# I-Introduction

## 1.1-*Vangueria species*

*Vangelia* is a genus of flowering plants in the family Rubiaceae. The genus is named for "'Voa vanguer", as *V. madagascariensis* is known in Malagasy. The genus contains over 50 species distributed in Africa south of the Sahara with one species occurring in Madagascar (*V. madagascariensis*). The centre of diversity is in East Africa (Kenya, Tanzania) and they are rare in West Africa[1].

Several *Vangueria* species - *V. latifolia*, *V. pygmaea*, *V. thamnus* - are known to cause gousiekte, a cardiotoxicosis of ruminants characterised by heart failure four to eight weeks after ingestion of certain rubiaceous plants[2].

### *Vangueria spinosa*

The column chromatographic fraction of ethyl acetate leaf extracts of *Vangueria spinosa* were screened for antibacterial activity and phytochemical analysis. The ethyl acetate fraction was significantly active at 4 to 64 mg/L against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* with minimum inhibitory concentration of 1.5625 to 3.1250 mg/mL. The active fraction revealed the presence of flavonoids. The active antibacterial agent in the most potent fraction (EA3) was isolated and identified as flavonoid (-)-epicatechin-3-O-β-glucopyranoside by thin layer chromatography (TLC) .The results support the ethnomedicinal use of leaf of *V. spinosa* for the treatment of bacterial diseases[3].

*Vangueria spinosa*

### *Vangueria infausta*

*Vangueria infausta*, the medlar or African medlar, is a species of plant in the family Rubiaceae, which is native to the southern and eastern Afrotropics. The fruits are consumed by humans and have a pleasant apple like flavor. The specific name *infausta* alludes to the misfortune believed to result from its use as firewood[4].

*Vangueria infausta*

The fruits are eaten raw and the pulp sometimes soaked in water and then dried to use later. The pulp, when mixed with sugar and water, makes a good substitute for apple sauce; it has sweet and slightly sour taste. Each 10-g fresh fruit contain 3.7 g vitamin C,1.4 g protein, 28 g carbohydrate, 28 mg sodium, 0.61 mg nicotinc acid and high levels of calcium and magnesium. Seeds can be eaten roasted.The laves of V. infausta are seldom browsed by cattle, but very much by goats . V.infausta is a good source of fire wood. The roots and leaves are used by traditional healers[4].

### *Vangueria madagascariensis*

*Vangueria madagascariensis* (J.F. Gmel)- Rubiaceae- is aprofusely branched shrub or small tree, 2-15m tall, with smooth

*Vangueria madagascariensis*

| | |
|---|---|
| Kingdom: | Plantae |
| *Clade*: | Angiosperms |
| *Clade*: | Eudicots |
| *Clade*: | Asterids |
| Order: | Gentianales |
| Family: | Rubiaceae |
| Subfamily: | Ixoroideae |
| Tribe: | Vanguerieae |
| Genus: | *Vangueria* |

grey bark and a whitish or cream slash. Leaves opposite, elliptic-ovate or rotundate, dark green above, paler beneath, glabrous or, rarely, slightly pubescent, with acuminate or, rarely, obtuse or acute apex and prominent venation below, 7-20 x 2-11 cm. Leaf stalks 5-10 mm long. Flowers greenish-yellow, yellow or cream, fulvous-pubescent, borne indense axillary cymes, sweet scented. Fruit globose, very smooth and shiny, 3-4.5 x 2.5-4.2 cm, greenish when immature, changing to yellowish-brown when ripe, contain 4-5 woody seeds up to 1.6 cm long.The generic name 'Vangueria' is derived from a Malagasy word[4].

Flowering takes place in the rainy season, while fruit ripening occurs during the dry season. Fruit takes 6-8 months from flower fertilization to ripening, depending on locality[4].

*V. madagascariensis* is commonly found in evergreen forest, riverine forest, wooded bush and and wooded grassland throughout Africa and into Asia and Australia. It grows naturally in riverine-lowland forests. The species is fire sensitive.

Biophysical limits: altitude: 600-2130 m ; mean annual temperature: 17-29° C ; mean annual rainfall: 930-1240 mm ; soil type: volcanic ash soils; light yellowish-brown to reddish-yellow, gritty, sandy clay loams and red to dark red friable clays with lateritic horizon[4].

The plant is native to: Angola, Benin, Botswana, Cameroon, Central African Republic, Congo, Democratic Republic of Congo, Eritrea, Gabon, Ghana, Kenya, Lesotho, Mozambique, Namibia, Nigeria, South Africa, Swaziland, Tanzania, Togo, Uganda, Zambia, Zimbabwe , Australia, India, Singapore.

The ripe fruit pulp is edible and has a pleasant chocolate-like flavour. The fruit is also used to add flavour to beer. The pleasant-smelling flowers of *V. madagascariensis* attract bees, and are therefore a suitable honey source.This multi-branched shrub or tree is popular as a source of both firewood and charcoal. Wood is suitable for building construction, tool handles and carving[4].

Roots and bark are used in traditional medicine; for example, in Tanzania an extract from the roots is used to treat worm infections[4].

### 1.2- *Acacia* species

*Acacia* is a genus of shrubs and trees belonging to the subfamily *Mimosoideae* of the family *Fabaceae*, first described in Africa by Swedish botanist Carolus Linnaeus in 1773. The plants tend to be thorny and pod –bearing, with sap and leaves typically bearing large amounts of tannins[5].

*Acacia* species is indogenous to Sudan, Ethiopia, East Africa, Angola, Iran, Afaghnistan and India. The *Acacia* genus include more than 1,200 species[6]. of flowering trees and shrubs, some of them are used in folk medicine.

The traditional circumscription of the genus *Acacia* is not monophyletic. This discovery has led to the breaking up of *Acacia* into five genera along with the much debated re-typification of the genus with an Australian species instead of the original African type species[7].

Nine sub- species are known for *Acacia nilotica* . Hyberidization between the various *Acacia* species occurs and has been influenced by human`s seed dispersal. Within the *Acacia nilotica* complex the pods are very variable[8].

*Acacia nilotica*

The bark and seeds are sources of tannins, while the gum, bark, roots and flowers are used for medicinal properties[9]. A 19th century Ethiopian medical text describes a potion made from an Ethiopian species of Acacia as a cure for rabies[10].An astringent medicine high in tannins, called catechu is procured from several species, but more especially from *Acacia catechu*.

