

Review

# Bioactive Secondary Metabolites from Plant Sources: Types, Synthesis, and Their Therapeutic Uses

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**Abstract:** Plants are the source of various photochemicals; metabolites are used in medicinal and environmental sectors as well as being widely used in commercial and pharmaceutical products. Although they produce a number of medicinal products, either already on the market or under trial, the amounts obtained from plant sources are very minute or difficult to synthesize at an industrial level due to the complex chemical composition and chirality exhibited by these compounds. However, plant cell cultures offer a good alternative for the consistent production of desired secondary metabolites under the influence of precursors and elicitors. In this review, we discuss the various aspects of secondary metabolites, production synthesis, and sources of medical products from plant sources.

**Keywords:** metabolites; medicinal effect; synthesis; precursor and elicitors



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## 1. Introduction

Secondary metabolites are small organic molecules originated from primary metabolites during the embolism of plant; they essentially have molecular masses of less than 3000 da. The chemical nature and composition of metabolites in plants varies among species. There is no clear differentiation between primary and secondary metabolites and this is quite confusing to define, since most metabolites in natural products of plants are secondary metabolites. Secondary metabolites are interesting for various diverse reasons as, in the literature, they have been found to be interesting due to their structural diversity and their potency as a drug candidate and/or antioxidants. There are few examples of chemical diversity of plant metabolites, thus they are complexes that cannot be synthesized by the industry [1,2].

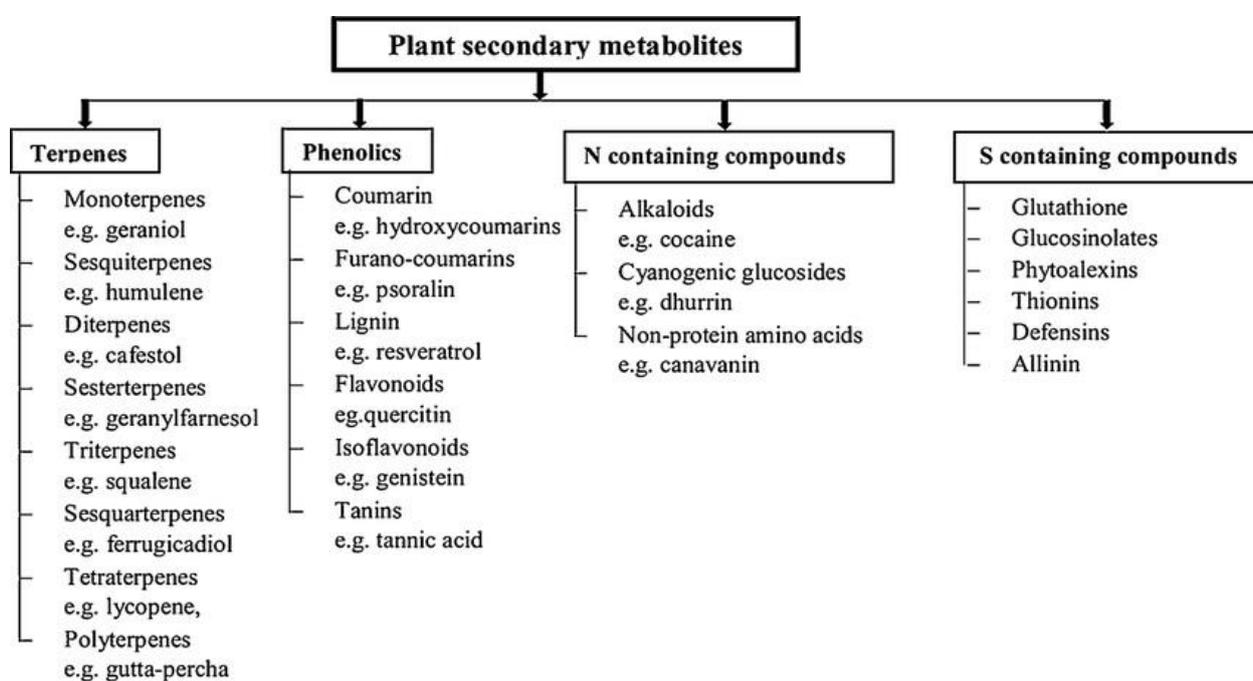
The use of plant metabolites started as far back as 2600 BC, and in the following 4000 years, secondary metabolites originating from plants were used mainly for medicinal and poison purposes as well as food. Morphine was the first natural product isolated from the opium poppy (*Papaver somniferum*) in 1806 and it opened a new era in secondary metabolite research [1]. It was then established that plant extract activity is attributed to a single organic compound [3], which has its own individual identity and also can be purified. Thus, these research outcomes initialized natural product research and, to date, plant secondary metabolites have played a major role, which is demonstrated by the fact that more than 30% of medicinal products derive directly or indirectly from natural products [4–6]. In the past 100 years, there has been a rapid growth in plant metabolite research due to the availability of hi-tech research equipment.

