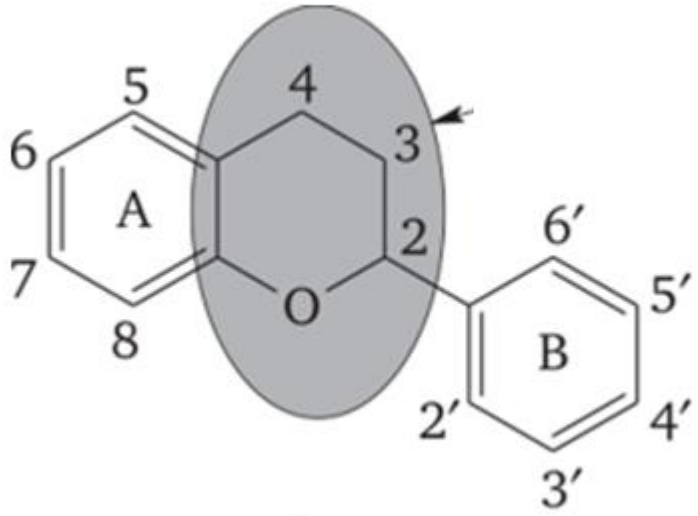
Flavonoids. Classification of flavonoids. Distribution in nature, isolation, methods of identification.

Flavonoids are phenolic compounds that contain a defenylpropane fragment in their structure, which can be represented as a C6 - C3 - C6 skeleton.
At the base of all flavonoids is a compound called flavan, which is 2-phenyl-chromane or 2-phenyl-benz-γ-pyran.



Classification of flavonoids

The modern classification of flavonoids is based on:

1. position of the phenyl side radical;
2. the degree of oxidation of the propane moiety;
3. the size, presence or absence of heterocycle.

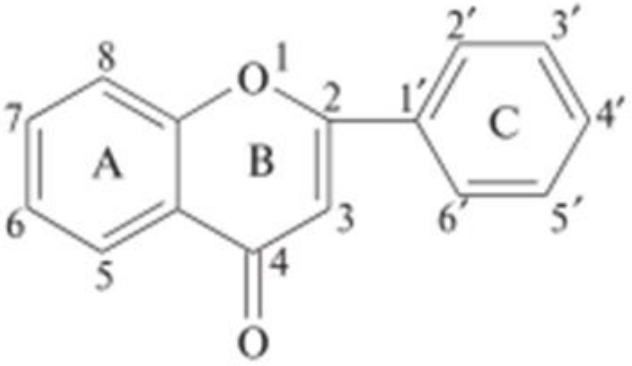
Flavonoids are divided into 4 groups depending on the attachment site of the side phenyl radical:

•Flavonoids proper (**euflavonoids**). The lateral phenyl radical is attached at positionThe most numerous group (about 400 aglycones are known). There are 10 main classes of euflavonoids.

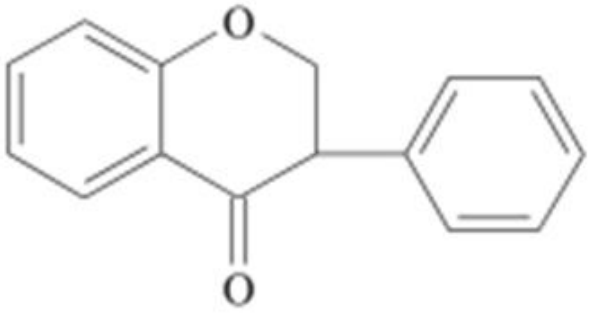
•**Isoflavonoids**. The lateral phenyl radical is attached at position About 60 compounds are known, characteristic mainly for representatives of the legume family.

•**Neoflavonoids**. The lateral phenyl radical is attached at position C4.

