

**METABOLITES IN PLANTS AND ITS CLASSIFICATION**

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Article Received on  
11 Oct 2014,

Revised on 06 Nov 2014,  
Accepted on 02 Dec 2014

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**ABSTRACT**

Metabolites such as carbohydrates, organic and amino acids, vitamins, hormones, flavonoids, phenolics, and glucosinolates are essential for plant growth, development, stress adaptation, and defense. Besides the importance for the plant itself, such metabolites determine the nutritional quality of food, colour, taste, smell, antioxidative, anticarcinogenic, antihypertension, anti-inflammatory, antimicrobial, immunostimulating, and cholesterol-lowering properties. Secondary metabolites are organic molecules that are not involved in the normal growth and development of an organism. While primary metabolites have a key role in survive of the species, playing an active function in the photosynthesis and respiration, absence of secondary metabolites does not result in immediate death, but rather in long-term impairment

of the organism's survivability, often playing an important role in plant defense. This review is focused on plant metabolites and classification.

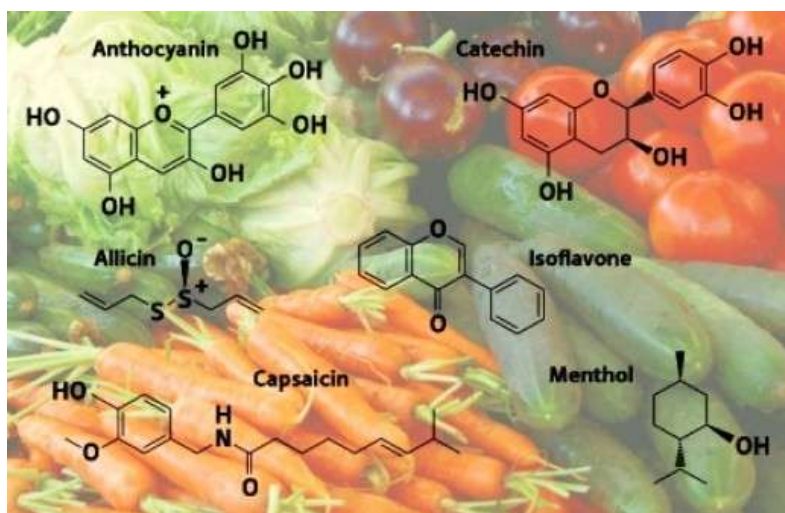
**KEY WORDS:** Primary Metabolites, Secondary Metabolites.

**INTRODUCTION**

A substance essential to the metabolism of a particular organism or to a particular metabolic process is called metabolites. <sup>[1]</sup> Over the centuries humans have relied on plants for basic needs such as food, clothing, and shelter, all produced or manufactured from plant matrices (leaves, woods, fibers) and storage parts (fruits, tubers). Plants have also been utilized for

additional purposes, namely as arrow and dart poisons for hunting, poisons for murder, hallucinogens used for ritualistic purposes, stimulants for endurance, and hunger suppression, as well as inebriants and medicines. The plant chemicals used for these latter purposes are largely the secondary metabolites, which are derived biosynthetically from plant primary metabolites (e.g., carbohydrates, amino acids, and lipids) and are not directly involved in the growth, development, or reproduction of plants. <sup>[2]</sup> Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts. <sup>[3]</sup> In India, almost 95% of the prescriptions were plant based in the traditional systems of Unani, Ayurveda, Homeopathy and Siddha. <sup>[4]</sup> The study of plants continues principally for the discovery of novel secondary metabolites. Around 80% of products were of plant origin and their sales exceeded US \$65 billion in 2003. <sup>[5]</sup>

### Primary and Secondary Metabolites



**Fig. 1. Structure of Anthocyanin, Catechin, Allicin, Isoflavone, Capsaicin And Menthol**

A plant cell produces two types of metabolites: primary metabolites involved directly in growth and metabolism (carbohydrates, lipids and proteins), and secondary metabolites considered as end products of primary metabolism and not involved in metabolic activity (alkaloids, phenolics, sterols, steroids, essential oils, lignins and tannins etc). They act as defense chemicals. Their absence does not cause bad effects in the plants.

