants, structure-activity relationships and biological lifespan.

6.4 Neuroprotective and Psychopharmacological Activity

The cases of neurodegenerative diseases, especially Alzheimer's disease, wherein plant alkaloids receive considerable attention as leads for developing new drugs. For the compounds that might be able to inhibit AChE, activate NMDA receptors and be neuroprotective, Ng et al. (2015) have summarized galantamine, huperzine A and vincamine. The traditional use of valerian species in epilepsy has been confirmed by using murine models, in which isovaleramida, extracted from Valeriana pavonii, has been determined to be an anticonvulsant (Giraldo et al., 2010). The essential oil of Citrus aurantium blossom (neroli) exhibited anticonvulsant activity with gamma-aminobutyric acid (GABA) related mechanism which can be explored for herbal drug development as anxiolytic and antiepileptic drugs (Azanchi et al., 2014). Another significant therapeutic use of the leaf extract of Moringa oleifera is the wound healing effect which was demonstrated in albino rats (Rathi et al., 2006).

6.5 Metabolic Regulation and Enzyme Inhibition

Plant secondary metabolites are important secondary metabolites that play a role in metabolic regulation e.g. diabetes. As described in the review of Sales et al. (2012), plant-derived α-amylase inhibitors are a natural way to decrease postprandial hyperglycemia. A range of therapeutic targets such as cyclooxygenase (COX), lipoxygenase (LOX), kinases are targeted by the enzyme inhibitory activities of flavonoids and their derivatives (Nile et al., 2018). The relation between plant growth retardants and production of secondary metabolites (Rani and Sudhakar, 2018) and nutritional factors and PSM content in edible plants such as hazelnut (Pourfarzad and Mehrpour, 2017) indicate that phytochemistry and agriculture science are mutually related with human nutrition. Other specific trained plants are also utilized for their production of other beneficial compounds, e.g., squalene-rich extracts to prevent the occurrence of skin cancer (Ghimire et al., 2016) and polyphenol-enriched cosmetics for body care (Zillich et al., 2015), where again PSM has proven to be versatile in the health and wellness industry. It is noteworthy, that the bioactive compounds of Saussurea laniceps, a medicinal plant has a very high antibacterial activity and known as "snow lotus", illustrated the ongoing discovery of pharmacologically active PSMs from medicinal plants that have traditionally been used (Chen et al., 2016).

Table 3. Therapeutic Applications of Representative Plant Secondary Metabolites: Mechanisms, Targets, and Clinical Significance

Compound	Class	Mechanism of Action	Therapeutic Target	Key References
Vincristine	Indole alkaloid	Microtubule disruption; mitotic arrest	Leukemia, lymphoma	Matsuura and Fett-Neto (2017); Kumar (2014)
Indole-3-carbinol	Indole derivative	ERα receptor targeting; cell cycle arrest	ERα+ breast cancer	Caruso et al. (2014)
Lupeol	Triterpenoid	AKT/ERK pathway modulation; apoptosis induction	Pancreatic, drug-resistant cancers	Liu et al. (2015);

				Nyaboke et al. (2018)
Asiatic acid	Triterpenoid	Mitochondrial destruction; caspase activation	Lung cancer, breast cancer	Tian et al. (2017); Hsu et al. (2005)
Ursolic acid	Triterpenoid	JNK/Akt/mTOR signaling modulation	Breast cancer metastasis	Yeh et al. (2010)
Quercetin	Flavonoid	ROS scavenging; COX/LOX inhibition	Inflammation, cardiovascular disease	Nile et al. (2018); Dai and Mumper (2010)
Galantamine	Alkaloid	Acetylcholinesterase inhibition	Alzheimer's disease	Ng et al. (2015)
Cinnamaldehyde	Phenylpropanoid	Membrane disruption; quorum sensing inhibition	Bacterial infections	Vasconcelos et al. (2018); Martelli and Giacomini (2018)
Dichamanetin	Flavonoid	ROS-mediated mitochondrial apoptosis	Cancer cell proliferation	Yong et al. (2013)
α-Amylase inhibitors	Various PSMs	Starch digestion retardation	Diabetes mellitus type 2	Sales et al. (2012)
Geraniol	Monoterpene	Antimicrobial; anti-inflammatory	Skin care, infections	Chen and Viljoen (2010); Mukherjee et al. (2011)

7. Ecological and Environmental Significance

Plant secondary metabolites are the currency of plant-environment interactions that influence the interactions between plants and herbivores, pathogens, competitors, mutualists and the abiotic environment (Freeman and Beattie, 2008; Loreto et al., 2014). From molecular defenses to high-level ecosystem processes, the ecological roles of PSMs are vast and studying this interaction has important implications in both ecology as well as agriculture applications.

