Flavonoids

Prof. Dr. Ali Hikmet Meriçli

INTRODUCTION

Flavonoids in the broad sense of the term are virtually universal plant pigments. Almost always water-soluble, they are responsible for the color of flowers, fruits, and sometimes leaves. Examples are yellow flavonoids (chalcones, aurones, and yellow flovonols) and red, blue, or purple anthocyanins. When they are not directly visible, they contribute to the color by acting as copigments : for example, colorless flavone and flavonol copigments protect anthocyanins.
All flavonoids – approximately 4000- have a common biosynthetic origin, and therefore possess the same basic structural element, namely the 2-phenylchromane skleton. We can classify them :

2-phenylbenxopyriliums, i.e., anthocyanins

2-phenylchromones

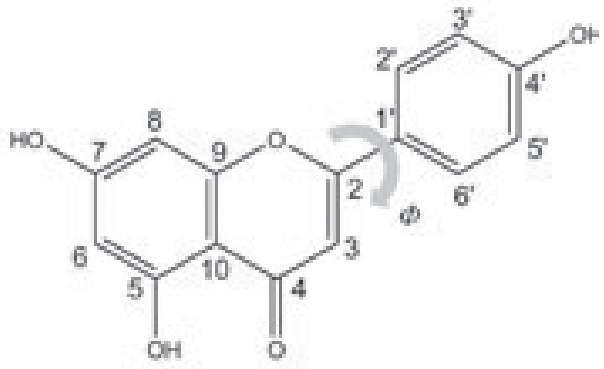
- flavones, flavonols, and their dimers
- flavanones, and dihydroflavonols
- isoflavones, isoflavanones

2-pheylchromane

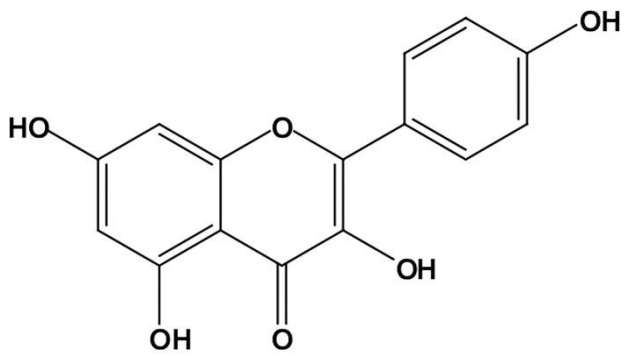
- flavans
- flavan-3-ols, flavan-3,4-diols

Chalcones and dihydrochalcones (the pyran ring opens)

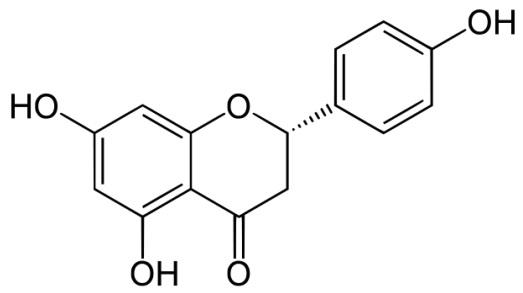
2-benzylidene coumaranones (= aurones)



FLAVONE

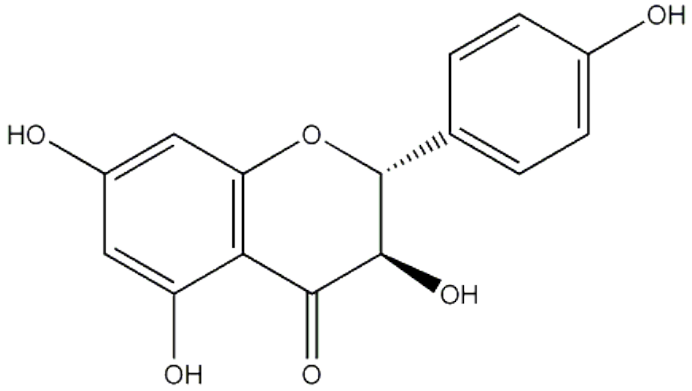


FLAVONOL



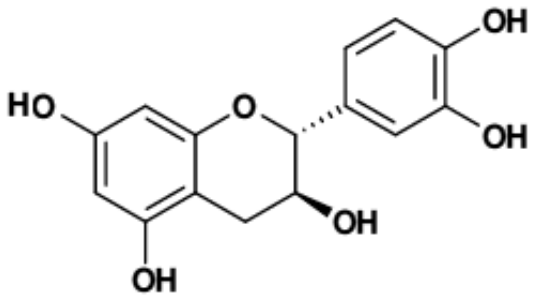
Naringenin

FLAVANONE



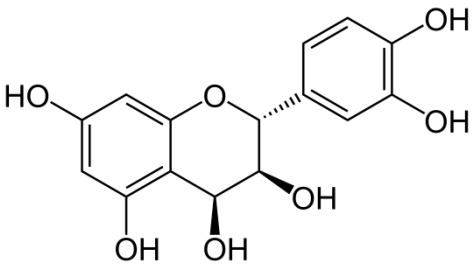
Dihydrokaempferol

DIHYDROFLAVONOL



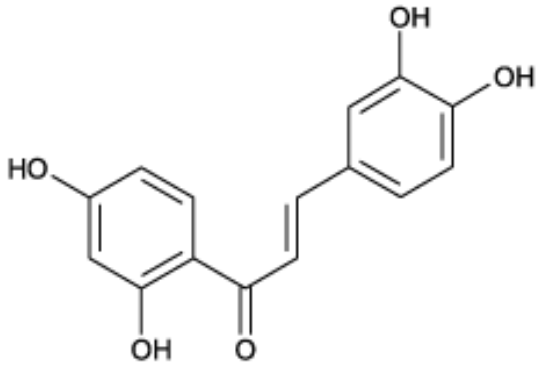
Catechin

FLAVAN-3-OL



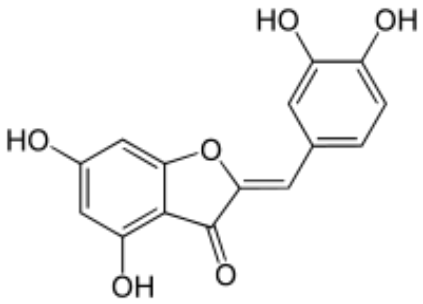
Leukocyanidin

FLAVAN 3,4-OL



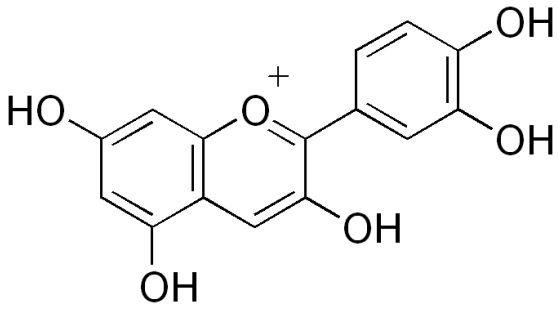
Butein

CHALCONE



Hispidol

AURONE



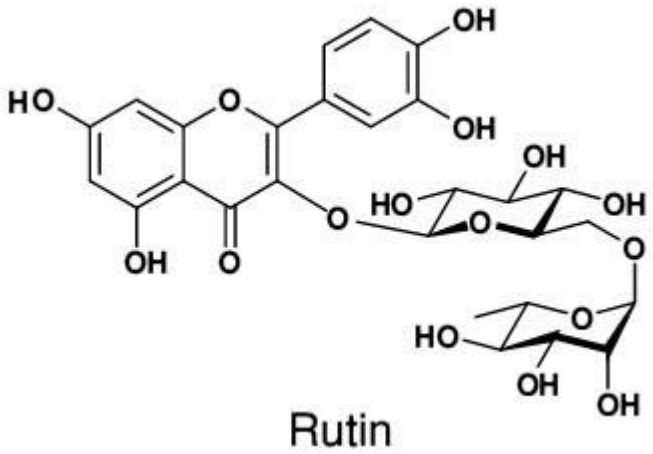
Cyanidin

ANTHOCYANIDIN

Except algae, flavonoids are wide distributed in the plant kingdom. They occur free or as their O- and C-glycosides and O-uronic derivatives. The glycosidic forms of flavonoids are water-soluble, accumulate in vacuoles, and depending on the species, either concentrate in the epiderm of the leaves or spread in both the epiderm and the mesophyl. In flowers, they are concentrated in epidermal cells.

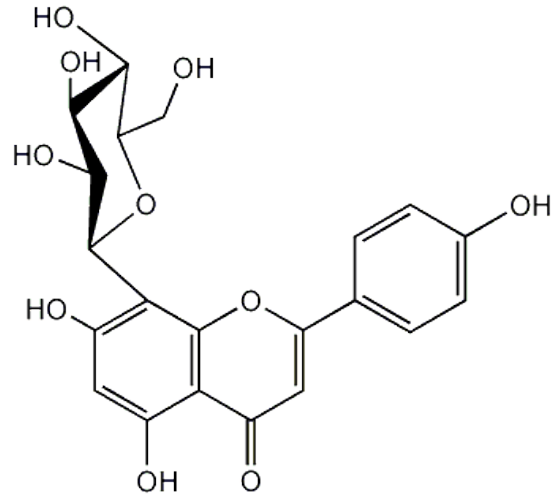
CHEMICAL STRUCTURE AND CLASSIFICATION

All flavonids contain mostly a OH, OCH_3, or O-gl at the 5,7 and 4' positions. A flavonol always contains a substituent on the 3. position. Flavanones are characterized by the absence of 2,3 double bond. C-Glycosylflavonoids are not rare. The bond is established between the asymmetric carbon on the sugar, and the C-6 or C-8 of the aglicone.