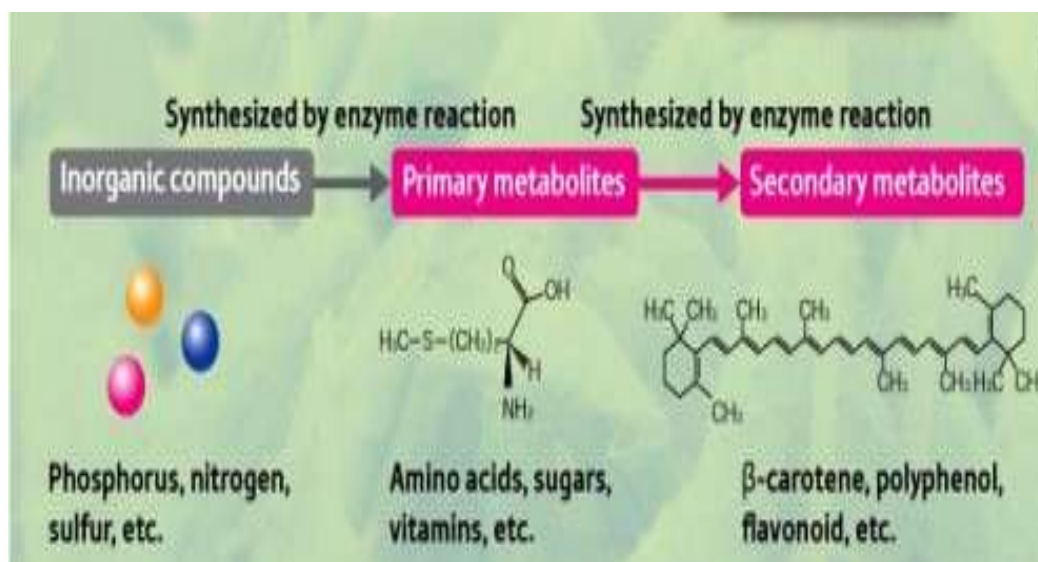


Fig. 2. Flow Diagram

### Definition

Secondary metabolites are substances which are produced by plants as defense chemicals. Their absence does not cause bad effects to the plants. They include alkaloids, phenolics, steroids, essential oils, lignins, resins and tannins etc.

Secondary metabolites are compounds biosynthetically derived from primary metabolites. Secondary metabolites or secondary compounds are compounds that are not required for normal growth and development, and are not made through metabolic pathways common to all plants. In plant kingdom they are limited to occurrence and may be restricted to a particular taxonomic group genus, species or family. Secondary metabolites are accumulated by plant cells in smaller quantities than primary metabolites. These secondary metabolites are synthesized in specialized cells at particular developmental stages making extraction and purification difficult. <sup>[6]</sup>

### Classes and Number of Secondary Metabolites

Table No. 1. -Number of Secondary Metabolites reported from Higher plant

Type of secondary metabolite	Approximate numbers
<b>Nitrogen-containing Secondary metabolites</b>	
Alkaloids	21000
Non-protein amino acids (NPAAS)	700
Amines	100
Cyanogenic glycoside	60
Glucosinolates	100
Alkamides	150

Lectins, peptides, polypeptide	2000
<b>Secondary metabolites without nitrogen</b>	
Monoterpenes including iridoids	2500
Sesquiterpenes	5000
Diterpenes	2500
Triterpenes, steroids, saponins	5000
Tetraterpenes	500
Flavonoids, tannins	5000
Phenylpropanoids, lignin, coumarins, lignans	2000
Polyacetylenes, fatty acid, waxes	1500
Anthraquinones and other polyketides	750
Carbohydrates, organic acids	200

Based on their biosynthetic origins, plant secondary metabolites can be divided into three major groups:

1. Terpenoids
2. Flavonoids and allied phenolic and polyphenolic compounds,
3. Nitrogen-containing alkaloids and sulphur-containing compounds. <sup>[7]</sup>

### 1. Terpenoids

The terpenes, or isoprenoids, are one of the most diverse classes of metabolites. The *Dictionary of Natural Products* lists over 30000, mainly of plant origin, encompassing flavours and fragrances, antibiotics, plant and animal hormones, membrane lipids, insect attractants and antifeedants, and mediators of the essential electron-transport processes which are the energy-generating stages of respiration and photosynthesis. <sup>[8]</sup>

Terpenoids are the largest and most diverse family of natural products, ranging in structure from linear to polycyclic molecules and in size from the five-carbon hemiterpenes to natural rubber, comprising thousands of isoprene units. All terpenoids are synthesized through the condensation of isoprene units (C5) and are classified by the number of five-carbon units present in the core structure. <sup>[9]</sup>

Many flavor and aromatic molecules, such as menthol, linalool, geraniol and caryophyllene are formed by monoterpenes (C10), with two isoprene units, and sesquiterpenes (C15), with three isoprene units. Other bioactive compounds, such as diterpenes (C20), triterpenes (C30) and tetraterpenes (C40) show very special properties.

#### 1.1 Monoterpenes and Sesquiterpenes (Plant volatiles)

Plant volatiles are typically lipophilic liquids with high vapor pressures. Non-conjugated plant volatiles can cross membranes freely and evaporate into the atmosphere when there are

no barriers to diffusion. The number of identified volatile chemicals synthesized by various plants exceeds 1000 and is likely to grow as more plants are examined with new methods for detecting and analyzing quantities of volatiles that are often minute. <sup>[10-11]</sup> studying the volatile fraction requires analytical methods and technologies that not only evaluate its composition exhaustively but also monitor variations in its profile and detect trace components characterizing the plant being investigated. <sup>[12]</sup> The gas chromatography (GC and GC-MS) is a very powerful analytical tool for the identification of essential oil components. However, GC-MS has its limitations. Isomers usually give very similar mass spectra. This is particularly true for terpenes and even more for sesquiterpenes. Therefore, a favorable match factor between mass spectra is not sufficient for identification. <sup>[13]</sup> Retention indices have been used, together with mass spectrometry, for the proper identification of essential oils composition. Misidentification is not rare, however, either if a non-authentic mass spectra library is used, which means a database built with data from the literature, not from the analysis of real standards, or by misuse of retention indices. <sup>[14]</sup> CC has been applied to solve this problem. After isolation, a NMR analysis can be performed for the unknown or suspect compound, so that the correct mass spectrum and retention index can be recorded. Despite the advances in analytical methods to evaluate the composition of essential oil, CC remains a powerful technology for separation and characterization of specific compounds of interest. Column chromatography has been largely used and reported for searching and identification of new molecules, sometimes associated with their antimicrobial, antibacterial and antifungal activities. Also, this technique has been successfully used to obtain sufficient amounts of a substance for the investigation of its biological properties and allowing the detection of its olfactory properties. Isolation is also applied to and, very important for volatiles, to evaluate its odor. <sup>[15]</sup>