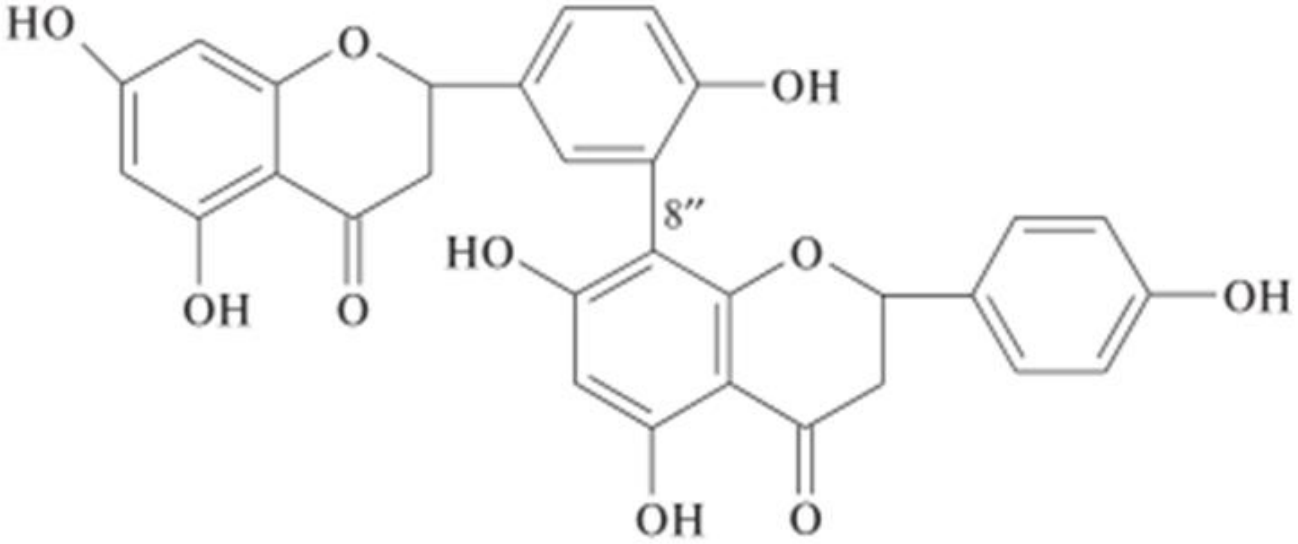
Other classes of flavonoids include xanthones, flavolignans, coumaroflavonoids, and biflavonoids. These compounds are widely studied and have high biological activity.

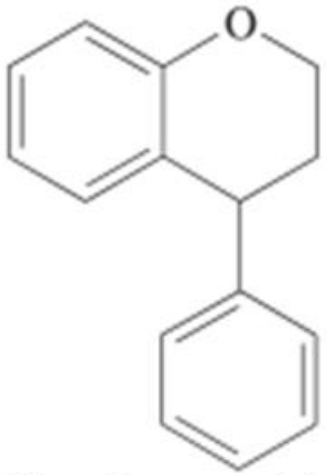


euflavonoids



Isoflavonoids

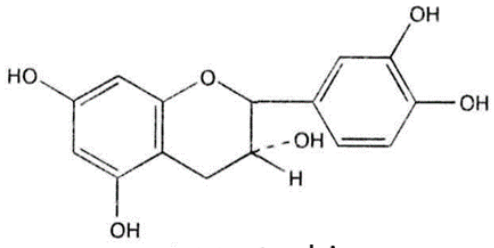




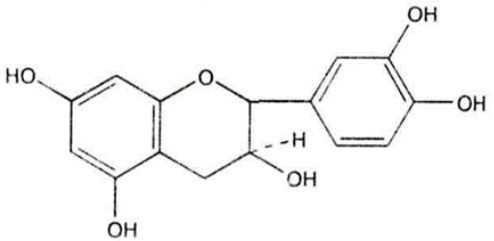
Neoflavonoids

•According to the degree of oxidation of the propane fragment, flavonoids proper (euflavonoids) are divided into: oxidised and reduced.

Reduced (flavan derivatives) are divided into 5 groups.
Catechins (flavan-3-ols). The most reduced flavonoid compounds.



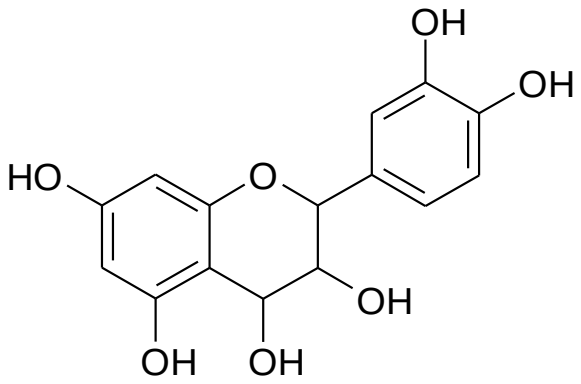
(+)-catechin



(−)-catechin

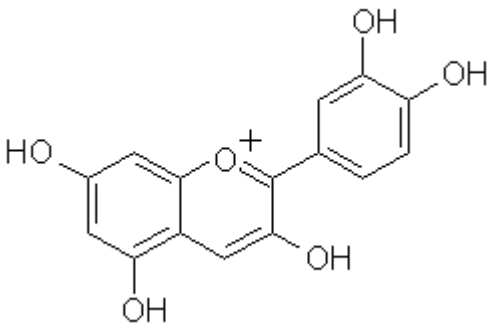
The flavan-3-ols molecule contains two asymmetric carbon atoms in the pyran ring (C2 and C3) and, therefore, four isomers and two racemates are possible for each molecule. For example, the known isomeric compounds (+)-catechin and (-)-epicatechin differ in the configuration of the hydroxyl group of the third carbon atom, they also differ in physical properties (melting point, specific rotation, etc.). Epicatechin has greater biological activity. It is accumulated in the leaves of Chinese tea.