The biological functions of metabolites were widely unrecognized due to the frequently low concentrations of metabolites in plants, and earlier, they were recognized as metabolic waste or detoxification products. Knowledge about secondary metabolites, known at earlier times for their toxic effects on animals cells, but later for their ecological importance and numerous other benefits, has emerged over the past four decades [7,8].

## 2. Types of Secondary Metabolites

Plants naturally produce a variety of products of different chemical natures, which are used for the growth and development of plants. Primary metabolites provide the supplies required for processes, such as photosynthesis, translocation and respiration. The products derived from primary metabolites, not directly involved in growth and development, are considered secondary metabolites. In general, secondary metabolites are the product of primary metabolites and are produced from biosynthesis modifications, including methylation, glycosylation and hydroxylation. Secondary metabolites are certainly more complex in structural composition and side chains compared to primary metabolites [9,10].

There are three major classes of plant metabolites (Figure 1) based on the biosynthetic pathway: (i) phenolic groups (composed of simple sugars and benzene rings), (ii) terpenes and steroids (composed mainly of carbon and hydrogen), and (iii) nitrogen-containing compounds [11].



**Figure 1.** The four major types of secondary metabolites that have the major terpenes and phenolics.

Phenolic compounds derive from the phenylpropanoid pathway in plants and they have diverse structures and supply flowers, fruits and vegetables with color. Alkaloids and flavonoids are large groups of secondary metabolites with highly diverse biological functions. A variety of phenolic metabolites seems to have several properties, such as anti-oxidant, anti-carcinogenic and anti-inflammatory effects [12]. Alkaloids are other major metabolites that often have pharmacological and recreational effects. There is a third group of nitrogen-based organic compounds, which contain a heterocyclic ring of aromatic amino acids that is synthesized from the shikimic acid pathway. Interestingly, the aromatic amino acids phenylalanine and tryptophan are common metabolic precursors for both phenolic compounds and alkaloids [1].

## 3. A Brief Description of Various Secondary Metabolites: Phenolic Compounds

### 3.1. Flavonoids

To date, more than 4000 different flavonoids have been identified from plant origin. These are more commonly found in green plants [13], and are present mainly as glycosides in leaves, flowers, stems and roots. Flavonoids consist of two benzene rings [14]; thus, chalcones, flavones, flavanols, flavanones, anthocyanins, and isoflavones are major flavonoids that are often brightly colored [14].

### 3.2. Phenylpropanoids

Phenylpropanoids are only used by plants and microorganisms and derive from the shikimate pathway, producing essential aromatic amino acids, such as phenylalanine and tyrosine. As these are mostly required by animals and humans, plants become a part of the food chain for humans to obtain essential amino acids.

### 3.3. Terpenes

These are a structurally diverse and large metabolite group with at least more than 35,000 different terpenes having been characterized to date [15]. Terpenes consist of isoprene units that can be modified by cyclisation reactions, making them easily recognizable. They are classified in different groups based on the number of isoprene units in their carbon skeleton, as indicated in Figure 1 [16]. Terpenoids include metabolites with antitubercular [17], anticancer activities [15], anxiolytic, and mutagenic active molecules [18].