## 1.2 Diterpenes and Sesterterpenes

The diterpene compounds arise from geranyl diphosphate, and present 20 carbon units in their basic skeletal type. One of the simplest and most important of the diterpenes is phytol, a reduced form of geranylgeraniol, which forms the lipophilic side-chain of the chlorophylls. <sup>[16]</sup> Cyclization reactions of geranyl diphosphate led to many structural types of diterpenoids, presenting a large range of polarity nature, from apolar hydrocarbons such as cembrene, <sup>[17]</sup> a 14-membered ring, to fully oxidized skeleton of virescenoside, isolated from marine fungus *Acremonium striatisporum*. <sup>[18]</sup>

Sesterterpenes (C<sub>25</sub>) may be the least common group of terpenoids. This class of compounds arises from geranylgeranyl diphosphate, which by cyclization can give rise to various skeletal types, presenting different oxidation levels and several biological activities. Although many examples of these natural terpenoids are known, they are primarily isolated from fungi and marine organisms.

Considering the large range of polarity nature presented by both diterpene and sesterterpene, the isolation and purification techniques vary and can be classic TLC, preparative thin-layer chromatography (PTLC), CC, flash chromatography (FC), or modern high performance liquid chromatography (HPLC), multiflash chromatography, vacuum liquid chromatography (VLC), solid-phase extraction and others.<sup>[19]</sup>

### 1.3 Tetraterpenes (Carotenoids)

More than 650 carotenoids (C<sub>40</sub>) are found in nature, constituting the largest group of natural dyes. The carotenoids are substances with very special properties possessed by no other group of substances; these form the basis of their many varied functions and actions in all kinds of living organisms. Carotenoids are biosynthesized by plants, algae, fungi, yeasts and bacteria. The carotenoids are isoprenoid compounds, biosynthesized by tail-to-tail linkage of two geranyl diphosphate molecules. This produces the parent C<sub>40</sub> carbon skeleton from which all the individual variations are derived. This skeleton can be modified:

- A. by cyclization at one end or both ends of the molecule to give the seven different end groups
- B. by changes in hydrogenation level, and
- C. by addition of oxygen-containing functional groups.

Carotenoids that contain one or more oxygen functions are known as xanthophylls, the parent hydrocarbons as carotenes.<sup>[20]</sup> After being absorbed through human diet, some carotenes, among them beta-carotene are pro-vitamin A; other, such as lycopene are important due to their antioxidant properties.<sup>[21]</sup> Carotenoid extracts have been screened by TLC and separated by CC, involving liquid-solid chromatography (adsorption). Various adsorbents have been applied in carotenoid analysis, including Al<sub>2</sub>O<sub>3</sub>, silica, magnesium oxide (MgO), calcium hydroxide [Ca (OH)<sub>2</sub>], calcium carbonate [CaCO<sub>3</sub>], siliceous earth as hyflosupercell and others. In normal phase CC, the adsorption affinity depends on the number of conjugated double bonds, cyclization and the presence of oxygen substituents.<sup>[21]</sup> CC has been used for separations of mixtures of carotenes and xanthophylls, aiming for mainly analytical

determinations, standard purifications, biological evaluations of carotenoids and the purification of synthesized carotenoids, especially by flash chromatography. Separations on basic adsorbents such as MgO and Ca (OH)<sub>2</sub> are mainly determined by the number and type of double bonds in the carotenoid molecules. [22] The procedure for isolating and purifying carotenoid standards was established because of the difficulty in obtaining standards commercially. The procedure consists of carotenoid extraction with cold acetone, partition to petroleum ether in a separatory funnel with addition of water, concentration in a rotatory evaporator and chromatographic separation of carotenoids on CC developed with petroleum ether containing increasing percentages of ethyl ether and acetone. [21]