•***Leucoanthocyanidins (flavan-3,4-diols).***

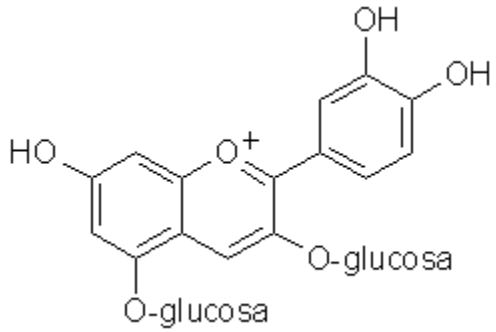


Twelve compounds are known. Leucoanthocyanidins as monomers accompany catechins in Chinese tea leaves. Dimers and polymers of catechins and leucoanthocyanidins are structural units of condensed tannins. Unlike other flavonoids, catechins and leucoanthocyanidins generally do not form glycosylated forms. Leucoanthocyanidins are labile compounds that are easily oxidised to the corresponding anthocyanidins when heated with acids.

•***Anthocyanidins (flavylium cation derivatives).***



Cyanidin

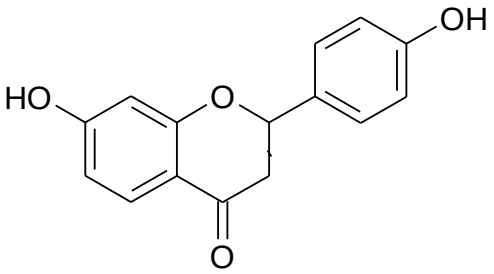


Cyanine

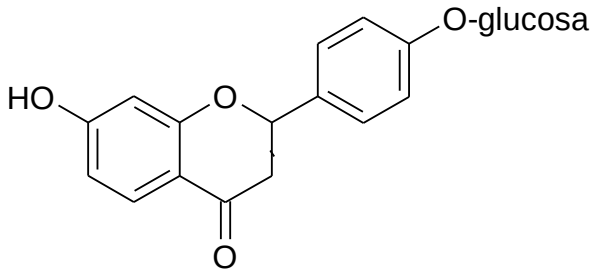
Six aglycones were found. In acidic medium form salts of pink to crimson colour, in alkaline medium, acting as anions, give salts of olive-green colour. They are able to form salts with metals. Salts of Ca, Mg - blue colour, K - from purple to black-violet. Contained in the fruits of blueberries, flowers of cornflower, herb of violet tricolour. Anthocyanidin glycosides are called anthocyanins, e.g. cyanine, a 3,5-diglucoside of cyanidin, is found in the flowers of cornflower.

•***Flavanones (flavan-4-ones).***

About 30 aglycones have been isolated.A small group of flavonoids whose structure is based on an unstable dihydro-γ-pyrone ring. In the presence of alkalis, the ring opens and chalcones are formed. Found in the families of *Rosaceae*, *Lamiaceae* and *Brassicaceae.*

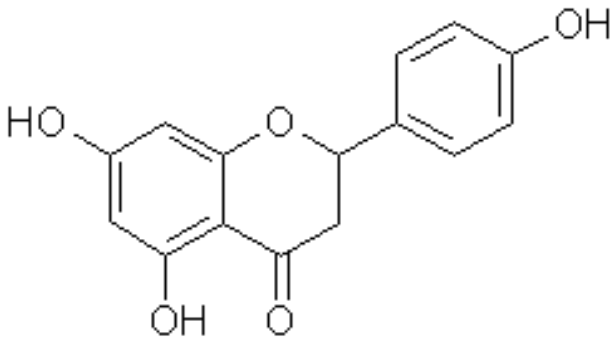


Liquiritigenin



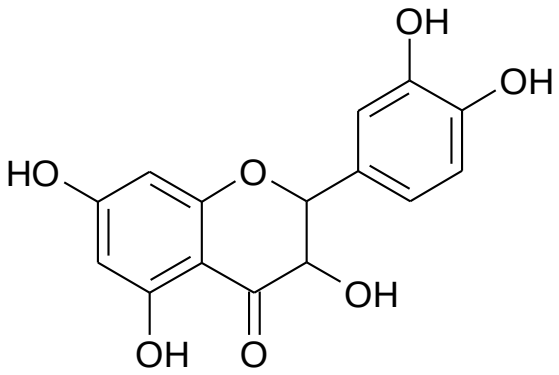
Liquiritin

Naringenin - 5,7,4´-trihydroxyflavanone and its 5-monoglycosides - salipurposide and helichrysin are found in the flowers of *Helichrysum arenarium*.



Naringenin

Flavanols (flavanone-3-ols). They differ from flavanones by the presence of a hydroxyl group at C3 and, like catechins, contain two asymmetric carbon atoms in the molecule. They are very labile and therefore do not accumulate in plants in significant amounts.

