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DETERMINATION OF INULIN IN THE ROOTS OF ARCTIUM LEOSPERMUM L.

Temirov Alisher Smatillaevich
Karimova Yulduz Zafarzhon kizi
Nuridullaeva Kamola Negmatilloevna
State Institution 'Centre for the Safety of Pharmaceutical Products' under the Ministry of Health of the Republic of Uzbekistan
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ABSTRACT

The article presents the results of research on the development of a method for the quantitative determination of inulin in the roots of Arctium leospermum L. growing in Uzbekistan. For this purpose, a spectrophotometric method was used. The data obtained were statistically processed.

Introduction

Scientific research is being conducted worldwide in the field of 'pharmaceutical remake', i.e. comprehensive research into medicinal plants that have been widely used in folk medicine for centuries, studying their chemical composition, establishing quality standards and pharmacological activity, and creating original, effective and safe medicines. A number of studies are being conducted to justify their use in official medicine. In this regard, plants containing inulin are of particular interest, since inulin is a linear polysaccharide, polyfructose, consisting of a large number of fructose residues (from 10 to 36). It belongs to dietary fibres and is considered a 'reserve' carbohydrate for plants. Like other plant-based fibres, inulin has the ability to bind radionuclides and various toxic substances (slags, toxins, heavy metal salts, and others). Thanks to these properties, inulin is used as an enterosorbent in therapeutic nutrition and is a component of dietary supplements. One of the main properties of inulin is its ability to lower blood sugar levels, which is why medicines and dietary supplements containing it are used as adjuvants in the treatment of type II diabetes to compensate for and stabilise carbohydrate metabolism, reduce the dose of the main hypoglycaemic drug, and for secondary prevention of diabetes, namely the prevention of diabetic nephropathy. [1-4].

The aim of the study is to develop a method for the quantitative determination of inulin in the roots of Arctium leospermum L.

Materials and methods. The study materials were samples of Arctium leospermum L. roots harvested at the end of their growing season. For quantitative analysis, spectrophotometry (SP) in the visible range was used, based on measuring the optical density of the products of the interaction of fructose, formed after the breakdown of inulin, with resorcinol in an acidic environment.

Results and discussion.

As is known, under the action of hydrochloric acid, inulin breaks down to form fructose and glucose. However, only fructose interacts with resorcinol [5]. Obviously, there is a direct relationship between the concentration of inulin in solution and the fructose formed as a result of hydrolysis. For this reason, fructose was used as a standard substance in spectrophotometric determination, especially since the absorption spectra of the products of the interaction of fructose and purified extract from the roots of Arctium leospermum L.with resorcinol in an acidic environment coincide (Fig. 1).

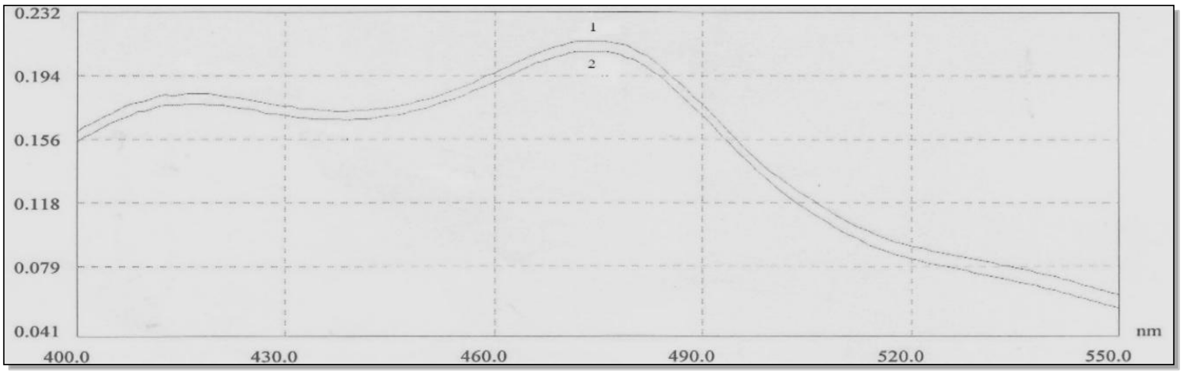

Figure 1. Absorption spectra of the interaction products of fructose (1) and purified extract from the roots of Arctium leospermum L. (2) with resorcinol in an acidic environment.