The gum is used for toothache and for healing wounds. The pods are used for diabetes , leprosy , tuberculosis , cough , colds ,diarrhea , dysentry ,inflammations , ophthalmia , syphilitic alcers , and fever[11,12] .

It was demonstrated that the aqueous extract of *Acacia nilotica* possesses anti-inflammatory, analgesic and antipyretic activities[13].The aqueous and alcoholic extractives were also tested against human pathogenic bacteria. The extracts were more effective against Gram positive cocci than Gram negative bacilli. Though the aqueous and alcoholic extractives failed to show any detectable anti-fungal activity, the n-hexane extracts demonstrated a satisfactorily significant activity against *Candida albicans*[14] .

*Acacia nilotica* var. *adansonii* is another important species occurring in Algeria, Burkina Faso, Cameroon, Cape Verde, Chad, French Guiana, Gambia, Ghana, Guinea Bissau, India, Iran, Ivory Coast, Libya, Mali, Niger, Nigeria, Oman, Pakistan, Senegal, Somalia, Sudan, Togo.

The plant is occasionally used for firewood and good quality charcoal in the Sahelian regions, and is a preferred firewood in Tanzania. It has a hard heavy heartwood, with a density of 0.945 and a sapwood with a density of 0.827, it is resistant to water and termites.The uses include construction work, boat-building, fencing, tool handles and art objects. Foliage and pods are browsed by camels, horses, sheep and goats. The subspecies is a source of gum and tannin, the bark and pods are used for tanning leather and as a dye source. The gum is locally used for making ink. The subspecies is commonly planted as a shade tree in the Sahelian regions[4].

In Burkina Faso, the leaves are used against diarrhoea, the grilled and crushed seeds for treatment of haemorrhoids and gingivitis, and the powdered bark as a local aemostatic.

A decoction of the pods is used for coughs, and swallowing the juice formed by chewing is said to treat dry cough. The flowers are a source of pollen and nectar for bees[4].

*Acacia nilotica* var. *adansonii*

*Acacia nilotica* var. *adansonii*

## 1.3-Flavonoids

Flavonoids are a large group of polyphenolic secondary metabolite compounds occurring in plants ,a group containing more than 8000 known compounds arising from the great structural diversity possible from the various hydroxylation, hydrogenation, methoxylation, malonylation, glycosylation, sulfation and acylation patterns of the core flavonoid structure[15].

Originally ,flavonoids were discovered as the pigments responsible for plant colour, ranging from red ,orange ,yellow to violet in flowering plants .Anthocyanins were the first group of flavonoids documented[16] .Later ,flavonoids were discovered as important factors for plant growth ,development and defence ,for example ,attracting animals for pollination and seed dispersal ,providing ultraviolet protection ,inducing nitrogen fixation[17].Consumption of flavonoids appears to bestow potential benefits for human health .This has provoked tremendous interest that has developed continuously and is now a large and important research are a in food chemistry and nutrition with interactions with pharmacy[18]**.**

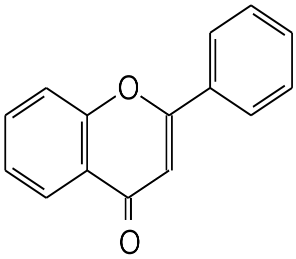
Flavonoids are believed to be endowed with biological activities, such as anti- inflammatory, anti-allergic, anti-ischemic, anti-platelet, immunomodulatory and anti- tumoral activities[19]. They have also been shown to inhibit some enzymes, including lipoxygenases and cyclooxygenases, mono-oxygenases, xanthine oxidase, mitochondrial succinoxidase, reduced nicotinamide-adenine dinucleotide (NADH) oxidase, phospholipase A2, topoisomerases and protein kinasesi[20].The biological activities of flavonoids were thought to be mainly due to their antioxidant properties, which are displayed by limiting the production of reactive oxygenspecies (ROS)and/or scavenging them . Now it is thought that inhibition of enzymes has at least an important role.

Flavonoids are components of the diet of herbivores and omnivores, including humans. They are principally found in all types of fruits, vegetables and plant-based food including grains, nuts, stems, leaves and popular drinks suchtea, coffee, and flavonoid intake may reach 1g/day[21].

Flavonoid consumption may have important implications for human health. In fact, foods like apple and onions were known for their beneficial effects on health long before flavonoids were isolated, purified and characterised[22].

## 1.4-Basic structure of flavonoids

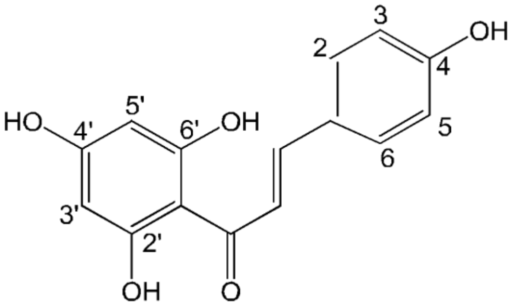
The flavonoids constitute one of the most characteristic classes of compounds in higher plants, generally categorized as phenols or polyphenols, which are a group of low molecular weight substances. Their chemical structures are based on a $C_{15}$ skeleton with a chromane ring bearing a second aromatic ring B in position 2, 3 or 4. The skeleton can be represented as a $C_6$-$C_3$-$C_6$ system. Fig. (1) shows the general structure of flavonoids. The left-hand ring, which is derived from the acetate malonate path way, is referred to as the A ring. The right hand ring, which is derived from the ring carbons of phenylalanine, is referred to as the B ring. The heterocyclic ring between the two rings is referred to as C ring the.



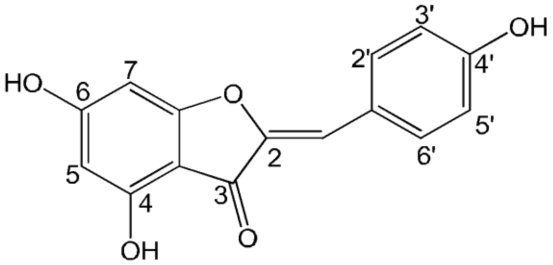
(1)

The complete structure is numbered from the heterocyclic oxygen, which is designated a sposition 1, clockwise around the C and A ring to position 8. The B ring is numbered separately with primed numbers, starting from C ring bond as 1' to the 6' position clockwise .Variations in the chemistry of the C ring give rise to a number of distinct sub-classes of flavonoids, of which 6 are prominent in the human diet.

