



Biological significance of secondary metabolites: A review

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Abstract

Plant secondary metabolites, also known as phytochemicals, natural products are unique source for pharmaceuticals, food additives, flavours, industrially important bio-chemicals and responsible for medicinal properties of plants to which they belong. Their classification is based on chemical structure, composition, their solubility in various solvents, or the pathway by which they are synthesized. Four major classes are generally considered; phenolics, terpenes, alkaloids and steroids. Secondary substances have many different functions; of interest in this exercise to their role as chemical signals. Chemical signals act on different levels in a living world. Substances produced by one tissue that influence another tissue within the same organism are hormones. Those produced by one individual and influencing another individual of same species are pheromones. Secondary compounds have no apparent function in a plants primary metabolism but often have an ecological role; they are pollinator attractants, represent chemical adaptations to environmental stresses, or serve as chemical defenses against micro-organisms, insect and higher predators, and even other plant. Secondary metabolites that are used commercially as biologically active compounds (pharmaceuticals, flavors, fragrances and pesticides) are generally higher value lower volume products than the primary metabolites. Thus, secondary metabolites can be considered as special materials and fine chemicals.

Keywords: metabolites, terpenoids, phenolics, alkaloids, steroids, pheromones

Introduction

Secondary metabolites are an assortment of many different compounds which serve variety of functions and are restricted to different species of plants and often accumulate in different parts of plants. These molecules are known to play a major role in the adaptation of plants to their environment, but also represent an important source of active pharmaceuticals. Secondary plant metabolites have traditionally been regarded as toxic and protective against predators, or acting as insect attractants. These are the chemicals that are not part of such major organic compounds viz. carbohydrates, proteins, fats and nucleic acids (Harborne 1998; Mann 1980; Petrovaska 2012) [38, 51]. They are also called “natural products” or phytochemicals, do not participate directly in growth and development, are chemically diverse natural products, not synthesized outside the plant kingdom, are accessories to primary metabolites and act as hormones and in plant defense mechanism, can have both toxicological, behavioral and attractant effects on many different species, including interesting properties in the mammalian central nervous system as stimulants by acting on the cardiovascular system (Bell 1980; Alves 1988; Tang and Eisenbrand 1992; Verpoorte 1998; Sumner *et al.*, 2003) [81, 5, 2, 78, 75].

Biochemical origin of secondary metabolites

Secondary metabolites are more complex than primary metabolites. This is because secondary metabolites are derived from the primary products, such as amino acids or nucleotides, by modification, such as methylation, hydroxylation, and glycosylation. The pathways of biosynthesis are responsible for the occurrence of both primary and secondary metabolites (Sumner 1983; Herbert

1989; Lancini and Lorenzetti 1993; Maffei *et al.*, 2011; Michal and Schomburg 2013) [43, 51, 57, 60]. Biosynthetic reactions are energy consuming, fuelled by the energy released by glycolysis of carbohydrates and through the citric acid cycle.

Oxidation of glucose, fatty acids and amino acids results in ATP (adenosine triphosphate) formation, which is a high-energy molecule formed by catabolism of primary compounds. ATP is recycled in fuel anabolic reactions involving intermediate molecules on the pathways. Whereas, catabolism involves oxidation of starting molecules, biosynthesis or anabolism involves reduction reaction. Hence, the need of reducing agent or hydrogen donor, which is usually the NADP (nicotinamide adenine dinucleotide phosphate). These catalysts are known as coenzymes and the most widely occurring is CoA (coenzyme A) made up of ADP (adenosine diphosphate) and pantetheine phosphate (Maffei *et al.*, 2011) [57]. The most common pathways taken for biosynthesis are performed through the pentose for glycosides, polysaccharides; shikimic acid for phenols, tannins, aromatic alkaloids; acetate-malonate for phenols and alkaloids and mevalonic acid for terpenes, steroids and alkaloids (Lancini and Lorenzetti 1993; Zhong 2001) [51, 91]. As showed in the Fig. 1, the scheme outlines how metabolites from the process of photosynthesis, glycolysis and Krebs cycle are tapped off from energy-generating process to provide biosynthetic intermediates. By far, the important building blocks employed in the biosynthesis of secondary metabolites are derived from acetyl-CoA (acetyl coenzyme A), shikimic acid, mevalonic acid and 1-deoxylulose 5-phosphate (Michal 2013) [60].