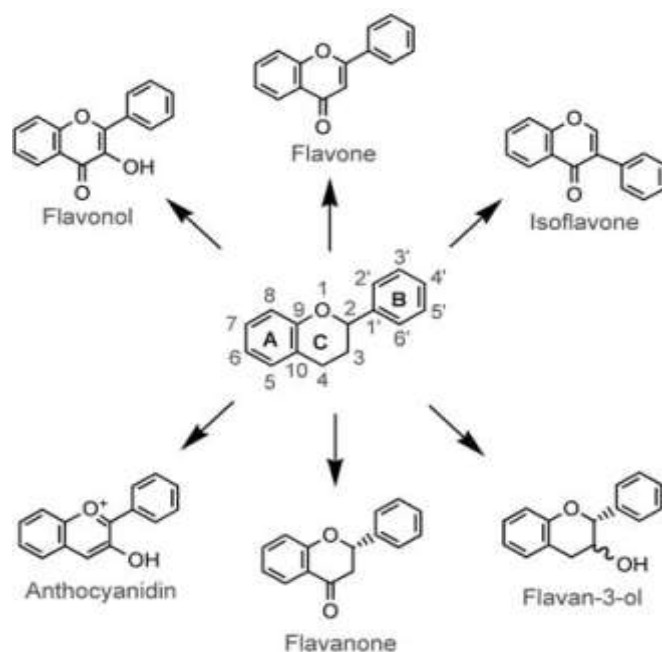
A variety of carotenoid standards have been isolated and purified using MgO: Hyflosupercel (1:1) CC developed with 2-8% ethyl ether in petroleum ether and 2-95% acetone in petroleum ether: 98% β-carotene (isolated from carrot), 94% lycopene, 99% β- cryptoxanthin, 91% γ-carotene and 91% rubixanthin (from pitanga). [23]

## 2. Phenolic Compounds

Phenolics are characterized by having at least one aromatic ring with one or more hydroxyl groups attached. In excess of 8000 phenolic structures have been reported and they are widely dispersed throughout the plant kingdom. [24] Phenolics range from simple, low molecular-weight, single aromatic-ringed compounds to large and complex tannins and derived polyphenols. They can be classified based on the number and arrangement of their carbon atoms and are commonly found conjugated to sugars and organic acids. Phenolics can be classified into two groups: the flavonoids and the non-flavonoids.

### 2.1 Flavonoids

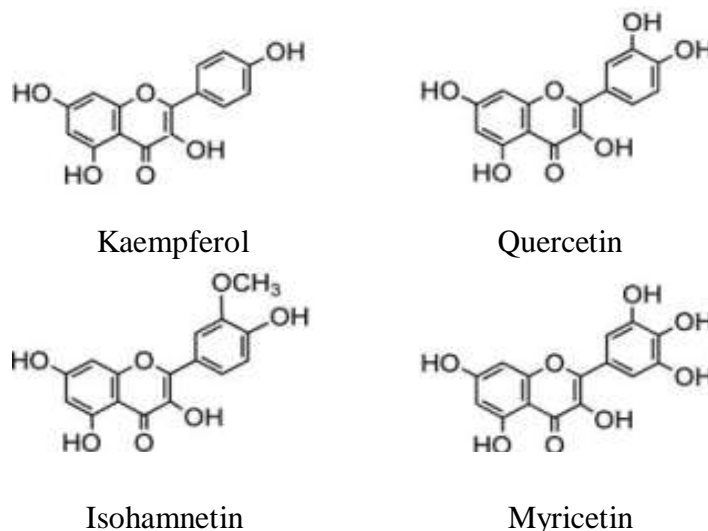
Flavonoids are polyphenolic compounds comprising fifteen carbons, with two aromatic rings connected by a three-carbon bridge.



**Fig. 3. Generic Structure Of Major Flavonoid**

They are the most numerous of the phenolics and are found throughout the plant kingdom. <sup>[25]</sup> they are present in high concentrations in the epidermis of leaves and the skin of fruits and have important and varied roles as secondary metabolites. In plants, flavonoids are involved in such diverse processes as UV protection, pigmentation, stimulation of nitrogen-fixing nodules and disease resistance. <sup>[26]</sup> The main subclasses of flavonoids are the flavones, flavonols, flavan-3-ols, isoflavones, flavanones and anthocyanidins (Figure 3). Other flavonoid groups, which quantitatively are in comparison minor components of the diet, are dihydroflavonols, flavan-3,4-diols, coumarins, chalcones, dihydrochalcones and auronnes . The basic flavonoid skeleton can have numerous substituents. Hydroxyl groups are usually present at the 4, 5 and 7 positions. Sugars are very common with the majority of flavonoids existing naturally as glycosides. Whereas both sugars and hydroxyl groups increase the water solubility of flavonoids, other substituents, such as methyl groups and isopentyl units, make flavonoids lipophilic. <sup>[27]</sup> There is information on the levels of flavonols found in commonly consumed fruits, vegetables and beverages. <sup>[28]</sup> However, sizable differences are found in the amounts present in seemingly similar produce, possibly due to seasonal changes and varietal differences. <sup>[29]</sup>

