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Secondary metabolites and their multidimensional prospective in plant life

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Abstract

Plants wield an arsenal of structurally diverse chemical compounds called secondary metabolites, which equip them strategies to deter enemies, fend off pathogens, supersede competitors and surpass environmental constraints. These chemicals are produced under specific abiotic stresses and pathogenic attacks, therefore impart survival tactics to plants. A large number of such compounds and their biosynthetic pathways have been discovered so far from plant kingdom. Owing to their diverse biological and physio-chemical properties secondary metabolites are of great interest to man and impart uses as drugs, oils, waxes, perfumes, flavoring agents, dyes and many other commercially important materials. Secondary metabolites led to the emergence of a new research discipline of plant metabolomics, committed to detection and identification of biosynthetic pathways of these compounds, their structural elucidation and applications. The review provides an insight into the diversity of plant secondary metabolites, their classification, biosynthesis, biological properties and their multidimensional prospective in plant life.

Keywords: Secondary metabolites, bioactive compounds, plant defense, phytochemicals

1. Introduction

“Necessity is the mother of invention” seems true, if we look at the plants, which being non-motile and lacking immune system are not left helpless against a wide variety of biotic and abiotic stresses, instead wield an arsenal of chemicals to deter enemies, fend off pathogens, supersede competitors, and surpass environmental constraints^[1, 2].

These chemicals were named “secondary metabolites” by A. Kossel in 1891, who described these organic compounds as incidentally occurring and not of paramount significance to plant life. A great majority of these compounds do not directly participate in growth, development and reproduction of plants hence named “secondary metabolites”. These compounds are deployed as taxonomic markers because of having limited distribution in taxonomic groups. In contrast, primary metabolites include carbohydrates, acyl lipids, phyto-sterols and organic acids, which are found in all tissues of plants and perform metabolic activity necessary for growth and development of plants. There are no clear cut defined boundaries between the two categories and both cannot be separated on the basis of their chemical structure, precursor molecules and biosynthetic origin e.g. amino acid proline is a primary metabolite while its C₆ analogous molecule, pipercolic acid is alkaloid. Similarly diterpenes and triterpenes both classes contain primary and secondary metabolites^[3, 4].

These secondary metabolites are major contributors of specific odour, color and taste of plant parts. In the past these organic compounds were thought to be biologically insignificant and therefore plant biologist gave little attention to them. However, their chemical structures and properties were studied extensively by organic chemists since the 1850s. It has now become evident that such beliefs were misleading and inaccurate and that secondary metabolites play an active and key role in potential defence mechanisms, especially in the chemical warfare between plants and their pathogens^[3, 5]. Some of these compounds have also been elucidated to have role against herbivores and to attract pollinators, allelopathic agents, and protection against toxicity, UV-light shielding and signal transduction. In view of their current prospective the term secondary metabolite seems inappropriate because it poses them as unimportant despite having a multidimensional role in plant life^[6-9].

From the last few decades secondary metabolites have got great attention due to their potential role in human nutrition, cosmetics, drugs and their indispensable role in plant defense. This drastic change in interest of secondary metabolites is not purely academic but also of commercial nature.

There commercial importance as dyes, drugs, polymers, waxes, glues, fibers, antibiotics, herbicides, insecticides also fueled the growing interest of secondary metabolites. The growing attention of their multidimensional role in plant and human life led to the reevaluation of their possible roles especially in ecological interactions^[10,11].

2. Classification of Secondary metabolites

Plant secondary metabolites are classified into four major categories as classified by British Nutrition Foundation^[12]. These four categories include terpenoids (such as carotenoids, sterols, cardiac glycosides and plant volatiles), phenolics (such as lignans, phenolic acid, tannins, coumarins, lignins, stilbenes and flavonoids), nitrogen containing compounds (such as non-protein amino acids, cyanogenic glucosides and alkaloids) and sulphure containing compounds (such as GSH, GSL, phytoalexins, thionins, defensins and lectins)^[13].

2.1. Terpenes

Terpenoids are structurally most diverse class of secondary metabolites. They contain more than 40,000 structurally diverse compounds and form the largest class of plant metabolites^[14]. Their name terpene or terpenoid was used for the reason because there first member was isolated from turpentine oil. They were monoterpenes (C₁₀) discovered in 1850s and are considered as the base units from which subsequent nomenclature is derived. All terpenoids are derived from repeated branched isopentane skeleton, which are generally referred to as isoprene units. Thermal decomposition of most terpenoids yield isoprene gas as a product. Under suitable chemical conditions these 5-carbon isoprene units can be polymerised to form variety of terpenoids and for this reason terpenoids are often called as isoprenoids³ (Croteau *et. al.* 2000). In vivo terpenes are not synthesized from isoprene units instead all have a biosynthetic origin from acetyl-coA or its intermediates^[15-20].