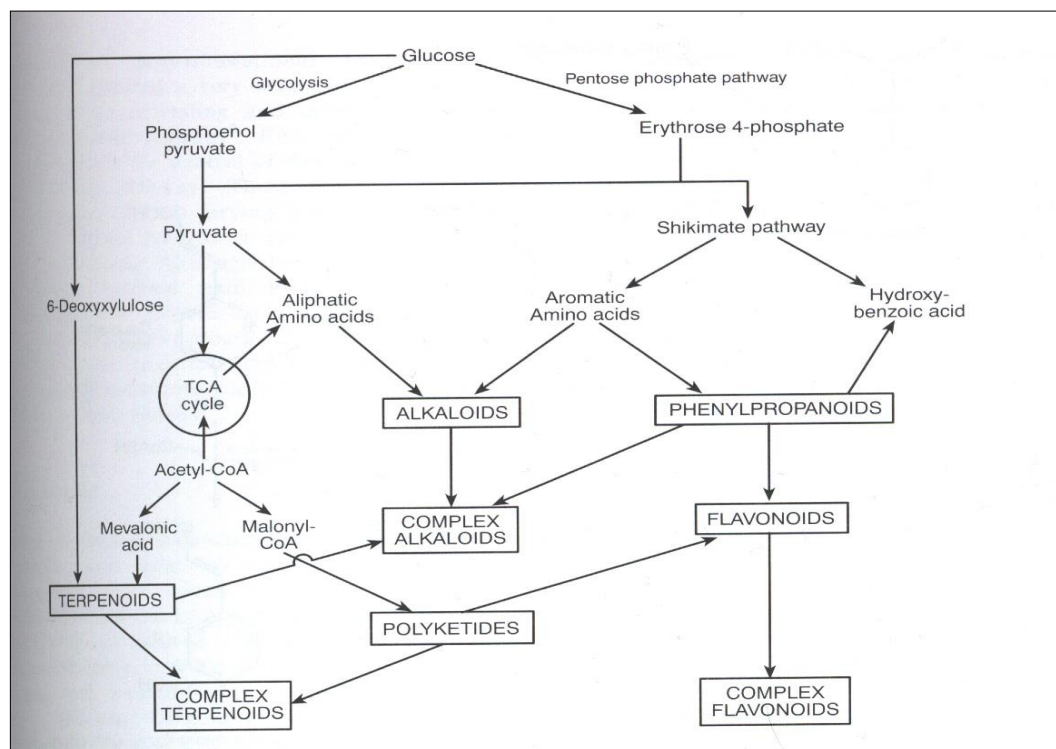


Fig 1: Overview of biosynthetic pathway of secondary metabolites

Major classes of secondary metabolites

Secondary metabolites are classified into four major classes. They are:

Terpenoids

Terpenes are derived from 5-carbon isoprene unit (C₅H₈). These are the largest group of natural products from plants with over 20,000 known structures, comprising essential oil (used in perfumes and medicines), flavors, fragrances and lipid soluble plant pigments. The terpenes are hydrocarbon that usually contains one or more C-C double bonds. The terpenoids are oxygen-containing analogs of the terpenes. These hydrophobic compounds are usually stored in plants in resin ducts, and oil cells. Examples of Terpenes include α -pinene and β -pinene, the primary component of turpentine this give rise to its characteristic odor. Camphor and menthol are example of terpenoids. Both of these have a fragrant, penetrating odor and taste cool. Camphor is used as a moth repellent. Menthol is a mild anesthetic that is added to some brands of cigarettes. Although the Terpenes and terpenoids discussed so far have very different structures, they have one important property in common: They all contain 10 carbon atoms, neither more nor less. Each of these compounds can be traced back to a reaction in which a pair of five-carbon molecules is fused. Thus, it is not surprising that we can find sesquiterpenes (15 carbon atoms), diterpenes (20 carbon atoms), diterpenes (30 carbon atoms), and so on. Carotenoids are a group of terpenoids that include pigments such as β -carotene and lycopene (the red color in tomato). Other terpenoids include essential oils, taxol (cancer drug), digitalis (heart medicine) and rubber (Pant 1979; Harborne 1999; Gershenzon and Dudareva 2007) [61, 39, 28].

