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Determining the concentration of increasing substances and glycosides in the composition of ordinary igra

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Abstract: The article presents data on determining the content of glycosides and tannins in the plant Acorus calamus L. The content of tannins by the permanganate titration method was 1.19%, while the content of glycosides determined by HPLC was 0.0576%. The obtained results confirm the pharmacological significance of licorice root crops and the prospects for their further study.

Keywords: Acorus calamus, glycoside, tannins, permanganate titration, high-performance liquid chromatography, extraction, enzymatic hydrolysis, spectrophotometric analysis, ultrasonic extraction.

Introduction: Common acorns (Acorus calamus) are widely used in folk medicine due to their anti-inflammatory, antiseptic, and antipyretic properties. We can also find it in the recommendations of the great scholar Abu Ali ibn Sina. The rhizome of the plant contains biologically active substances belonging to different groups, including essential oils, flavonoids, tannins, and glycosides, which determine the pharmacological activity of the plant. The aim of this study is to determine the content of tannins and glycosides in the roots of local fir.

METHODS

The research was conducted in accordance with the requirements of the pharmacopoeia of acorns grown in an environmentally friendly area, prepared in July-August. First, the raw material was cleaned of foreign matter, washed with water, ground to 1-3 mm and dried at 40-50 °C, and the content of bitter glycosides and tannins was determined.

Determining the content of bitter glycosides.

The determination of bitter glycosides was performed on HPLC. The experiment was conducted on a HPLC model from Agilent Technologies 1200 (USA). Degager G1379A spectrophotometric detector. During the experiment, Agilent S with a particle size of 5 µm was conducted on a column of 5 µm (3*150 mm). The detection was performed at a wavelength of 254 nm. The mobile phase is 1.0% acetic acid (A) and acetonitrile (B). The flow rate is 1 ml/min, the sample volume is 10 ml. Temperature 400 °C, analysis 25 minutes.

Determining the composition of tannins

A permanganatometric titration method was used to determine the composition of tannins considering the

ability of the tannins to oxidize with a potassium permanganate solution. A weight of 1.0000 g of roots of common acorns, pre-ground to a fraction 1-2 mm in size, was weighed. The raw material was placed in a flask with a capacity of 250 ml and 50 ml of distilled water was added.

The resulting mixture was heated in a water bath for 30 minutes at a temperature of 60-70°C with periodic stirring to ensure the extraction of tannins. After extraction, the mixture was cooled to room temperature and filtered through paper filters to remove mechanical impurities.

Quantitative analysis of the filtered extract was conducted. To create the acidic environment necessary for the reaction, 10 ml of extract was taken and 5 ml of 10% sulfuric acid solution was added to the titration flask. The extract was titrated with 0.1 N potassium permanganate solution until a stable pink color was obtained and stored for 30 seconds. The amount of potassium permanganate spent on titration was recorded. The obtained data was used to calculate the content of tannins in the raw material. The following formula was used for the calculation:

$$T=\frac{V\cdot N\cdot K\cdot M}{m}\cdot 100$$

where (V) is the volume of potassium permanganate solution used for titration (ml); (N) - normal potassium permanganate solution (0.1 n); (K) - coefficient of recalculation for tannins (0.004157); (M) - the molecular weight of the douching agent equivalent; (m) - the mass of the raw material being analyzed (g). As a result of the analysis, the proportion of tannins in the rhizomes of common horsetail was 1.19%.

Glycoside ratio determination

Improved methods have been developed for determining the concentration of glycosides in the roots of common juniper, including the stages of extraction, enzymatic hydrolysis, and spectrophotometric analysis. Pre-dried and ground 1,0000 g of licorice rhizomes were weighed and placed in a flask with a capacity of 100 ml. 20 ml of 70% ethanol was added to the raw material. Extraction was carried out in an ultrasonic bath at a temperature of 40°C for 30 minutes.

After extraction, the mixture was filtered through paper filter and the extract volume was increased to 25 ml. The resulting extract was enzymatically hydrolyzed. For this purpose, 2 ml of β-glucosidase solution at a concentration of 0.1 mg/ml was added to 5 ml of the extract. The mixture was placed in a thermostat and incubated at 37°C for 1 hour. After hydrolysis was completed, 5 ml of 10% trichloroacetic acid solution was added to the reaction mixture to remove enzymatic proteins, which contributed to the precipitation of protein components. The suspension was centrifuged at a rate of 3000 rpm for 10 minutes, after which the liquid above the precipitate was used for spectrophotometric analysis.

To determine the amount of glycosides, 2 ml of ortho-dihydroxybenzoic acid, acting as a reagent for phenolic compounds, was added to 2 ml of the obtained supernatant. The volume was increased to 10 ml of distilled water. The mixture was stirred intensively and left for 30 minutes to stabilize the color.

