Lesson 9.

Topic of the lesson: «Quantitative and qualitative analysis of medicinal raw material containing different groups of biologically active substances (polysaccharides)».

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

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

3. To learn how to perform quantitative analysis of polysaccharides by spectrophotometry.

Work 1. Qualitative analysis for polysaccharides of the medicinal plant material "Elecampane rhizomes and roots".

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

To identify polysaccharides (inulin) in medicinal plant material, carry out the following reactions:

1. When applying 2-3 drops of iodine solution (Lugol's reagent) to a cross section of the rhizome, no blue staining should be observed.

2. When 2-3 drops of 20 % alcohol solution of alpha-naphthol and 1 drop of concentrated sulphuric acid are applied to a transverse section of rootstock, a red-violet or orange-red staining should be observed, respectively.

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

Work 2. Moisture determination of the medicinal plant material "Elecampane rhizomes and roots".

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

Measure the moisture content and calculate the percentage of moisture in the sample of raw material to be analysed.

Compare the result with the pharmacopoeial article "Elecampane rhizomes and roots". Write a conclusion.

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

Work 3. Quantitative analysis for polysaccharides of the medicinal plant material "Elecampane rhizomes and roots".

Study the method of determining the amount of polysaccharides (fructosans and fructose converted into inulin) by spectrophotometry in medicinal plant material.

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

Determine the amount of polysaccharides according to the method and calculate the percentage of polysaccharides in the sample of raw material.

Compare the obtained result with the pharmacopoeial article "Elecampane rhizomes and roots". Write a conclusion.

Write the results of the study 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[i]:
_____
_____
_____

Determination of moisture:

Regulatory moisture content:
Calculations:

Quantification of the sum of fructosans and fructose converted to inulin:
Standartization of raw materials by normative documentation:
Calculations**:**

Conclusion:_____
_____

Method for quantitative determination of polysaccharide sum (fructosans) by spectrophotometry

For quantitative determination of polysaccharide sum (fructosans) an analytical sample of the raw material was ground to particle size, passing through a sieve with a hole diameter of 1 mm. About 1.0 g (precise weight) of the crushed raw material was placed in a 250 ml flask, 60 ml water was added and the sample was heated in a boiling water bath (immersion flask) for 45 min, then cooled at room temperature for 5 min.

The obtained extraction is filtered through cotton wool into a 200 mL volumetric flask so that the raw material particles do not get on the filter. The flask is washed with 10 ml water and filtered into the same flask. The extraction with water is repeated twice more (first time heated for 45 min with 30 ml water, second time for 15 min with 30 ml water), filtering the extraction into the same volumetric flask. Then the raw material is transferred to cotton wool, the flask is rinsed with 10 ml water, filtering the wash through the cotton wool. The cotton wool with the seed is squeezed out. To the obtained extraction 1 ml of lead (II) acetate solution 10% is added to the volumetric flask, stirred and allowed to stand for 10 min.

Then 2 ml of anhydrous dinatrium hydrophosphate 5% solution is added to the flask, stirred and allowed to stand for 5 min. Then the volume of the solution in the flask is brought to the mark with water and stirred. The contents of the flask is filtered through a paper filter, discarding the first 10-15 ml of the filtrate.

2 mL of the filtrate is placed in a 100 mL volumetric flask, the volume of the solution is brought to the mark with water, stirred (solution A of the test solution).

In each of two 50 ml conical flasks 5 ml of 0.1% alcoholic resorcinol solution and 10 ml of 30% hydrochloric acid are placed . Then 5 mL of test solution A is added to the first flask and 5 mL of water (test solution A) is added to the second flask. Both flasks are heated in a water bath at 80℃ for 20 min and then cooled to room temperature. The contents of the flasks are transferred quantitatively to the appropriate 25 mL volumetric flasks and the volume of the solutions are adjusted to the mark with 30% hydrochloric acid, stirred (solution B of the test solution, comparison solution B).

After 15 minutes measure the optical density of solution B on a spectrophotometer at a wavelength of 483 nm in a cuvette with a layer thickness of 10 mm relative to the reference solution B.

The content of fructosans and fructose in terms of inulin in absolutely dry raw material in percent (X) is calculated according to the formula:

$$x = \frac{A \times 200 \times 100 \times 25 \times 100}{A_{1см}^{1\%} \times \alpha \times 2 \times 5 \times (100 - W)} = \frac{A \times 5000000}{A_{1см}^{1\%} \times \alpha \times (100 - W)}$$

where A is the optical density of solution B of the test solution;

A_1cm^(1%) - specific absorption index of products of reaction of inulin with resorcinol in acid medium at a wavelength of 483 nm, equal to 498;

α - weight of raw material, g;

W - loss in weight during drying of raw materials in per cent.