In a few cases, the six-membered heterocyclic ring C occurs in an isomeric open form or is replaced by a five-membered ring, for example, chalcones (2) and aurones (3).Note that the carbon numbering in chalcones is different compared with the numbering system of the basic structure of other classes.



(2)



(3)

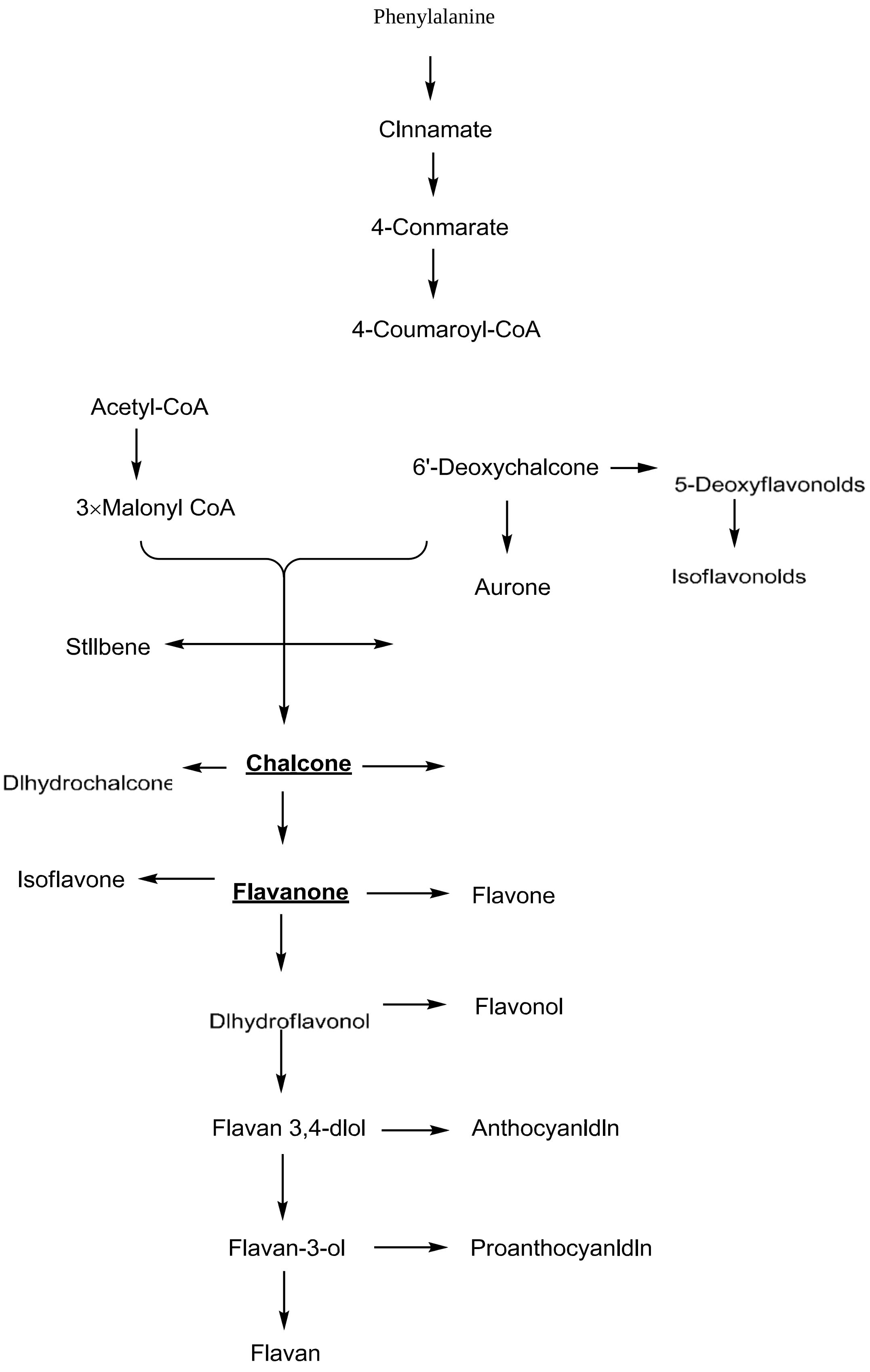
## 1.5-Biosynthesis of flavonoids

Flavonoids are ubiquitous non-nutrient secondary metabolites with important functions. Their biosynthesis is probably the best characterized of all the secondary metabolic pathways.

The flavonoid path way is part of the larger phenylpropanoid path way ,which produces a range of other secondary metabolites ,such as phenolic acids,lignins, and stilbenes ,and involves numerous enzymes.The key flavonoid precursors are phenylalanine from shikimate and arogenate path ways and malonyl-co enzyme A (CoA) from citrate produced by the TCA cycle [23]. Phenylalanine is transformed to *trans*-cinnamic acid and then hydrolysed to 4-coumaric acid. This structure forms the flavonoid B ring and central 3-carbon fragment .The addition of three carbon malonyl-coenzyme A (CoA) units provides the A ring .The resultant molecule is chalcone which is the first flavonoid, formed by chalcone synthase, which may be enzymically isomerised to a flavanone. The flavanone is the main intermediate for forming other sub-classes of flavonoids. The general phenylpropanoid and flavonoid biosynthetic is shown in Scheme 1.1.

Nearly all flavonoids (except catechins) do not occur in plants in the unsubstituted form. The most frequently occurring forms are the O-glycoside derivatives[24]**.**Flavonoid molecules not attached to sugar moieties are referred to as aglycones,whereas flavonoid molecules with sugar moieties are called flavonoid glycosides.Glycosylation is either *O*- or *C*- linked ,and leads to an increase in the hydrophilicity of the flavonoid molecule. Glycosylation also results in increased complexity of the molecule [25]. The level of glycosylation varies from mono-, di- ,or

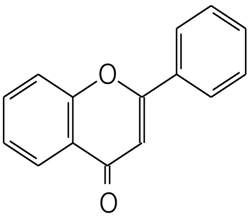
higher. The types of monosaccharides involved include D-glucose, L-rhamnose , glucorhamnose , galactose, xylose , and arabinose[26,27].