7.1 Defense Against Herbivores and Pathogens

The chemical plant defence against herbivore attack and pathogen invasion is the most basic component of the rest of the plant defence system and can be either constitutive or inducible. Constitutive defence (presence of pre-formed toxic compounds and structural defence, both with the assistance of secondary metabolites) can be seen as protection against all attacks, irrespective of intensity. Represented by the anatomical and chemical defenses of conifer bark against the attack of bark beetles and other insects: specialized anatomical structures (resin ducts) that store chemical defenses that are toxic or deterrent to the attacking insect, which can be easily released as the insect attacks.

Inducible defenses is activated by a specific biotic or abiotic stress signal(s). The function of low molecular weight antimicrobial compounds synthesized after recognition of the pathogen, known as phytoalexins, in limiting the proliferation of pathogen in host tissues is extremely significant (Ahuja et al., 2012). This diversity of structure is representative of the number of different phytoalexins found within the plant families and shows how evolution has evolved as an arms race between plant and pathogen. One of the most complex and potent naturally occurring insect antifeedants elaborated from Azadirachta indica (neem), the structure of azadirachtin has been difficult to total synthesis for over 20 years (Veitch et al., 2008). Peptide and phytochemical elicitors have been demonstrated to induce phenolics, antioxidants and antimicrobial activity in dark germinated mung bean sprouts by Randhir et al. (2004), which suggests potential for eliciting these activities in mung bean sprouts.

7.2 Plant Volatiles and Chemical Communication

VOCs emitted by plants serve a number of ecological functions such as herbivore deterrence, the attraction of natural enemies of herbivores, attract pollinators and inter-plant signaling (Loreto et al., 2014). In short, the study of the comprehensive review of Loreto et al. (2014), revealed that plant volatiles and the environment is very complex. They were able to demonstrate the influence of plant volatiles emission on both genetic and environmental considerations. Some of the most significant plant-insect signalling plants are monoterpenes such as geraniol and sesquiterpenes such as farnesol (Chen and Viljoen, 2010; Caputi and Aprea, 2011).

Plant volatiles are also directly involved in fruit selection and dispersal in addition to the plant's defenses against herbivores. Valenta et al. (2013) show that mouse lemurs consider colour and odour (PSM product) as a criterion when selecting fruits, highlighting the ecological role of PSMs in the process of reproductive success and propagation of species. Some of the secondary metabolites serve two useful purposes: they help against enemies, and they help to communicate with mutualists.

7.3 Allelopathic Interactions

The negative direct or indirect impact of one plant on another because of the release of secondary metabolites into the environment is called allelopathy; it is an important ecological mechanisms that affect plant community structure and succession. John and Sarada (2012) reviewed the role of phenolics in allelopathic interactions and showed how the phenolics produced by the root exudates and seed litter of plants can suppress the germination of competing plants, as well as the growth of seedlings. The allelochemical effects of phenolics could be related to the environmental factors that affect the persistence and availability of phenolics in the rhizosphere, such as pH, microbial activity and moisture level.

7.4 Adaptation to Abiotic Stress

Plant adaptation to abiotic environmental stress is closely related to production of secondary metabolites. When plants are exposed to UV radiation, especially UV-B (280–315 nm), the concentration of UV-absorbing phenolics in epidermal plant tissues increases, which gives them photoprotection and may lead to an increase in the antioxidant capacity of plant tissues (Verdaguer et al., 2017). The accumulation of phenolics in germinating grapevine seeds under cold stress and subsequent recovery upon stress alleviation (Weidner et al. 2014) was used as an example to show the dynamic nature of the so-called secondary metabolism in response to stress and its recovery upon stress removal. The combinations of abiotic factors (temperature, CO_2 concentration, soil moisture) differently affected the production of PSM from five species of woody plants, suggesting a promising shift in the chemical ecology of plants under climate change, which has significant implications for the quality of medicinal plants, according to Berini et al. (2018). Lin et al. (2010) further showed the relationship between environmental adaptation and secondary metabolism through initial screening tests of high-phenolic plant clonal systems in the removal of nitrate in cold latitudes. Berdy (2005) reviewed the role of bioactive microbial metabolites in plant health and their application for minimizing stress in plants, and discussed the interactions between secondary metabolites produced by plants and microbes in the rhizosphere.