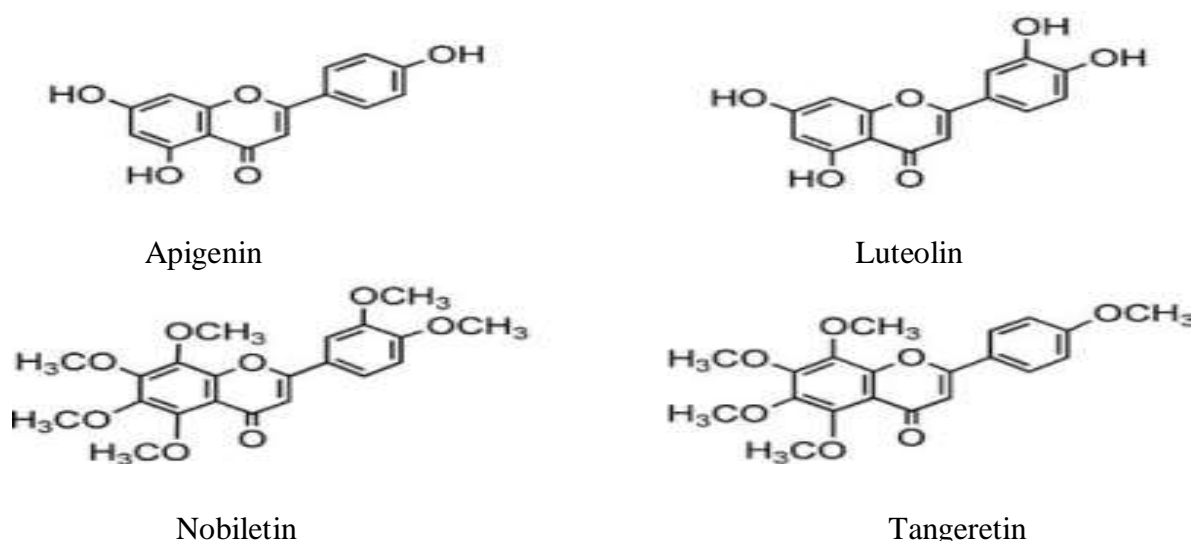




**Fig. 4. The flavonol aglycones Kaempferol, Quercetin, Isorhamnetin and Myricetin.**

### 2.1.1 Flavones

Flavones have a very close structural relationship to flavonols. Although flavones, such as Luteolin and Apigenin, have A- and C-ring substitutions, they lack oxygenation at C3 (Figure 5). A wide range of substitutions is also possible with flavones, including hydroxylation, methylation, *O*- and *C*-alkylation, and glycosylation. Most flavones occur as 7-*O*-glycosides. Unlike flavonols, flavones are not distributed widely with significant occurrences being reported in only celery, parsley and some herbs. In addition, polymethoxylated flavones, such as nobiletin and tangeretin, have been found in citrus species. Flavones in millet have been associated with goitre in west Africa. <sup>[30]</sup>



**Fig.5. The flavones Apigenin and Luteolin, and the polymethoxylated flavones Nobiletin and Tangeretin.**

### 2.1.2 Flavan-3-ols

Flavan-3-ols are the most complex subclass of flavonoids ranging from the simple monomers (+)-catechin and its isomer (-)-epicatechin, to the oligomeric and polymeric proanthocyanidins, which are also known as condensed tannins. Unlike flavones, flavonols, isoflavones and anthocyanidins, which are planar molecules, flavan-3-ols, proanthocyanidins and flavanones have a saturated C3 element in the heterocyclic C-ring, and are thus non-planar. The two chiral centres at C2 and C3 of the flavan-3-ols produce four isomers for each level of B-ring hydroxylation, two of which, (+)-catechin and (-)-epicatechin, are widespread in nature whereas (-)-catechin and (+)-epicatechin are comparatively rare. <sup>[31]</sup>

### 2.1.3 Isoflavones

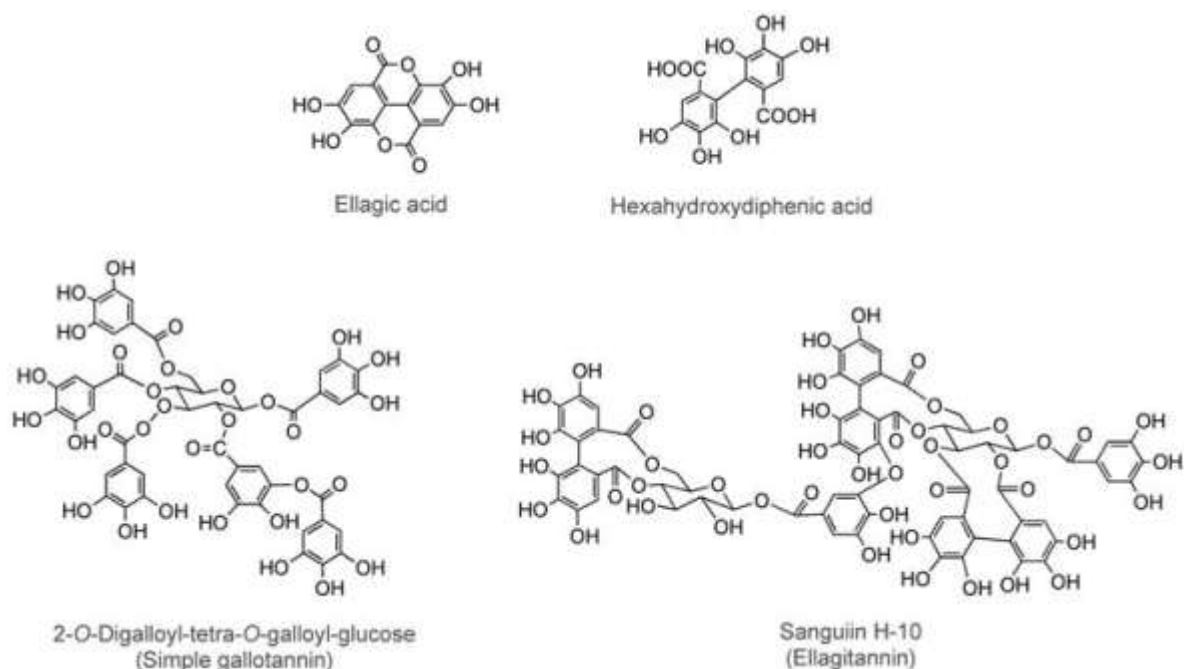
Isoflavones are characterized by having the B-ring attached at C3 rather than the C2 position. They are found almost exclusively in leguminous plants with highest concentrations occurring in soyabean (*Glycine max*). <sup>[32]</sup>