### 3.4. N-Containing Compounds

Alkaloids contain nitrogen in their cyclic organic compounds, but they have very limited presence in nature [19]. These are mostly soluble in aqueous solutions; thus, they are conveniently extracted in water upon the protonation of the nitrogen. This group have some of the most famous and infamous compounds, such as caffeine, nicotine, cocaine and morphine, which are known for their anxiolytic, analgesic and hallucinogenic effects [15], and often have physiological effects on the central nervous system. Although it is a small group of metabolites, 50% of plant-derived pharmaceuticals are alkaloids [20].

## 4. Induction of Plant Secondary Metabolism and Production by Inducer and Precursor

It has been broadly documented that plant-rich sources of a variety of secondary metabolites can be used for medicinal and therapeutic purposes and there have been efforts to commercialize the production for human benefit. However, sometimes it is not possible to use the biochemical synthesis pathway of secondary metabolites at an industrial level due to the complexity of the metabolic pathways; they are complicated structures to synthesize in a laboratory and the chirality exhibited by these compounds and most plants accumulate secondary metabolites in small amounts in specialized tissues in their life cycle (Figure 2). In that case, the production of such metabolites is enhanced in various ways. As these secondary metabolites are mainly regulated by the transcriptional activities of the gene cassette encoding particular enzymes in the biosynthetic pathway for desired products, the catalytic activities of these genes or enzymes are kept at very low levels naturally and gene active status can be triggered by the use of elicitors [21]. The exposure of plant cells to elicitors or precursors may also result in the accumulation of secondary products [22]. The most used and tested elicitors are jasmonic acid, and its methyl ester called methyl jasmonate, tryptophan, phenylalanine, and plant-specific messenger molecules originating from arachidonic acid [22]. Effective elicitors enhance the production of useful metabolites in plant cells via the upward regulation of the biochemical mechanisms by which these elicitors regulate the biosynthesis of the natural products. Although it is known which particular elicitors should be used for peculiar metabolites, one must know how an elicitor is recognized by plant cells and how the signals are transformed in cells expressing and controlling the biosynthesis of related genes to produce secondary metabolites. To explore these questions, attempts should be made to explore the biosynthetic pathway of secondary metabolites.

If the metabolite is not feasible to scale up through chemical synthesis, the only remaining option is to produce it through the enhancement of cell cultures, which include being treated with elicitors, precursors, and signaling compounds. Precursors are related compounds or intermediates of a secondary metabolite from the beginning of the biosynthetic pathway [23], such as *Salvia officinalis* and *Taxus*, which are cell suspension cultures that are stimulated by taxol production and rosmarinic acid, respectively, with the addition

of phenylalanine [24,25]. Elicitors have been developed to improve the yield of secondary metabolites via signal triggering [26,27].

