Practice No. 7

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ANALYSIS OF DRUGS CONTAINING SAPONINS

Saponins are a group of glycosides which are characterized by their ability to form foamy solutions with water (the name is derived from the latin word sapo = soap), they are also characterized by their hemolytic activity.

Depending on the structure of aglycone or sapogenin, saponins can be divided into two large groups – steroid saponins (sarsapogenin, diosgenin, digitonin etc.) and pentacyclic triterpenoid saponins (quillaic acid, glycyrrhizic acid, primula-saponin etc.).

The following reactions are used for the identification of saponins:

Precipitation reactions

Saponins form clots with reagents, such as magnesium hydroxide, barium hydroxide, copper and lead salts, as well as with cholesterol, Nessler's reagent etc.

Determination method:

About 0,5 g of saponin containing drug is dissolved in 2 ml of water and mixed with 2 ml of the reagent in a test tube to form clots.

Color reactions

The most commonly used agents are concentrated sulfuric acid or a mixture of equal parts of concentrated sulfuric acid and ethanol. Dehydrogenation and the emergence of other double bonds usually occurs during these reactions. The disadvantage of the listed precipitation and color reactions is that they are not specific for saponins. These reactions also give positive results with other triterpenes and steroids present in the drug. Therefore, saponins need to be isolated from the drug first (eg. by microsublimation) for the reaction to take place and colors to be visible.

Salkowski reaction

We subject one of the drugs containing saponins (Flos primulae) to microsublimation. We dissolve the microsublimate in 2 ml of chloroform and add few drops of concentrated sulfuric acid. Yellow color appears, and later passes into various shades depending on the drug used.

Liebermann - Burchard reaction

Heat approximately 5 mg of the saponin drug with 1 ml of acetic anhydride, then add several drops of concentrated sulfuric acid. Yellow color appears, later turning into red shades.

Rosenthaler reaction

Approximately 5 mg of the saponin drug produces red to purple color with a 1% solution of vanillin in concentrated hydrochloric acid. The reaction must be heated.

The evidence of saponins on blood agar

The characteristic feature of saponins is the ability to induce hemolysis of red blood cells. Hemolytic activity is not an exclusive property of saponins, although in many cases it is the only guideline for identification and quantitative evalutation. When coming into contact with saponins, red blood cells are hemolyzed and hemolysis (release of hemoglobin) takes place. When performing *in vitro*, the initially cloudy opaque suspension of erythrocytes changes into a clear, bright red solution and the non-hemolyzed blood cells settle at the bottom of the tube during the partial hemolysis.

Hemolysis can also be performed on blood gelatin or on blood agar. Saponins extracted from the drug diffuse into the gelatin or agar and cause the hemolysis of blood cells, resulting in brightening.

Determination method:

1,00 g of the powdered drug is boiled in a test tube with 10,0 ml of isotonic phosphate buffer. After cooling the mixture is filtered. Cork borer is used to carve openings with a diameter of approximately 10 mm into the agar plate. These openings are filled with the drug filtrate (up to 3 drops). Saponines diffuse into the blood agar and they creates the haemolytic effect. Its size will be evaluated after 2 and 24 hours.

Determination of the foaming number according to Kofler

One of the most striking properties of saponins, which makes them different from other glycosides, is the surface activity. Saponin solutions can greatly reduce the surface tension compared to water, and are characterized by strong foaming. The surface activity of their solutions decreases with concentration and is highly dependent on the accompanying ingredients. Therefore, evaluation of saponin drugs based on the surface tension of their extracts is only of limited use, on a relative scale.

The **foaming number** is a value, which can be used for approximately expressing the quality of the saponin drugs. The number indicates the largest dilution of the drug extract, which creates a ring of foam of 1 cm height in the tube after shaking.

Drugs that contain Saponines:

Saponariae radix

Saponaria officinalis, Caryophylaceae
Content compounds: a mixture of saponins known as saporubrin, the main saponins are saponoside A and D, the main sapogenin is quillaic acid.

Senegae radix

Polygala senega, Polygalaceae
Content compounds: a mixture of saponins = senegin (saponins with an aglycone derived from presenegin), the fresh root contains the phenyl glycoside primveroside, lipids, di- and tetra-carbohydrate esters, salicylic acid, valeric acid methylester, traces of essential oils.

Herniariae herba

Herniaria glabra, Herniaria hirsuta, Caryophylacea

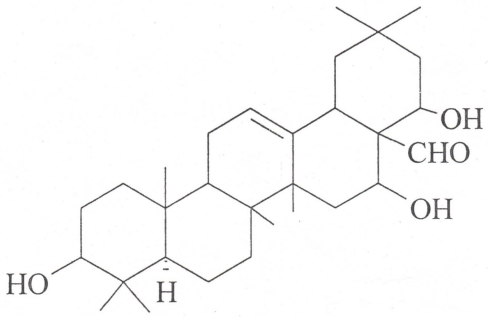
Content compounds: neutral saponins (a mixture of medicagenic and gypsogenic acid derivatives etc.), acidic saponins, flavonoids, coumarins, (umbelliferone and herniarin = methylumbelliferone), tannins.

Primulae radix

Primula veris, Primula elatior, *Primulaceae*
Content compounds: triterpenic saponins with aglycones derived from priverogenin A and B, primulagenin etc., phenolic glycosides (primulaverin), carbohydrates, tannins.

Primulae flos

Primula veris, Primula elatior, *Primulaceae*
Content compounds: triterpenic saponins with aglycones derived from primulaverin A and B, primulagenin etc., essential oils.