## 2.2 Non-flavonoids

The main non-flavonoids of dietary significance are the C6–C1 phenolic acids, most notably gallic acid, which is the precursor of hydrolysable tannins, the C6–C3 hydroxycinnamates and their conjugated derivatives, and the polyphenolic C6–C2–C6 stilbenes.

### 2.2.1 Phenolic acids

Phenolic acids are also known as hydroxybenzoates, the principal component being gallic acid. The name derives from the French word *galle*, which means a swelling in the tissue of a plant after an attack by parasitic insects. The swelling is from a build up of carbohydrate and other nutrients that support the growth of the insect larvae. It has been reported that the phenolic composition of the gall consists of up to 70% gallic acid esters. <sup>[33]</sup>



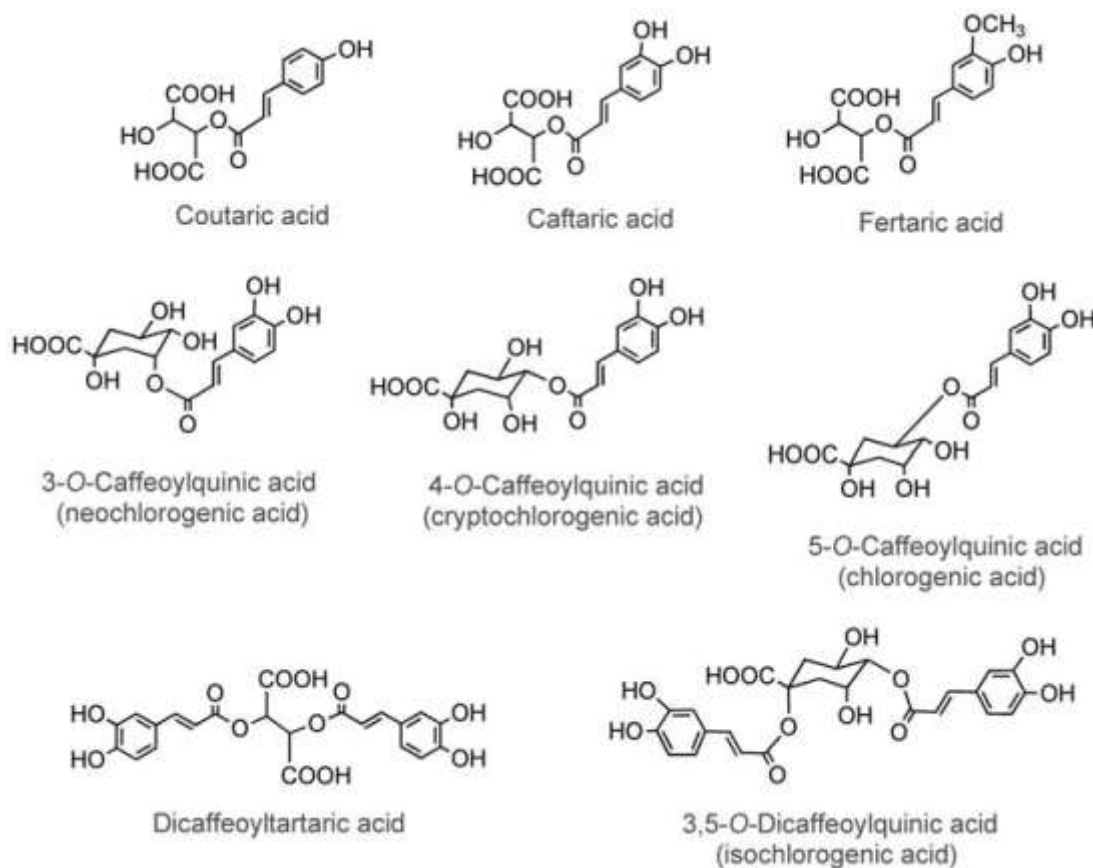
**Fig. 6. Ellagic acid Hexahydroxydiphenic acid 2-O-Digalloyl-tetra-O-galloyl-glucose Sanguine H-10**

