Practice No. 6

ANALYSIS OF DRUGS CONTAINING FLAVONOIDS

Flavonoids are a large group of naturally occurring phenolic compounds that are derived from benzopyran (chromane) with a side phenyl substituent at position C-2, C-3 or C4. They often occur in dicotyledonous plants as yellow or red pigments in flowers, leaves and fruits.

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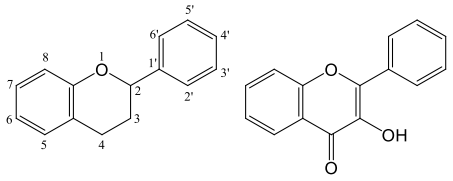


Flavon Flavonol

In the organism they are probably connected to the red-ox. processes, because of their effect on the metabolism of arachidonic acid they have anti-inflammatory and anti-allergic properties, as well as anti-thrombotic and vaso-protective effects. Many plants that contain flavonoids are used as diuretic and spasmolytic drugs e.g. parsley, liquorice.

Flavonoids are relatively very reactive compounds. Their reactivity is due to the location of the reactive group in the molecule. The most reactive flavanoids contain an aglycone derived from a flavonol.

There are two main possibilities of how flavonoids can react, due to presence of an easily dissociable hydroxyl groups in the molecule. The first one is the possibility to change their oxidation state during the hydrogenation or dehydrogenation processes, the second is the possibility of chelate formation.

As a common qualitative reaction, we use a reduction test, in which aglycones and glycosides of flavons, flavonols or flavanons in acidic ethanolic condition, change the color into red to purple in the presence of magnesium or zinc. Zinc and magnesium cause the hydrolytic cleavage of the flavanoid glycosides into aglycones and sugars, the free aglycone changes into a compound similar to anthocyanidins.

Flavonoids form chelate complexes with metals. Formation of the chelate is possible for 3- or 5-hydroxyflavons e.g. the complex of *rutin* with lead is orange and it is insoluble in neutral or slight basic water solutions.

Boron test is based on the reaction of boric acid with flavonols where the hydroxy-group is close to the carbonyl-group. Flavonols form complexes with boron (= two hydroxyl group of boric acid are substituted by an organic residue)

Thin layer chromatography

Tested solution: 1 g of the powdered drug is mixed with 10 ml of methanol and heated for 5 minutes in a water bath at 60 ºC. After cooling down, the extract is filtered.

Reference solution: rutin in methanol

Elution mixture: ethylacetate : formic acid : water (80 : 10 : 10)

Detection reagent: 15 ml of 3% boric acid solution and 5 ml of 10% oxalic acid solution mixed immediately before use!!!

20 µl of both solutions are apllied separately on the TLC plate and it is eluted for a distance of 10 cm. The chromatogram is dried at room temperature, covered by detection reagent and heated for 10 - 15 minutes at 120 °C. Evaluation will be under ultraviolet light. Flavonoids give yellow-green fluorescent spots.

Determination of content of flavonoids by colorimetric methods

Colorimetric methods are based on the color changes which arise when flavonoids react with different metals (chelate). The zirconia and aluminum salts are the most commonly used. Formation of complexes between flavonoids and aluminum chloride is preferable in a solvent which consists of acetic acid and pyridine. In this mixture, there don't form precipitates, which might absorb a part of the active compounds on their surface and finally cause a decrease in the absorbance. Accompanying compounds (e.g. chlorophyll), which can interfere in the determination, can be removed by separating the extract with carbon tetrachloride. The measurement results refer to the standard - rutin, hyperoside or isoquercetin.

Procedure of determination:

0.2 g of the powdered drug is heated with 1 ml of 0.5 % methenamine solution, 20 ml of acetone and 2 ml of HCl in a flask under reflux for 30 minutes. After cooling, the mixture is filtered into a 100 ml volumetric flask using a piece of cotton wool. The drug and cotton wool are returned back to heating flask and heated twice with 20 ml of acetone for 10 minutes. After cooling down, the extracts are filtered into same volumetric flask. The volume is completed to 100 ml by acetone and shaken (solution A).

20 ml of solution A are mixed with 20 ml of water in a separatory funnel and performed liquid-liquid extraction at first with 15 ml and then three times with 10 ml of ethylacetate. The united ethylacetate layers are washed two times by 50 ml of water in a separatory funnel. The ethylacetate layer is filtered over 10 g of anhydrous sodium sulfate into a 50 ml volumetric flask and the volume is completed to 50 ml by ethylacetate (solution B).

Tested solution: 10 ml of solution B is placed into a 25 ml volumetric flask, 1 ml of 2 % aluminum chloride solution in a mixture of acetic acid and methanol (1 : 19) is added and completed by the mixture of acetic acid and methanol (1 : 19) up to mark and shake thoroughly.

Reference solution: 10 ml of solution B is placed into a 25 ml volumetric flask and complete by mixture of acetic acid and methanol (1 : 19).

The absorbance is measured after 30 minutes at 425 nm in 10 mm layer against the reference solution.