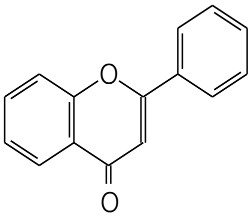
Scheme 1.1: General phenylpropanoid and flavonoid biosyntheticpathways

## 1.6-Major sub-classes of flavonoids

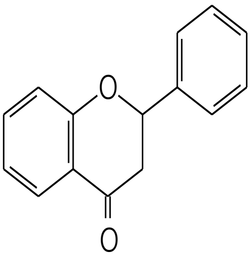
Flavonoids are a very large group of polyphenolic natural products. There are different ways to classify flavonoids, for example, according to their biosynthetic origin, according to whether the central heterocyclic ring is unsaturated or not, according to their molecular size[28]. The most common way is according to the variation of the heterocyclic C ring. From the flavonoid basic structure, a heterocyclic pyrone C ring can be derivatised to the flavones, flavonoles, flavanones and isoflavones; a pyran C ring produces the flavanols and anthocyanins[29].The basic structures of each sub-class are shown below:



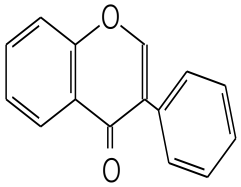
Flavone



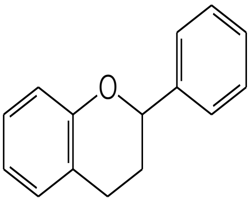
Flavonol



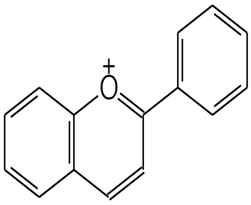
Flavanone



Isoflavone



Flavan

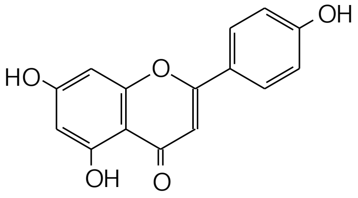


Anthocyanin

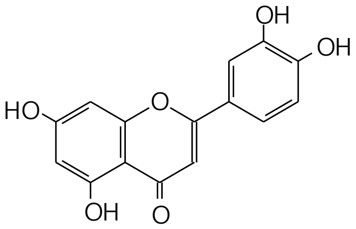
## 1.7-Flavones

In flavones, the C ring bears a >C=O bond at the 4 position, which is the common structural feature of the flavones, flavonols, flavanones and isoflavones, and a double bond between the 2 and 3 positions.

Common members of the flavones are apigenin and luteolin, which are most abundant in parsley, celery and capsicum pepper, though flavones are found in many other plants and notably exist in grains and herbaceous families, e.g. labiatae, umbelliferae and compositae[30]. Apple skin, onions, tea, olive, celery and red pepper are good sources of flavones.



Apeginin



Luteolin

Flavones mainly occur as 7-O-glycosides in plants and the most frequently bound sugar is glucose although a variety of other sugar moieties may be attached to an aromatic carbon atom as C-glycosides [27].

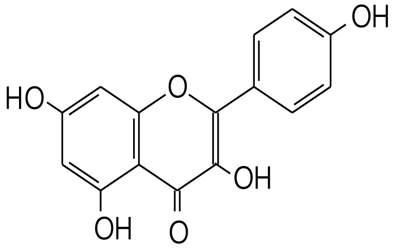
## 1.8- Flavonols

Flavonols generally exist in woody angiosperms but are also predominant in vegetables, fruits and beverages. Onions, berries, cherries, broccoli, apple, grapefruit, tea and red wine are rich sources of flavonols such as quercetin , myricetin, kaempferol and tamarixetin[31]. Flavonols are closely related to the flavones, their structures differing from flavones only in the presence of a hydroxyl group at the 3 position on the C ring.

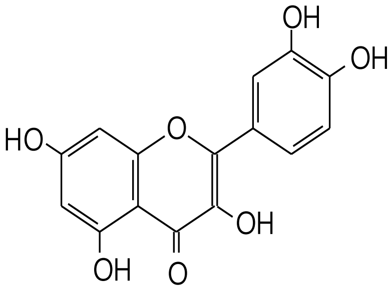
Another major difference is the position of Glycosylation. Flavones are characteristically present as the 7 –glycoside whereas flavonols are generally glycosylated at the 3 position, less frequently the 7 position,and only in rare cases the *C*-4ˋ,*C*-3 and*C*-5 positions[27].Quercetin is the main representative of the flavonols in the human diet, and it usually occurs as *O*-glycosides ,with D-glucose as the most frequent sugar residue. More than 170 different quercetin glycosides have been identified[32].High concentrations of quercetin can be found in onions as quercetin-3-glucoside, quercetin-4'-glucoside, quercetin-3,4ˋ-diglucoside and isorhamnetin-4ˋ- glucoside[33].

In green beans, quercetin exists mainly as quercetin-3-*O*-glucuronide[34].Other notable members of this sub-class are kaempferol, found in leafy vegetables and herbs, and myricetin, found in berries and tea[35]. Flavonols are nearly always encountered as glycosides in plants, but quercetin may be found as an aglycone in the waxy coating on leave and leaf buds[36].Quercetin and kaempferol are also found in the free form in some species of aquatic algae and bacteria and may be synthesized in large amounts to protect against UV radiation[37].

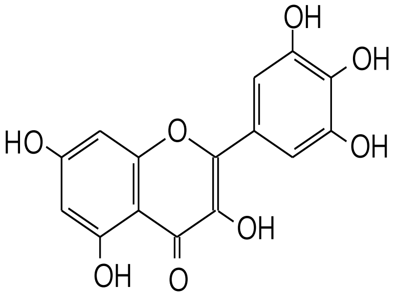
Flavone and flavonol *O*-glycosides make up one of the largest classes of flavonoids with over 2000 known structures. There are 279 glycosidic combinations of the most common flavonol aglycone, quercetin, and 347 kaempferol *O*-glycosides that had been identified up to December 2003[38].The group includes any bound form of flavones or flavonols such as acylated and sulfated derivatives and not only those conjugates with sugar. Thus, the number of possible combinations is enormous because of the wide variation in possible substitutions .However ,only a few esters of flavones are known .By comparison ,a series of mostly monoacylated flavonols is known and recent reports increased the number slightly[28]. As a result, many researchers have counted dietary flavonoids based mostly on the content of only 3 flavonols — quercetin, myricetin and kaempferol — and two flavones — apigenin and luteolin[30].