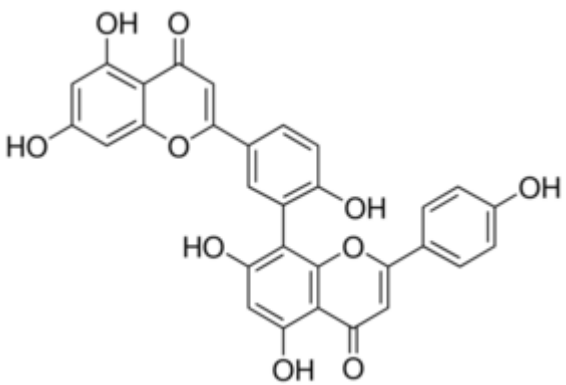
Rutin

Flavon 3-O-glycosid (flavonol)



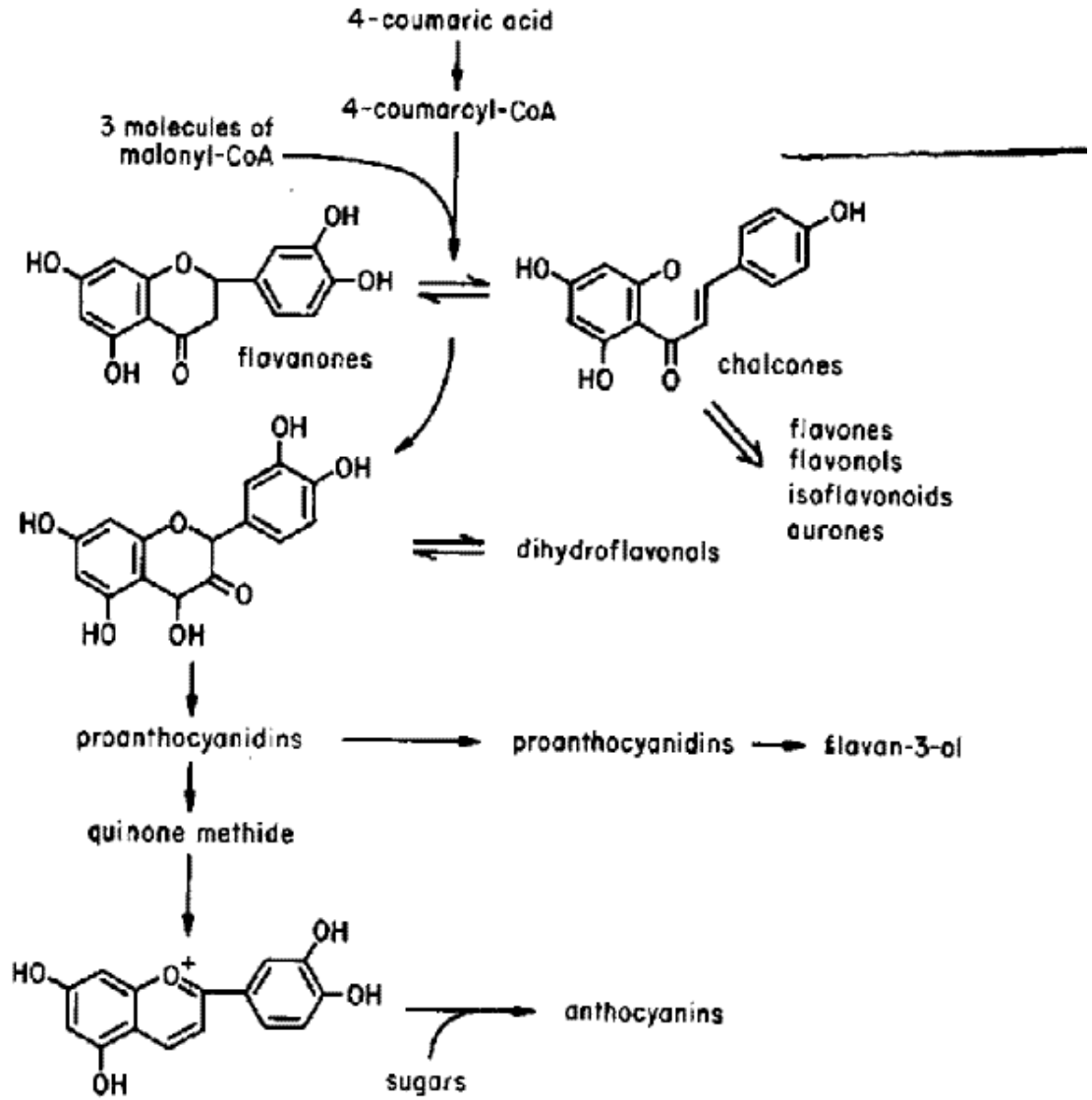
Vitexin

Flavon 8-C-glycosid



Amentoflavone (biflavonoide)

BIOSYNTHETIC ORIGIN



PHISYCO-CHEMICAL PROPERTIES, EXTRACTION, CHARACTERIZATION, AND QUANTITATION

Although,as general rule, glycosides are water-soluble and soluble in alcohols. Aglycones are, for the most part, soluble in apolar organic solvents; when they have at least one free phenolic group, they dissolve in alkaline solutions. Lipophilic flavonoids are directly extracted by solvents of medium polarity (e.g., dichloromethane); next they must be separated from the waxes and fats extracted simultaneously (of course, a preliminary hexane wash is possible; but the selectivity of this solvent is not absolute). The glycosides can be extracted, most often at high temperature, by acetone or by alcohols (ethanol, methanol) mixed with water.

Solvent evaporation under vacuum can be the next followed, when only the aqueous phase is left, by a series of liquid-liquid extractions by nonmiscible solvents : petroleum ether which eliminates lipids, toluene which eliminates chlorophyl, chloroform or diethyl ether which extract free aglycones; and ethyl acetate which dissolves the majority of glycosides. The free saccharides remain in the aqueous phase with the most polar glycosides when these are present.

The separation and purification of the different flavonoids is based on the usual chromatographic techniques (on polyamide, cellulose, or sephadex gel). They also can be isolated by HPLC.

Characterization

Although several color reactions allow the characterization of aglycones and glycosides in crude extracts, preliminary work on these extracts is conventionally dominated by TLC analysis.

- directly, since chalcones and aurones are usually visible, and turn orange and red respectively, in the presence of ammonia vapors;
- by examination under UV light before and after spraying with aluminium trichloride, or before and after exposure to ammonia vapors;
- after spraying with a 1% solution of the ester of 2-aminoethanol and diphenylboric acid, in other words the «Naturstoff Reagenz A (NA)»by examination under UV light;

• after spraying with ferric chloride, anisaldehyde, diazotized sulfanilic acid or other general reagents for phenols;

• by utilizing more or less specific reactions or properties, such as :

- Reaction known as cyanidin (or Shibata) reaction, with magnesium powder, or with zinc (Shinoda) both in the presence of hydrochloric acid.

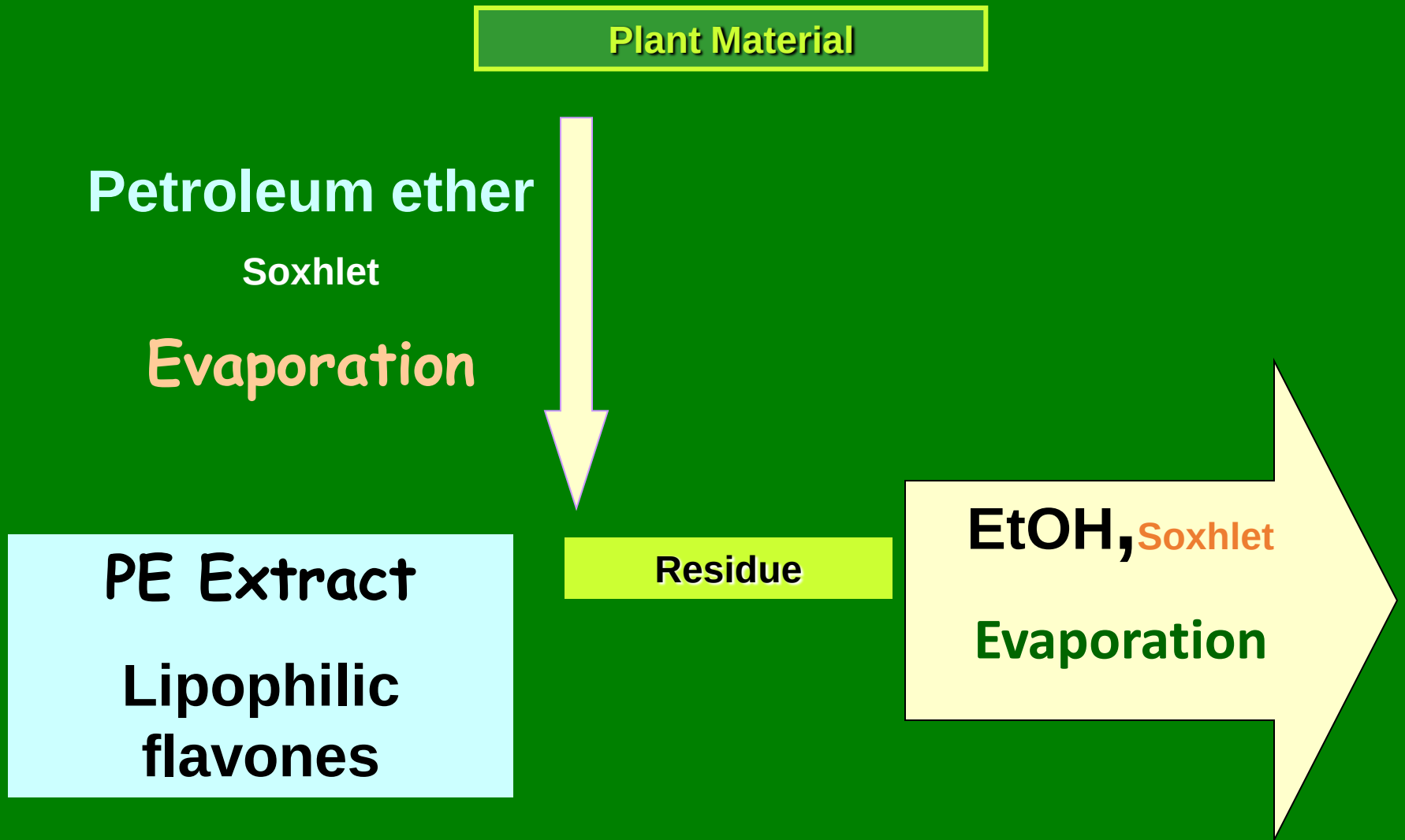
Flavones → orange

Flavonols → red ,

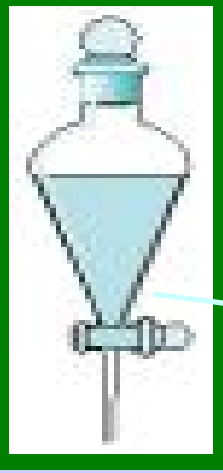
Flavanones → purple colors.