Terpenoids play diverse functional role in plants like phytol and carotenoids as photosynthetic pigments, ubiquinone, plastoquinone as electron carriers, gibberellins, abscisic acid as hormones and sterols as structural components of cellular membranes. Xanthophylls, α -carotenes, β -carotenes and lycopene are red, orange and yellow colored lipid-soluble pigments are also carotenoids. In most of the green leafy vegetables they are masked by chlorophyll. In tomatoes, carrots, pumpkins and sweet potatoes their bright colors are due to carotenoids. Carotenoids are not only photo-oxidative protectants for other pigments but are also precursors of abscisic acid, which modulates developmental and stress responses^[21]. Some important terpenes are shown in figure.1.

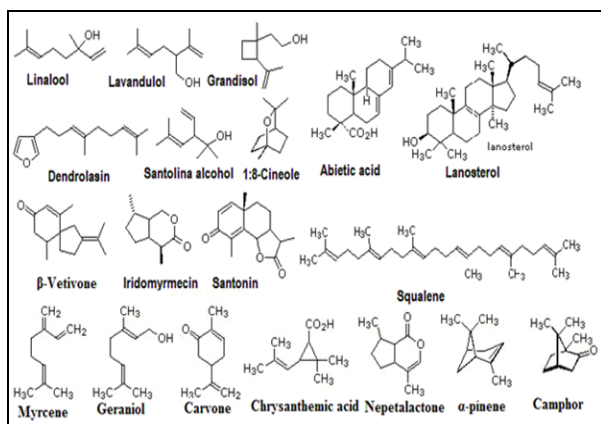


Fig 1: structures of some common terpenes

2.1.1 Nomenclature of terpenes

The size of Terpenes ranges from five carbon hemiterpenes to large size complexes including rubber containing thousands of isoprene units. All terpenes are classified according to five-carbon isopentane units of the core structure. Terpenes with 5-C are named hemiterpenes (half terpenes), with 10-C are monoterpenes, 15-C are sesquiterpenes (one and half terpenes), 20-C are diterpenes, 25-C are sesterterpenes (two and half terpenes), 30-C are triterpenes, 40-C are tetraterpenes and above are polyterpenes (C₅)_n^[11,22].

a. Monoterpenes (C₁₀): Terpenes comprising 10-C are monoterpenes. Many derivatives of monoterpenes are important tools in plant defense against pathogens e.g. pyrethroids are monoterpenes esters which occur in leaves of *Chrysanthemum* species and pose neurotoxic insecticidal activities for many insects like bees, wasps, beetles and moths. They are also used in commercial insecticides for being having low toxicity for mammals. Monoterpenes are also present in resin ducts of gymnosperms in the form of α -pinene, β -pinene, myresine and limonene, which render them toxic for serious pests especially bark beetle^[23].

b. Sesquiterpenes (C₁₅): Terpenes comprising 15-C are sesquiterpene (one and half terpenes). Despite their presence in essential oils, numerous sesquiterpenes act as phytoalexins, antibiotic compounds produced during microbial attacks, and as antifeedants that deter herbivory. Costunolides found in family asteraceae have five membered lactone ring and provide strong deterrence to herbivores^[24]. Abscisic acid the plant hormone is a 15-C sesquiterpene, it is produced from Xanthoxin (15-C precursor) which is formed by asymmetric cleavage of a carotenoid (C-40). Abscisic acid plays many physiological roles in plants including transcriptional activator and modifying membrane properties in response to water stress, and maintaining of seed and bud dormancy^[25, 26]. It also causes increase in calcium concentration and alkalination of cytosol^[27, 28]. It also causes increase in concentration of UV-B absorbing quercetin, kaempferol, flavonols, and two hydroxy-cinnamic acids, ferulic and caffeic acids. Thus all of the above mentioned roles of ABA and changes stimulate defense system of plants against UV-B^[29].

c. Diterpenes (C₂₀): Terpenes comprising 20-C are diterpenes. Diterpenes include phytol a hydrophobic tail of chlorophyll which helps them to anchor in thylakoid membranes^[30], gibberellin hormones which are involved in seed germination, leaf expansion, act as growth promoters, involved in flower and fruit set and bio mass production^[31], CO₂ fixation, stomatal conductance, assimilate translocation, phloem loading^[32-34], phytoalexins, resin acids found in conifers and legumes and pharmacologically active metabolites including taxol found in bark of *Taxus* species (Yew) used as anticancer agent and forskolin used to treat glaucoma^[3]. Some other diterpenoids include abietic acid, which is a diterpene present in leguminous and pines trees. In pines it is found in resin canals which when pierced by pathogens may physically lock them by outflow^[35].

d. Triterpenes (C₃₀): Terpenes comprising 30-C are triterpenes, which are a large class arising from squalene, formed by coupling of two farnesyl diphosphate units^[36, 37]. A large number of structurally diverse triterpenes like oleanane, ursane, lupine types result from cyclization of squalene units^[38]. A number of biologically active important products like

steroidal saponins, sterols, bile acids, mammalian sex hormones, cardioactive glycosides and corticosteroids result from skeletal modifications of side-chains [37]. Sterols are also the important components of cell membranes that act as ion channels and regulate permeability of small molecules especially by decreasing the movement of fatty acids. The milkweeds yield several sterols which deter herbivores and insect pathogens [36]. Saponins are another group of triterpenoids, covalently attached to one or few sugar moieties [39]. Another group the phytoecdysones, play their role as insect deterrent by disrupting moulting and also impart lethal consequences to many other developmental and physiological processes [40]. Limonoids are also triterpenes which are bitter substances present in citrus fruits of family Rutaceae and deter herbivores. Azadirachtin is another limonoid found in *Azadirachta indica* which is toxic and deter feeding pathogens [41].