Kaempferol



quercetin

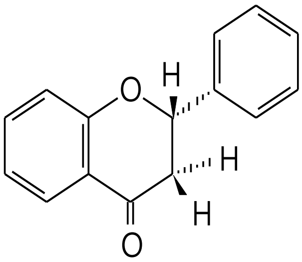


Myrecin

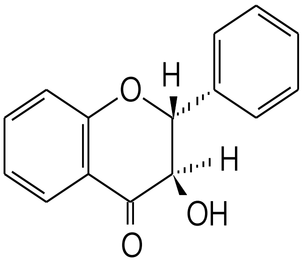
## 1.9-Flavanones are dihydroflavones

Flavanones are dihydroflavones, and like dihydroflavonols are referred to as flavanonols (and 3-hydroxylflavanones as well). In the past ,flavanones and dihydroflavonols have been put into the category of minor flavonoids with chalcones ,dihydrochalcones and aurones since there were few compounds belonging to these flavonoid sub-classes. Nevertheless, the term minor flavonoids is no longer appropriate because the number of known "minor flavonoids" has increased considerably[39].

Compared to flavones and flavonols, the structural feature of flavanones and dihydroflavonols is the absence of the double bond between the 2 and 3 positions on the heterocyclic C ring ,which is present in flavones and flavonols[40].Thus,in flavanones, C-2 bears one hydrogen atom in addition to the phenolic B ring, and C-3 two hydrogen atoms .Two stereoisomeric forms of each flavanone structure are possible ,since C-2 is a centre of asymmetry (epimeric centre). Consequently ,the B ring can be either in the (2*S*)-or(2*R*)-configuration.The great majority of the flavanones isolated from plants are laevorotatory(–)-or(2*S*)-flavanones ,because the enzymatic reaction catalyzing the conversion of chalcones to flavanones is stereospecific**[39]**.In dihydroflavonols, the C-3 atom bears both a hydrogen atom and a hydroxyl group ,and is therefore an additional centre of asymmetry .Hence ,four stereoisomers are possible for each dihydroflavonl structure ,(2*R*,3*R*), (2*R*,3*S*),(2*S*,3*R*), and(2*S*,3*S*). All four configurations have been found in naturally occurring dihydroflavonols ,but the (2*R*,3*R* ) -configuration is by far the most common**[39]**.

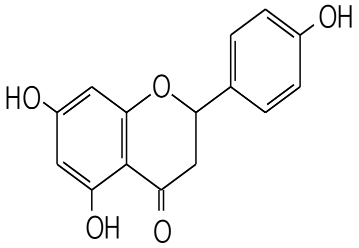


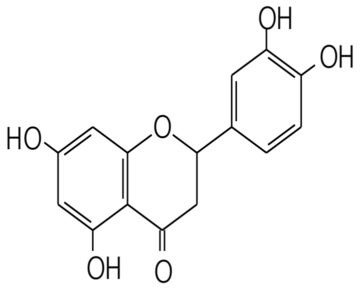
(2S)-Flavanone



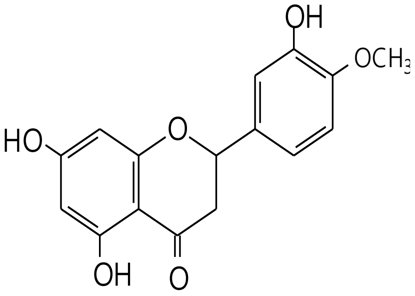
(2R,3S)-Flavanol

As in all other flavonoids ,there is structural variation in flavanones and dihydroflavonols because of variation in hydroxylation , methylation , methylation , prenylation, benzylation, glycosylation, etc. of suitable carbon atoms in the skeleton, i.e., C-5, C-6, C-7,and C-8 of the A-ring, C-2', C-3', C-4',C-5',and C-6' of the B ring, and C-2 of the C ring in both flavanones and dihydroflavonols[39].In addition, the hydroxyl group at C-3 in dihydroflavonols can be methylated, glycosylated, or esterified .Flavanones substituted by hydroxyl ,methoxy , methylenedioxy ,and *C*-methyl or related groups could conveniently be called “simple flavanones”, in contrast to flavanones bearing more complex substituents such as prenyl and benzyl groups[39]. dihydroflavonols are mainly conjugated as *O*-substitutions, but *C*-conjugations are also known . Flavanones exist in citrus fruits such as grapefruit, oranges and lemons .The most common members are naringenin, hesperetin and eriodictyol[41]. Biochemically ,chalcones are the immediate precursors of flavanones ,and some flavanones are isomerized by ring opening into chalcones during isolation from plants or after chemical treatment with alkali[40].In turn, flavanones are intermediates in the biosynthesis of most other flavonoid groups, including flavones, flavonols, and isoflavonoids.





naringenin eriodictyol



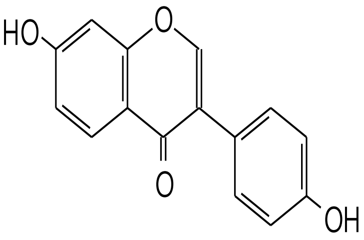
Hesperetin

## 1.10-Isoflavones

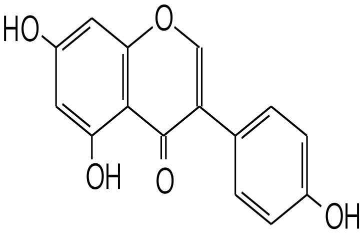
Most of the flavonoids (flavanones, flavones, flavonols, flavanolsand anthocyanins) are bearing B in position 2 of the heterocyclic ring .However, in isoflavonoids, ring B occupies position 3. It has been established that acetate gives rise to ring A and that phenylalanine, cinnamate and cinnamate derivatives are incorporated into ring B and C-2, C-3, and C-4 of the heterocyclic ring[42].