Structure elucidation : especially UV and NMR techniques are very useful.

ISOLATION PROCEDURE



EtOH Extract



+ H_2O

Shaking with toluene

Toluene phase

chlorophyll+side compounds

Aqueous phase

chloroform

Chloroform phase

AGLYCONES

Aqueous phase

Separating funnel (EtOAc)

EtOAc phase

GLYCOSIDES

$CHCl_3$ phase

AGLYCONES

EtOAc phase

GLYCOSIDES

Evaporation under vacuo

Column chromatography

Pure flavones

Identification and reactives:

Shibata reaktion : Drog: Extraction with EtOH, adding Shibata reactive (EtOH + H_2O + conc. HCl 1: 1 : 1) and **Mg** . **After oxydoreduction**

FLAVONES

FLAVONOLS

FLAVANONES

Isoflavones, chalcones and aurones do not react.

$FeCl_3$ reactive :

Mostly green, sometimes dark blue

(side by side trihydroxy groups)

Flavanones Clared red

KOH reactive : **Yellow - orange**

Special reactives : NA reactive

BIOLOGICAL PROPERTIES

The main property that is recognized for flavonoids is «venoactivity», in other words their ability to decrease capillary permeability and fragility. Because of this property they were referred as «**vitamin P**». Vitamin P is more active together with vitamin C.

Flavonoids and Free Radicals

Many properties, shown in vitro, could explain the actions of flavonoids. Initially, it was postulated that they act on the reduction of dehydroacorbic acid via glutathione by acting as hydrogen donors. The more reducing the flavonoid, the greater is the ascorbic acid sparing.

It is now more generally accepted that the phenols that flavonoids scavenge free radicals formed under different circumstances :

- anoxia
- inflammation
- lipidic autoxidation

Biochemically, free radicals are thought to be responsible for nucleic acid alterations, mutations, initiations and promotion of carcinogenesis, and cellular damage, because of their ability to react with membrane phospholipids, among other reason. The antagonist effect towards free radical production can be studied experimentally. This has spurred research, including epidemiplogic studies, on the potential role of antioxidants (i.e., free radical scavengers), such as flavonoids, some lignans, and other metabolites found in the daily diet, in preventative therapy.

Other Properties

Flavonoids have antispasmodic, diuretic, anti-inflammatory properties. They are also enzyme inhibitors in vitro.

USES OF FLAVONOID-CONTAINING DRUGS

Some crude drugs are used for the industrial extraction of flavonoids, for example total citroflavonoids, diosmin, hesperidin, rutin (diosmin occurs in *Citrus* but is obtained by semisynthesis). Others, the activity of which is due to several active principles, are used as titrated extracts (*Ginkgo*). Flavonoids are also always found in herbal teas.

CHIEF FLAVONOIDS ON THE MARKET
Citroflavonoids (Bioflavonoids)
Flavonoids from the Fruits of Various *Citrus species*
(Citri pericarpium)

Citrus aurantium **var. *amara* - turunç (bitter orange)**
Citrus aurantium **var. *dulcis* - portakal (orange)**
Citrus aurantium **var. *bergamiae* – bergamot (bergamot**
Citrus limonum **– limon (lemon)**
Citrus reticulata **– mandalina (mandarin)**
Citrus paradisi **– greyfurt (grapefruit)**

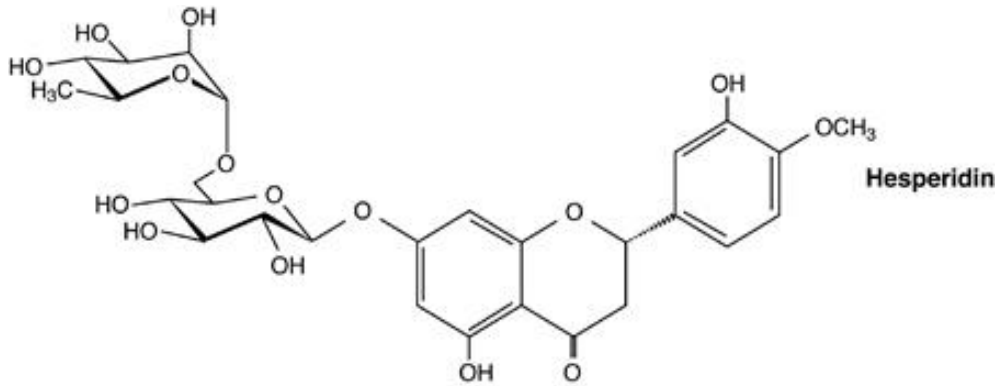
Citrus species, widely used for their essential oil, they are also a source of pectins and flavonoids. These are very abundant in the pericarp, and are mainly flavanone glycosides (hesperidin or hesperetin 7-O-rutinoside, neohesperidin, naringin, eriodyctin, eriocitrin). The pericarps also contain flavone glycoside (diosmin). Neohesperidin and naringin are found in bitter orange, hesperidin, in sweet orange, and grapefruit is rich in naringin. Citroflavonoids are extracted from pericarps and pulps with water, and isolated using different procedures. Currently the pharmaceutical industry uses:

A mixture of total citroflavonoids

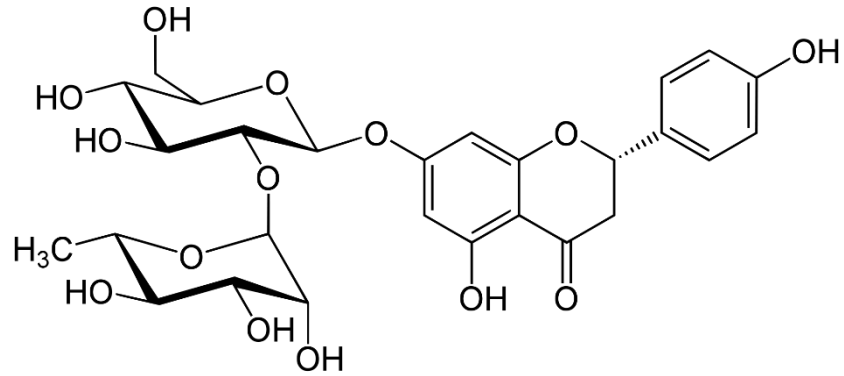
Glycosides of pure flavanones : hesperidin, naringin

Semisynthetic derivatives such as hesperidin methyl chalcone

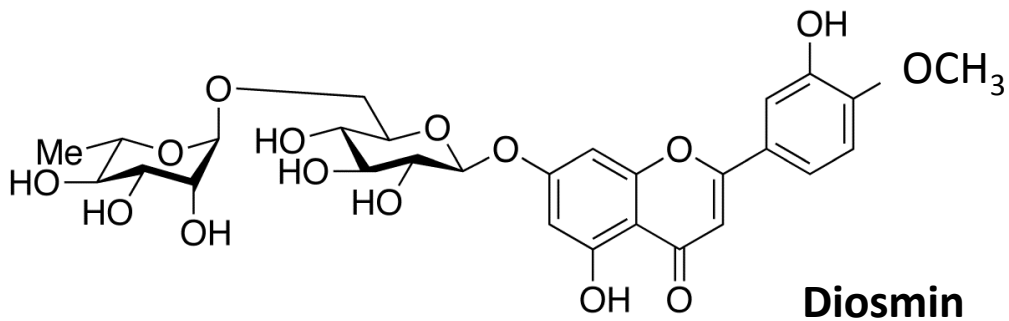
A flavone glycoside obtained by semisynthesis :diosmin



Hesperidin



Naringin



Diosmin

All of these flavonoids are used pure (diosmin, naringin) or in combination (with ascorbic acid, aesculetin, ruscosides, and more).

The accepted indications for preparations containing high doses of citroflavonoids are to improve the symptoms of venous and lymphatic vessel insuffiency, for the adjunctive treatment of the functional signs of capillary fragility, and to treat the functional symptoms of the acute attack of piles.

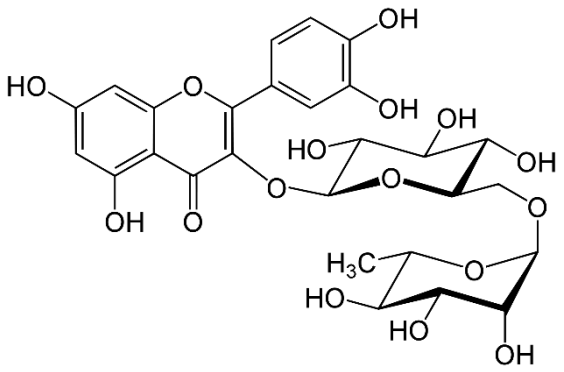
DRUGS RICH IN RUTIN

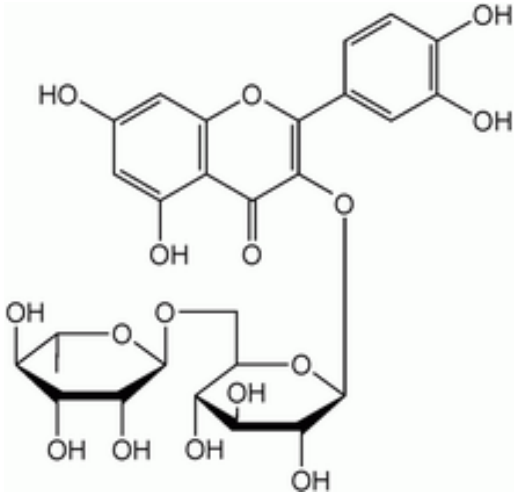
Sophorae flos Japanese pagoda tree sofora

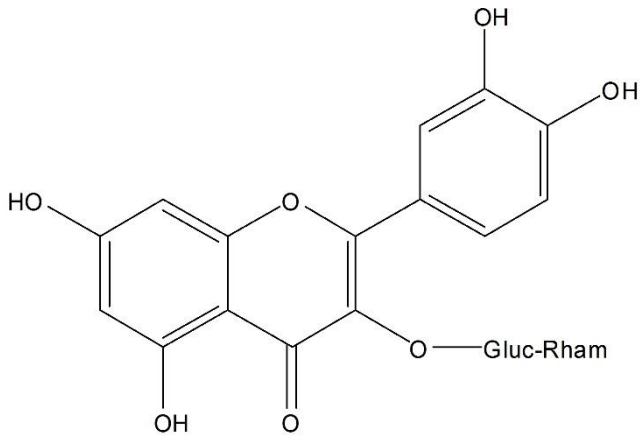
Sophora japonica **Fabaceae**

Rutin :Qercetin 3-O-rutinoside

Sources of rutin : Although rutin is reletively abundant in plants, only a small number of drugs contain quantities sufficient for industrial extraction.







