Topic of the lesson:

«Qualitative and quantitative analysis of medicinal plant raw materials containing various groups of biologically active substances (tannins) »

Aims:

1. to learn the methods of qualitative and quantitative analysis of medicinal plant raw materials containing tannins.

2. To learn how to conduct qualitative reactions on tannins in order to identify this group of biologically active substances.

3. to learn how to conduct quantitative analysis of tannins by titrometry.

Work 1. Qualitative reactions for the detection of tannins in the medicinal plant material "Oak bark".

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

Proceed with the extraction of the sum of tannins according to the following procedure: 5 g of crushed raw material is poured into 100 ml of boiling water, heated in a water bath for 15 min, filtered through a folded filter. Qualitative reactions are carried out with the filtrate.

To identify tannins in medicinal plant material, carry out the following reactions:

1.General qualitative reactions.

a) Precipitation with gelatin. To 3-5 ml of extraction add 2-3 drops of a 1% gelatin solution in 10% sodium chloride solution. In the presence of tannides a precipitate or turbidity appears from the formed gelatinanthannates (look at the black background, comparing to the broth), soluble in an excess of the reagent.

b) Reaction with potassium bichromate. To 3-5 ml of extraction add 2-3 drops of 5% potassium bichromate solution. If tanides are present, a darkening of the solution or a yellow-brown precipitate is observed.

c) Precipitation with basic acetic acid lead. To 3-5 ml of extraction add a solution of lead basic acetic acid. A precipitate precipitates in the presence of tannides.

2.Reactions for the discrimination of groups of tannides.

a) Colour reaction with 3-valent iron salts: Add 3 drops of 1% ironammonium alum solution to 2-3 ml of the broth. Hydrolysable tannins give a black-blue colouring and condensed tannins give a black-green colouring.

b) Sample with bromine water. Add a few drops of bromine water to 5 ml of extraction and bring the liquid to the boil (under draft!). The results are ascertained after 5 minutes. Condensed tannins give an immediate yellow-orange precipitation with bromine water. Hydrolysable tannides form soluble compounds which precipitate only with an excess of bromine and gradually.

c) Assay with medium lead acetate in acetic acid medium. To 3 ml of extraction add 6 ml of 10% acetic acid and 3 ml of 10% solution of medium lead acetic acid. In the presence of hydrolysable tannides a white precipitate precipitates. Filter the precipitate and add 10 drops of 10% ferric-ammonium alum solution and 0.5 g sodium acetate to the filtrate (do not shake!). If the raw material contains condensed tannins, the filtrate turns black-green.

d) Sampling with formaldehyde and concentrated hydrochloric acid. Add 10 ml of 40% formaldehyde solution and 5 ml of concentrated hydrochloric acid to 50 ml of extraction and boil for 30 minutes in a reflux condenser flask. Observe whether a brick red precipitate forms; its appearance indicates the presence of gallic acid or tannins of the condensed group. After cooling down, filter the liquid, pour 10 ml into a test tube, add 1 ml of 1% ironammonium alum solution and a few pieces of crystalline or fused sodium acetate (do not shake the solution after adding sodium acetate). If tannins of the hydrolysable group are present, the liquid near the sodium acetate pieces will turn blue or violet.

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

Work 2. Determination the moisture content of the medicinal plant material "Oak bark".

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

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

Compare the obtained result with the data of the pharmacopoeial article "oak bark". Write a conclusion.

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

Work 3. Quantitative analysis of the amount of tannins in terms of tannin of medicinal plant material "Oak bark".

Study the method of quantitative determination of the amount of tannins by permanganatometric method (method 1) in medicinal plant raw materials.

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

Determine the amount of tannins in terms of tannin according to the method and calculate the percentage of tannins in the sample of raw material to be tested.

Compare the result with the pharmacopoeial article "oak bark". Write a conclusion.

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

Method for the quantitative determination of tannins in terms of tannin (method 1).

Quantitative determination of tannins is carried out by permanganatometric method according to the method of GF XIV.

2 g (exact sample) of crushed raw materials, sifted through a 3 mm sieve with holes, placed in a conical flask with a capacity of 500 ml, pour 250 ml of water heated to boiling and boil with a reflux condenser on an electric cooker with a closed spiral for 30 minutes under periodic stirring. The obtained extraction is cooled to room temperature, filtered through cotton wool into a 250 ml volumetric flask so that particles of the raw material/precipient do not fall into the flask, add water to the solution to the mark and stir. 25.0 ml of the obtained water extract is placed in a 1000 ml conical flask, add 500 ml of water, 25 ml of indigo sulphonic acid and titrate under constant stirring with 0.02 M potassium permanganate solution until the colour turns golden yellow.

In parallel, a control experiment is carried out: 525 ml of water, 25 ml of indigo sulphonic acid solution is placed in a 1000 ml conical flask and potassium permanganate solution is titrated with 0.02 M solution under constant stirring until the colour turns golden yellow. 1 ml of potassium permanganate solution of 0.02 M corresponds to 0.004157 g of tannins in terms of tannin. The content of the amount of tannins converted into tannin in absolutely dry raw materials in percentage (X) is calculated by the formula:

$$X = \frac{(\mathrm{V}-\mathrm{V1})*0{,}004157*250*100*100}{\mathrm{m}*25*(100-\mathrm{W})}$$

where V - the amount of potassium permanganate 0.02 M solution consumed in the titration of aqueous extract, ml,

V1 - the amount of potassium permanganate 0.02 M solution consumed in the titration of the control experiment, ml;

0.004157 - the number of tannins, corresponding to 1 ml of potassium permanganate 0.02 M solution (in terms of tannin), g;

a - weight of raw material or medicinal plant preparation, g;

W - moisture content of medicinal plant raw material or medicinal plant preparation, %;

250 - total volume of aqueous extract, ml;

25 - volume of aqueous extract taken for titration, ml.

Protocol of analysis of medicinal raw materials

Date_____

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

Results of qualitative reactions:
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Determination of moisture:
Regulatory moisture content:
Calculations:

Quantification of tannins in terms of tannin:
Standardization of raw materials according to normative documentation:
Calculations:

Conclusion:_____
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