Isoflavonoids include isoflavanones ,isoflavones and isoflavonol s ,with isoflavones constituting the largest group of natural isoflavonoids.There are over 350 known isoflavones, making them the largest group of compounds in the class

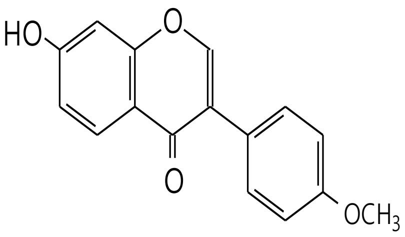
of isoflavonoids[43]. The best known isoflavones are daidzein, genistein, which occur mainly as the β-glycoside forms. Daidzin and genistin are abundant in soybeans and consequently in a wide range of soy-derived foods and to a lesser extent in other legumes. Traditional soy foods are made from soy beans and include both fermented and non-fermented foods. Non-fermented soy foods contain isoflavones mostly present as the β-glycoside forms, while fermented soy foods contain mostly unconjugated isoflavones[44].In addition, glycitein, formononetin, and biochanin A are also important isoflavones. These are commonly found to occur as the glucoside, glucoside malonate esters, or free aglycones. The structures of the 5 common isoflavones are shown below:



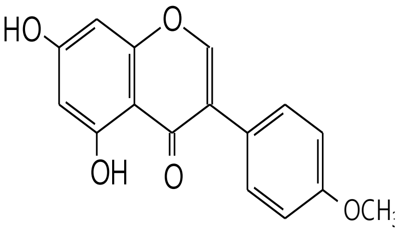
Daidzein



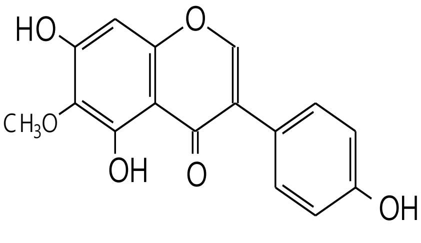
Genistein



Formononetin



Biochanin



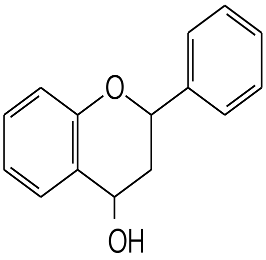
Glycetein

Differing from flavonoids,the distribution of the isoflavones in the plant kingdom is largely restricted to the family Leguminoseae, with soybeans being the primary human food rich in these compounds[45,46].Broad bean and red clover are also sources of isoflavones but at much lower levels.

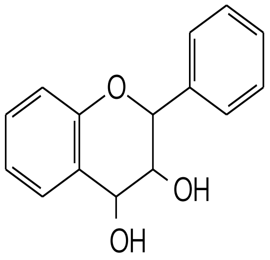
## 1.11-Flavanols

Flavanols are also referred to as proanthocyanidins, flavan-3-ols or simply catechins, which represent a large group of flavonoids with similar structures . However, the definition of this group is a little bit arbitrary. Generally speaking, flavans also include flavan-4-ols and flavan-3,4-diols, of which the latter two are also named as leucocyanidins from which anthocyanidins can be produced, and flavan-3- ols which are also called catechins that are the best representatives of this flavonoid sub-class[47].Much research on this sub-class has been carried out on catechins. Frequently, flavan itself is used to name that type of substance in this group without any hydroxyl group on the heterocyclic C ring. All four types are monomeric flavonoids .Proanthocyanidins are referred to as oligomers and polymers,most of the cases indicate flavan-3-ol oligomers excluding di- and tri-flavonoids[48].

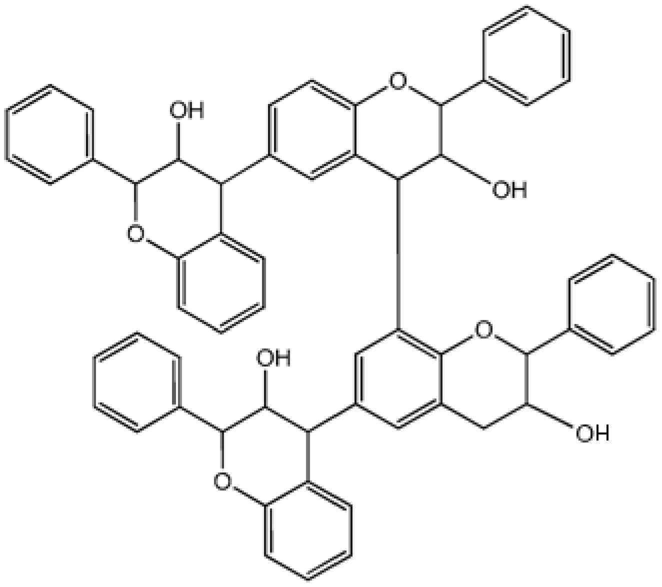
The discriminating structural feature of flavanols ,which they have in common with anthocyanidins only, is the lack of an oxygen group at the 4 position of the heterocyclic C ring[48].The lack of a double bond between the 2 and 3 positions and the presence of a 3-hydroxyl group create two centres of asymmetry(carbons at positions 2 and 3).Thus,in principle, two stereoisomeric forms are possible .Nevertheless ,only flavanols with a 2*R* configuration have been found in nature.The predominating flavanols are (+) . Some representative flavans are shown below.



Flavan-4-ol

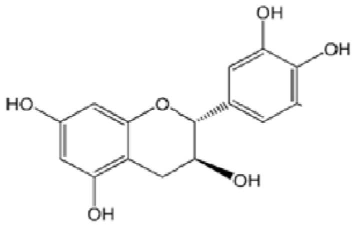


Flavan-3,4-diol



proanthocyanidins

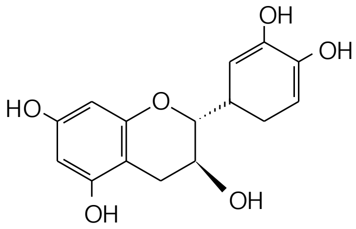
Flavanols can associate with each other to give rise to dimers, oligomers and polymers. Those molecules, linked by bonds between carbons atoms of the rings of the monomers, are known as proanthocyanidins. The carbon atom at the 4 position on a monomer is bound to the next monomer at the 8 or 6 position[49]. Catechin polymers contribute a bitter taste and brown pigments to foods such as tea and dark chocolate[50]. The structure of a common catechin is shown below:



Catechin

As opposed to other flavonoids which exist in nature as glycosides, flavanols exist in nature as aglycone forms while glycoside forms are rare[50].