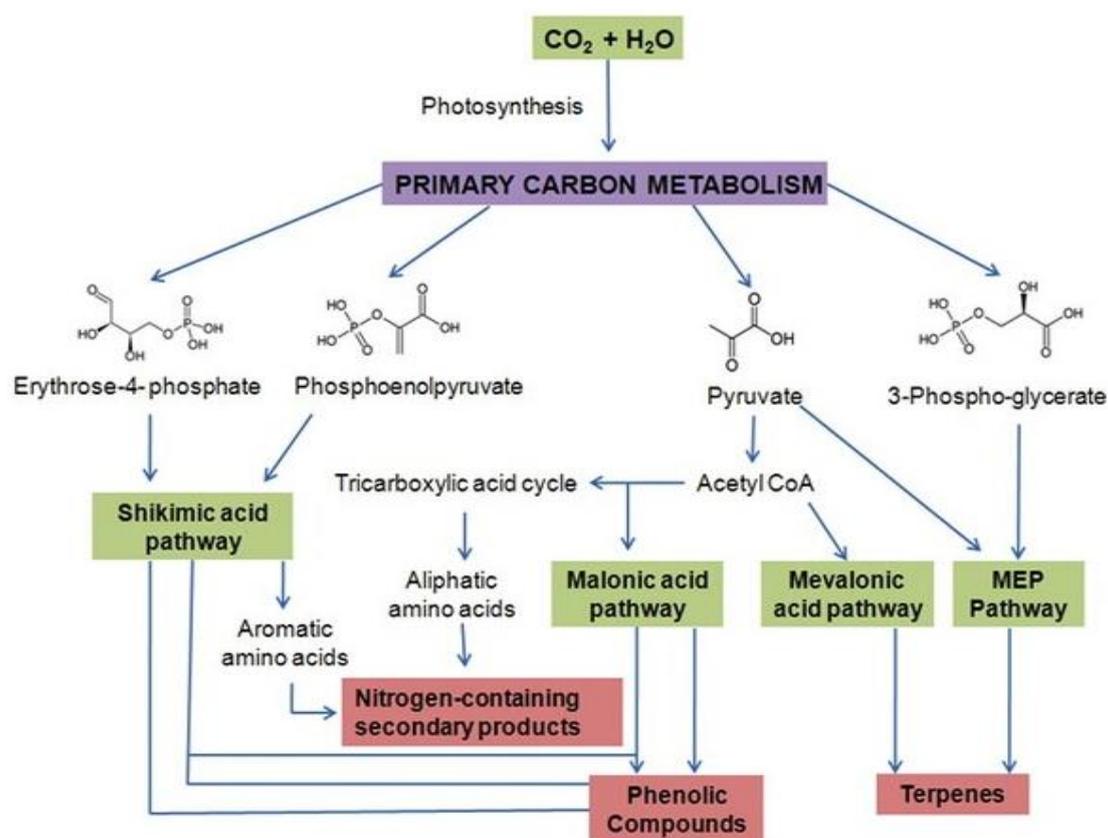
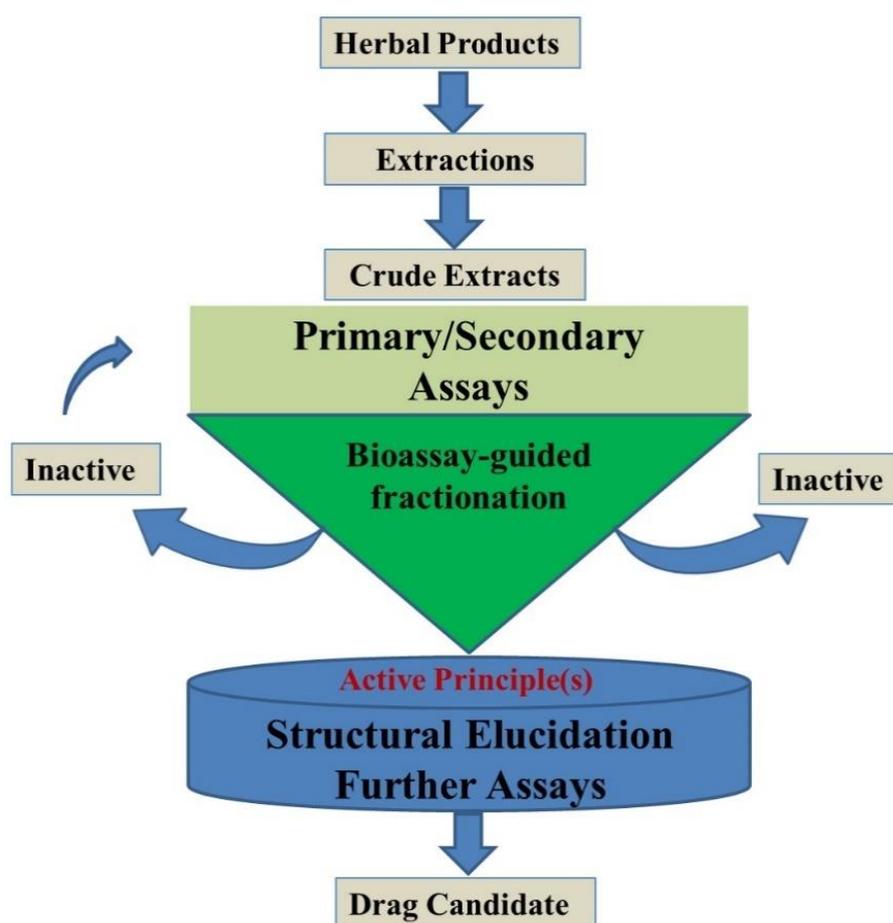