Gallic acid is the base unit of gallotannins whereas gallic acid and hexahydroxydiphenoyl moieties are both subunits of the ellagitannins. Gallotannins and ellagitannins are referred to as hydrolysable tannins, and, as their name suggests, they are readily broken down, releasing gallic acid and/or ellagic acid, by treatment with dilute acid whereas condensed tannins are not. Condensed tannins and hydrolysable tannins are capable of binding to and precipitating the collagen proteins in animal hides. This changes the hide into leather making it resistant to putrefaction. Plant-derived tannins have, therefore, formed the basis of the tanning industry for many years. Tannins bind to salivary proteins, producing a taste which humans recognize as astringency. Mild astringency enhances the taste and texture of a number of foods and beverages, most notably tea and red wines. <sup>[34]</sup>

### 2.2.2 Hydroxycinnamates

Cinnamic acid is a C6–C3 compound that is converted to range of hydroxycinnamates which, because they are products of the phenylpropanoid pathway, are referred to collectively as phenylpropanoids. The most common hydroxycinnamates are *p*-coumaric, caffeic and ferulic acids which often accumulate as their respective tartrate esters, coutaric, caftaric and fertaric acids. Quinic acid conjugates of caffeic acid, such as 3-, 4- and 5-*O*-caffeoylquinic acid, are common components of fruits and vegetables. 5-*O*-Caffeoylquinic acid is frequently referred to as chlorogenic acid, although strictly this term is better reserved for a whole group of

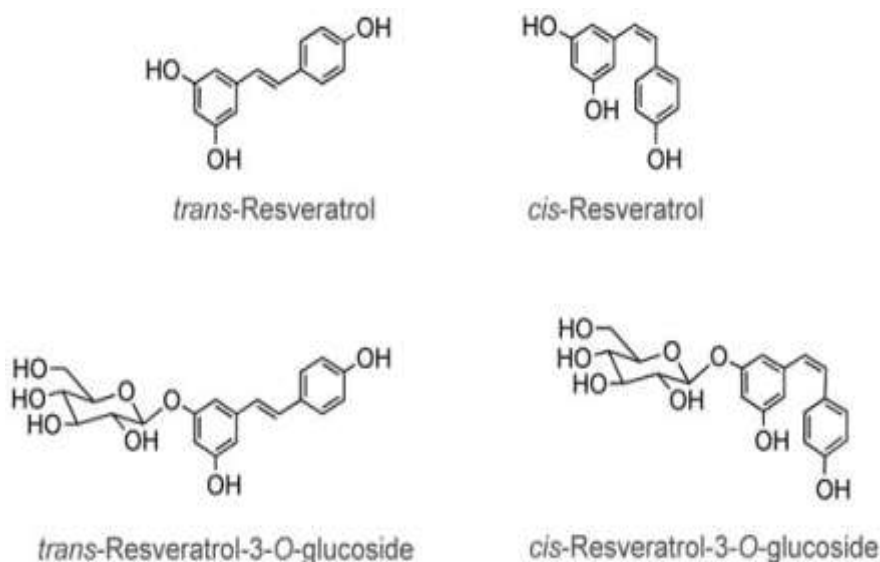
related compounds. Chlorogenic acids form ~10% of leaves of green mate (*Ilex paraguariensis*) and green robusta coffee beans (processed seeds of *Coffea canephora*). Regular consumers of coffee may have a daily intake in excess of 1 g.



**Fig. 7. Structures of conjugated hydroxycinnamates.**

### 2.2.3 Stilbenes

Members of the stilbene family which have the C<sub>6</sub>–C<sub>2</sub>–C<sub>6</sub> structure, like flavonoids, are polyphenolic compounds. Stilbenes are phytoalexins, compounds produced by plants in response to attack by fungal, bacterial and viral pathogens. Resveratrol is the most common stilbene. It occurs as both the *cis* and the *trans* isomers and is present in plant tissues primarily as *trans*-resveratrol-3-*O*-glucoside which is known as piceid and polydatin. A family of resveratrol polymers, viniferins, also exists. The major dietary sources of stilbenes include grapes, wine, soya and peanut products. *Trans*-resveratrol and its glucoside are found in especially high amounts in the Itadori plant (*Polygonum cuspidatum*), which is also known as Japanese knotweed. <sup>[35]</sup>



**Fig. 8. Structures of the stilbenes *trans*- and *cis*- Resveratrol and their glucosides.**

### 3. Alkaloid

The alkaloids are a large and structurally diverse group of compounds; some of them are not entirely distinguishable from amines (e.g., ephedrine). The names of these molecules tend to end in the suffixes *-ine* or *-in*. Many are derived from amino acids, but others result from modification of various classes of molecules including polyphenols, terpenes, or steroids. With a few notable exceptions, alkaloids are most soluble in hydroethanolic media and they generally occur as salts (e.g., chlorides or sulfates) and or as N-oxides in the plants. Most of them have a heterocyclic nitrogenous ring or ring system and a basic (alkaline) character. They are often dramatic compounds, having noticeable physiological effects – whether therapeutic or detrimental. Among the alkaloids we find potent medicinal molecules as well as toxic or even potentially fatal ones.