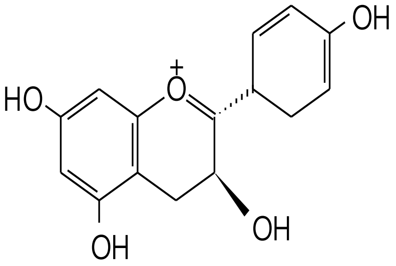
Flavanols are widespread in plant foods especially in woody and some herbaceous plants. They have been reported in tea, fruits and legumes. Tea is probably the most important flavanol source in many countries. It combines a high level of consumption with relatively high flavanol content. Furthermore, it is the only plant food for human consumption found so far which contains (–)-epigallocatechin gallate(EGCG)[48]. The most abundant monomeric flavanols of black tea are (–)- epicatechin gallate(ECG), (–) -epigallocatechin(EGC). Only a small part of the flavanol content of teas is constituted by (+)-catechin, (–)-epicatechin and (+)- gallocatechin. Flavanols are sometimes conveniently called tea flavonoids, and tea has been tested extensively for its biological actions in *in vitro* and *in vivo* animal experiments. Besides tea, flavanols have also been determined at high level in chocolate, black grape, cherry and other fruits[51].



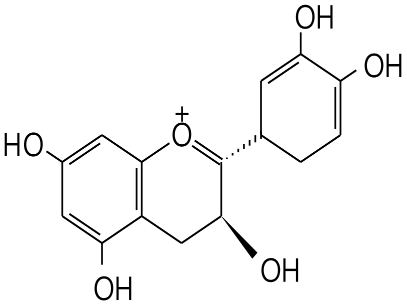
Monomeric catechin

## 1.12- Anthocyanins

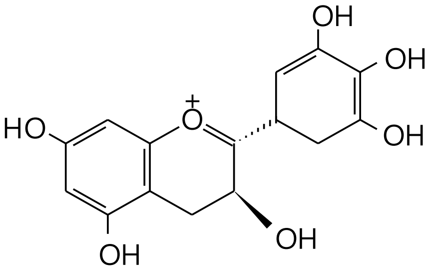
The anthocyanins constitute a major flavonoid group that is responsible for cyanic colours ranging from salmon pink through red and violet to dark blue of most flowers, fruits, and leaves of angiosperms. Strong pigmentation usually indicates high anthocyanin content. They are sometimes present in other plant tissues such as roots, tubers, stems, bulbils, and are also found in various gymnosperms, ferns, and some bryophytes[52].They are of great economic importance as fruit pigments and thus are used to colour fruit juices and some beverages. The past twenty years has witnessed a renaissance in research activities on and general interests in these water-soluble pigments[53]. The most common anthocyanins are shown below.



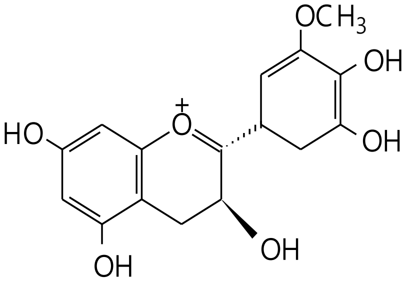
Pelargonidin



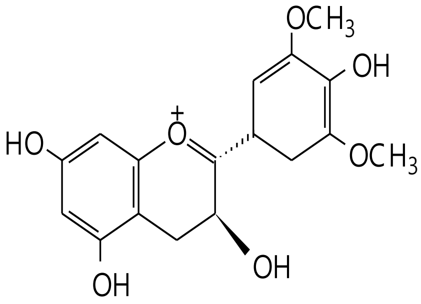
Cyanidin



Delphinidin



Petunidin



Malvidin

The term anthocyanins refer to the glycosylated flavonoids whereas the aglycones are termed anthocyanidins. As for most types of flavonoids, anthocyanins are always glycosylated in nature. Most anthocyanins contain two, three, or just one monosaccharide unit. The most common forms are 3-glycosides and 3,5-diglycoside**[14]**. However, as many as seven glycosyl units have been found. Altogether 240 and 24 anthocyanins have been reported to contain a disaccharide and a trisaccharide respectively, while no tetrasaccharide has been found yet. The sugar moieties commonly are connected to the anthocyanidins through *O*-linkages; however, 8-*C*-glucosylcyanidin3- [6-(malonyl)glucoside] has been isolated[54]. This was the first report of a natural *C*-glycosyl anthocyanin although *C*-glycosylation is common in other flavonoids, especially flavones**[54]**.

Anthocyanins are possibly charged and exist in different chemical forms at different pH values through alteration of the carbon backbone. Therefore, colour is pH- dependent. They are usually red in acid conditions and blue in alkaline conditions and chelate with metal ions like $Ca^{2+}$and $Mg^{2+}$under alkaline conditions.

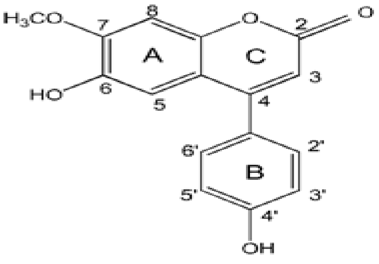
Besides the major sub-classes, there are other sub-classes of flavonoids, which have skeleton structures differing from the flavonoid basic structure(seefigure1-1). Few compounds have been found in these groups.

## 1.13 -Neoflavonoids

Neoflavonoids are agroup of chromane derivatives with ring B in position4(4- phenyl-coumarins = neoflavonoids). They are biogenetically derived by rearrangement to the flavonoid 2-pheyl chroman system by means of a 1,2-aryl

rearrangement[27].The neoflavonoids can be considered to be formed from isoflavonoids following a further aryl rearrangement from the 3 position to the 4 position. The isoflavonoids and the neoflavonoids are regarded as abnormal flavonoids[55].

Neoflavonoids are glycosylated mostly as 5-*O*-forms though occasionally 7-*O*- glycosides may present. By contrast to the distribution of isoflavonoids restricted to the family Leguminoseae, neoflavonoids are concentrated in many plant families :the Guttifereae , Leguminosae, Rubiaceae, Passifloraceae, Polypodiaceaeand Compositeae. The notable activity and the reported presence of neoflavonoids in plants of traditional medicine have focused on their isolation and synthesis.A typical neoflavonoid is melanin isolated from heartwood of *Dalbergia mwlanoxylon*(Leguminosae)[41] the structure of which is shown below.