(**rutin**, quercetin-3-rutinoside)

RUTIN

Fagopyri folium **Buckwheat** **karabuğday yaprağı**

Fagopyrum esculentum **Polygonaceae**

Other sources :

Eucalyptus macrorrhyncha (folium)

Dimorphandra spec. (fructus)

Rutin extraction from Sophorae flos does not present any special difficulties : extraction by boiling water and crystallization upon cooling, recrystallization from ethanol.

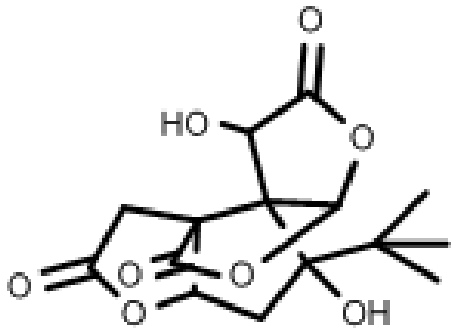
Rutin alone or in combination (with citroflavonoids,ascorbic acid, aesculetin, ruscosides, and more) is promoted for the symptoms of venous and lymphatic vessel insuffiency, for the adjunctive treatment of the signs of capillary fragility, and to treat the functional symptoms of the acute attack of piles.

DRUGS FOR WHICH PART OF THE ACTIVITY MAY BE DUE TO FLAVONOIDS

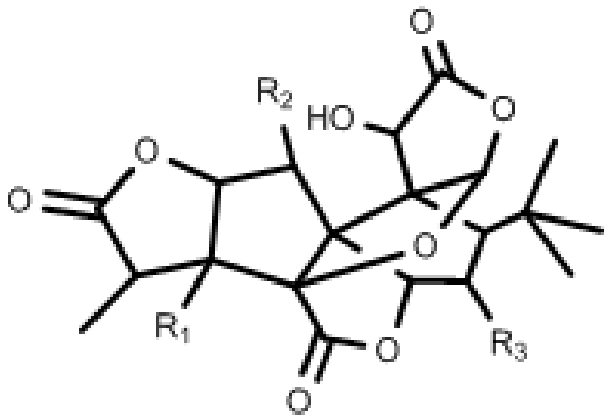
Ginkgo folium maidenhair tree gingko, mabet ağacı yaprağı

Ginkgo biloba **Ginkgoaceae**

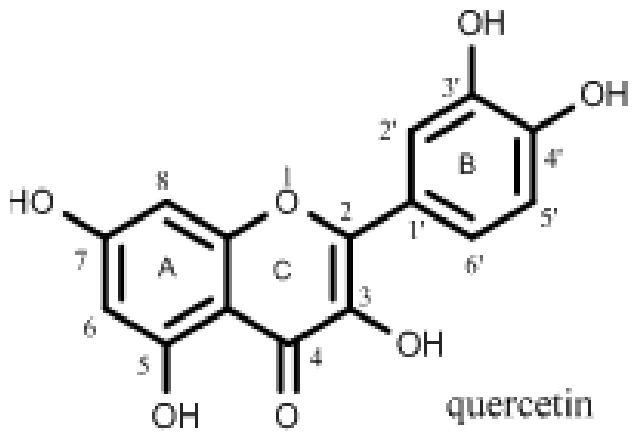
The main principles of Ginkgo folium are flavonoids (0.5-1%), and terpenoid lactones (diterpenoids : ginkgolides A-M (up to 0.5%), and a sesquiterpenoid : bilobalide (0.1%). The flavonoids are represented by about twenty flavonol glycosides, namely O-glycosides, quercetin and kaempferol 3-O-rhamnosides and 3-rutinosides. The ginkgo leaf also contains flavan-3-ols, proanthocyanidins, and biflavonoids which are 3'→8'' biflavones (amentoflavone, bilobetol, ginkgetin sciadopitysin). Known as ginkgolides A, B, C, J (and M in the roots), ginkgo diterpenes have a very specific hexacyclic structure, and three lactone rings.



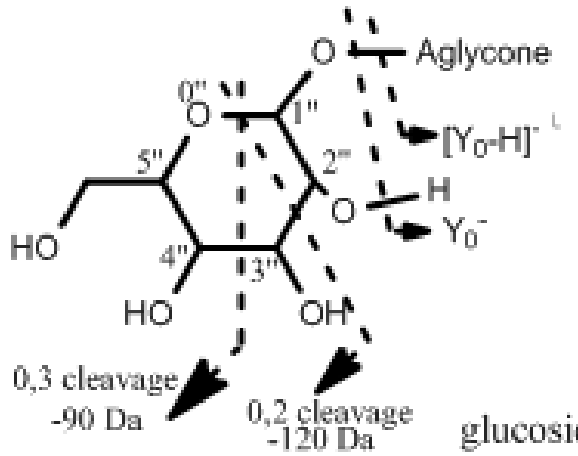
bilobalide



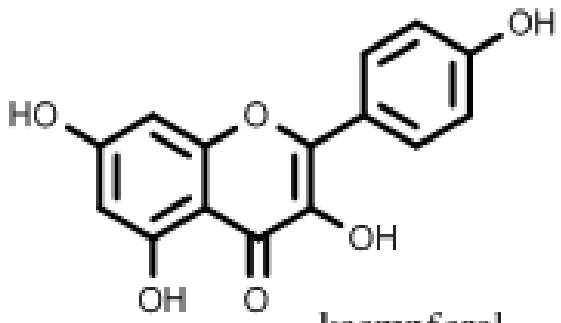
	R_1	R_2	R_3
Ginkgolide A	OH	H	H
Ginkgolide B	OH	OH	H
Ginkgolide C	OH	OH	OH
Ginkgolide J	OH	H	OH



quercetin

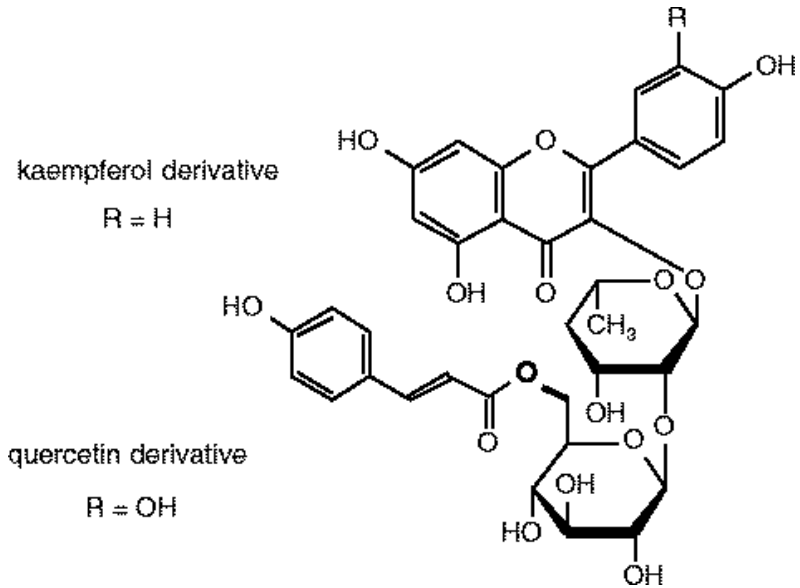


glucoside

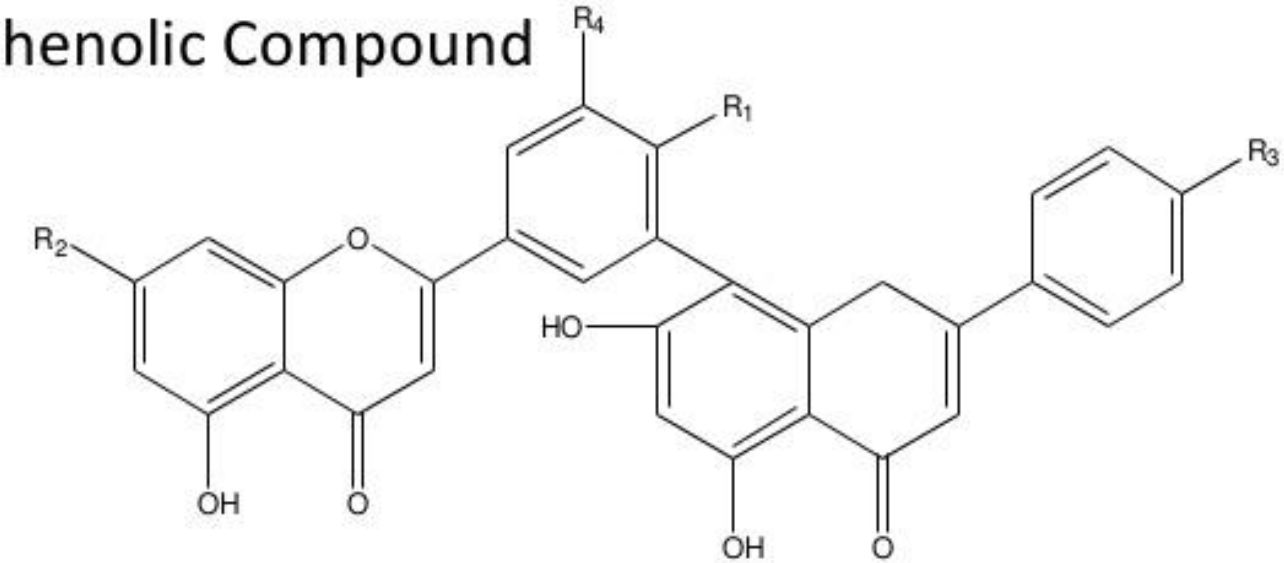


kaempferol

Structures



- Phenolic Compound



Amentoflavone:	$R_1=R_2=R_3=R_4=H$
Bilobetin:	$R_1=OCH3, R_2=R_3=OH, R_4=H$
Ginkgetin:	$R_1=R_2=OCH3, R_3=OH, R_4=H$
Isoginkgetin:	$R_1=R_3=OCH3, R_2=OH, R_4=H$
5'-methyloxybilobetin:	$R_1=R_4=OCH3, R_2=R_3=OH$
Sciadopitysin:	$R_1=R_2=R_3=OCH3, R_4=H$

Pharmacological activity : Ginkgolide B is an inhibitor of the platelet activating factor (= PAF). This anti-PAF activity and the activities of flavonoids, particularly as free radical scavengers, may explain the numerous properties of ginkgo extract that have been observed. This extract is said to be a vasoregulating agent (an arterial vasodilator and a venous vasoconstrictor able to decrease capillary fragility), an inhibitor of cyclo-oxygenase and lipoxygenase, and an inhibitor of platelet and erythrocyte aggregation. It decreases capillary hyper-permeability, improves irrigation, and activates cell metabolism, particularly in the cortex (by increasing glucose and oxygen uptake). The terpene-containing fractions prolong the survival of hypoxic rats; they protect neurons and astrocytes from damage by transient ischemia.