There are many different ways of classifying alkaloids; here we use a system based mainly on either the type of ring structure or the botanical taxa in which the alkaloids are found. By this method, there are some sixteen major groups of alkaloids; eighteen if you include the methylxanthines (e.g., caffeine) and phenethylamines. Some of these, like the indole or the isoquinoline alkaloids, contain several subcategories and a large number of therapeutic compounds; others have only a few molecules of interest to botanical medicine. In alphabetical order, these are:

- a) Amaryllidaceae alkaloids
- b) Betalain alkaloids
- c) Diterpenoid alkaloids

- d) Imidazole alkaloids
- e) Indole alkaloids
- f) Isoquinoline alkaloids
- g) Methylxanthines
- h) Monoterpenoid alkaloids
- i) Peptide alkaloids
- j) Phenethylamines
- k) Piperidine alkaloids
- l) Pyridine alkaloids
- m) Pyrrolidine alkaloids
- n) Pyrrolizidine alkaloids
- o) Quinoline alkaloids
- p) Quinolizidine alkaloids
- q) Steroidal alkaloids
- r) Tropane alkaloids

The true alkaloids are derived from amino acids, are basic and contain nitrogen in a heterocyclic ring, e.g. nicotine and atropine. Common alkaloid ring structures include the pyridines, pyrroles, indoles, pyrrolidines, isoquinolines and piperidines. The pseudoalkaloids are basic but are not derived from amino acids, e.g. caffeine and solanidine. The protoalkaloids are derived from amino acids, are basic but the nitrogen is not in a heterocycle, e.g. the phenylethylamine derived alkaloids such as mescaline. The alkaloids are often unevenly distributed in plant families. One exception to this are plants from the Papaveraceae; all genera studied contained at least one alkaloid. Common alkaloid containing plants can be found in the Leguminosae, the Liliaceae, the Solanaceae and the Amaryllidaceae. As well as the large number of useful pharmacological properties of the alkaloids utilized by man, alkaloids have been shown to be important resistance factors against herbivorous pests. The evidence for this role is increasing and there are several good examples. It has been shown that a group of quinolizidine alkaloids were effective feeding deterrents against a number of herbivores including insects, molluscs and mammals.<sup>36</sup> Furthermore these alkaloids were also toxic against some fungi and bacteria. However, it has yet to be demonstrated if the concentrations used in the bioassays occur in the plants. In Cultivars of *Locustra angustifolius* grown in Western Australia a reduction in the concentration of alkaloids below 5 mmol kg<sup>-1</sup> led to a significant loss of the crop by increased

aphid feeding. <sup>[36]</sup> The role of the alkaloids in potato in relation to insect-resistance has been assessed by several groups; especially resistance against the Colorado beetle (*Leptinotarsa decemlineata*) and the potato leafhopper (*Empoasca fabae*). The common potato (*Solanum tuberosum*) contains the alkaloid solanine, which has no effect on Colorado beetle. Potato species (*Solanum demissum*) resistant to this pest were found in South America. The resistance appeared to be dependent on the concentrations of the alkaloid demissine. This alkaloid is structurally related to solanine but the beetles were unable to detoxify it. The importance of *Solanum* alkaloids, particularly those related to solanidine, was demonstrated in resistance to the leafhopper. <sup>[37]</sup> Increasing concentrations of the alkaloids in various cultivars were positively correlated with a reduction in leafhopper infestation. In barley the concentration of the alkaloid gramine in the leaves was shown to be associated with resistance to the aphid *Schizaphis graminum*. <sup>[38]</sup> As barley plants mature the foliar concentration of gramine decreases and the rate of feeding and survival of the aphid increases. If the aphids are given diets supplemented with gramine, similar to concentrations found in the young plants, feeding and development are severely retarded. When tall fescue (*Festuca arundinacea*) was colonized by the fungal endophyte *Acremonium coenophialum*, there was a significant reduction in feeding and survival of two aphid species; *Rhopalosiphum padi* and *Schizaphis graminum*. <sup>[39]</sup> Analysis of various extracts from control and endophyte-colonized plants demonstrated a role for the pyrrolizidine alkaloids. In the colonized plants, the concentrations of the alkaloids were greatly elevated. Various feeding experiments using extracts from control and endophyte-colonized plants showed that the alkaloids were very potent antifeedants and were also extremely toxic to *R. padi* and *Oncopeltus fasciatus* (a milkweed bug). The number of examples of insect-resistance in plants related to alkaloid concentrations are increasing. There are a number of elegant examples of coevolution of the host plant and the insect pests, especially with regard to specialist and non-specialist feeders. <sup>[40]</sup>

## CONCLUSION

The medicinal plants appear to be rich in metabolites, widely used in traditional medicine to combat and cure various ailments. The distribution of metabolite within a plant, both between tissues and during growth and development, is rarely uniform. These compounds may elicit a long range of different effects in man and animals. Plants with potent metabolites are often characterized as both poisonous and medicinal and a beneficial or an adverse result may

depend on the amount eaten and the context of intake. These efforts may provide presence of metabolites in plant and its classification.

### CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

### ACKNOWLEDGEMENT

The authors are grateful to Prof. (Dr.) S.K. Prajapati H.O.D in the Department of Pharmacy Bundelkhand University, Jhansi, India for providing necessary laboratory facilities and we are also thankful with our deepest core of heart to Dr. Raghuv eer Irchhaiya for his valuable guidance.

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