8. Conclusion and Future Perspectives

This is a review of in-depth phytochemical fingerprinting and therapeutic value of phytochemicals from plant secondary metabolites from the year 2001 to 2018, based on 94 peer reviewed publications. The wide variety of PSM chemical constituents such as phenolics, terpenoids, alkaloids and sulfur chemistry compounds point out the plants' evolutionary smartness to develop their molecular solutions to solve ecological problems, and their great potential for application in pharmacology (Kroymann, 2011; Singh and Sharma, 2015; Dewick, 2009). The following main conclusions can be drawn from this synthesis. First, the regulation of PSMs biosynthesis is complex and interconnected and integrates a multitude of signals from gene, developmental and environmental origin that modulate the production of these metabolites (Vranova et al., 2012; Ramakrishna and Ravishankar, 2011; Berini et al., 2018). This versatility is achieved through two parallel biosynthetic pathways for the formation of isoprenoids (MVA and MEP pathways) and through the interaction and cross-talk between the biosynthetic pathways of phenolic biosynthesis and alkaloid biosynthesis and also by the compartmentalization of the biosynthetic enzymes in cellular compartments within the cell (Vranova et al., 2012; Olivieto et al., 2017).

The method of extraction of phytochemicals will definitely affect the phytochemical profile and biological activity. With the advantages of high efficiency, high selectivity and environmental protection, the use of advanced technology like MAE, UAE, PLE, and SFE has greatly enhanced the extraction efficiency, selectivity and environmental friendliness of food products (Ingle et al., 2017; Azwanida, 2015; Kaufmann and Christen, 2002). However, cross-study comparisons and meta-analyses are constrained by the lack of consistency in the extraction of the data from the different studies (Pandey et al., 2014; Sasidharan et al., 2011), which is extremely difficult to achieve in translational research. Third, PSMs have therapeutic uses in virtually all the major disease categories, ranging from cancer to neurodegeneration to metabolic diseases. Plants contain a series of compounds that have significant impact on cancer cells through various mechanisms such as induction of apoptosis, cell cycle arrest, disruption of mitochondria and modulation of signaling pathways including lupeol, asiatic acid, indole-3-carbinol and dichamanetin (Gali-Muhtasib et al., 2015; Liu et al., 2015; Yong et al., 2013; Tian et al., 2017). Other medicinal applications along with their antioxidant and anti-inflammatory properties (Nile et al., 2018; Martins et al., 2016) and the potential of PSMs as antibiotic adjuvants to combat multidrug resistance (Mikulasova et al., 2016; Vasconcelos et al., 2018) underscore their versatility.

There are also fascinating areas of knowledge where PSM studies are in the early stages, that help support these advances. The bioavailability and metabolic clearance of PSMs in vivo following oral administration is not well defined. As already mentioned by Velderrain-Rodriguez et al., (2014) and Martins et al., (2016) there is a critical difference between in vitro bioactivity and actual physiological effects and, therefore, additional phytochemical and pharmacokinetic and bioavailability studies are needed (Wang et al., 2018; Surco-Laos et al., 2016). Second, a systems biology approach needs to be integrated with high throughput metabolomics platforms (Hall et al., 2008) to produce a comprehensive secondary metabolite map of model plants, as well as to understand pathway level regulation. Third, synthetic biology and metabolic engineering promise solutions for a sustainable, high-value PSMs production, requiring more understanding in regulating and controlling the pathways (Vanhercke et al., 2013; Ghimire et al., 2016). From the ecological and medicinal plant functional point of view, the response of MSM to climate change (Berini et al., 2018; Verdaguer et al., 2017), could be of significant importance and should be studied with an emergency.

Secondary metabolites of plants continue to be an abundant source of therapeutic bioactive compounds to humans. Discovering phytochemicals and making them clinically viable will be a complex journey which involves interdisciplinary collaboration and a quest to exploit the diversity of chemicals in nature for therapeutic benefit.

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