Figure 2. Layout of drug discovery process: from nature to market.

### 5. Applied Methods for the Isolation and Structure Elucidation of Metabolites: Improved Biomass and Secondary Metabolite in Culture Environment

To obtain highly concentrated secondary metabolites, supporting factors, such as media components, physical factors (pH, temperature, light, etc.) and plant growth regulators, play an important role [28,29]. The selection of source explants as inoculums to the production is crucial, which should accumulate large number of secondary metabolites (Figure 3). Medium standardization is known to influence the biomass yield of secondary metabolites [30]; although [31] it is a widely used medium for the rapid growth of callus, it is not ideal for inducing secondary metabolites.

There have been several techniques developed to extract metabolites, including conventional and new hi-tech techniques. Conventional techniques include using organic solvents, such as hexane, methanol, acetone, ethanol, etc., and/or water, and the procedure is carried out at room temperature, which mostly allows for the dissolution of the soluble metabolites excreted into the solvent during the growth processes. This process relies on the combined action of heat, mixing and on the extracting capacity of the solvents [32], which are necessary for the differentiation of active components by using the appropriate solvents. Several novel extraction methods, such as microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), pressurized liquid extraction (PLE) and supercritical fluid extraction (SFE), have been developed. These techniques reduce the volume of the solvent, the extraction time, and improve yield [33].



**Figure 3.** Steps required for the characterization of a bioactive compound from natural sources.

## 6. Identification and Characterization of Techniques

To identify the desired secondary metabolites, the pure compound is separated from the mixture and identified through chromatographic and non-chromatographic techniques. Chromatographic techniques are used in both the separation and/or identification of the active components in a mixture. Chromatographic techniques are used on an industrial scale and/or for academic purposes to separate and purify the products in various syntheses. There are various chromatography techniques, from the simplest thin-layer chromatography (TLC) to advanced gas chromatography and liquid chromatography with mass spectroscopy [14,34].

## 7. The Role of Plant-Derived Natural Products in Drug Innovation and Plants as Sources of Bioactive Natural Medicinal Products

A plant product ranges from a simple skeleton to far more complex molecules that are even not possible to synthesize in laboratory conditions. These metabolic derivatives have highly specific activities via a unique mechanism (Figure 4). These are the reasons why plant metabolites are potentially important in drug development, especially nowadays, when in-depth knowledge is emerging through the human genome sequencing of multiple new molecular targets that have been tested for their potential use as drug targets [35]. The isolation and discovery of quinine became a landmark in the field of pharmacognosy [36] and since then, many active ingredients of plant source have been identified and characterized.