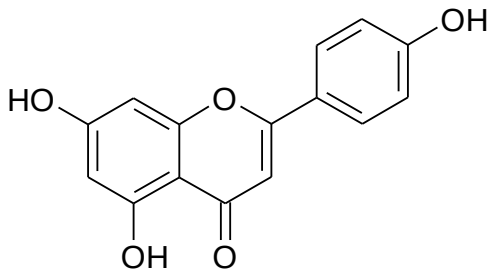


Taxifolin

Taxifolin (dihydroquercetin) and aromadendrin (dihydrocampferol) are rare and oxidise very quickly. They are found in the wood of coniferous (*Picea*, *Pinus*) and deciduous (*Eucalyptus*) trees, as well as in *Equsetum* (herb of *Equisetum arvense*).

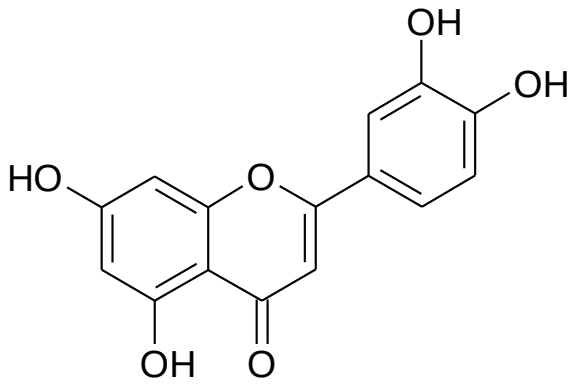
Oxidised (flavone derivatives) are divided into 2 groups.

1. ***Flavones. About 20 aglycones have been isolated.***
2. a) Apigenin is a 5,7,4′-trihydroxyflavone and its 5-glycosides.



7-C-glucoside-4′-acid para-coumaric apigenin - quinqueloside is found in motherwort herb. 8-C-glucoside of apigenin - vitexin is contained in fruit of *Crataegus* and herb of *Viola*.

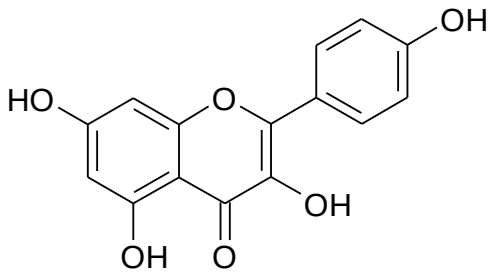
b) Luteolin - 5,7,3′,4′-tetrahydroxyflavone and its 5-glycosides.



Apigenin and luteolin in the form of 5-glycosides are contained in the herb of *Equsetum*, *Bidens tripartita*, in the flowers of and *Helichrysum arenarium*.

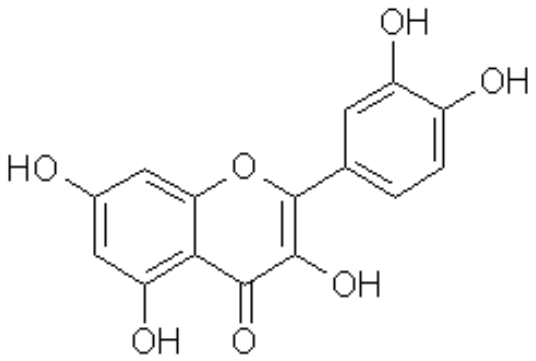
Flavonols (flavon-3-ols). The most numerous and widespread group. About 210 aglycones have been identified, of which the most common are kaempferol and quercetin.

a) Kaempferol is 3,5,7,7,4′-tetrahydroxyflavone, or 5,7,4′ trihydroxyflavonol-3.



Equisetrin - 7-fructose-arabinoside kaempferol is found in the herb of *Equisetum arvense*.

б) Quercetin is 3,5,7,7,3′,4′-pentahydroxyflavone, or 5,7,3′,4′-tetrahydroxyflavonol-3. It is found as an aglycone in the herb of *Astragalus*.



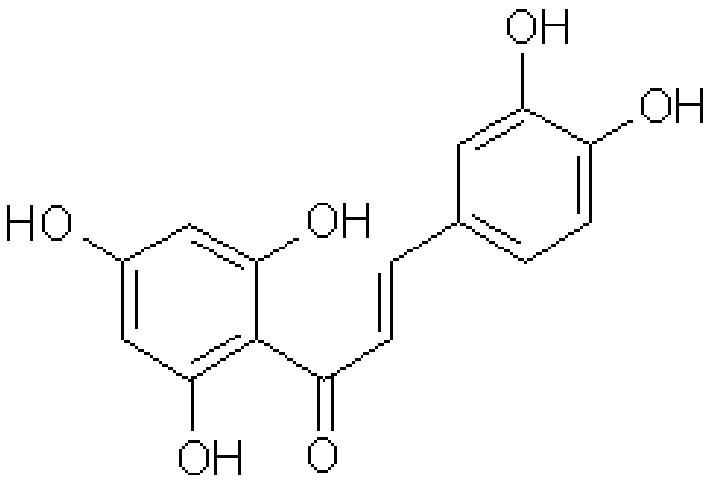
Quercetin glycosides:

- rutin - 3-rutinoside (glucoramnoside) of quercetin is found in the herb of *Viola*, *Leonurus*, *Hypericum*, fruits and buds of *Sophora japonica*, fruits of *Aronia melanocarpa*;
- avicularin - 3-arabinoside of quercetin is contained in the herb of *Polygonum aviculare*;
- hyperoside- 3-galactoside quercetin contained in the herb of *Hypericum*, flowers and fruits of *Crataegus*;
- quercitrin- 3-rhamnoside of quercetin is contained in the herb of *Hypericum*, flowers of *Crataegus*.

