Class 4

Phytochemical analysis of raw materials containing flavonoids.

PURPOSE OF THE STUDY:

1. To master the techniques for the isolation of simple phenols, anthracene derivatives and flavonoids.

2. To learn to carry out qualitative reactions on these groups of substances.

3. To study the methods of quantitative determination of simple phenols, anthracene derivatives and flavonoids in medicinal plant raw materials.

QUESTIONS:

1.Chemical classification of flavonoids using basic structural formulae.
2.Physico-chemical properties of flavonoids.
3.Methods of isolation from plant raw materials.
4.Qualitative analysis and quantitative analysis of raw materials containing flavonoids.
5.Formulas: flavan, catechin, leucocyanidin, anthocyanidin, flavanonol, flavanone, flavone, flavonol, chalcone, dihydrochalcone, auron, isoflavone, quercetin, rutin, hyperoside, luteolin, apigenin, kaempferol, naringenin, avicularin, sulfuretin.

Work 1. Qualitative analysis of raw materials containing flavonoids.

1 g of dried and crushed raw material of bird's throat (or St. John's wort/ immortelle) and 20 ml of ethanol put into a flask and heated on a water bath to boiling. Shake the flask several times, cork it and leave it for 0.5-1 hour, stirring its contents from time to time. Filter the extract through cotton wool and carry out qualitative reactions.

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

Synod test (cyanidine test)

Add 3 drops of concentrated hydrochloric acid to two test-tubes containing equal quantities of the extract. Then in one of the test tubes add a few grains of magnesium or zinc. Heat both test tubes on a water bath to boiling and leave for 5 to 10 minutes. If flavonoids are present, an orange or bright red colouration appears in the test tube with magnesium. If anthocyanins, chalcones, aurones and catechins are present in the extract, they give colouring with concentrated acid without the addition of metallic magnesium due to the formation of oxonium salts.

Flavones usually give dim orange-red colouring, flavonols and flavanones develop deep pink, scarlet or crimson colouring.

The colouring develops because of the reduction of flavones and flavonols to anthocyanidins, which form coloured oxonium salts in an acidic medium.

test with 1% alcoholic solution of aluminium chloride.

To 0.5 ml of the alcoholic extract add a few drops of the reagent. Flavonoids form a yellow colouring (yellow-green).

alkaline reaction

To 0.5 ml of alcoholic extract add a few drops of 10% alcoholic alkali solution. Flavones and flavonols dissolve in alkalis to form a yellow colour. Chalcones and aurones immediately form red or purple solutions with alkalis (this reaction is very specific for them).

sample with 0.5 % alcoholic ferric chloride solution

Ortho-dioxyphenolic groups in flavonoid molecules cause green colouring, and trioxyphenolic groups in the row position cause blue colouring.

reaction with basic lead acetate solution

Add 3-5 drops of 2% basic lead acetate to 1 ml of the extract. The appearance of yellow-orange colouration indicates the presence of flavonoids.

boron-citric acid reaction

5-oxyflavones and 5-oxyflavanols interact with boric acid in the presence of citric acid (or oxalic acid) to form a bright yellow staining with yellow-green fluorescence.

reaction with ammonia solution

Flavones, flavanones, flavonols and flavanonols give a yellow colouration when heated, turning orange or red. Chalcones and aurones immediately give red or purple colouring. Pure catechins do not give colouring, but the presence of even a small amount of impurities (oxidation products) causes the appearance of yellow colouring. Anthocyanins in the presence of ammonia or sodium carbonate give blue or violet colouring.

reaction with 1% vanillin in concentrated hydrochloric acid

Catechins form a red-maple colouring (derivatives of floroglucin and resorcin).

Write down the results of the reactions in the workbook and conclude about the presence of flavonoids in the analysed raw materials.

Work 2. Quantitative analysis of raw materials containing flavonoids.

Study the methods of quantitative determination of the studied groups of BAS in medicinal plant raw materials. Fill in the table for raw materials: blue cornflower, hawthorn, immortelle, horsetail, wheatgrass, St John's wort, motherwort, violet.

Methods for the quantitative determination of active substances

Medicinal plant raw material	Biologically active substances	Method of determination
Blue cornflower flowers		
Hawthorn flowers		
Hawthorn fruit		
Sand immortelle flowers		
Horsetail herb		
Horsetail herb		
St John's wort		
Motherwort herb		
Violet herb		

SELF-MONITORING EXERCISES:

1. Which qualitative reactions are specific for flavonoids?

2. Which qualitative reactions can be used for the quantitative determination of flavonoids?