The content of flavonoids in percentage is calculated according to following scheme :

$$\frac{A * 1.25}{m}$$

A – absorbance of solution in maximum at 425 nm

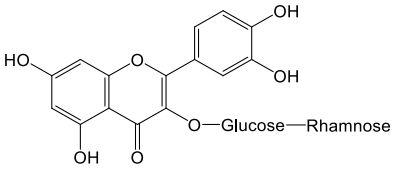
m – weight of drug in grams

Drugs that contain flavonoids

Sambuci flos

Sambucus nigra, *Caprifoliaceae*

Content compounds: quercetin glycosides (rutin, isoquercitrin, hyperoside etc.), amines, essential oils with high content of free fatty acids and n-alkanes, organic acids (chlorogenic acid, *p*-coumaric acid, caffeic acid, ferulic acid and their glycosylesters), traces of the glycoside sambunigrin, esterified triterpens and triterpenic acids, mucilage, tannins



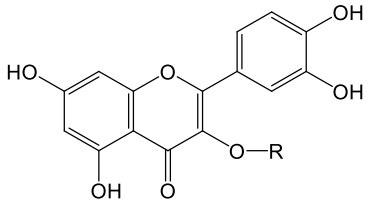
Rutin

Colorimetric determination of the content of flavonoids: calculated as isoquercetin

Tiliae flos

Tilia cordata and *Tilia platyphylos, Tiliacae*

Content compounds: quercetin glycosides (rutin, isoquercitrin, hyperoside etc.), Caempherol glycosides (astragalin, tiliroside), myricetin glycosides, mucilage, tannins, organic acid (chlorogenic acid, p-coumaric acid, caffeic acid), essential oils (content alkanes, farnesol, geraniol and eugenol as a base of smell)

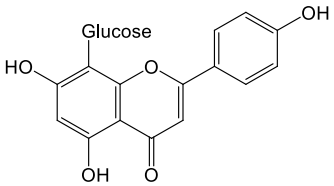


R = H = Quercetin
R = glucose = Quercitrin

Crataegi folium cum flore

Crategus oxyacantha, Crategus monogyna, Rosaceae

Content compounds: flavonoids, especially quercetin and apigenin glycosides (rutin, hyperoside, quercetin-rhamnogalactoside, vitexin), dimeric protoanthocyanidins, (-)/ -epicatechin, mixture of triterpene acids, choline, amines, purines and catechol tannins



Vitexin

Colorimetric determination of the content of flavonoids: calculated as hyperoside.

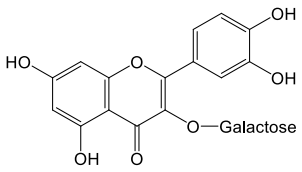
Crataegi fructus

Crategus oxyacantha a *Crategus monogyna, Rosaceae*

Betulae folium

Betula pendula, Betula pubescens, Betulaceae

Content compounds: flavonoids, especially quercetin glycosides (hyperoside, quercetin-3-O-arabinoside, quercitrin, rutin), essential oils, catechin tannins



Hyperoside

Colorimetric determination of the content of flavonoids: calculated as hyperoside

Calendulae flos

Calendula officinalis, Asteraceae

Content compounds: flavonoids, especially quercetin and isorhamnetin glycosides, essential oils, hemolytic active glycosides of oleanolic acid - calendulosides, triterpenic alcohols, carotenoid pigments, phenolic acids, bitter compounds, polyacetylenes
Colorimetric determination of the content of flavonoids: calculated as hyperoside

Propolis

Resinous substances from the buds of some trees or pollen transformed in honey bee stomach (*Apis mellifica*).

Content compounds: more than 100 different compounds, resins, waxes, essential oils, pollen, flavonoids, aromatic aldehydes and alcohols, vitamins, mineral compounds

Identification:

0.7 g of drug is heated with 15 ml of methanol for 15 minutes under reflux and the solution is filtered after cooling down. The filtrate is used for following tests:

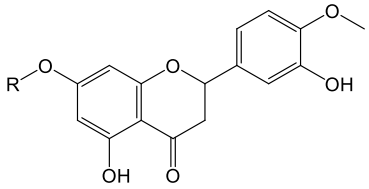
1- 0.1 g of zinc powder and 2 ml of 6 M HCl is added to 5 ml of the filtrate. After a few minutes, the color is changed into red.

2- 0.5 ml of lead acetate solution and 0.5 ml of 6 M ammonia solution is added to 5 ml of the filtrate. It forms an orange precipitate (proof of presence of rutin).

3- 5 ml of the filtrate is dried in a ceramic dish and 3 ml of 3 % boric acid solution and 1 ml of oxalic acid solution are added to the residue. The mixture is dried again and it is left to stand for 5 minutes in a water bath. The residue is extracted by ether. Etheric solution fluoresceinates under yellow-green color.

Aurantii dulce pericarpium

Dried outer pericarp of *Citrus aurantium L.subsp. aurantium*, *Rutaceae*
Content compounds: flavonoid glycosides (hesperidin, neohesperidin, naringin, eriocitrin, rutin), essential oils, hemicelluloses, pectin, starch, vitamin C, carotenoids (violaxanthin)



R = H = Hesperetin
R = rhamnoglucosyl = Hesperidin

Identification

1- Several pieces of the drug are shaken with 5 ml of diluted sodium hydroxide solution in a tube; the color solution is changed into intensive yellow color (hesperidin)

2- Several pieces of the drug is shaken with 5 ml of concentrated sulfuric acid; the color of the solution is changed into yellow, and when slight heated, it changes into red-brown (hesperidin)