Flavonoid groups are also distinguished:

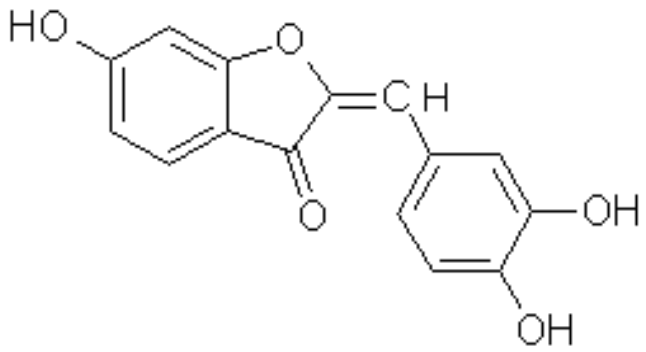
I) with a broken heterocycle :

Chalcones and dihydrochalcones. Occur together with flavanones.
a) Butein - 5,7,3′,4′-tetrahydroxychalcone is found in the herb of *Bidens tripartita* in free form and as glycosides.



II) with a five-membered heterocycle :

Aurons. They are distributed mainly in the families of the *Asteraceae*, *Lamiaceae* and *Scrophulariaceae*. Sulphuretin and its 7-glucoside are found in the herb of *Bidens tripartita*.

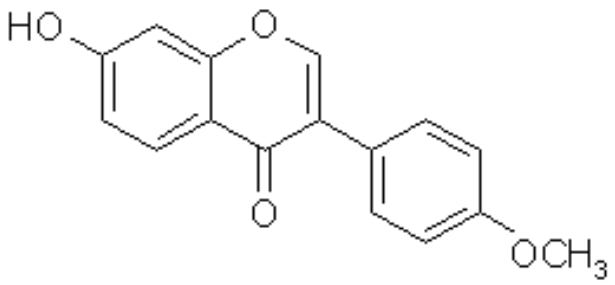


Sulfuretin

Isoflavonoids in plants occur mainly as isoflavone derivatives.

Ginestein - 5,7,4′-trihydroxyisoflavone and daidzein - 7,4′-dihydroxyisoflavone are found in bean, roots of *Ononis arvensis* and other plants of the *Fabaceae* family.

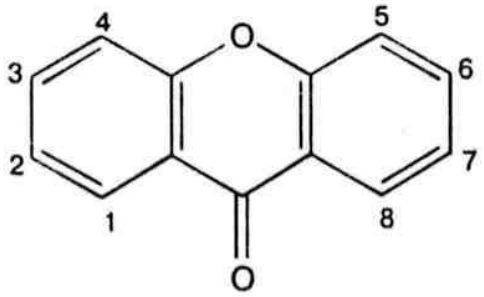
Formononetin - 7-hydroxy-4′-methoxyisoflavone and its 7-glucoside ononin are found in the roots of *Ononis arvensis*.



Formononononetin

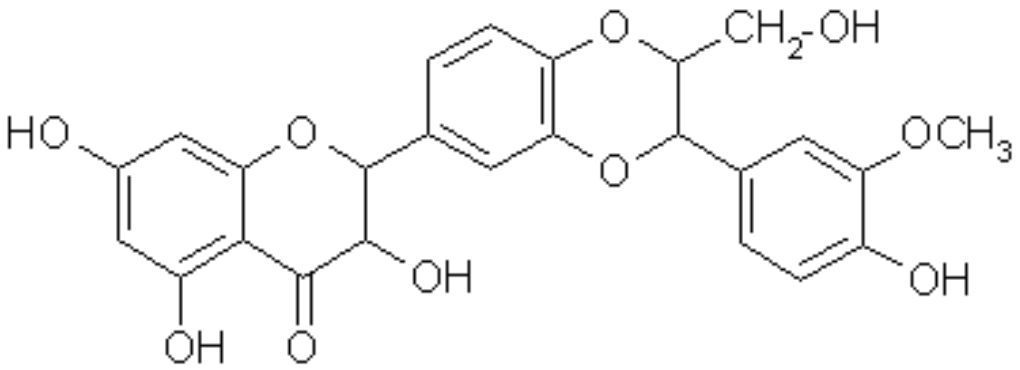
Other classes of flavonoids :

Xanthones are a class of natural compounds having the structure of dibenzo-gamma-pyrone. The name is derived from Greek "xanthos" - yellow, as natural xanthone derivatives have yellow or cream colouring. The first representative of this series - genzizin - was isolated from gentian yellow (*Gentiana*) in 1921. Currently, there are up to 300 xanthone derivatives isolated from plants. They are found in free form and in the form of O- and C-glycosides mainly in the families of *Gentianaceae*, *Hypericum* and some others.