Melanln

## 1.14-Chalcones

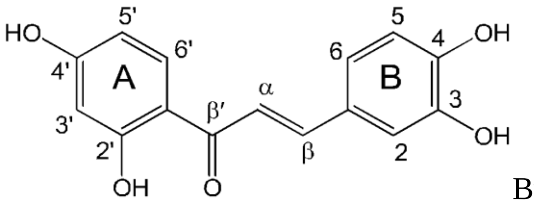
Chalcones, and the closely related dihydrochalcones, are unique in the flavonoid family in lacking a heterocyclic C ring **[55]**. They are open-chain $C_6$-$C_3$- $C_6$ compounds which are the first $C_{15}$ intermediates in flavonoid biosynthesis. The numbering system of chalcones differs from that of ring-closed flavonoids, which has been shown in Figure1-1. In chalcones and dihydrochalcones the prime positions are on the A ring, as opposed to the B ring in all cyclic flavonoids.

The atom numbering of chalcones, dihydrochalcones, and aurones remains a potential source of confusion when compared to that of other classes of flavonoids. The A and B rings of all the flavonoids have the same origin in biosynthetic terms, with the A rings derived from the acetate pathway and the B rings from the shikimate pathway. The crucial difference is in the style of atom numbering, in which primed numbers are used to refer to the A ring of chalcones and dihydrochalcones, but to the B ring of other flavonoid classes, including the aurones. Similarly ,the B rings of chalcones and dihydrochalcones carry the non-primed numbers instead of the A ring. The numbering scheme followed for chalcones and dihydrochalcones is also different, because the C 3 unit linking the A and B rings is referred to only in terms of carbonyl(β'), α-and β-carbons, whereas the equivalent carbon atoms of the heterocyclic C rings of other flavonoids are numbered together with the rest of the molecule. For the B ring, the carbon can be numbered either clockwise or counterclockwise .The aurone numbering system is an ominous because of the five-membered C ring .The result is that the A ring positions equivalent to other flavonoids (excluding chalcones and dihydrochalcones) bear a number one less in value.

The common structures of chalcones and dihydrochalcones are all hydroxylated to varying degrees and many are also *O*-methylated as well.Chemically chalcones can be classified into two groups.The first are chalcones with varying hydroxylation. They may be partly *O*- methylated and some have prenyl substitution. They may occur as glycosides but glycosidic variation is limited with glucose being the common sugar.

The second group are those with complex structures involving in many cases extra furano or pyrano rings fused to either the A or B ring . Chalcones have a limited but scattered occurrence. They are abundant in the Leguminosae, for example being present in heartwood of trees or flowers of gorse, and in the Compositeae

,where they provide yellow flower colour in *Coreopsi s*and related species. They are also present in the crystalline deposit of fronds of certain fern species. Chalcones can co-occur sometimes with the related flavanones and may be accompanied in flowers by related yellow aurone pigments[37].Apart from providing their yellow colour, no other clearcut functions have yet been elucidated in plants for the chalcones. However, they are of medicinal interest. Butein is a common example.



Butein

and somes tructures have anti-peptic or anti-hepatotoxic activities[30], more recently,chalcones have been found with anti-angiogenic effect[55]. Atypical chalcone is butein found in many leguminous plants and in *Anacardiaceae* and *Compositeae* as well.

Dihydrochalcones are directly related to the chalcones and are derived from them by reduction of the chalcone α, β-double bond. The best known dihydrochalcone is phloridzin, which occurs in the skin of apple[30].

Most of the dihydrochalcones are hydroxylated naturally. A number are glycosidic, as in the case of phloridzin are known. Dihydrochalcones are relatively small group of flavonoids and have a somewhat erratic distribution[37].They have been recorded in about 28 plant families, notably in species of *Ericaeae* and *Rosaceae*. They may also accompany chalcones in exudates of *Pityrogramma* and other fern species.Aspecial property of some dihydrochalcones is their extremely sweet taste and there has been much interest in developing them as foods weeteners.

## 1.15-Aurones

Aurones are highly coloured flavonoids based on the 2-benzylidenecoumaranone structure .Auronols which could be considered in the chemical sense as hydrated aurones ,are 2-hydroxy-2-benaylcoumaranones[37] . The conventional numbering system for aurones and auronols has positions 4 to 7 on the A-ring ,where position 4 is biosynthetically equivalent to position 5 in normal flavonoids and position 2' in chalcones .

The first aurone was isolated as a yellow pigment from *Coreopsis grandiflora* in1943 named leptosidin. Since then a limited number of other aurones have been detected not only in flowers but also in bark ,wood and leaves[30].A well-known aurone is bracteatin which was discovered in snap dragon(*Antirrhinum majus*) [56,57].

A typical member of auronol is carpusin from *Pterocarpus*.

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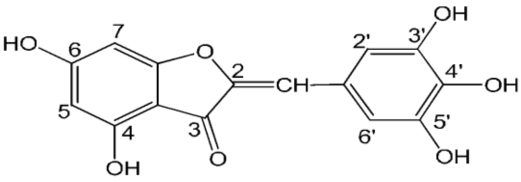

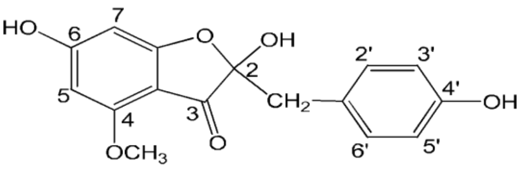

Bracteatinty- typical aurone

Carpusin-typical auronol

Aurones are glycosylated in nature with the glucose as the most usual sugar but many aglycone forms are also found .Aurones have a scattered occurrence in the flowering plants and provide yellow to brown colours .

Chalcones and aurones are best known as the yellow to orange coloured flower pigments of somes pecies .The distribution of these compounds is not restricted to flowers, but can be found in many different plant tissues [57].The chalcones are structurally one of most diverse groups of flavonoids ,as witnessed by the formation of a wide range of dimers, oligomers and conjugates of various kinds. At the same time, they are of great significance biosynthetically as the immediate precursors of all other classes of flavonoids[23].Underlying these important attributes is the unique feature that distinguishes chalcones and dihydrochalcones from other flavonoids, the open-chain three-carbon structure linking the A and B rings in place of a heterocyclic C ring. In plants, chalcones are converted to the corresponding(2S)-flavanones in a stereospecific reaction catalyzed by the enzyme chalcone isomerase. The close structural and biogenetic relationship between chalcones and flavanones explains why they often co- occur as natural products[3]