Alkaloids

Alkaloids are the aromatic molecules containing nitrogen which affects the human nervous system. At least 10,000

alkaloids have been isolated from plants. Many names end in-ine, e.g. morphine, cocaine, nicotine, atropine. The majorities of alkaloids are known as “mind altering drugs” and are synthesized from amino acids. Alkaloids are generally present in plants as salts of carboxylic acids (such as citric acid, lactic, oxalic, acetic etc.). Alkaloids and amines often affect neuroreceptors as agonists or antagonists or modulate other steps in the signal transduction e.g. ion channels. The alkaloids are used in drugs, medicines, and poisons e.g. Morphine and Codeine are alkaloids synthesized by opium poppy as been recognized to most deter herbivores to have a lessened sensation of pain. Alkaloids are well known for potent pharmacological activities, such as analgesics, ant malarial and treatment of hypertension, mental disorders and tumors (Cordell 1981; Rueffer 1988; Harborne 1999) [39, 17, 67].

Steroids

Steroids are triterpenes or triterpenoids. Triterpenes are a group of molecules that contain 30 C-atoms and are generated by the polymerization of six isoprene units. It is of some importance whether two of these are in a cis- (i.e. at the same side of the cyclic system) or in a trans-position (at opposite sites). The steroids are compounds that have basic structure formed by fusing three- six member rings and a five member rings. The most important property of this molecule is the fact that, with the exception of the -OH group on the lower-left hand corner of the molecule, there is nothing about the structure of this compound that would make it soluble in water.

The steroids include the progestogens, estrogens and androgen. This hormone plays a vital role in pregnancy. Estrogens hormones, such as estone and estradiol, they participate in both the ovarian and estruses cycles, and are therefore another model for the design of oral contraceptives. Anabolic steroids help build muscle tissue and increase body mass by acting like the body natural male

hormone, testosterone (Weinberg 1971; Harborne 1999; Dewick 2002) [86, 39, 20].

Phenolics

Compounds based on benzene rings with an attached -OH group. Many serve a protective role as UV “sunscreens”, antimicrobial compounds, anti-herbivory compounds and antioxidants. These are the group of natural products over 8000 known structures. Most Phenolics compounds belong to the flavonoids (have pharmacologically useful antioxidant properties), lignin (include wide range of biological activities including fungal growth inhibition, fish toxicity, insect antifeedant functions) the primary substances of wood, is the most common member of this group. Tannins are bitter tasting compounds which probably act as deterrents to herbivores. They are mainly found in bud and foliage tissues, seeds, bark, roots, sapwood and heartwood, but bark and heartwood often contain the largest levels. Bark of many forest trees, such as silver birch, cherry, larches etc. provide a rich source of polyphenols and tannins in particular alder bark and fruit have been reported to contain around 20% tannins (Haslam 1989; Harborne 1989; Harborne 1999) [41, 39].

Functions of secondary metabolites and the process of their formation

Secondary metabolites may be formed as the result of detoxication of substances accumulating in primary metabolism e.g. In animals, for instance, steroids hormones and epinephrine are transformed to other secondary metabolites which may be excreted more easily and are removed from the urine or bile (Luckner 1990) [55]. Secondary metabolites may possess physiological significance e.g. as coenzymes or cosubstrates, they may increase membrane rigidity, are involved in light perception and regeneration, in the storage of nitrogen etc. (De vries 1992; Edward and Gatehouse 1999) [18, 22]. Secondary metabolites may be used as chemical signals in the co-ordination of cell metabolism in multicellular organisms e.g. hormones, neurotransmitters (Golds and Wheeler 1985; Colho *et al.* 2013) [32]. Secondary metabolites may co-ordinate the activity of different organisms of same species (pheromones) and they may be involved in the ecological relations between the different groups of organisms (Schildnicht 1976; Bell 1980; Waterman 1992; Demain and Fang 2000) [69, 5, 85, 19].