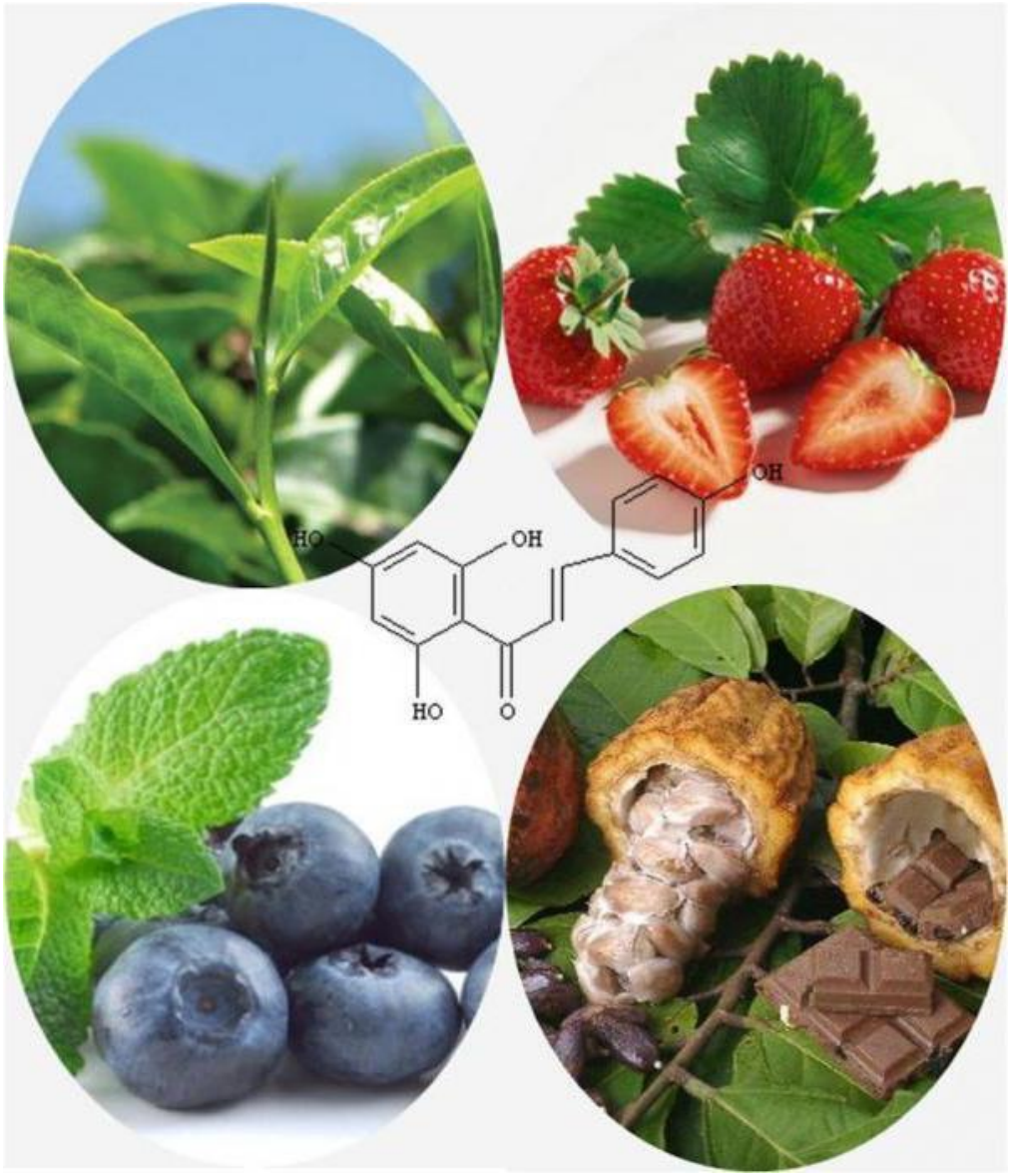
Xanthone

Flavolignans **-** silibin, silidianin, silichristin are found in fruits of *Silibum marianum.*



Silibin

Distribution and localisation of flavonoids in the plants



Factors influencing the accumulation of flavonoids in plants

-The main ones are age and phase of plant development.
-The greatest amount of flavonoids is accumulated in many plants in the flowering phase, while in the fruiting phase it decreases.
-Environmental factors (light, soil, moisture, altitude, etc.) also have a significant effect on the accumulation of flavonoids.
-In southern and highland areas, under the influence of light and on soils rich in trace elements, the flavonoid content increases.

Biological role of flavonoids

1. Flavonoids act as filters in plants, protecting tissues from the harmful effects of UV rays.

2. According to the hypothesis of Russian biochemist V.I. Palladin, flavonoids are hydrogen carriers in the respiratory chain of plant cell mitochondria.