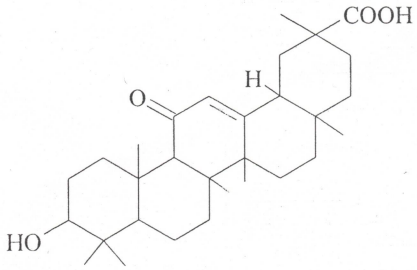
priverogenin A

Identification

Perform the proof of presence test of saponins on blood agar for the above selected drugs.

Liquiritiae radix

Glycyrrhiza glabra, Fabaceae
Content compounds: triterpenoid saponins, the main compound is glycyrrhizin (calcium and potassium salt of glycyrrhizinic acid, whose aglycone is the glycyrrhetinic acid), other glycosides are derived from liquiritic acid and glabrinic acid, besides saponins it contains also flavonoids (liquirtin, isoliquirtin), carbohydrates and polysaccharides.



Glycyrrhetinic acid

Identification

1- Approximately 0,5 g of powdered drug is boiled with 5 ml of water and is filtered after cooling, the filtrate foams by shaking and the foam is still visible within 1 hour.
2- 5 ml of concentrated sulfuric acid are added to 0,05 g of powdered drug, both drug and the acid turn orange, later red (glycyrrhizin).
r aP 2,0 ml of 50% sulfuric acid solution is poured over 0,01 – 0,02 g of powdered drug. Acid turns orange-yellow within 30 minutes (flavonoid glycoside liquirtin)

Determination of glycyrrhizinic acid content by titration

Weigh 2,0 g of the radix liquiritiae drug on an analytical scale, mix it in the 100 ml boiling flask with 20 ml of 3% acetone nitric acid solution and heat in a water bath whose temperature must not exceed 60°C under a reflux condenser for 10 minutes. The cooled extract is filtered using a Büchner funnel. Extraction by the acetone solution in the water bath is repeated three more times. The drug is then washed with such amount of acetone, that the total volume of the acetone extract is 100 ml. The extract is then poured into 200 ml beaker. 40 ml of 60% ethanol is added and while continuously stirring, another 10 ml of 25% ammonia is slowly added too. After mixing, the mixture is cooled with ice to 15°C. The resulting precipitate (ammonium glycyrrhizin) is filtered using a suction filter and washed with acetone until the flowing acetone is colorless (50 – 100 ml). The precipitate is left to dry in the air on a filter and then dried further in an oven at 60°C. The dried precipitate is completely dissolved in 50,0 ml of water and then 20 ml of neutral formaldehyde is added to the aqueous solution. Titration is carried out using 0,1 M sodium hydroxide solution until phenolphtalein turns red.

Calculate the percentage of glycyrrhizinic acid in the drug.

1 ml of 0,1 M sodium hydroxide solution is equivalent to 0,0273 g of glycyrrhizinic acid.

Ononidis radix

Ononis spinosa agg., Fabaceae
Content compounds: flavonoids, essential oils, tannins, organic acids

Identification
1- Sublimate, often consisting of twisted, radially arranged needles, which turn blue-violet (onocol) after a while when drops of ethanolic solution of vanilin are poured on it, arises through microsublimation at about 220°C (ČsL 4).
2- Cross-section of the root is moistened with a drop of diluted ammonia solution, it turns yellow.

Thin-layer chromatography on silica gel (CL 2002)

Tested solution: 1 g of powdered drug is mixed with 5 ml of methanol and heated for 2 minutes on a water bath. After cooling, it is filtered.

Reference solution: 40 mg of cholesterole is dissolved in 5 ml of chlorofom.

Developing mixture: 96% alcohol : toluene : chloroform ~~(10: 40: 40)~~ 10 : 45 : 45

Detection reagent: anisaldehyde II solution (add 90 ml of 96% ethanol to 10 ml of anisaldehyde, mix, add 10 ml of sulfuric acid and mix again).

Apply 20 µl of both solutions and develop over a distance of 15 cm on the layer. The layer is dried on air and observed under UV light. Spots of standards are visible on the the chromatogram of the reference solution. There is an intense blue stain and other less intense spots on the chromatogram of the tested solution at 365 nm.

spray with the detection reagent and dry for 5 to 10 minutes in an oven at 100 – 105°C. The purple spot on the chromatogram of the tested solution is (onocol). There are two spots on the chromatogram of the reference solution, on is gray-violet (vanillin) and the other is red (resorcinol).

Verbasci flos

Verbascum phlomoides, Verbascum densiflorum, Scrophulariaceae
Content compounds: saponins, flavonoids, iridoids, carotenoids, mucilages, carbohydrates, essential oils

Thin-layer chromatography on silica gel (CL 2002)

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

Reference solution: 5 mg of rutin and 5 mg of hyperoside is dissolved in 5 ml of chloroform.

Developing mixture: formic acid, ~~acetic acid~~, water, ethyl acetate (~~11: 11: 27: 100~~) BUTANOL 40 : 10 : 30 : 50

Detection reagent: 15 ml of 3% boric acid solution and 5 ml of 10% oxalic acid solution

We apply 20 µl of the reference solution and the sample separately. The layer is dried on air, sprayed with detection reagent and dried for 5 – 10 minutes in an oven at 100 – 105°C. There is a stain corresponding to rutin by location and color, and another one, corresponding to hyperoside by location. Immediately above and below this spot are two spots fluorescing orange, in the vicinity of the head is an intense yellow stain and under this one, there is another one, fluorescing in blue.