Uses : **Gingko leaves are used to produce an extract titrated 24% flavonoids and 6% ginkgolides-bilabolide**. This extract has undergone several dozen human clinical tirials, especially to assess its efficacy for «cerebral insuffiency».

Main pharmacological properties of ginkgo

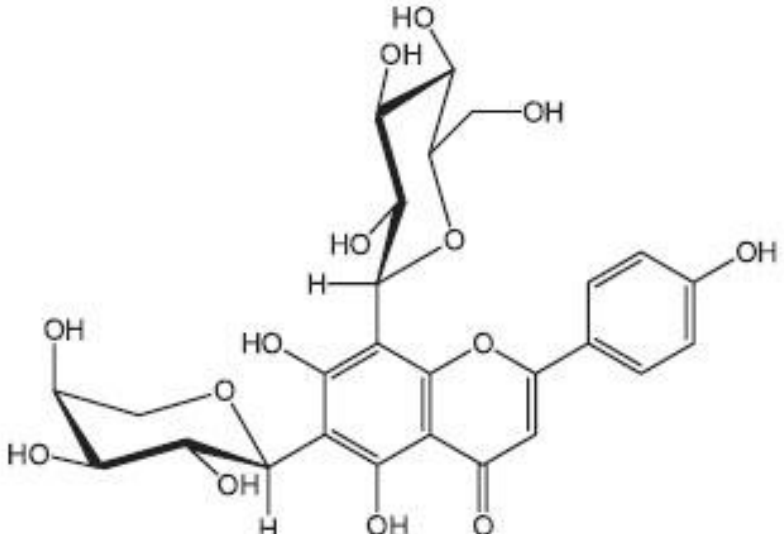
Antioxidant properties
Anti platelet activating factor (anti-PAF) activity
Anti-Ischemic properties
Dementia
Alzheimer's Disease
Tinnitus
Interminent claudication
Macular degeneration

Passiflorae herba **passion flower** **çarkıfelek**

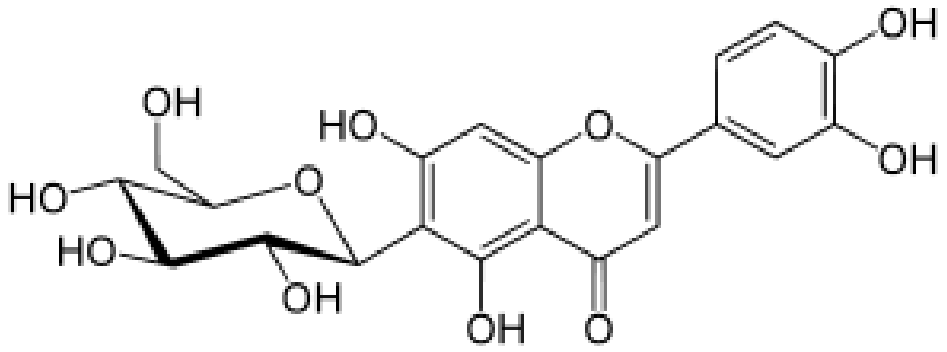
Passiflora incarnata **Passifloraceae**

Chemical Classifictation : Next to phenolic acids, coumarins, phytosterols, and traces of indole alkaloids (harman, harmol, harmine), the drug can contain up to 2.5% flavonoids. The major ones are flavone di-C-glycosides : schaftoside and isoschaftoside (apigenin C-glucosyl-C-arabinosides, 8,6 and 6,8 isomers) together with other flavone C-glycosides (isovitexin, isoorientin, vicenin…)

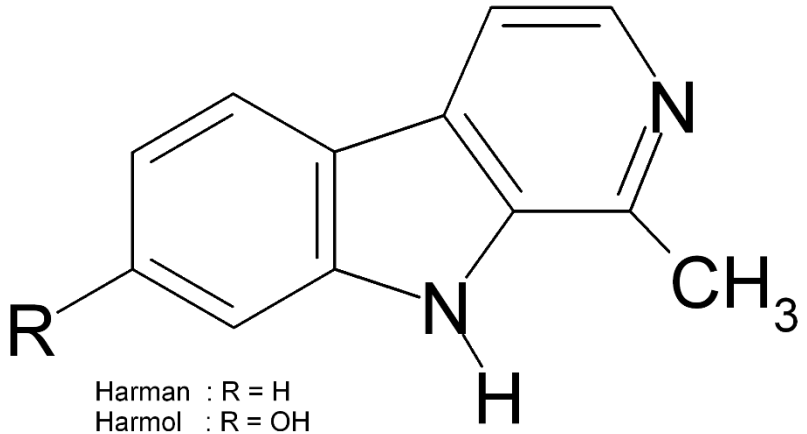
Pharmaceutical activity and Uses : Tradition attributes to the drug sedative, antispasmodic, and tranquilizing properties. All the compounds are together responsible for the acivities (flavonoids, alkaloids, even in minor amounts, and other compounds). The drug (in infusions), its galenical preparations (powders, extract, tincture), and the phytopharmaceuticals containing it are traditionally used by the oral route to treat abnormalities of the cardiac rhythm in the adult (normal heart) and to treat the symptoms of nervousness in adults and children, particularly minor sleeplessness. In Germany (Commission E),: the drug can be used for nervous restlessness, and for mild sleeping difficulties and gastrointestinal signs of nervous origin.



Isoschaftoside



Isoorientin



Harman : R = H
Harmol : R = OH
Harmin : R = O-Methyl

Helichrysum **spec.**
everlasting

Asteraceae

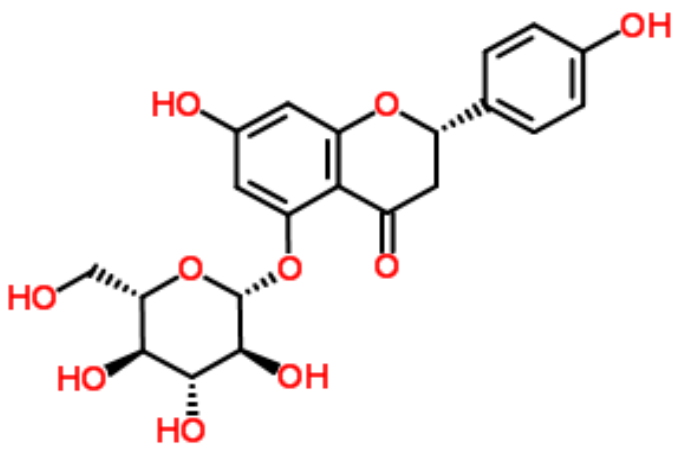
Helichrysi flos
ölmez çiçek

Helichrysum plicatum

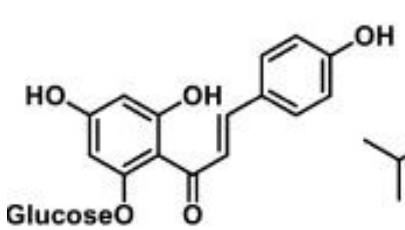
H. graveolens

H. orientale

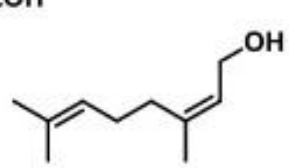
Helichrysum species have been used traditionally to treat urinary stones, especially in Anatolia , in the form of herbal teas (infusions). The capitula (flowers) are rich in flavonoids (helichrysin A and B, isosalipurposide, astragalin, naringenin, apigenin, apigenin 7-glucoside). Especially *Helichrysum plicatum*, *Helichrysum orientale* and *Helichrysum graveolens* are very rich in flavonoids (more than 5%). No specific side-effects have been reported.



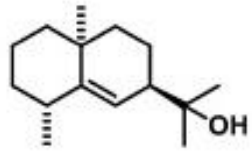
Helichrysin A (-)-naringenin-5-O-glucoside
Helichrysin B (±)-naringenin-5-O-glucoside



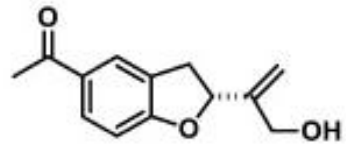
Isosalipurposide



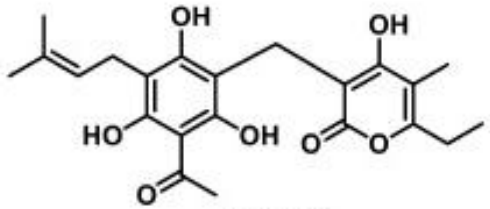
Nerol



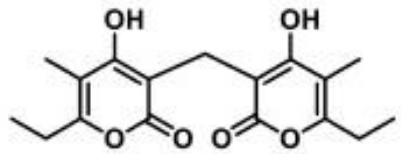
Rosifoliol



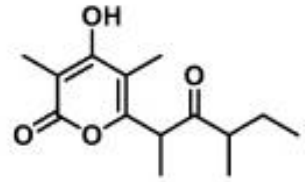
10-Hydroxytremetone



Arzanol



Helipyrone



Mycropyrone

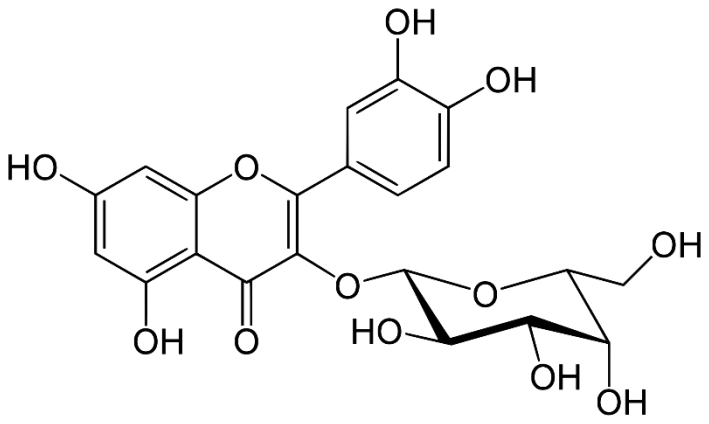
Helichrysum conglobatum
grows in Cyprus

H. sanguineum
rich in anthocyanins

Betulae folium **birch** **huş ağacı yaprağı**

Betula alba, B. pendula **Betulaceae**

The drug contains about 2-3% flavonoids (especially hyperoside = quercetin-3-O-galactoside), and saponins. It is used for its diuretic activity as urinary tract cleanser. The drug is not recommended for heart and kidney diseases.