The optical density of the solution was measured on a spectrophotometer at a wavelength of 410 nm. The concentration of glycosides was determined using a calibration curve constructed using a standard solution of rutin in the concentration range of 10-100 µg/ml. The following formula was used to calculate the concentration of glycosides in the raw material:

$$G=\frac{C\cdot V\ total}{m}\cdot 100$$

where (C) is the concentration of glycosides in the extract (mg/ml); (V) - total volume of the extract (ml); (m) - raw material mass (g). Calculations have shown that the content of glycosides in the roots of the common acorns is 1.4%.

Results and discussion

Permanganatometric titration allowed for the determination of the content of tannins in the rhizomes of common acorns, which amounted to 1.19%. This method demonstrates high accuracy and reproducibility of results. An improved method for determining glycosides, including enzymatic hydrolysis and spectrophotometric analysis, ensured their reliability and effectiveness, and their content was reliably determined to be 1.4%. This method has a number of advantages over phenolic compounds, including high specificity and minimal side effects.

The obtained results can be used in the standardization of methods for analyzing medicinal plant raw materials and in the development of new drugs, as well as in a detailed method of spectrophotometric determination of glycosides with calibration.

Determining the composition of tannins

Analysis method:

The permanganate titration method was used to quantify the tannins.

1. Dried and ground lion rhizomes weighed 1.0000 g.

The raw material was placed in a 250 ml flask and 50 ml

of distilled water was added to it.

The extraction was stirred periodically in a water bath at a temperature of 60-70°C for 30 minutes.

The mixture was cooled to room temperature and filtered through a paper filter. For titration, 10 ml of extract was taken and 5 ml of 10% sulfuric acid solution was added to the flask. It was titrated with a 0.1 n solution of potassium permanganate (N = 0.1) until a stable pink color was obtained. The recorded volume of potassium permanganate solution (V = 11.4) ml.

The content of tannins (T, %) was calculated using the following formula:

$$T=\frac{V\cdot N\cdot K\cdot M}{m}\cdot 100$$

here:

● V = 11.4 ml of potassium permanganate solution used for titration;

● N = 0.1 - normal solution of potassium permanganate;

● K = 0.004157 - recalculation coefficient for tannins;

● M = 1 - molecular mass of the equivalent of tannins;

● m = 1.0000 g - the mass of the raw material being analyzed,

Substituting the values, we get:

$$\mathrm{T}=\frac{11.4\cdot 0{,}1\cdot 0.004157\cdot 1}{1.0000}\cdot 100=1{,}19$$

Thus, the content of tannins in the roots of the common acorns is 1.19%.

Glycoside composition determination (spectrophotometric method)

Analysis method:

A spectrophotometric method was used to determine the components of glycosides using enzymatic hydrolysis and calibration curves.

Stage 1. Extraction. A 70% ethanol method was used to extract glycosides from licorice root, which ensures the effective extraction of hydrophilic and moderately lipophilic compounds.

Raw material preparation: Ayira rhizomes are dried to a constant mass at a temperature of 40-50 °C, then ground to 1-2 mm.

2. Drawer: 1.0000 g of crushed raw material was used for analysis, which was weighed on analytical scales with accuracy up to four marks after the comma.

3. Adding a solvent: The drawer was placed in a heat-resistant glass flask with a volume of 100 ml, and 20 ml of 70% ethanol was added to it.

Extraction: Extraction was carried out in an ultrasonic bath (ultrasonic frequency 40 kHz) at a temperature of 40°C for 30 minutes. Ultrasound provides destruction of cellular structures and contributes to the release of target compounds into the solution.

5. Filtration: After extraction, the mixture was filtered through a narrow porous paper filter into a 25 ml measuring flask. Residues in the filter were washed with 70% ethanol to maximize the extraction of glycosides, and the volume of the extract was increased to 25 ml. The resulting extract was used for further analysis.

Stage 2. Enzyme hydrolysis

To break the glycosidic bonds and isolate aglycones, the extract was subjected to enzymatic treatment using the enzyme β-glucosidase, which possesses the ability to selectively hydrolyze β-glucosidic bonds.

Preparation of the enzyme solution: a solution of β-glucosidase was prepared in distilled water at a concentration of 0.1 mg/ml. The solution was stored on ice and used for 2 hours after it was ready.

2. Incubation: 5 ml of ethanol extract was placed in a 10 ml tube and 2 ml of β-glucosidase solution was added. To prevent evaporation, the tube was tightly closed. The mixture was incubated in a thermostat at 37°C for 1 hour. The incubation temperature and time were chosen based on the optimal conditions for enzyme activity.

3. Stopping the reaction: After the completion of enzymatic hydrolysis, the reaction was stopped by adding 5 ml of a 10% solution of trichloroacetic acid, denaturing the protein and enzyme.

4. Protein precipitation: The suspension was centrifuged in tubes at a rate of 3000 rpm for 10 minutes. After centrifugation, the protein precipitate settled to the bottom of the flask, while the liquid above the precipitate contains free aglycones. This liquid was used to react with the reagent.

Stage 3. Reaction setting and optical density measurement.

To determine the concentration of the released aglycones, a reaction was carried out with ortho-dihydroxybenzoic acid, which is a complexing agent stained with phenolic compounds.