In addition to inulin, l roots contain fructofuran, mono- and disaccharides. Inulin is known to be soluble in water but insoluble in 95% alcohol. This property forms the basis of the method we used, according to which two extracts were obtained from the raw material – water and alcohol. Inulin with accompanying inulides and fructosides passed into the first extract, and only fructosides passed into the second. The difference between the two determinations was used to calculate the total content of inulin and inulinides, designated as the total fructosan content.

When developing the methodology, we also studied the influence of various factors on the extraction of active substances from the raw material (Table 1).

Table 1

Study of optimal conditions for extracting active substances from the roots of Arctium leospermum L.

Extraction conditions	Fructose content in raw materials, %				
	batch 1	batch 2	batch 3	batch 4	batch 5
Ethanol	*Effect of the extractant*				
95%	2,38	2,65	2,63	2,57	3,30
70%	14,92	14,25	14,6	14,15	8,82
40%	13,34	13,73	14,92	14,42	14,93
25%	13,35	14,97	14,06	14,65	14,04
12,5%	14,20	15,30	14,68	14,97	14,66
Water	17,90	18,97	18,29	18,32	19,5

	The influence of raw material fineness				
More than 7 mm	14,80	16,80	17,61	17,00	14,61
7 mm	14,90	15,43	15,05	18,02	16,42
5 mm	13,50	15,60	16,64	18,83	18,83
3 mm	14,40	14,12	16,50	17,91	17,03
2 mm	14,70	14,90	16,10	15,82	17,22
1 mm	18,30	18,90	18,23	18,74	19,04
	The influence of extraction temperature conditions				
Without heating	19,8	20,72	20,3	19,68	18,8
Heating in a water bath: 40^0C	15,2	16,7	15,1	14,4	17,0
60^0C	16,1	17,3	16,2	16,0	17,5
80^0C	16,5	17,7	17,05	17,4	16,9
100^0C	17,4	18,2	18,35	17,8	15,4
Open fire	16,9	17,5	18,0	17,2	22,0
	Effect of extraction time				
15 min	14,3	15,2	14,1	15,1	18,0
30 min	15,8	16,5	16,5	15,9	217,9
45 min	17,98	18,29	17,9	16,6	12,3
1 hour	16,8	18,29	17,7	15,8	12,35
1,5 hour	16,8	17,0	17,5	15,5	17,9
2 hour	14,8	16,4	15,7	15,5	16,9
	The influence of the ratio of raw material to extractant				
1:50	15,0	15,3	15,8	14,6	20,8
1:100	16,3	17,0	16,9	17,5	16,4
1:200	17,9	18,4	17,6	18,2	17,8
1:250	17,3	17,5	17,0	17,8	16,7

As can be seen from the data in the table, optimal extraction is achieved when water is used as the extractant, the raw material is ground to a particle size that passes through a sieve with a hole diameter of 1 mm, heating in a boiling water bath for 45 minutes, and a raw material to extractant ratio of 1:200. Studies have shown that when using 3-fold extraction, the extraction of inulin from the raw material is complete. [6].

Methodology for quantitative determination.

1. *Determination of the amount of fructosides and fructosans.* The analytical sample of raw material is ground to a particle size that passes through a sieve with holes 1 mm in diameter. Approximately 1 g (precise weight) of raw material is placed in a 300 ml conical flask, 60 ml of water is added, and the mixture is heated in a boiling water bath for 45 minutes. The resulting extract is filtered while warm through cotton wool into a 200 ml volumetric flask so that no raw material particles get onto the filter. The flask is rinsed with 10 ml of water and filtered into the same volumetric flask. The extraction is repeated twice (the first time heated for 45 minutes with 30 ml of water, the second time for 15

minutes with the same amount of water). Next, the raw material is transferred to a filter with cotton wool, the flask is rinsed and the residue on the filter is washed, using 10 ml of water each time. The cotton wool with the raw material is squeezed out.

Add 2 ml of a 10% solution of basic lead acetate to the extract, mix and leave for 10 minutes. Then add 2 ml of a 5% solution of sodium phosphate, mix and leave for 5 minutes. Bring the volume of the solution in the flask to the mark with water and mix. Filter the extract through a paper pleated filter, discarding the first 10-15 ml of filtrate. Place 2 ml of the filtrate in a 100 ml volumetric flask, bring the volume of the solution to the mark with water and mix (solution A).