Physiological activities of secondary metabolites

- **Support and mechanical strength:** Cellulose and hemicellulose polysaccharides form the cell wall i.e., the “skeleton” of higher plants. Lignins stabilize the wall. Wall forming compounds also cover and protect the cell of molds, bacteria, as well as the pollen grains of higher plants and the spore’s micro-organisms. Haploids increase the membrane rigidity in certain prokaryotes and replace there the sterols and carotenoids which have the same function in most other groups of organisms (Fengel and Wegener 1984; Gottlieb and Yoshida 1989) [25, 33].
- **Improvement of resistance:** Surface lipids like alkanes and alkenes, waxes, cutin and suberin improve the physical resistance of plants and the resistance to microbial attack. Gums form a barrier at lesions

hindering the invasion of micro-organism (Prestwiten 1984; Wallace 2004; Bohme *et al.* 2014) [64, 83].

- **Photosynthesis and light perception:** Chlorophylls play a key role in photosynthesis. Carotenoids and open chain tetrapyrrols act as accessory pigments. Retinal is involved in light perception in micro-organisms and animals. An open chain tetrapyrrol is the prosthetic group of phytochrome, the most important light sensitive pigment in plants. Secondary products may act, however, also as simple light screens in all groups of organisms (1990 Vinning; Sumner *et al.* 2003; Zhaoa *et al.* 2005) [75, 48].
- **Storage of nutrients:** Secondary compounds participate in the storage and transport of carbon in micro-organisms, plants and animals. Starch, glycogen, fatty oils, polyhydroxybutyric acid and fats participate in the storage and transport of carbon in micro-organisms, plants and animals. Serotonin, allantoin and allantoic acid are stores and transport forms of nitrogen. Calcium oxalate is a store for Ca²⁺ ions in micro-organisms and plants (Kirtikar and Basu 1918; Harborne 1977; Harborne 1991; Kesseler and Baldin 2001) [50].

Secondary metabolites mediating ecological relations:

The mutual relation between the different group of organisms which cause the formation of characteristic communities of living being in forests, deserts, pools, rivers, sea etc. (Harborne 1977; Harborne 1991; Colelho *et al.* 2013)

Some examples are:

1. The leaf-cutting ants have obviously mastered the science and art of growing a fungal garden on a heap of fallen leaves, petals and even refuse. They do this by producing right kind of chemicals at the right time and in the correct proportion. Entomologists have identified two types among these ants. The smaller kind belong to ‘worker class’ and the larger constitute ‘army’. The head of worker ants secrete a mixture of about twenty terpenoids which include citral, phenyl ethyl alcohol and geranic acid etc. citral has been identified as alarm pheromone and these individual members of group of worker ants communicate with each other through these chemicals (Heil and McKey 2003) [42].
2. Fungi and plants are rich sources of thousands of secondary metabolites. The genetically coded possibilities for secondary metabolite production, the stimuli of the production, and the special phytotoxins basically determine the microscopic fungi-host plant interactions and the pathogenic lifestyle of fungi. The review introduces plant secondary metabolites usually with antifungal effect as well as the importance of signaling molecules in induced systemic resistance and systemic acquired resistance processes. The review also concerns the mimicking of plant effector molecules like auxins, gibberellins and abscisic acid by fungal secondary metabolites that modulate plant growth or even can subvert the plant defense responses such as programmed cell death to gain nutrients for fungal growth and colonization. It also looks through the special secondary metabolite production and host selective toxins of some significant fungal pathogens and the plant response in form of phytoalexin production (Keller *et al.* 2005; Tunde *et al.* 2015) [49, 80].

Secondary metabolites involved relationship between plant and micro-organisms

Chemical communications between plant and insect in any ecosystem can both mutually benign and antagonistic. Plant requires some insect for the dispersal of their seeds and for protection against certain other pests. They therefore possess insect attractants. They also have insect repellents to ward off the unfriendly among the insect community in the neighborhood (Walton and Brown 1999; Demain and Fang 2000; Singer *et al.* 2013) [84, 19]. e.g.

- Plants of cabbage family produce glycosides of mustard oils which are highly toxic to large majority of insects. At the same time these compound seems to stimulate the adult female cabbage butterfly, pieris brassicae to lay her egg on the plant. These compounds serve as feeding stimulants for the larvae of butterfly. Thus the cabbage seems to have given butterfly while repelling other insects through the agency of isothiocyanate glycosides (Tabashnik 1987) [77].
- Certain plant herbivorous insects and caterpillars form an interesting triangle of symbiotic interaction. The female riodinid butterfly, this be irenea, deposits eggs on sapling of genus eroton. The caterpillars emerging from these eggs start feeding on the tree, the leaves of which have exudates at the base. This nectary exudates attracts ants which protect this be caterpillars from predators like in social wasps. The latter are warded off by the ants which assume an aggressive posture due to the release of alarm pheromones by the caterpillars. In this case, the plant provides hospitality to the caterpillars and ants and protects itself from other predators (Agrawal, and Weber, 2015) [1].