e. Tetraterpenes (C₄₀): Terpenes comprising 40-C are tetraterpenes, which constitute a large group of natural dyes and possess a variety of functions. Despite plants these are also formed by bacteria, algae and fungi. The most important tetraterpenoids are carotenoids which are biologically synthesized through tail-to-tail linkage of the two geranylgeranyl diphosphate molecules. These parent carotenoids are then modified by variations in three different ways. First by cyclization at one or both ends, Second by the addition of oxygen-containing functional group, Third by changes in hydrogenation level. Carotenes are pure hydrocarbons and act as accessory pigments during photosynthesis, oxygen containing carotenes are called xanthophylls which are also accessory pigments. Some carotenes e.g. β -carotenes when absorbed through human diet act as pro-vitaminA, others e.g. lycopene act as antioxidants [42].

f. Polyterpenes (C_n): Terpenes comprising more than forty carbons are polyterpenes. Rubber is a polyterpene which contains 1500-15000 isopentenyl units, and all of the C-C double bonds have cis-configuration whereas in gutta rubber all the double bonds are in trans-configuration. In long vessels called laticifers the rubber found provides protection against herbivores and a mechanism for wound healing [43].

2.1.2 Biosynthesis of Terpenes

All terpenes are biosynthesized from same precursor isopentyl diphosphate (IDP), and its isomer dimethyl-allyl diphosphate (DMAPP or DMADP) [14]. IDP and DMADP are synthesized from two different pathways i.e. chloroplast pathway and cytosolic pathway (Fig.2). The cytosolic pathway is mevalonic acid pathway (MVA) which works within most eukaryotes and synthesizes sesquiterpenes, triterpenes etc. The second is newly discovered pathway, 2-C-methyl-D-erythritol-4-phosphate/1-deoxy-d-xylulose-5-phosphate pathway (MEP / DOXP) which works in most prokaryotes, also in the plastids of eukaryotes and synthesizes hemiterpenes, monoterpenes, diterpenes and tetraterpenes(carotenes), [42, 44, 45].

Mevalonic acid pathway starts from condensation of three molecules of acetyl-coA, which subsequently form mevalonic acid. Upon phosphorylation mevalonic acid is converted to mevalonate 5-phosphate which is then converted to precursor isopentyl diphosphate (IDP) [45]. In chloroplast pathway condensation of pyruvate and glyceraldehydes-3-phosphate results in formation of 2-C-methyl- D-erythritol- 4-phosphate(MEP), which further converts into 4-hydroxy- 3-

methylbut- 2-enyl diphosphate (HMBDP), which is further converted into IDP and DMADP [10]. Both IDP and DMADP are interconvertible by a reversible reaction catalyzed by isoprenyl diphosphate isomerase (IDI) enzyme [46].

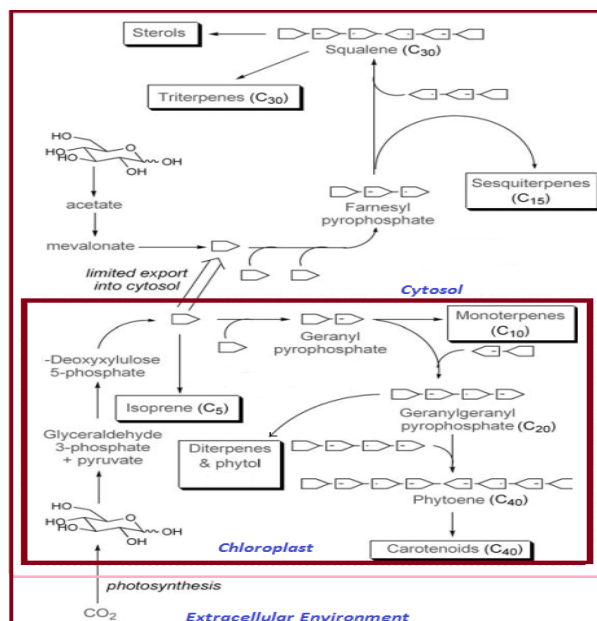


Fig 2: Two different pathways of terpene biosynthesis. Each block represents 5-carbon isoprenyl units (DMAPP or IPP) [10].

2.2 Phenolics

These compounds consist of at least one aromatic ring attached with one or more hydroxyl groups [47]. Chemical structure of phenols varies from simple phenols (i.e. catechols and hydrobenzoic acid derivatives) to catechol melanins (C₆)₆ long chain polymers having high molecular weight, condensed tannins (C₆-C₃-C₆)_n and lignins (C₆-C₃)_n. Flavonoids (C₆-C₃-C₆) and stilbenes (C₆-C₂-C₆) having intermediate molecular weights are also phenolic compounds. Fig. 3 describes basic structures of phenolic compounds. Flavonoids which are formed from chalcone include anthocyanins, flavonols (i.e. quercetin and myricetin), isoflavones (i.e. daidzein and genistein) [11].