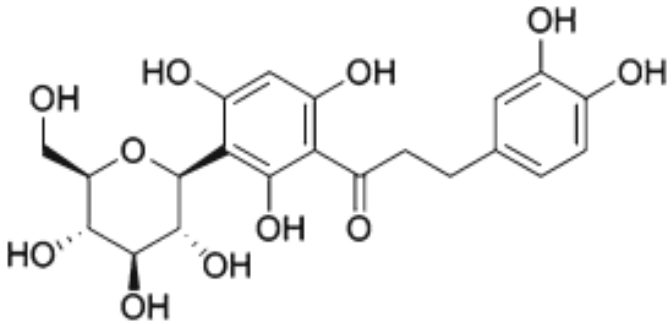
hyperoside

Aspalathi folium **rooibos tea** **roybos çayı**

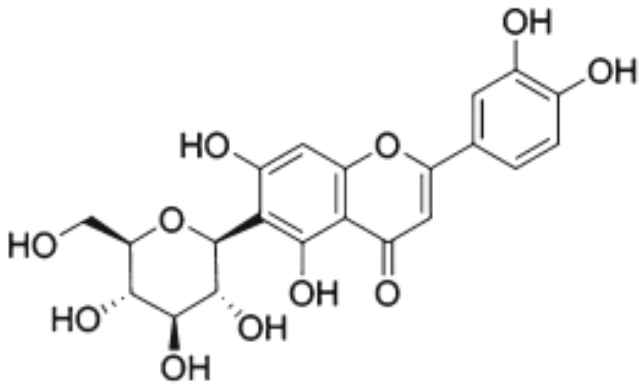
Aspalathus linearis **Fabaceae**

The young leaves, fermented, and dried are used as an alternative to tea, particularly in South Africa. They are reputed to be sedative and to promote digestion and sleeping, it is also used in weight-loss programs. The consumption of this tea is currently spreading, including in Europe, especially because some believe that it has antioxidant properties.

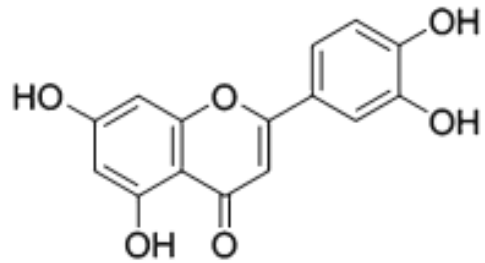
The stems and leaves do not contain caffeine, but they contain ascorbic acid, phenolic acids, and flavonoids (C-glycosides : aspalathin, orientin, isoorientin). Aspalathin is a dihydroxychalcone C-glycoside. Characteristic of the fresh plant, it disappears completely during fermentation. The fermented product is rich in quercetin. The literature contains no reports of toxicity or side effects.



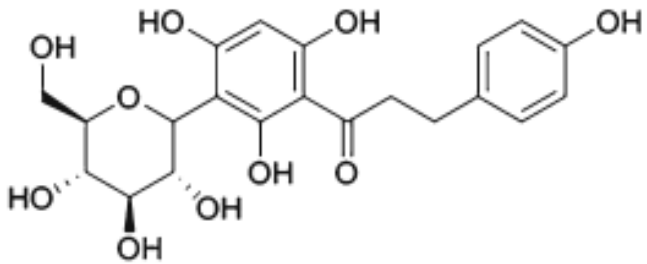
Aspalathin



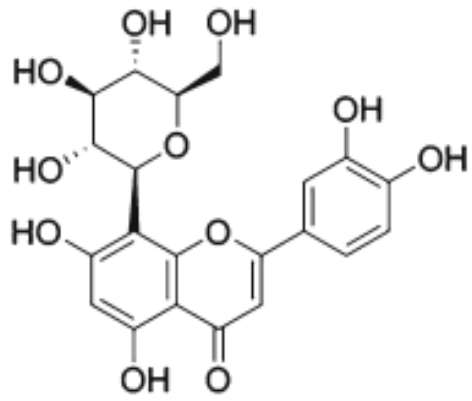
Isoorientin



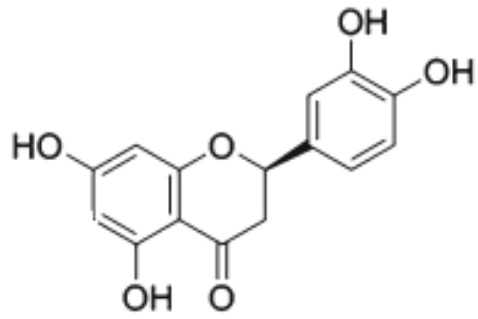
Luteolin



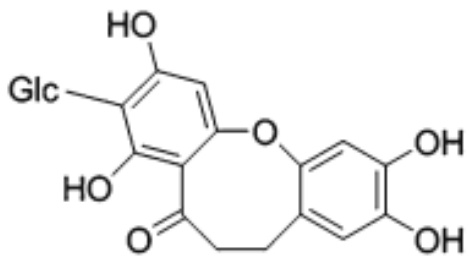
Nothofagin



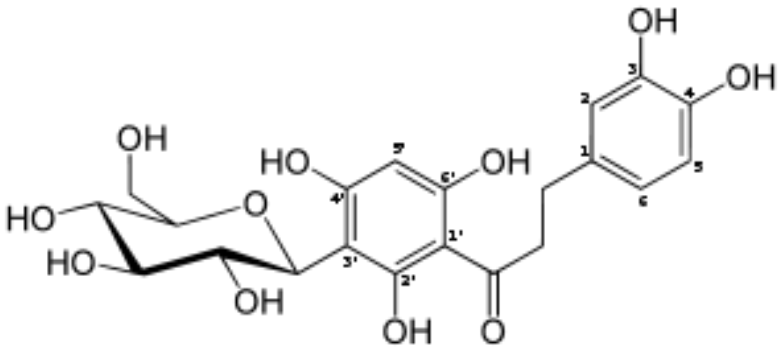
Orientin



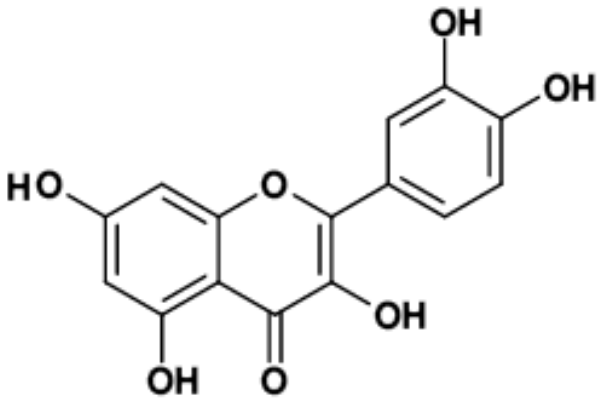
Eriodictyol



Aspalalinin



Aspalathin



Quercetin

Other plants/Drugs rich in flavonoids

Thymus vulgaris kekik (thyme) herba spasmolytic

Chamaemelum nobile roman papatyası (roman chamomile) flos anti-inflammatory

Achillea millefolium civan perçemi (yarrow) flos spasmolitic

Equisetum arvense at kuyruğu (horsetail) herba diuretic

Isoflavonoids

Isoflavonoids are characterized, like flavonoids, by a C_{15} skleton of the Ar-C_3-Ar type, but one which is now rearranged to be a 1,2-diphenylpropane : all molecules in this group can be related to the skleton of 3-phenylchromane. The distribution of these flavonoids is rather limited : they are in fact almost specific to the Fabaceae. The most common compounds are isoflavones, which occur in the free state, or, less commonly, as glycosides (O-glycosides, or exceptionally C-glycosides).

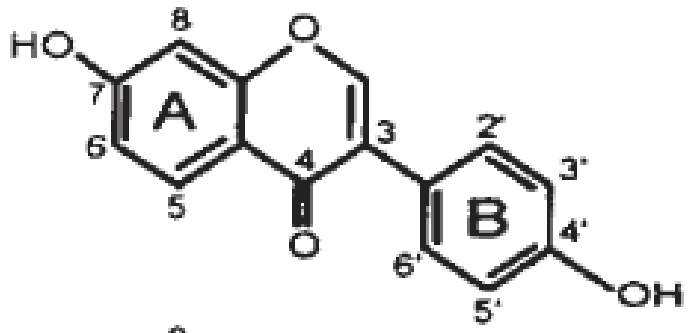
BIOLOGICAL ACTIVITY : In plants a good number of isoflavonoid structures are phytoalexins, in other words substances produced by the plant in response to an infection by a pathogenic agent most often fungal in nature. Estrogenic properties are also known, the other activity is the insecticide activity of rotenoids.

Sojae semen **soybean** **soya fasulyesi tohumu**

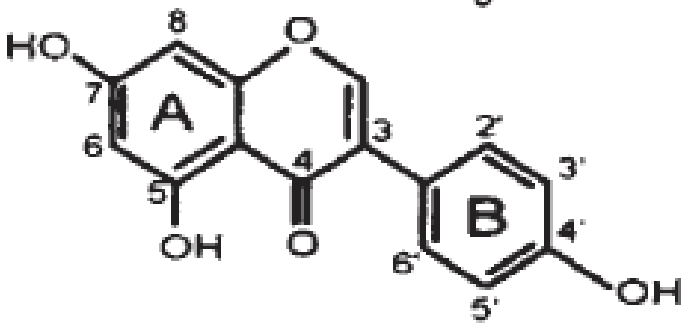
Glycine max **Fabaceae**

Estrogenic activity of isoflavonoids : The occurence of isoflavonoids raises the question of their potential impact on human health. In the soybean, the concentration of daidzein (7,4’-dihydroxyisoflavone), genistein (5,7,4’-tri hydroxyisoflavone), and their glycosyl derivatives can reach 3 g/kg. These isoflavones bind to estrogen receptors, and most, they have a weak estrogenic activity. They are also tyrosine-kinase inhibitors which may have a role in the transformation and cell polyferation phenomena. Pure genistein is also an anticarcinogen.