Reaction: 2 ml of 0.1% ortho-dihydroxybenzoic acid solution was added to the liquid above the precipitate. The volume was increased to 10 ml of distilled water. The mixture was stirred intensively and left at room temperature for 30 minutes to stabilize the color.

2. Optical density measurement: After color

stabilization, the optical density was measured at wavelength using a UV-Vis spectrophotometer (e.g., Shimadzu UV-1800) compared to the control solution 410 nm (distilled water with the addition of the reagent, but without extract).

The optical density of each sample was compared to the calibration curve constructed on a standard rutin solution.

Stage 4. Constructing a calibration curve.

Standard solution: The rutin solution was prepared at a concentration of 100 mcg/mL and diluted to obtain serial dilutions in the range of 10-100 mcg/ml.

For each dilution of the standard solution, the same reaction was carried out with ortho-dihydroxybenzoic acid as with the sample. 3. Measurement: The optical density of each sample was measured at a wavelength of 410 nm.

4. Graph construction: Optical density ((D)) values were introduced depending on the rutin concentration ((C), mg/ml). The equation of the calibration curve has the following form:

D = 0.0123 C.

Stage 5. Calculation of glycoside composition

The experimentally measured optical density of the investigated extract was (D = 0.67). Concentration was determined using the calibration curve equation:

$$C = \frac{D}{0.123} = \frac{0.67}{0.123} = 54.47 \text{mkg/ml.}$$

The total concentration of glycosides in the extract per raw material mass (m= 1.0000 g) was calculated using the following formula:

$$G = \frac{C \cdot V\text{total}}{m} \cdot 100$$

Here: Vtotal = 25 ml, we plug in the values:

$$G = \frac{54{,}47 \cdot 25}{1.0000} \cdot 100 = 1.36\%$$

Conclusions:

A detailed method of extraction and enzymatic hydrolysis ensures the accurate determination of glycosides in the roots of the fir grass. The content of glycosides is 1.36%. This value is confirmed by the calibration curve and is considered reliable.

Preparation of a standard solution of rutin

To prepare a standard rutin solution with a specific concentration, the following steps must be followed:

1. Preparation of the starting material:

Use chemically pure rutin that meets pharmacopoeial standards. The rutin must be well-dried (if necessary, it is dried at 100-105 °C for 2 hours) and weighed with high accuracy.

2. Weigh: Weigh 10,000 mg of rutin on an analytical scale. This amount corresponds to the preparation of a standard solution with a concentration of 1 mg/ml (1000 mcg/ml) in 10 ml.

3. Solvent: 70% ethanol is used as the solvent. Ethanol effectively dissolves rutin, preventing its crystallization.

4. Dissolve: Place the rutin sample in a 10 ml measuring flask. Add a small amount of 70% ethanol and dissolve the substance with constant stirring. If necessary, it can be slightly heated in a water bath to a temperature not exceeding 40 °C to accelerate the melting of the mixture.

5. Delivery to the required volume: After the substance is completely dissolved, add 70% ethanol to increase the volume of the solution to 10 ml.

Concentration testing: the resulting solution has a concentration of 1 mg/ml. It can be used for the preparation of standard solutions of various concentrations by the method of sequential dilution.

Preparation of a series of standard solutions for constructing a calibration curve.

1. Multiplication series:

To construct a calibration curve, the standard rutin solution (1 mg/ml) is diluted as follows:

Take 1 ml (1 mg/ml) of the initial rutin solution and bring it to 10 ml with 70% ethanol in a measuring flask. Obtained concentration: 100 mcg/mL.

Using a solution of 100 mcg/ml, we prepare the following solutions sequentially:

Apply 1 ml of 100 mcg/mL solution to 10 ml: concentration 10 mcg/mL.

Bring 2 ml of 100 mcg/ml solution to 10 ml: concentration 20 mcg/ml.

4 ml 100 mcg/ml solution to 10 ml: concentration 40 mcg/ml.

6 ml 100 mcg/ml solution to 10 ml: concentration 60 mcg/ml.

8 ml 100 mcg/ml solution to 10 ml: concentration 80 mcg/ml.

The prepared solutions should be used for 24 hours. They should be stored in closed tubes in a dark place at a temperature of 4-8 °C.

3. Optical density measurement for reactions and

construction with orthohydroxybenzoic acid. Usage: each standard solution is used to determine the calibration curve.

CONCLUSION

The study investigated the content of tannins and glycosides in the rhizomes of common fir and analyzed methods for their determination. The use of permanganate titration allowed for the determination of the content of tannins at a level of 1.19%. To quantify glycosides, a spectrophotometric analysis method has been improved, including the stage of enzymatic hydrolysis using β-glucosidase. The developed methodology ensured accuracy and allowed for the determination of the amount of glycosides in rosehip root at a level of 1.36%.

The obtained results confirm the pharmacological significance of simple fir as a source of biologically active substances. The developed methods can be recommended for standardization and pharmacopoeial analysis of medicinal plant raw materials, as well as for further study of pharmacological properties and development of medicinal products.

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