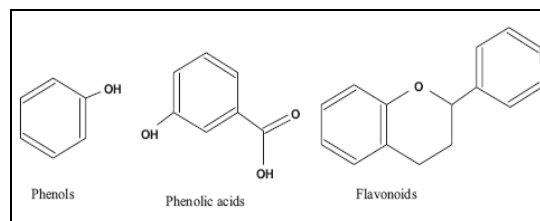


Fig 3: Some Common Phenolic Compounds

Phenolic compounds protect plants from herbivory, pathogen attack and other animals due to their deterrent abilities. Their high concentration also imparts fungal resistance [48]. Phenolic compounds often found attached to sugars which reduces their endogenous toxicity. They also shield plants from UV radiation and cold stress [49].

2.2.1 Classification of Phenolics

More than 8000 phenolic compounds have been discovered so far. Based upon their structure, phenolics have been divided into two main categories i.e. flavonoids and non-flavonoids [10].

A) Flavonoids

These are polyphenolic compounds containing 15-carbon atoms with two aromatic rings connected through a 3-carbon bridge. Fig. 4 describes the parent structure of flavonoids.

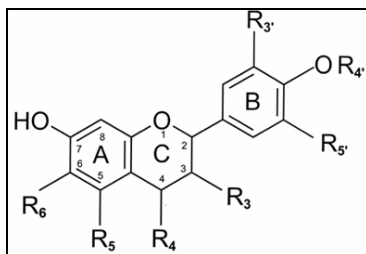


Fig 4: Basic Structure of Flavonoids

They are present in skin of fruits and epidermis of leaves and impart many important functions. They impart pigmentation to plant parts, shield against UV-radiations and defense against diseases [50, 51]. Important flavonoids include flavonols, flavones, flavan-3-ols, isoflavones, anthocyanidins and flavanones. Other flavonoid found in minor quantities include coumarins, aurones, chalcones flavan-3,4-diols, and dihydrochalcones [10].

a. Flavonols: Flavonols are most widely spread flavonoids mostly occurring as *O*-glycosides (having oxygenation at carbon 3) include quercetin, kaempferol, myricetin and isorhamnetin. At the 3 position of the C-ring conjugation occurs but substitutions can also occur at the 3,4,5, and 7 positions of the carbon ring [52] (Figure 5). They are present in commonly consumed vegetables, fruits and beverages. They are found in vegetables like onions, leeks, endives, and broccoli [53, 54].

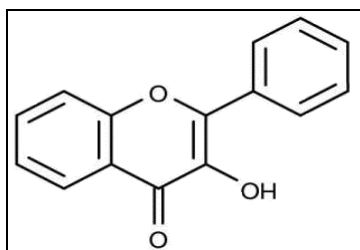


Fig 5: Basic Structure of Flavonol

b. Flavones: Unlike flavonols flavones lack oxygenation at carbon 3, (Figure 6). A number of substitutions are possible with flavones, like methylation, hydroxylation, glycosylation and *O*- and *C*-alkylation. Most of these flavones occur as 7-*O*-glycosides. They are not commonly found like flavonols but are limited only to certain plants including in parsley, thyme, and celery. Common examples of flavones include polymethoxylated flavones like tangeretin and nobiletin, have been present in citrus species, 3 flavones such as luteolin, chrysoeriol and apigenin are found in celery and thyme [55].

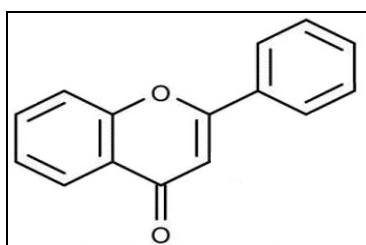


Fig 6: Basic Structure of Flavone

c. Flavan-3-ols: They are the most complex subclass of flavonoids, ranging from monomeric to oligomeric and polymeric forms. They have saturated C3 element just like flavanones and proanthocyanidins (Figure 7). They are abundantly present in green tea as (-)-epigallocatechin gallate, (-)-epigallocatechin and (-)-epicatechin gallate. Other examples include (-)-epicatechin, (+)-Catechin and (+)-Epicatechin [53].

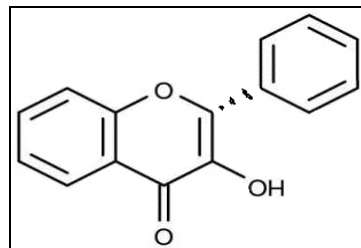


Fig 7: Basic structure of flavan-3-ol

d. Anthocyanidins: They are principally found as anthocyanins which are their conjugated sugar derivatives, and are widely distributed in plant kingdom. Except other plant parts anthocyanins are mostly found in flowers and fruits where they impart red, blue and purple colors to attract pollinators and protect them against harms of excessive sunlight. Their basic structure is shown in figure 8. They also develop their conjugates with other compounds like organic acids etc. The most commonly found anthocyanidins are cyanidin, peonidin, malvidin, pelargonidin, delphinidin, and petunidin, present in red cabbage, radish, and red lettuces [10].