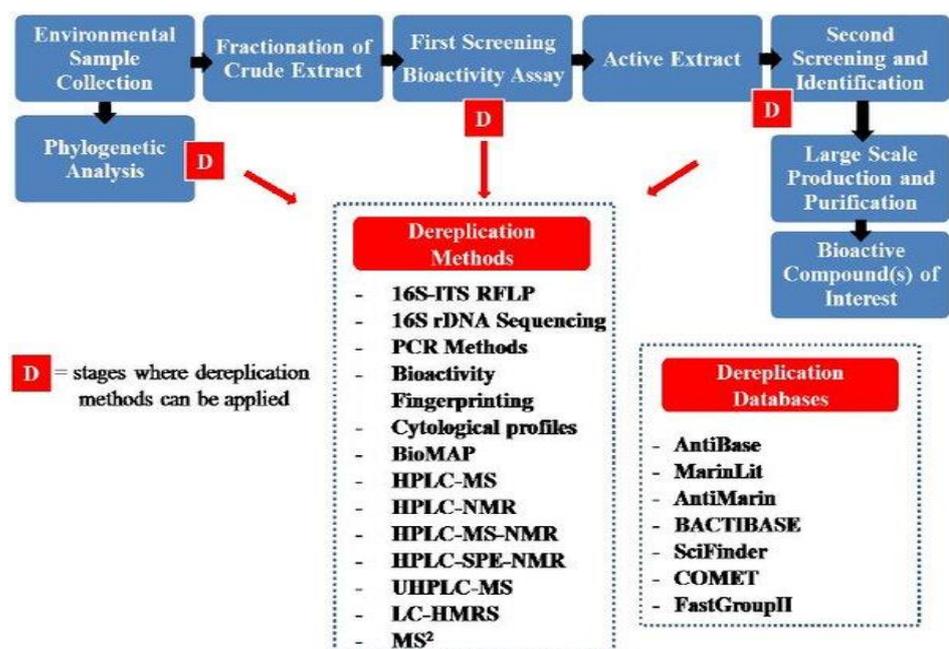


Figure 4. The various steps and techniques required during the characterization of plant products.

## 8. Function and Use of Secondary Metabolites

In the early days, plant extracts were considered to have the function of waste or detoxification products [37]. Until then, most research efforts had not been initiated and research started once technology was used to explore the applications of secondary metabolites for their intended biological functions in nature. The functions of the vast majority of secondary metabolites, however, are still unknown. Secondary metabolites are most importantly used as pharmaceuticals, food additives and cosmetics ingredients.

## 9. Current Plant-Derived Natural Products in Therapeutic Use

There are various plant derivatives of natural products that are in regular use as therapeutic agents (Table 1).

Table 1. Important plants species and their metabolites with medicinal effects.

Drug	Source	Target Disease	Reference
Metformin	<i>Galega officinalis</i>	Anti-diabetic	[38]
Galantamine	<i>Galanthus woronowii</i>	Alzheimer's disease	[39]
Artemisinin	<i>Artemisia annua</i>	Malignant cerebral malaria	[36]
Paclitaxel	<i>Taxus brevifolia</i>	Antimitotic agent-Variou cancer	[40]
Calanolide A	<i>Calophyllum lanigerum</i> ,	Type-1 HIV	[35]
Crude extract	<i>Sarcotheca griffithii</i>	Cough	[41]
Crude extract	<i>Piper retrofractum</i>	Fever, hypotension, abdominal pain, anthelmintic	[42,43]
Crude extract (acrylamides and phaeophytins)	<i>Talinum triangulare</i>	Cuts, wound, scabies and peptic ulcer	[44,45]
Crude extract (monoterpenes and sesquiterpenes)	<i>Amorpha fruticosa</i>	Antibacterial, insecticidal, cytotoxic	[46–48]
Crude extract	<i>Alpinia officinarum</i>	Anti-microbial and anti-inflammatory	[49]

Table 1. Cont.