Several recent studies suggest that isoflavones and soybean decreases the symptoms of menopause (hot flashes and others) and reduce the risk of osteoporosis.

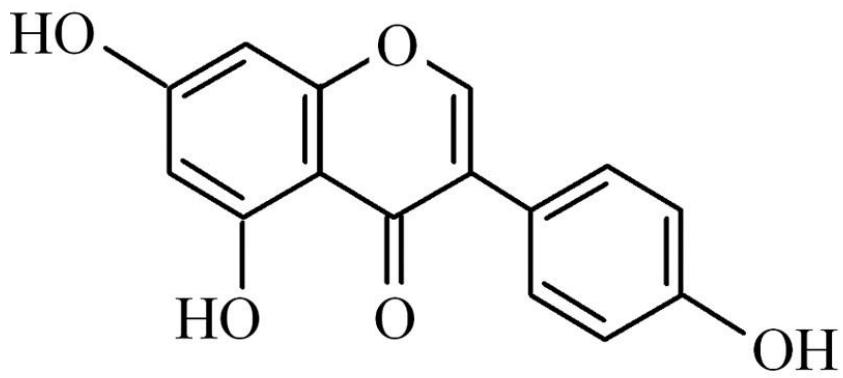


Daidzein
(4',7-dihydroxyisoflavone)

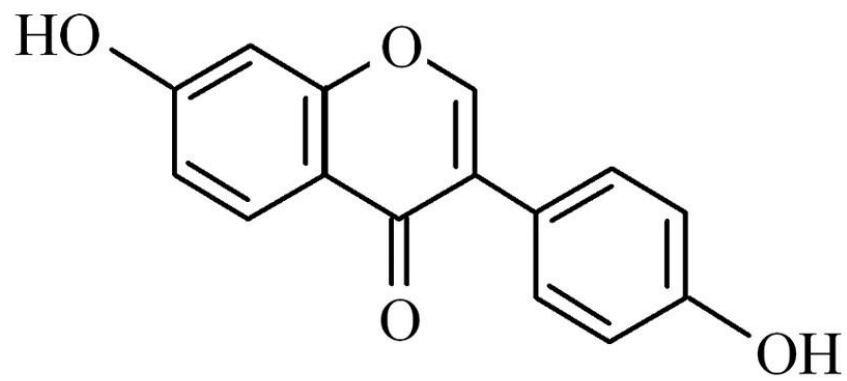


Genistein
(4',5,7-trihydroxyisoflavone)

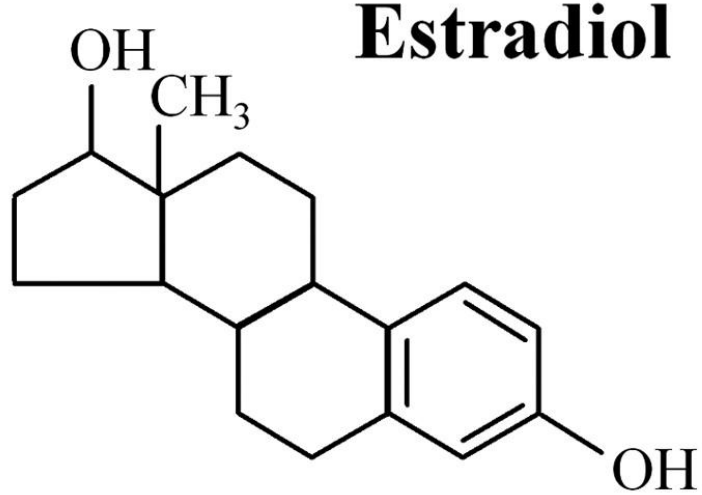
Genistein



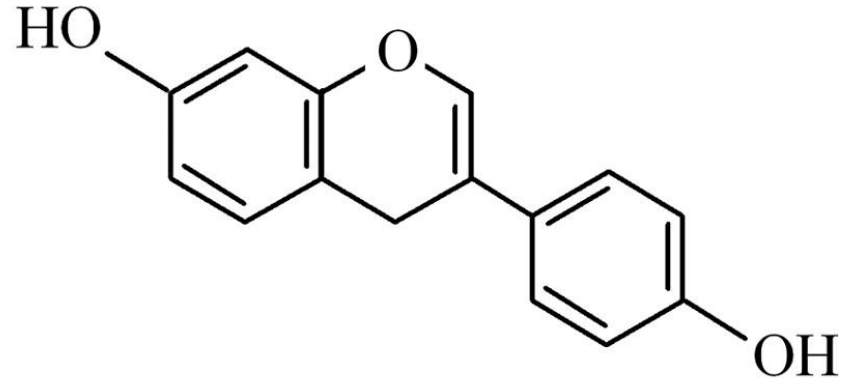
Daidzein



Estradiol



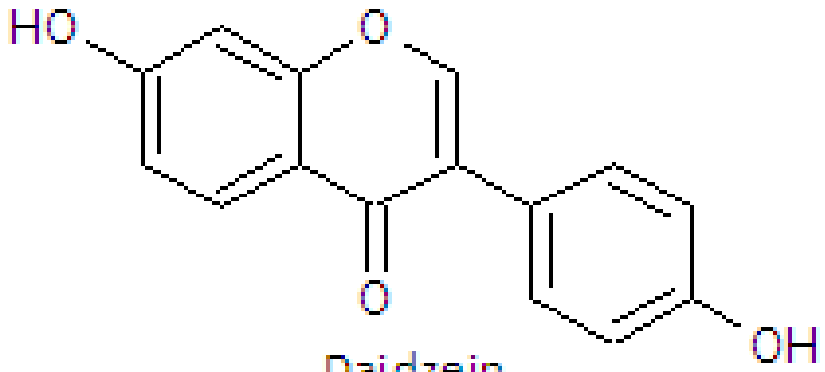
Equol



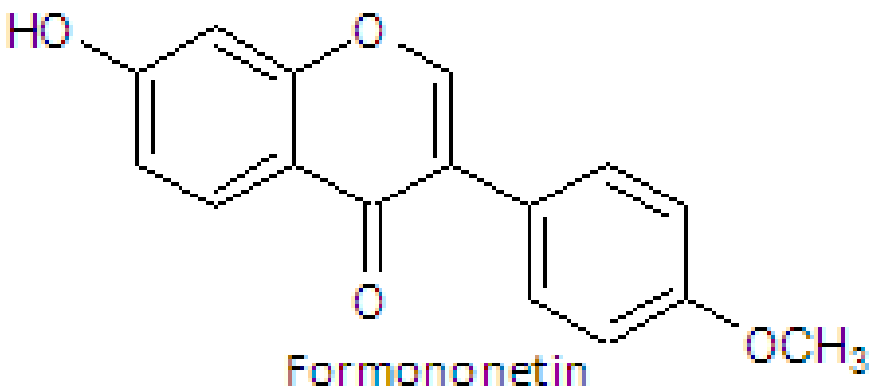
Trifolii pratensi herba **red clover** **kırmızı yonca**

Trifolium pratense **Fabaceae**

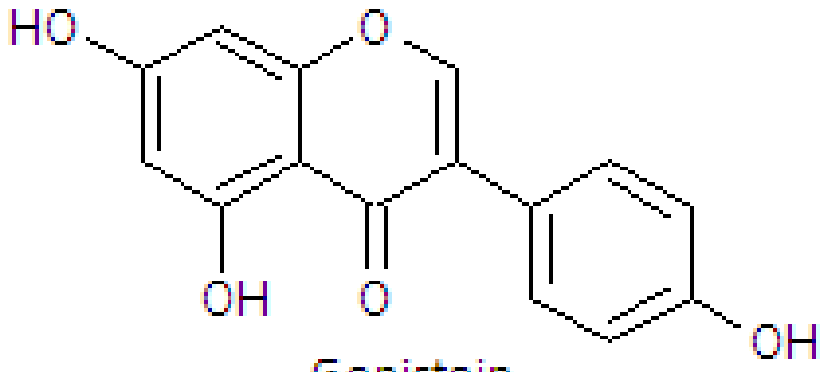
Like soybean, this drug is also rich in isoflavonoids (daidzein, genistein, formononetin, biochanin A..) and is used for the same purposes. By using of this drug, one should be careful, because it also contains some coumarins with anticoagulan activity.



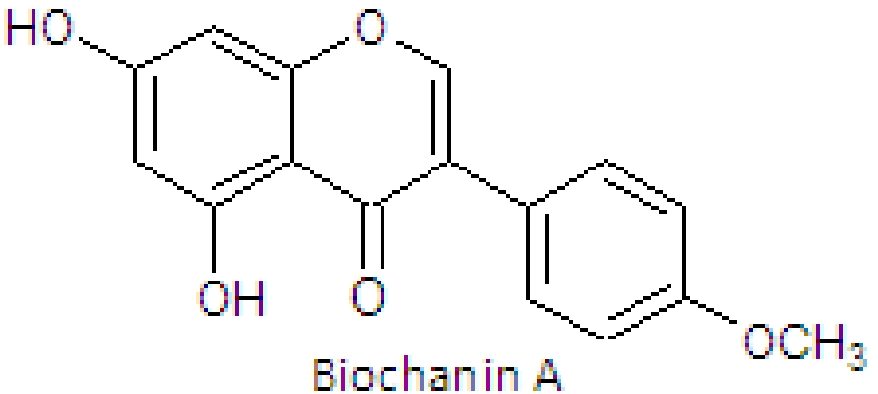
Daidzein



Formononetin



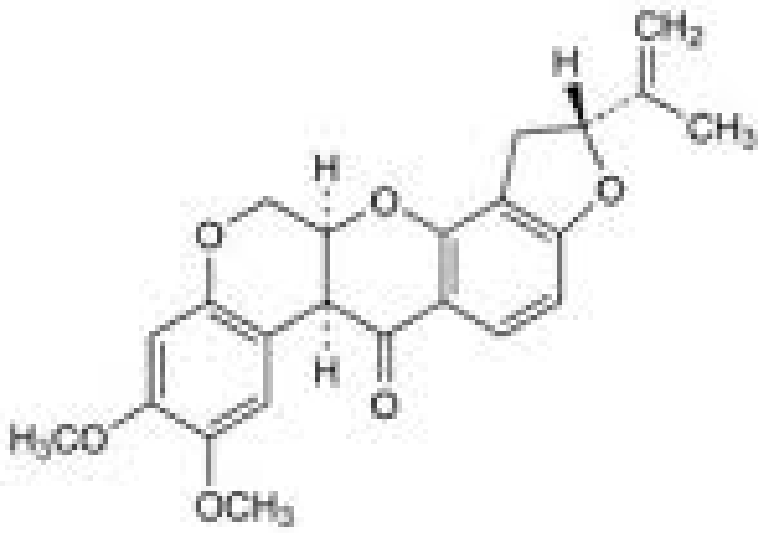
Genistein



Biochanin A

Rotenoids : These compounds, biogenetically related to isoflavonoids, have in common a four-ring structure: a chromanochromanon. The chief representative of the group is rotenone, the major active principle in the roots of various tropical Fabaceae.