Defensive function of herbivore-induced plant volatile emissions in nature

Wild tobacco plants are attacked by caterpillars of the moth *Manduca quinquemaculata*. This attack induces the tobacco plants to emit volatile organic compounds that attract natural enemies of the caterpillars such as big-eyed bugs (*Geocoris pallens*) caterpillars of the moth *Manduca quinquemaculata*. (Harborne 1991; Foley and Moore 2005) [26].

Table 2: Secondary metabolite and their occurrence

Group of Compound	Individual substances (occurrence)
monoterpenes	Limonene (citrus), geraniol (rose)
Sesquiterpenes	β -ionone (violets), bisabolol (orange)
Benzoic acid derivatives	Vanillin (vanilla, orchids)

Table 3: Pollinators close to flowers are attracted by secondary metabolites absorbing visible or UV radiation

Coloration of flower secondary products used as pigments	Pollinations attracted
Flavones e.g. luteolin, quercetin	Bees and other insects (recognizing uv absorption) bats (mostly colorblind)
Yellow: carotenoids, betaxanthins, yellow flavonols	Bees, butterflies
Red, violet, blue: Betacyanins, anthocyanins	Bees, birds, butterflies, moths

Influence of secondary metabolites on seed dispersal:

Colors, smell and taste of ripe fruits indicate to animals that they are suitable as food. The animals swallow the fruits (including the seeds they contain), digest the flesh and scatter the seeds together with the feces (Barnea *et al.* 1993; Cipollini and Levey 1997b; Harborne 1997) [3, 15, 37].

- Smell of fruits is caused by mono and sesquiterpenes i.e. component of essential oils.
- The sweet taste is brought about by different mono

Plant-plant interactions caused by secondary metabolites

Allelopathy occurs when one plant produce chemicals that inhibit the growth of other plants. Plant competes with each other for moisture, light and nutrients part of this competition is chemical interaction, which in most cases is brought about by secondary products. The substances involved once in leaves, stems and roots (Rice 1954; Whittaker and Feeny 1971; Putnam 1983; Chou, 1999) [54, 87, 65, 14]. e.g.

- The walnut tree which has the ability to protect its territorial rights with considerable vigor. The leaf canopy of this tree promises a cover under which other plants don't grow. This is due to juglone or 5- hydroxy naphthoquinone.
- Another example is the leaves of salvia leucophylla produce a volatile oil comprising cineole, camphor and related compounds. The oil gets volatilized into the atmosphere (like the oil of the Eucalyptus species) and then gets absorbed by the dry soil. Even low conc. of these compounds which thus get incorporated in the soil are enough to Inhibit the germination and the growth of grass and herbs which would have otherwise formed a thick under growth soon after the onset of the winter rains

Secondary metabolites and interaction of plant and animals

Secondary plant metabolites play an outstanding role in the mutual connection between plants and animals. They are involved in such general fields as predation, pollination and dispersal of seeds.

Attraction of plant pollinators by secondary metabolites

The co-evolution of plants and insects, birds and bats w.r.t. the pollination of flower has given rise to the formation of special morphological features and chemical characteristics. Pollinators visit flowers is to gather nectar, pollen as nutrients, which contain carbohydrates, fats, proteins etc. w.r.t. the secondary metabolites. Scents are of special significance for pollination of flowers by night- flying animals. At a greater distance pollinators may be lured by flower scents. These flower volatiles cover a wide range of chemical structures (Britton and Hornero-Mendez 1997; Choonhoven *et al.* 2005; Parachnowitsch *et al.* 2015) [10, 13, 62].

sacchrrides, sugar alcohols or oligosacchrrides.