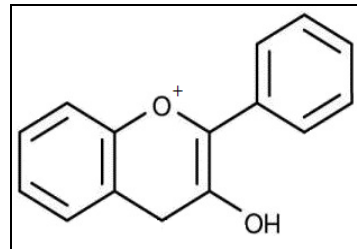


Fig 8: Basic Structure of Anthocyanidin

e. Chalcones and Dihydrochalcones: They are a major class of flavonoids. Chalcones and dihydrochalcones are present mostly in vegetables. Their basic structure is presented in figure 9. Chalcones, 1, 3-diphenylpropanones, have widespread distribution in vegetables, fruits, tea and soy where they impart their characteristics colours [56]. Their important roles include antioxidant, anticancerous, antiproliferative and anti-inflammatory effects. Acetophenones and benzaldehydes via the Claisen-Schmidt condensation using polar solvents form chalcones which are the precursors of flavones and flavanones [57-59].

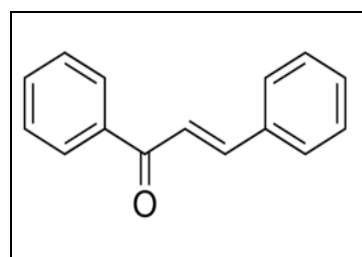


Fig 9: Basic Structure of Chalcone

f. Isoflavonoides: These are flavonone intermediates and their basic structure is presented in figure 10. They play their role in plant defense and development. These are very effective against reactive oxygen species (ROS), many studies reveal that phenolic compounds are the primary substrates for peroxidases, there by defend plants against various environmental stresses [60]. These are also synthesized in leguminous plant and are involved in formation of root nodules by nitrogen fixing bacteria [61].

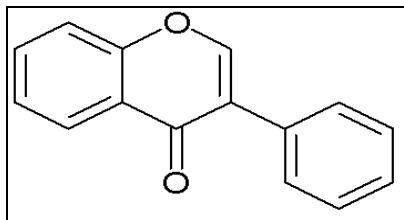


Fig 10: Basic Structure of Isoflavone

g. Coumarins: These compounds are an important component of plant defense mechanism and protect plants from herbivores, pathogenic insects, bacteria and fungi [62]. They are formed as a result of shikimic acid pathway and their basic structure is depicted in figure 11. They possess high antimicrobial activity against bacteria and fungi [62, 63]. They may be present as halogenated coumarins or hydroxylated coumarins and has more stability than pure coumarins. These coumarin derivatives exhibit antifungal activity [64].

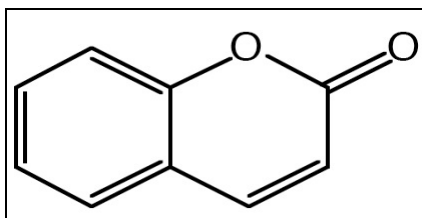


Fig 11: Basic Structure of coumarin

h. Furano-coumarins: These are a type of coumarins having attached a furan ring (Figure 12), which exhibit notable phytotoxicity and are abundantly present in the members of Apiaceae family. They only show toxicity when activated by UV-A light due to high energy electronic state and become inserted into the double helical DNA and bind with pyrimidine bases, there by block transcription and ultimately cause cell death [65] common example of furano-coumarin is Psoralin, which is used in fungal defense of plants [66].

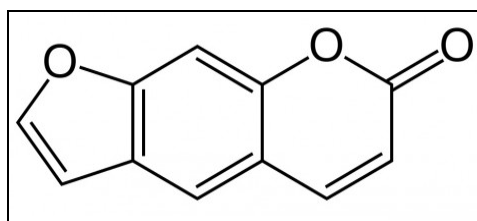


Fig 12: Basic Structure of Furano-coumarin

i. Lignin: It's a highly branched polymer of propanoid group. When alcohols like coniferyl, coumaryl or sinapyl are oxidized by peroxidases and simultaneously react they form lignin. Different proportion of these monomers impart differential properties to lignin. The physical toughness of lignin not only deters herbivory but also prevents microbial

growth when lignifications immediately starts after infection or wounds [67-69].

B) Non-flavonoids

These include phenolic acids, tannins, polyphenolics, hydroxycinnamates, stilbenes and their conjugated derivatives.

a. Phenolic acids and Tannins: These are also called as hydroxybenzoates and their main component is gallic acid. The term gallic acid is derived from a French word "galle" which means a swelling of plant tissue formed after parasitic attack. This swelling is formed of carbohydrates and other nutrient which promote larval growth of insect. 70% of this galle consists of gallic acid esters [70]. Fig. 13, illustrates some examples of phenolic acids.

