**Topic of the lesson:**

**Qualitative and quantitative analysis of medicinal plant raw materials containing various groups of biologically active substances (flavonoids)".**

**Aims:**

**1.To master the methods of qualitative and quantitative analysis of medicinal plant raw materials containing flavonoids.**

**2.To learn how to carry out qualitative reactions on flavonoids in order to identify this group of biologically active substances.**

**3. To learn how to conduct quantitative analysis of flavonoids by spectrophotometry.**

Work 1.Carrying out qualitative reactions for determination of flavonoids of medicinal plant raw material "Hyperici herba".

Take herb from your teacher for a sample of raw material.

Carry out the extraction of the amount of flavonoids using the following procedure: 3 g of dried and crushed raw material and 30ml of 70% ethanol are placed in a 100ml volumetric flask and extraction in a water bath with reflux condenser for 10-15 minutes. The extraction is cooled down, filtered through The extraction is cooled down, filtered through cotton wool and subjected to a qualitative reaction.

For identification of flavonoids in medicinal plant raw materials carry out the following reactions:

1. Sinode assay (cyanidine assay)

Two test tubes with the same amount of extract are taken, equal amount of extract and add 3 drops of concentrated acid in each tube of concentrated hydrochloric acid. Then in one of the test tubes a few rounds of magnesium or zinc grains. Both test tubes are heated in a water bath to boiling point and left for 5 - 10 minutes.

They are allowed to stand for 5 - 10 minutes. If there are flavonoids, an orange or bright red colour appears in the test tube with magnesium.

If anthocyanins are present in the extract anthocyanins, chalcones, aurones and catechins are present in the extract, they discolour due to the formation of oxonium salts.

Flavones generally give off orange-red colouring, the flavonols and flavanones develop a deep pink, scarlet or crimson colours.

The colouring develops as a consequence of the flavones and flavonols being reduced to anthocyanidins, which form coloured oxonium salts in an acidic environment.

2. Trial with 5% Alcoholic aluminium chloride solution.

To 0.5 mL of the alcoholic extract a few drops of aluminium chloride solution and a few drops of the reagent are added to a 0.5 ml alcohol extract. Flavonoids with 2 oxy groups at the C3 and C5 positions will form a citric yellow staining (yellow-green).

3.Reaction with an alkali .

To 0.5 mL of the alcoholic a few drops of a 10% alcoholic alkali solution are added to 0.5 ml of an alcohol extract. The flavones and flavonols dissolve in alkalis, producing a yellow colour. Chalcones and aurones immediately form red or purple solutions with alkalis (this reaction is very specific for them).

4. A test with 1% alcoholic ferric chloride solution

To 1 ml of filtrate 2 - 3% drops of a 1% ferric chloride solution are added to a mL of filtrate. The orthodioxyphenolic groups in flavonoid molecules cause the green colouring, whilst the trioxyphenolic groups in the nick positions cause the blue colouring at basic position cause their blue colouring.

5.Reaction with basic lead acetate solution

To 1 mL of extraction 3-5 drops of 2% basic lead acetate are added. The appearance of a yellow-orange staining flavonoids.

6.Boron-lemon 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.

7.Reaction with ammonia solution.

Add 3-5 drops of reagent to 1ml of filtrate. Flavones, flavanones, flavonols and flavanonols turn yellow when heated to orange or red. Halcones and aurones immediately give red or purple colouring. Pure catechins do not stain, but the presence of even a small amount of impurities (oxidation products) causes yellow

staining. Anthocyanins in the presence of ammonia or sodium carbonate give blue or purple colouring.

8.Reaction with 1% vanillin in concentrated hydrochloric acid

Catechins form a red or crimson colouring (derivatives of floroglucine and resorcinol).

Write the results of the test in the form of a protocol.

Work 2. Determining the moisture content of the medicinal plant material "Hyperici herba".

Take a sample of the raw material from the teacher for analysis.

Carry out moisture determination and calculate the percentage of moisture content in the sample of raw material to be examined.

Compare the result with the pharmacopoeial article "Hyperici herba". Draw a conclusion.

Write the results of the study in the form of a protocol.

Work 3. Carry out quantitative analysis of flavonoids of medicinal plant raw material "Hyperici herba".

Study the method of quantitative determination of flavonoids by spectrophotometry in medicinal plant raw material.

Take a sample of raw material from the teacher for analysis.

Determine the amount of flavonoids in terms of rutin according to the method and calculate their percentage in the studied sample of raw materials.

Compare the result with the pharmacopoeial article "Hyperici herba". Write a conclusion.

The results of the study write in the form of a protocol.

**Protocol of analysis of medicinal raw materials**

**Date**_____

Medicinal raw material
Eng/Lat._____
_____
Medicinal plant Eng/Lat._____
_____
Family Eng/Lat_____

**Results of qualitative reactions:**

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**Determination of moisture:**
Regulatory moisture content:
Calculations:

**Quantitative determination of flavonoids:**
Standardisation of raw materials according to normative documentation:
Calculations:

**Conclusion**:_____
_____

**Method of quantitative determination of flavonoids by spectrophotometry**

Quantitative determination of the amount of flavonoids is carried out according to the method FS.2.5.0015.15 “Hyperici herba”

Sampe of 1 g crushed raw materials to a particle size of 1 mm, placed in a 100 ml volumetric flask and add 30 ml of ethyl alcohol 50%. The flask is attached to a reflux condenser and heated in a water bath for 30 minutes from the boiling point of the extraction mixture. The hot extraction is filtered through a paper filter into a 100 mL volumetric flask so that the raw material particles do not get on the filter. Add 30 mL of 50% alcohol to the extraction flask. The extraction is repeated twice more and the extraction is filtered into the same flask. After cooling, the volume of the extraction is brought to the mark with 50% alcohol and stirred (test solution A).

Then 1 ml of the obtained test solution A is placed in a 25 ml volumetric flask, 2 ml of 2% alcoholic aluminium chloride solution is added, the volume of the solution is brought to the mark with 50% alcohol and stirred (solution B of the test solution).

At the same time a reference solution is prepared in the same way - in a 25 mL volumetric flask 0.1 mL of acetic acid diluted 30% is added to 1 mL of the test solution A and the volume is diluted to the mark with 50% alcohol.

The flask is left in a dark place for 40 minutes to allow the complexation reaction to proceed.

The optical density is measured by spectrophotometric method in the wavelength range of 200 - 600 nm.

The flavonoid content is determined in terms of the flavonoid whose spectral characteristics were similar to those of the maximum absorbance of the alcohol extraction obtained, using the specific absorbance index (rutin).

The amount of flavonoids is determined according to the following formula:

$$X = \frac{A_x \times 100 \times 25 \times 100}{A_{1см}^{1\%} \times \alpha \times 1 \times (100 - W)}$$

where $A_x$ is the optical density of the test solution;

$A_{1см}^{1\%}$ - Specific absorption index of rutin complex with aluminum chloride at a wavelength of 415 nm, equal to 248 (for the raw material Hyperici herba);

α - weight of the test raw material, g;

W - moisture content, %.