- Disappearance of toxic compounds e.g. alkaloids during ripening of fleshy fruits ensure the palatability of fruits to animals and the dispersal of seeds at an appropriate stage of seed development.
- In most angiosperm fruit coloration is caused be either by flavonoid or carotenoids.
- Fruits colored by anthocyanins are cherries, strawberries, plums etc.

- The wax layer of glucous fruits e.g. grapes and the fruits of prunus sp., juniper and figs, increases UV reflection and hence their visibility for birds.

Secondary plant metabolites attracting or repelling predators:

The influence of secondary metabolites on animals ingested with diet is complex. It may include the alteration of orientation, metabolic efficiency, growth, mating etc. and secondary metabolites may even induce death (Izhaki 2002; Schmelz *et al.*, 2009; Iason *et al.* 2012) [47, 71, 46].

- A toxic non-protein amino acid in mimosine which is produced by certain plants of the family mimosoideae. Mimosine itself is non toxic but becomes toxic after the micro-organisms present in the rumen convert it into 3-hydroxy-4 pyridone and alanine, presumably by a reductive cleavage of the C-N bond. The compound brings about liver damage, loss of hair and enlargement of the thyroid gland in affected animals.
- Some plants also synthesize organic cyanides, such as e.g. β -amino propionitrile which was first isolated from seeds of sweet pea, lathyrus odoratus as their γ -glutamyl derivatives. The compound causes skeletal deformation in young rats by inhibiting the enzyme which bring about the cross-linking of peptide chains in collagen and elastin

Influence of secondary metabolites on food preferences

Taste, flavor and color of food stuffs and beverages of

humans are dominated by secondary metabolites. They repel and attract human beings in the same way as other vertebrates (Guglielmo *et al.* 1996; Eriksson and Ehrlen 1998; Izhaki 2002; Gershenzon *et al.* 2012) [34, 23, 47, 29].

Table 5: Secondary metabolites are compiled which make food stuffs and beverages attractive to human:

Secondary products	Quality of taste or smell sweet taste of fruits
Citral	Smell of lemon
Piperine	Pungent principle of pepper
Eugenol	Smell of cloves
6 (s)- gingerol	Flavor of ginger
Ethyl-2 methyl butyrate	Smell of apple
Diacetylyle, acetoin	Flavor of butter

Secondary metabolites as active principle of drugs

Secondary metabolites have been used for the alleviation of diseases, and even today the active principles of many drugs are secondary metabolites of microbial, plant and animal origin. The vast bulk of early medicinal substances were plant derived. At least 25% of all prescription drugs sold in North America contains active substances which were originally isolated from plants. The bulk of plant-derived medicines can be categorized into a number of chemical families, including alkaloids, flavonoids, Terpenes, steroids as well as coumarins, quinines, salicylates and xanthenes (Caporale 1995; Bowey *et al.* 2003; Butler 2004; Beutler 2009) [12, 9, 11, 6].

Table 6: Plants-derived drugs

Drugs	Chemical type	Indication	Plant producer
Aspirin	Salicylate	Analgesic, anti-inflammatory	<i>Salix alba</i> (white willow tree) and <i>filipendula ulmaria</i> (meadow sweet)
Caffeine	Xanthine	Increases mental alertness, stimulate central nervous system	<i>Camellia sinensis</i>
Codeine	Alkaloid	Analgesic, cough suppressor	<i>Erythoxyleen coca</i> (coco leaves)
Morphine	alkaloid	Analgesic	<i>Papaver somniferum</i> (opium poppy)
Quinine	Alkaloid	Malaria	<i>Cinchora pubescens</i> (fever tree)
Taxol	Terpenoid	Ovarian, breast cancer	<i>Taxus brevifolia</i> (western yew tree)

Conclusion

Thanks to improvement of biochemical techniques and the rise of molecular biology, it has been clearly demonstrated that secondary products play a major role in the adaptation of plants to their environment. These molecules largely contribute to plant fitness by interacting with the ecosystems. They have been described as being antibiotic, anti-fungal and antiviral and therefore, able to protect plants from pathogens, and also anti-germinative or toxic for other plants. Economically important plants serve as source of industrial oils, resins, tannins, natural rubber, gums, waxes, dyes, pharmaceuticals and many specialty products. In the future biologically active plant derived chemical can be expected to play an increasingly significant role in the commercial development of new products for regulating plant growth and for insect and weed control

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