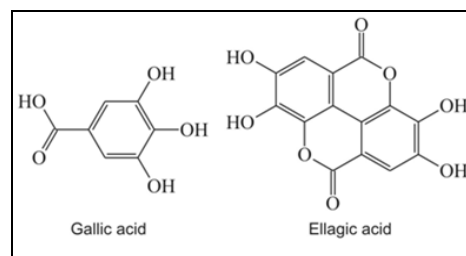


Fig 13: Cinnib Phenolic Acids

This Gallic acid also forms gallotannins, whereas gallic acid and hexa-hydroxydiphenoyl monomers unite to form ellagitannins. Tannins are of two types, hydrolysable tannins and condensed tannins. Hydrolysable tannins include ellagitannins and gallotannins, which can readily be hydrolysed with dilute acid to release gallic acid and ellagic acid monomers. Condensed tannins cannot be hydrolysed in this way. Tannins impart toxic properties and act as feeding deterrent for herbivore animals. They combine with salivary proteins and develop astringent sensation [71]. Hydrolysable tannins and condensed tannins can bind with collagen protein in animal hides and cause its precipitation. This changes animal hides to leather, which becomes resistant to putrefaction. Thus plant derived tannins have therefore formed the basis of tanning industry [72]. Some tannin like protocatechillic acid and chlorogenic acids are readily oxidized and impart disease resistant properties to plants e.g. prevent smudge in onions caused by *Colletotrichum circinans* [73, 74].

b. Hydroxycinnamates: Hydroxycinnamates are phenylpropanoids because they are derived from cinnamic acid (C_6-C_3), (Figure 14), which is the product of phenylpropanoid pathway. Their common examples include caffeic acid, ferulic acid, p-coumaric acid, which deposits as tartarate ester in fruits and vegetables [10].

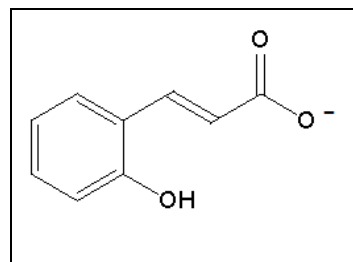


Fig 14: Hydroxycinnamate

c. Stilbenes: Stilbenes are polyphenolic compounds with (C₆-C₂-C₆) structure (Figure 15). They act as phytoalexins, which are produced by plants when attacked by bacteria, fungi, viruses and nematode pathogens. Their common examples include resveratrol, which exists in both *cis*- and *trans*- forms. Stilbenes are usually present in soya, peanuts, wine and grapes. *Trans*-resveratrol is abundantly present in *Polygonum cuspidatum* (Japanese knotweed, Idatori plant). Japanese knotweed is a very noxious plant has invaded most European and North American countries [75].