Add 5 ml of a 0.1% alcohol solution of resorcinol to two 25 ml flasks. Place 5 ml of solution A (the solution being analysed) in the first flask and 5 ml of water (the reference solution) in the second flask. Bring the volume of the solutions in the flasks to the mark with 30% hydrochloric acid solution and mix. Transfer the contents of the flasks to test tubes and heat in a water bath at 80°C for 20 minutes, cool to room temperature and mix.

The optical density of the analysed solution is measured on a spectrophotometer at a wavelength of 475 ± 2 nm in a cuvette with a layer thickness of 10 mm relative to the comparison solution. The content of the sum of fructosides and fructosans in terms of fructose and absolutely dry raw materials in percent (X) is calculated using the formula:

$$X = \frac{D \cdot 200 \cdot 100 \cdot 100}{95 \cdot 1 \cdot 2 \cdot m \cdot (100 - W)} = \frac{D \cdot 1000000}{95 \cdot m \cdot (100 - W)}, \quad (1)$$

where D is the optical density of the analysed solution; 95 is the specific absorption coefficient of the reaction products of fructose with resorcinol in an acidic environment; m is the mass of raw materials in grams; W is the loss in mass during drying of raw materials in percent.

2. Determination of fructosides. Approximately 1 g (precise weight) of crushed raw material is placed in a 300 ml round-bottom flask, 60 ml of 95% alcohol is added, and the mixture is heated in a boiling water bath with a reflux condenser for 45 minutes. Filter the resulting extract while warm through a layer of cotton wool into a 200 ml volumetric flask so that no raw material particles get onto the filter. Rinse the round-bottomed flask with 10 ml of 95% alcohol and filter into the same volumetric flask. The extraction is repeated twice more (the first time heated for 45 minutes with 30 ml of 95% alcohol, the second time for 15 minutes with the same amount of alcohol). Transfer the raw material to the filter, rinse the round-bottom flask, then wash the residue on the filter, using 10 ml of 95% alcohol each time. Squeeze the cotton wool with the raw material.

Add 1 ml of a 10% solution of basic lead acetate to the extract obtained, mix and leave for 10 minutes. Then add 2 ml of a 5% solution of sodium phosphate, mix and leave for 5 minutes. Bring the volume of the solution in the flask up to the mark with water and mix. Filter the extract through a paper pleated filter, discarding the first 10-15 ml of filtrate. Place 10 ml of the filtrate in a 100 ml volumetric flask, bring the volume of the solution to the mark with water, mix (solution A).

Proceed as described in the method for quantitative determination of fructosans above.

The fructoside content in terms of fructose and absolutely dry raw material in percent (X1) is calculated using the formula:

$$X_1 = \frac{D \cdot 200 \cdot 100 \cdot 100}{95 \cdot 1 \cdot 10 \cdot m \cdot (100 - W)} = \frac{D \cdot 200000}{95 \cdot m \cdot (100 - W)}, \qquad (2)$$

where the symbols are the same as in formula (1).

3. *Definition of fructosans.* The fructose content in terms of fructose and absolutely dry raw materials in percent (X2) was calculated using the formula:

$$X_2 = X - X_1 \qquad (3)$$

The results obtained are shown in Table 2. The results have been statistically processed.

Table 2

Results of quantitative determination of fructans in terms of fructose in the roots of Arctium leospermum L.

X_i,%	$\overline{X}$, mg/g	F	S^2	S	S_x	$\pm\overline{\varepsilon}$,%
X_1=18,84 X_2=18,93 X_3=19,60 X_4=19,79 X_5=19,56	19,344	4	0,184	0,429	0,192	2,754%

As can be seen from the data obtained, the relative error of determination at a confidence level of 95% does not exceed ±2.754%. The fructose content in terms of fructose and absolutely dry raw material in the roots of Arctium leospermum L. ranges from 18% to 20%.

Conclusions:

1. The optimal conditions for extracting fructans from Arctium leospermum L. roots have been determined.

2. A spectrophotometric method for the quantitative determination of fructans in Arctium leospermum L. roots has been developed. The relative error of determination at a confidence level of 95% does not exceed 3%.

3. Based on the results of quantitative determination, the standard fructan content in Arctium leospermum L. root raw materials has been established at no less than 18%.

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