3. Flavonoids participate in the process of photosynthesis and oxidative phosphorylation. Together with ascorbic acid participate in enzymatic processes of oxidation and reduction, contribute to the production of immunity.

4. Being plant pigments, flavonoids (in particular anthocyanins) give bright colours to flowers and fruits, which attract insect pollinators, birds and animals, and thus contribute to pollination and plant propagation.

Physico-chemical properties

Physical properties. Catechins, leucoanthocyanidins, flavanonols, isoflavones are colourless; flavanones, flavanones, flavonols are yellow; chalcones and aurones are orange; anthocyanidins are red, blue or violet amorphous or crystalline substances, odourless, bitter-tasting, with a certain melting point (glycosides - 100-180 ºC, aglycones - up to 300 ºC) depending on the reaction of the medium.Glycosylated forms of flavonoids, catechins and leucoanthocyanidins are well soluble in water, ethanol and methanol of various concentrations, insoluble in organic solvents (diethyl ether, chloroform, acetone). Free aglycones, except for catechins and leucoanthocyanidins, are insoluble in water, but well soluble in ethanol, methanol and other organic solvents (diethyl ether, chloroform, acetone). All flavonoids are well soluble in pyridine, dimethylformamide and alkalis.All flavonoids are optically active, able to fluoresce in UV light, have characteristic UV spectra, characterised by the presence of two absorption maxima, and IR spectra.

Chemical properties.

Chemical properties are determined by the peculiarity of flavonoids structure: presence of aromatic, pyran or pyrone rings, functional groups.

1. Glycosides undergo enzymatic and acid hydrolysis to aglycones and sugars. O-glycosides are hydrolysed more or less easily by dilute mineral acids and enzymes. C-glycosides are easily cleaved only under harsh conditions by strong acids (concentrated hydrochloric or acetic acids) or their mixtures (Kiliani mixture) under prolonged heating.
2. Thanks to rings A and B flavonoids are able to:Form complex compounds with metal salts (iron, aluminium, zirconium). With iron salts - depending on the amount of hydroxyl groups from green and blue to brown colouring; with aluminium salts - yellow colouring, with yellow-green fluorescence;react with diazonium salts to form azo dyes.

3. Flavonoids containing the pyrone cycle (flavones and flavonols) are capable of:
Reduce in acidic medium by atomic (free) hydrogen, obtained as a result of the reaction of interaction of acid with metallic magnesium or zinc, to anthocyanidins (Shinod's test, or cyanidine test);dissolve in alkalis to form water-soluble phenolates.
4. Flavonoids containing the pyran cycle (catechins, leucoanthocyanidins) can be easily oxidised to flavone and flavonol derivatives.
5. Flavonoids, when fused under harsh conditions with alkali, disintegrate into their constituent parts, which is used to establish their structure.
Physical and chemical properties are used in analysing raw materials for authenticity and quality.

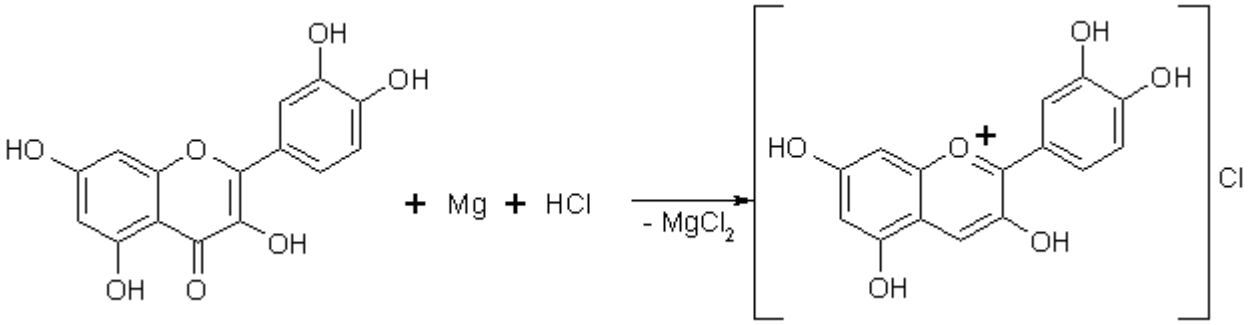
Methods of obtaining

To isolate flavonoids, plant material is extracted with ethanol. The alcohol extraction is evaporated, hot water is added to the residue and after cooling, non-polar compounds (chlorophyll, fatty and essential oils, etc.) are removed from the water base by chloroform or carbon tetrachloride. Specific methods exist for the isolation of individual flavonoids.

Qualitative reactions

There are no specific reactions for all groups of flavonoids. The following reactions are most commonly used.