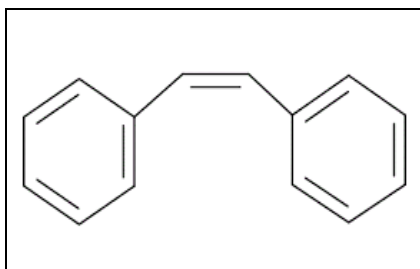


Fig 15: Basic Structure of Stilbene

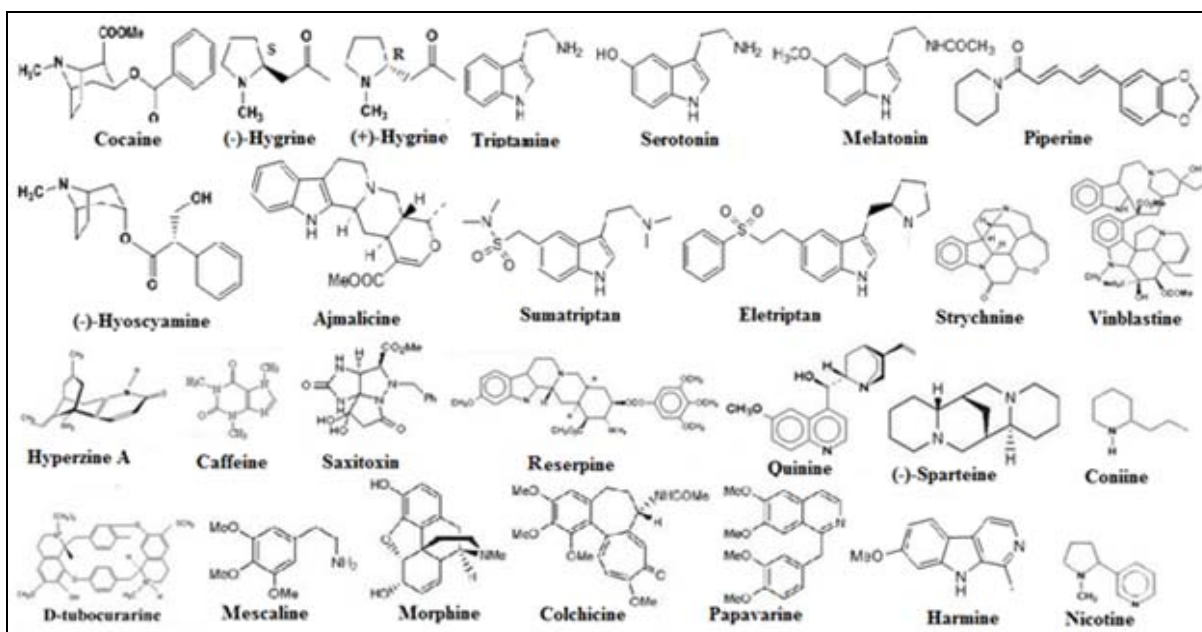


Fig 16: Structure of some common alkaloids

Due to a variety of biological activities about 12000 alkaloids are used as narcotics, pharmaceuticals and poisons [84]. Alkaloids are named either on the basis of genus name of plant (Atropine from *Atropa belladonna*), specific epithet of plant (Belladonine from *Atropa belladonna*), common name of plant (Ergotamine from ergot, Theine from Tea) etc. Commonly used plant-derived alkaloids as drugs include, vincristine, vinblastine and camptothecin used as anticancer, colchicin as gout suppressant, morphine and codeine as analgesic and scopolamine as sedative [10].

2.3.1 Classification of Alkaloids

Alkaloids are generally classified into three groups. a) Typical alkaloids having a heterocyclic ring with nitrogen atom and derived from amino acids. b) Biological amines which are non-heterocyclic. c) Steroidal alkaloids having heterocyclic ring with nitrogen atom but not derived from amino acids. Typical alkaloids are classified in following fourteen groups, Pyrrol and Pyrrolidine, Pyrrolizidine, Pyridine and Piperidine,

2.2.3 Biological properties of phenolics

Flavonoids have been found to prevent low density lipid peroxidation and thereby prevent atherosclerotic plaque formation, neurodegenerative diseases and ischemic injury [76-78]. They also capture free radicals and chelate copper and iron ions, which can promote free radical generation. They also stimulate enzymes which are involved in detoxification of cancer causing substances and inhibit inflammation and aging processes [54].

2.3 Alkaloids

These are nitrogen-containing compounds, which are derived from amino acids like tyrosine, lysine, tryptophan and aspartic acid [79]. They are present as secondary metabolites in about 20% of plant species and impart defensive role against herbivores and pathogen attacks [16, 80-81]. A large number of biologically active alkaloids have been isolated from plants (Fig. 16). At cellular level the action of alkaloids is quite variable, some effect nervous system, some effect protein synthesis, other affect membrane transport and enzyme activities [83].

Tropane, Quinoline, Isoquinoline, Aporphine, Norlupinane, Indole, Indolizidine, Terpenoid, Imidazole, Purine and steroid.

a. Benzyl-isoquinoline alkaloid: These are a pharmacologically active group of alkaloids present in Fumariaceae, Papaveraceae, Menispermaceae, Magnoliaceae and Ranunculaceae families of angiosperms. Most of these alkaloids help plants against pathogen attacks. Important examples of this group include berberine, tubocurarine, colchicines, morphine, sanguinarine etc. benzylisoquinoline alkaloids are synthesized from tyrosine [85].

b. Tropane alkaloids: These alkaloids contain an 8-azabicyclo [3.2.1] octane nucleus and are present in Convolvulaceae, solanaceae and erthroxylaceae families of angiosperms. Most of these alkaloids are toxic and are seldom pharmaceutically potent. Common examples of this group are atropine, hyoscyne and hyoxycyamine [86-87].

c. Nicotine: It is a simple alkaloid which is the active principle of most *Nicotiana* species. *Nicotiana tabacum* (Tobacco) is a native plant of America, and has been cultivated since 3000BC. Tobacco is chewed, sniffed, eaten, drunk, smoked and also used to kill parasites. Nicotine consists of a pyridine ring attached to a *N*-methylpyrrolidine ring, and it is formed from quinolinic acid and putrescine [88].

d. Purine alkaloids: These alkaloids include coffee, caffeine and theobromine. Coffee is obtained from *coffea arabica*, *Camellia sinensis* and *Ilex paraguariensis*. Theobromine is produced by *Camellia ptlophylla*, Cacao and *Camellia irrawadiensis* [89, 90]. Coffee and Tea are among the most widely used beverages in the world.

e. Pyrrolizidine alkaloids: These alkaloids are extremely toxic and pose deleterious effects to animals, therefore deter herbivores from plants. These are present in Asteraceae, Fabaceae, orchidaceae and boraginaceae families of angiosperms. These are mostly derived from polyamines (Putrescine and spermidine), and present as esters of basic alcohols also known as necine bases [91, 92].

f. Quinolizidine alkaloids: These alkaloids are toxic in nature and are mostly present in Leguminosae family. Most of these are produced by genus *Lupinus*, the seeds of which are used as protein rich food for humans and livestock. To remove these toxic alkaloids, seeds are soaked in water for long periods prior to consumption [93].

2.3.2 Biosynthesis of Alkaloids

Generally alkaloids are biosynthesized from aliphatic and aromatic amino acids, which are synthesized from two pathways, i.e. pyruvate pathway and shikimic acid pathway. Pyruvate and shikimate both are derived from phosphoenol pyruvate and erythrose 4-phosphate. All alkaloids are basically derived from L-amino acids like tyrosine, lysine, tryptophan and aspartic acid, but have some variation in biosynthetic pathway. For example purine alkaloids are synthesized from xanthosine, which is a purine nucleoside formed from purine nucleotides present in free pool. Pyrrolizidine alkaloids are formed from polyamines putrescine and spermidine. Spermidine is synthesized from decarboxylation of *S*-adenosylmethionine (SAM) [92]. Caffeine is also synthesized from *N*-methylation of xanthosine during 7-methylxanthosine formation using SAM as methyl donor. Quinolizidine alkaloids are developed from decarboxylation of lysine [94].