Drug	Source	Target Disease	Reference
Crude extract	<i>Ocimum Basilicum</i>	Anti-inflammatory activity	[50]
Crude extract	<i>Costus pictus</i>	anti-diabetic	[51]
Isopropanol	Synthesis	Used as alcohol	[11]
Isovaleric acid	Essential oils	Anticonvulsant and used in perfumery	[52]
Limonene	Essential oils	Fragrant, anti-carcinogenic and bacterial	[53,54]
Linalool	Essential oils	Antibacterial, effects center nervous system	[55,56]
Phytoalexins	At site of infections in plants	Anti-antimicrobial, anti-cancer and anti-oxidative	[55,57]
Gibberellin	<i>Gibberella fujikuroi</i>	Elongation of plant and promotes growth	[58,59]
Brassinosteroids	<i>Brassica napus</i> , <i>Lychnis viscaria</i>	Plant protection	[60,61]
Sterols	Naturally found in plants, fungi and animals	Medicinal effect and nutritional supplements	[60,61]
Carotenoids	Chloroplasts, carrots, and chromoplasts of plants	Antioxidants	[60,61]
Atropine	<i>Atropa belladonna</i> , <i>datura stramonium</i>	Adversary of muscarinic acetylcholine receptors, anti-myopia effects, anti-cholinergic,	[62,63]
Berberine	<i>Berberis</i> species	Anti-bacterial, antiviral, anti-inflammatory, anti-cancer and anti-diabetic	[56,64,65]
Codeine	<i>Papaver somniferum</i>	Analgesic, antidepressant, antitussive, anti-diarrheal, sedative and hypnotic properties	[66–69]
Coniine	<i>Conium macularum</i>	Poisonous, neurotoxin	[70,71]
Cytisine (baptitoxine, sophorine)	<i>Cytisus laborinum</i>	Smoking cessation drug, acetylcholine agonist	[72,73]
Morphine	<i>Papaver somniferum</i>	Edema, acute pulmonary and shortness of breath	[74–76]
Nicotine	<i>Solanaceae</i>	Insecticide, anti-inflammatory and stimulant, antiherbivore	[77–80]
Quinine	<i>Cinchona succirubra</i>	Antimalarial	[81–84]
Solanine	<i>Solanum tuberosum</i>	Antifungal, ant pesticide, sedative, anticonvulsant, anticarcinogenic, anti-inflammatory	[85–88]
Strychnine	<i>Strychnos nux-vomica</i>	Pesticide	[89–92]
Thebaine (paramorphine)	<i>Papaver bracteatum</i>	Analgesic	[48,93–95]
Tomatine	<i>Tomato</i>	Anticancer, immune effects, antifungal	[96–98]

Galantamine, an alkaloid originated from *Galanthus woronowii*, was approved for Alzheimer's disease treatment in 2001 by the FDA [15]. Galantamine inhibits acetylcholinesterase (AChE) and modulates nicotinic acetylcholine receptor (nAChR). Artemisinin from *Artemisia annua* has been used as an antimalarial drug for at least 2000 years. Artemisinin contains sesquiterpene lactone, which has bioactivity proven to treat *malignant*

cerebral malaria caused by *Plasmodium falciparum* [36]. Artemisinin had poor bioavailability and so artemether, a derivative of artemisinin, was later developed with enhanced bioavailability [35,40]. Paclitaxel, isolated from *Taxus brevifolia*, a highly oxygenated tetracyclic diterpenoid, works as an antimetabolic agent that inhibits the polymerization of tubulin to form microtubules. It is also used as an effective drug against ovarian and breast cancers and it has subsequently been approved for many other cancer treatments [40]. As the demand of paclitaxel is extremely high and its natural availability is limited, to meet the market demands several approaches have been used for its synthesis from starting materials, such as baccharin III and 10-deacetylbaicalin III. In addition to chemical synthesis, it was also produced in high amounts using the second approach with cell cultures of *Taxus* plants. *Calophyllum lanigerum* was used to obtain Calanolide A, a dipyrano-coumarin that works as a non-nucleoside reverse transcriptase inhibitor (NNRTi) of type-1 HIV and an inhibitor of AZT-resistant strains of HIV [35].

## 10. Conclusions

Plants contain secondary metabolites that are very specific and sometimes extremely toxic at a high concentration, and represent a fascinating library of bioactive compounds with a broad activity in the context of human cells, bacteria, fungi, and parasites. For economic reasons, the study of secondary metabolites has been the subject of intense efforts, contributing to the development of several areas of phytochemistry. This review article focuses on various aspects of secondary metabolites, production synthesis, and sources of medical products from plants sources.

So, this article will be a comprehensive reference for researchers or readers who are interested in secondary metabolites.

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