Rotenone



Derridis radix **derris**

Derris elliptica **Fabaceae**

Derris are vines growing in southeast Asia. In their area of origin, the roots of these vines are traditionally used as insecticidal and ichthyotoxic agents. The drug consists of the root, and on the market, an extract is frequently found which is enriched and titrated to contain about 30% rotenone.

Rotenone is responsible for the insecticidal peoperties. The main market outlet for rotenoid-containing Fabaceae (powder, extracts, rotenone) is phytopharmacy (treatment of house plants, and, sometimes, of vegetable gardens) and the extermination of ectoparasites of domestic animals.

Flavonolignans

Flavonolignans are addition products of a phenylpropanoid alcohol, onto a flavonoid. These compounds are very rare compounds. The only pharmaceutical drug containing these type of compouds is :

Silybi mariae fructus = Cardui mariae fructus.

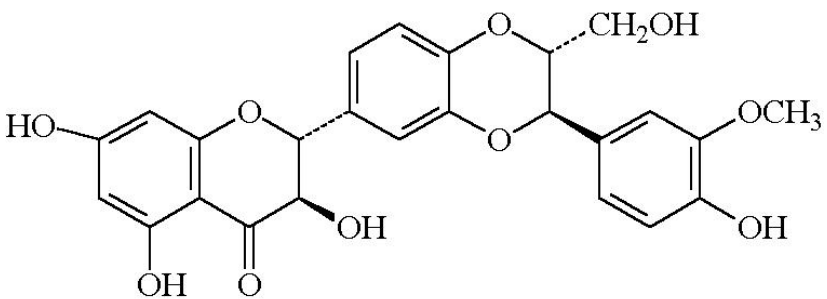
Silybi mariae fructus = Cardui mariae fructus **St. Mary thistle**

deve dikeni, meryemana dikeni

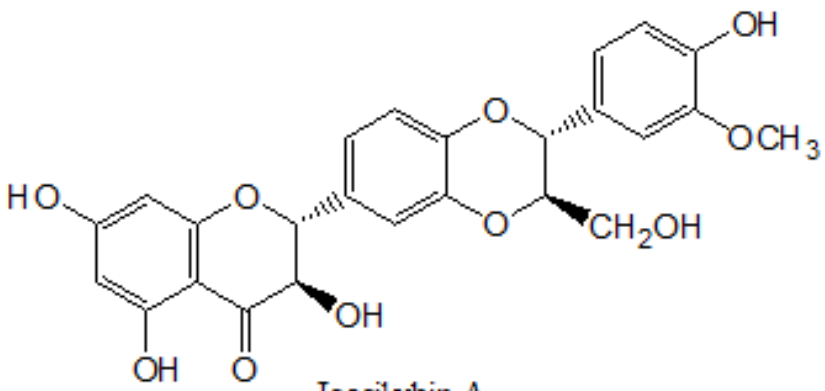
***Silybum marianum* (*Carduus marianus*)** **Asteraceae**

The plant is growing widespread in Cyprus

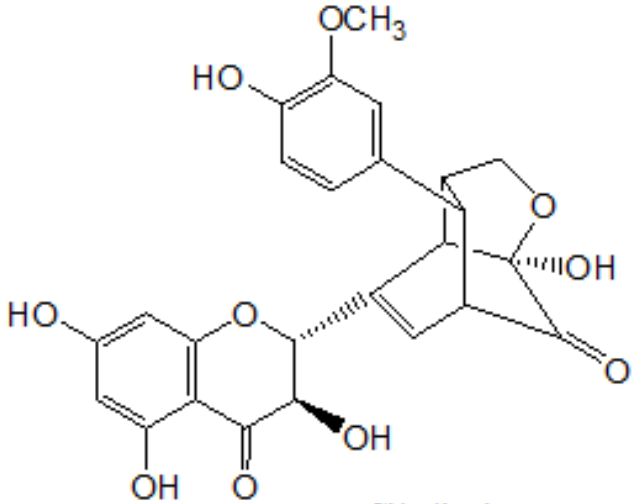
Chemical Composition : The drug contains 20 to 30% lipids, proteins, sugars, flavonoids (taxifolin, quercetin). The constituents responsible for the activity are **flavonolignans** initially isolated as a mixture of a phenylpropanoid alcohol (coniferyl alcohol), onto a 2,3-dihydroflavonol (taxifolin). **This mixture, commonly known as silymarin**, represents 1.5 to 3% of the weigth of the drug. Silybin (silibinin) is the major constituent of this mixture. The other constituents of silymarin are silydianin, silychristin and the simple flavonoid taxifolin.



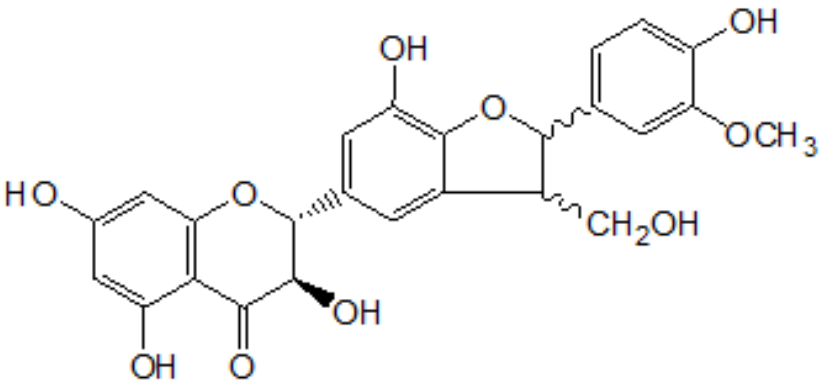
Silybin



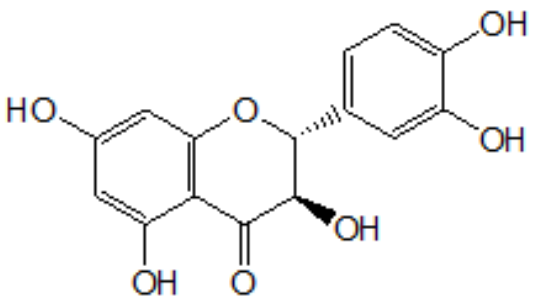
Isosilybin A



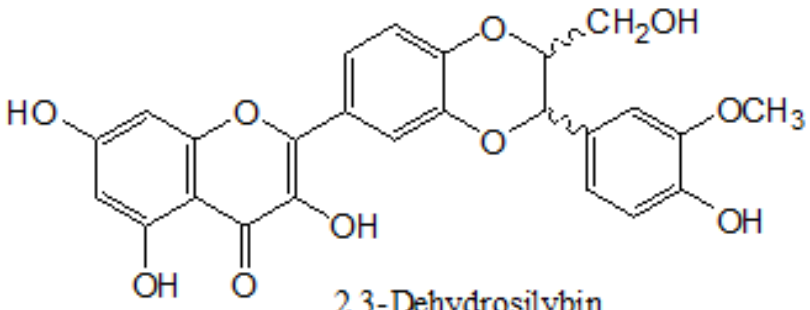
Silydianin



Silychristin



(+)-Taxifolin



2,3-Dehydrosilybin

Pharmacological Activity : Multiple experimental studies tend to demonstrate the **antihepatotoxic** activity of silymarin : prevention of the toxic effects of carbon tetrachloride, galactosamine, and other toxins at the level of the hepatic parenchima, **protection against the harmful effect of phalloidin administered parenterally**. Silymarin inhibits membrane lipid peroxidation, acts as a free radical scavenger, and inhibits the formation of leukotriene. It is thought to have a stabilizing effect on mebranes, and in the case of the *Amanita* toxin, it may compete for binding sites. It is devoid of acute or chronic toxicity and has practically no side effects.

Uses : In Germany and in other European countries, silymarin, or extracts titrated for silymarin are promoted as a treatment, per os for liver damage from poisoning and as an adjunctive treatment for chronic liver disease and cirrhosis; **an injectable form is used to treat *Amanita phalloides* poisoning**. The phytomedicines, containing the extracts of the achenes (fructus), are tradionally used orally for the symptomatic treatment of functional digestive signs thought to have a hepatic origin. The very low water solubility of the flavonolignans makes it unlikely that (very rare used) herbal tea forms have an antihepatotoxic activity.

Amanita phalloides

Reference Books :

Main Book

Bruneton, J., Pharmacognosy, Phytochemistry, Medicinal Plants, TEC & DOC Editions, Paris 1999

Other Books

- Hänsel, R., Sticher, O., Pharmakognosie – Phytopaharmazie, Springer Medizin Verlag, Heidelberg 2010
- Evans, W.C., Trease and Evans Pharmacognosy, Elsevier Limited, Edinburgh, London 2002
- Baytop, T., Farmakognozi I-II, İstanbul Üniv. Yay. No. 2783, Eczacılık Fak. No.29, İstanbul 1980
- Tanker, M., Tanker N., Farmakognozi I-II, Ankara Üniv. Eczacılık Fak. Yay. No. 63, Ankara 1990