1. Cyanidine assay (Shinode's assay). Flavonoids when reduced with hydrogen atom in the presence of magnesium (or zinc) and concentrated hydrochloric acid form a characteristic colouring. The reaction is very sensitive, based on the reduction of carbonyl group and formation of anthocyanidins. To carry out the reaction, 1 g of powder of raw material is poured 10 ml of 95% ethanol, heated on a water bath to boiling and insisted 3-4 hours. The alcoholic extract is filtered, evaporated to a volume of 2 ml, divided in half and poured into 2 test tubes; 3 drops of concentrated hydrochloric acid are added to each test tube. In the first test tube add 0,03-0,05 g of magnesium or zinc dust and heat on a water bath to boiling. The liquid is coloured red or bright pink. In the second test tube there is no colouring. Flavones give orange-red, flavonols pink to crimson coloured salts.



Quercetin — Cyanidine chloride

Anthocyanidins, chalcones and aurones in acidic medium immediately give coloured oxonium salts.

2. Briant's test. It is carried out in case of positive cyanidine reaction and is its modification. This test makes it possible to conclude about the presence of glycosides and (or) aglycones in the raw material.Octanol is added to the test tube where the Shinoda test was performed and shaken. If:the colouring has transferred to the organic layer - the raw material contains only aglycones, which are soluble in octanol;colouring remained in the aqueous phase - the raw material contains only glycosides;both layers were coloured - the raw material contains flavonoids both in the form of glycosides and aglycones.

3. Reaction with iron (III) salts. Complex compounds are formed with iron oxide chloride, coloured black-blue if the flavonoids are trihydroxy derivatives, and green if dihydroxy derivatives.4. Reaction with 2-5% alcoholic solution of aluminium chloride. Flavonoids having two hydroxy groups at C3 and C5 form chelate complexes due to hydrogen bonds between carbonyl and hydroxyl groups and aluminium ion, which are yellow with yellow-green fluorescence.Complexes with zirconium salts are formed similarly.

5. Reaction with 1% solution of basic lead acetate. Anthocyanidins give a blue amorphous precipitate.Flavones, chalcones and aurones give a bright yellow precipitate.

6. Reaction with 10% alcoholic alkali solution. Flavones, flavonols, flavanones and flavanonols dissolve in alkalis with formation of yellow phenolates, on heating the colour changes to orange or brown. Chalcones and aurones usually give red or bright yellow colouring when interacting with alkalis.Anthocyanidins form blue to olive green salts with alkalis.

7. Reaction of azo-combination with diazo compounds (sulfanilic acid or para-nitroaniline). An azo dye of orange, red or cherry red colour is formed.8. Boric-limonene reaction with Wilson's reagent (0.5 g each of boric and citric acids in methanol).Reaction for distinguishing flavonoids from furanochromones. Flavonoids give yellow coloured complexes with boric acid with bright yellow fluorescence, which are not destroyed by citric acid. Furanochromones do not react with a mixture of boric and citric acids.

Chromatographic study

For identification and separation of flavonoids, paper, column chromatography and thin layer sorbent chromatography methods are used.Different solvent systems are used:
for BC most often BUV (butanol - acetic acid - water) 4:1:5; 4:1:2;
for TLC - chloroform-methanol 8:3; 8:2.
Flavonoids are identified by characteristic luminescence on chromatograms in UV light before and after manifestation with chromogenic reagents.Catechins and leucoanthocyanidins do not fluoresce. Glycosides of flavones and isoflavones fluoresce blue or blue, flavonols fluoresce dark brown or black, aglycones of flavones fluoresce brown, flavonols fluoresce yellow, chalcones and aurones fluoresce yellow or orange.To show flavonoids on chromatograms, the following are used:

1) 25% ammonia vapour. There is an increase in the colour of the spots in UV light or a change of colour to yellow.
2) 2-5 % alcoholic solution of aluminium chloride. Yellow-green fluorescence is observed in UV light, yellow colouration of spots in visible light.
3) Catechins are shown by 1% solution of vanillin in concentrated hydrochloric acid, red staining is observed in visible light.Less frequently Wilson's reagent, 2 % methanol solution of zirconium chloride, antimony pentachloride solution in chloroform, diazo reagent are used.

Quantitative determination

For quantitative determination of flavonoids in medicinal plant raw materials, physicochemical methods of analysis (mainly photoelectrocolorimetric and spectrophotometric methods) are used.

Spectrophotometric method.
SFM is based on the ability of flavonoids or their coloured complexes to absorb monochromatic light at a specific wavelength.

Chromatospectrophotometric method.
Preliminary separation of flavonoids in a thin layer of sorbent (hawthorn flowers).
Stages of determination:

- Preparation of alcoholic extract;
- purification of the extract;
- chromatographic separation of flavonoids on "Silufol" plate in chloroform-methanol 8:2 solvent system together with hyperoside CO witness;
- identification of hyperoside and witness on the plates in UV light;
- elution of hyperoside and HSA with a 1:1 mixture of dioxane and water;
- measurement of the optical density of the test solution and SSO at a wavelength of 365 nm;
- calculation of hyperoside content.

Thank you for your attention