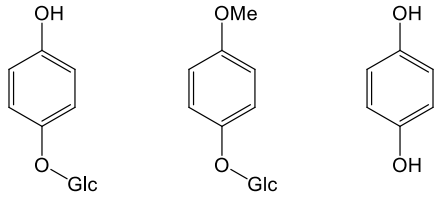
Practice No. 3

ANALYSIS OF DRUGS CONTAINING PHENOLIC GLYCOSIDES

Phenolic glycosides are a subgroup of a large group of natural compounds (glycosides). Glycosides are usually formed of two parts that are linked through a glycosidic linkage; one part is the sugar component, and the other part is the non-sugar aglycone (genin). In the case of phenolic glycosides, the aglycone is a derivative of monovalent, divalent or trivalent phenols or their homologues.

Uvae ursi folium - Bearberry leaf

The whole or cut sheet type of *Arctostaphylos uva-ursi L., Ericaceae*
Content compounds: methylarbutin and arbutin glycosides, which are hydrolyzed by dilute acids to hydroquinone, tannins, flavonoids and bitter substances.



Arbutin Methyl arbutin Hydroquinone

Myrtille folium - Blueberry leaf
Vaccinium myrtillus L. Vacciniaceae

Vitis idaeae folium - Cranberry leaf
Vaccinium vitis idaea L. Vacciniaceae

Identification

The qualitative analysis of phenolic glycosides is based on the fact that aglycones result from the hydrolysis by acids, which gives the appropriate response. For example, hydroquinone reduces ammoniacal $AgNO_3$ solution to the metallic silver. In addition, the aglycone reacts with molybdates and tungstates to form blue-green reduction products.

1- 0.5 g of the powdered leaves is boiled with 5 ml of water and the decoction is filtered after cooling. To 1 ml of the filtrate in a porcelain bowl, 4 ml of ammonium hydroxide and 1 ml of 10% solution of phosphomolybdic acid is added. There is a dark blue precipitate (arbutin).
2- 0.5 g of the drug is boiled with 5 ml of water and the decoction is filtered after cooling. To the filtrate add 1 drop of ferric chloride solution, there is a dark blue precipitate (tannins).

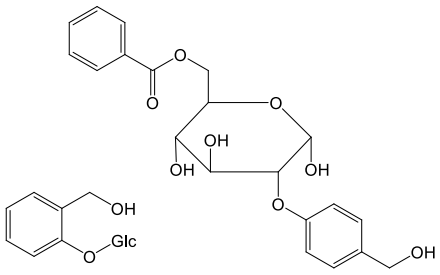
3- A leaf fragment is inserted into the solution of vanillin in concentrated hydrochloric acid, turns into intensive red (*Vaccinium myrtillus* and *Buxus sempervirens* are almost colorless).
4- The powdered drug is moistened by a drop of conc. HCl and subjected to microsublimation. At the temperature of 120-130 ° C hydroquinone sublimates. If a drop of ammoniacal $AgNO_3$ solution is added to the sublimate, the color turns black because of the reduced silver (hydroquinone) (it is done on one half of the slide only).
5- The second half of the sublimate on the slide is dissolved in 1 ml of hot water and a few drops of ferric chloride solution are added, there is a blue-green color, which quickly turns into brown-yellow color (hydroquinone).

Microsublimation
0.1 g of the powdered drug is moistened by 1 drop of conc. HCl and placed on the center of a watch glass with a diameter of about 7 cm. The watch glass is covered by a flat glass plate (it is possible to use microscopic slides that are closely packed together). The edge of glass plate should extend about approximately 1 cm beyond the edges of the watch glass. The beaker with cold water is placed on top.Tthe watch glass is heated on asbestos net using a light flame (minimally 10 cm under the net). It is possible to remove the glass plate a few times to see the changes during the microsublimation. The microsublimate is investigated under the microscope immediately after cooling down or it is used for the following experiments.

Salicis cortex – Willow cortex

genus *Salix*, *Salicaceae*

Content compounds: tannins, flavonoids, phenolic glycosides - salicin, populin and fragilin.



Salicin Populin

Identification

1- 10 ml of water and 0.5 g of powdered drug is mixed and boiled for a few minutes. The decoction is filtered after cooling down. To the filtrate 1 drop of ferric chloride solution is added. It forms brown-green precipitate (tannins).

2- The powdered drug is moistened by conc. H_2SO_4. The color is change to red (salicin).

Thin layer chromatography for phenolic glycosides

Thin layer chromatography on silica gel – A proof of the presence of hydroquinone

Tested solution: 0.1 g of the powdered drug is moistened by 1 drop of conc. HCl and sublimate at 160 °C for 15 minutes. The dry hydroquinone is dissolved in a small volume of methanol.

Reference solution: hydroquinone in methanol

Elution mixture: chloroform : methanol (95 ml : 5 ml)

Detection reagent I: phosphomolybdic acid

Detection reagent II: 20% solution of sodium bicarbonate

8-10 μl of the investigated and reference solutions are applied on a silica gel layer and it is left to elute for distance about 10-12 cm from the starting point. The layer is dried at room temperature and covered by detection reagent I and after 3 minutes by detection reagent II (stabilization of color).

Thin layer chromatography on silica gel – A proof of the presence of arbutin

Tested solution: 0.5 g of powdered drug and 5 ml of a mixture prepared from same volumes of methanol and water are mixed and heated under reflux condenser in a water bath for 15 minutes. The hot mixture is filtered and 0.2 ml of lead acetate solution is added to the filtrate and it is filtered again.

Reference solution: arbutin in methanol

Elution mixture: ethylacetate : methanol : water (100 : 17 : 13)

Detection reagent I: diazotized sulfanilic acid

Diazotized sulfanilic acid is prepared by mixing a few crystals of $NaNO_2$ and 5 ml of sulfanilic acid immediately before use!!!

Detection reagent II: ethanolic solution of KOH

10 μl of the investigated and reference solutions are apllied on TLC plate and it is left to elute for distance about 10 cm from the start. The layer is dried at room temperature and covered by detection reagent I and after 5 minutes by detection reagent II. The red spots emerge at the place where arbutin is located.

Quantitative determination of arbutin

The pharmacopoeial method for the quantitative determination of arbutin in drugs uses the reaction of divalent phenols with *p*-diazosulfanilic acid in an alkaline environment; it forms red azo-color. Arbutin can also be determined by the titration with iodine.

Determination of arbutin by titration

Weigh 0.500 g of the drug accurately on an analytic balance is mixed with 60 ml of water, boiled for 1 hour and the lost volume of water is always substituted. After cooling down the extract is filtered using a suction filter. The rest on a filter paper is washed by a small amount of water and added to the filtrate. To the filtrate in a 100 ml volumetric flask is added 10 ml of 3% lead acetate solution, it shaken and completed to 100 ml by water. The liquid is heated for a few minutes in a water bath and when the precipitate becomes condense, it is filtered using a suction filter. Then 0.5 ml of conc. sulfuric acid is added to the transparent filtrate and it is placed in a drying oven for 1 hour. The solution is filtered again and 0.1 g of zinc powder is added and shaken for 15 minutes. Solution is neutralized to litmus using 2 g sodium bicarbonate, filtered again after complete dissolving. 3 ml of starch solution is added to 50 ml of the filtrate (a half of starting material). It is diluted to approximately 250 ml by water and carefully titrated by 0.05 M iodine solution until a blue color appears which stays stable for 1 minute.

1 ml of 0.05 M of iodine solution accounts for 0.01361 g of arbutin and free hydroquinone, calculated as arbutin.

Calculate the percentage of arbutin in given drug