2.4 Sulphur containing secondary metabolites

These secondary metabolites are of two types derived from two important pathways. One group, formed from glucosinolate substrate hydrolyzed by myrosinase enzyme and this pathway is present in members of family cruciferae e.g. Nasturtium, Cabbage and broccoli. Second group, formed from alliin hydrolyzed by alliinase enzyme found in members of *Allium* genus e.g. *Allium cepa* (Onion), *Allium sativum* (Garlic) and *Allium porrum* (Leeks). Both these systems, glucosinolate-myrosinase and alliin-alliinase pathways seem to be evolved for herbivore defense. Presence of these pathways in both of these crop groups is no doubt one of the major reasons for use of these crops as vegetables, condiments, spices and in health care systems [90].

2.4.1 Classification of Sulphur containing secondary metabolites

a. Glucosinolates: This group consists of β -thioglucose unit, a sulphonated oxime unit and a variable side chain derived from an amino acid. A great variety of glucosinolates have been described with about 120 side chains, but only 16 side chains are most commonly found in crop plants. Seven of these side chains directly correspond to amino acids, Valine, leucine, isoleucine, alanine, tyrosine, tryptophan and phenylalanine [95]. Major understandings for the biosynthesis of glucosinolate pathway came from the biochemical study of model plant *Arabidopsis thaliana*. In the first step there is conversion of either a primary amino acid or a chain elongated amino acid to an aldoxime. The aldoxime then conjugates with a cysteine which donates sulphur, the complex is then cleaved by a C-S lyase enzyme resulting into a toxic thiohydroximate [96], which is detoxified by glycosylation by a uridine diphosphate glucose; thiohydroximate glucosyltransferase to produce desulphoglucosinolate [97, 98].

b. Alliinins: This group consists of compounds formed from hydrolysis of alkyl and alkenyl-substituted L-cysteine sulphoxides by the action of enzyme alliinase after tissue disruption. 2-propenyl L-cysteine sulphoxide or alliin is a characteristic of garlic and leeks, while 1-propenyl-L-cysteine sulphoxide or isoalliin is a characteristic of onions. Methyl and propyl-L-cysteine sulphoxides are also found in other *Allium* species. The alkyl and alkenyl-substituted L-cysteine sulphoxides are located in the cytoplasm, whereas alliinase is localized within the vacuole [99].

2.4.2 Biological properties of sulphur-containing secondary metabolites

Members of family cruciferae and *Allium* genus are rich source of sulphur containing compounds, most of which are volatile and impart characteristic aroma and flavor to these species. Hot and pungent flavor of many crucifer salads and condiments is due to the presence of isothiocyanates. Glucosinolates impart bitter taste to crucifers which prevents herbivory and pathogen attack. These compounds are also pharmaceutically employed against atherosclerosis and inflammatory diseases [90].

3. Conclusions and future prospects

Owing to the presence of this diverse variety and multidimensional role of secondary metabolites we can assume that these organic compounds are of immense importance for the growth, development, defense and survival of plants. Plants notably produce these compounds when they encounter herbivores or pathogen attacks. These compounds are also produced when plants face abiotic stresses i.e. salinity, drought, UV-radiations, heavy metals and harsh climate. Secondary metabolites possess diverse biological and physio-chemical properties and are therefore of great interest to man and commonly being used as drugs, oils, waxes, perfumes, flavoring agents, dyes and many other commercial applications. Secondary metabolites are produced from primary metabolites by various pathways under specific conditions. A summarized sketch shows that most secondary metabolites are derived either from amino acids or from acetyl co-A. Bioassay guided isolation of bioactive compounds and synthesis of their analogues is currently an effective approach for the development of plant derived products. A large variety of these compounds have been isolated and identified from

plants using these tools. Further exploration of these compounds can yield valuable pharmaceutical, medicinal and other commercially important materials.

The understanding of diverse array of secondary metabolites in plants led to the emergence of plant metabolomics, a new research discipline committed to detection and identification of biosynthetic pathways of these compounds, their structural elucidation and applications. Metabolomic tools like mass spectrometry, infrared spectrometry and nuclear magnetic resonance serve as an important platform for understanding the structural aspects of these compounds. Continued efforts in the past few decades in plant metabolomics led to the development of more comprehensive and highly sophisticated analytical methods. Now a day's metabolite finger printing and metabolite profiling are commonly being used for diagnostic analyses of plants^[100]. Despite this advancement a lot of work still has to be done for the understanding of these compounds. High throughput methods are required for rapid analysis of plant samples. More elaborate classification of these compounds is needed. Computational tools should be developed for analysis of large metabolite data sets.

4. Conflict of interests

The authors declare no conflict of interests.

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