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Determination of carbohydrate content in raw material of *Agastache foeniculum* and *Agastache urticifolia*

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Abstract
The content of polysaccharides and their individual fractions in Agastache foeniculum and Agastache urticifolia herb were determined. It was found out that the predominant fractions of the polysaccharide complex were water soluble polysaccharides, the contents of pectin substances and hemicellulose B was rather lower, and the hemicellulose A had the smallest contents. The content of mono- and disaccharides in A. foeniculum and A. urticifolia herbs was investigated by gas chromatography–mass spectrometry (GC-MS). The sucrose, glucose, inositol and fructose were dominant compounds in both raw materials.

Keywords: *Agastache foeniculum*, *Agastache urticifolia*, carbohydrates, polysaccharides, gas chromatography–mass spectrometry method (GC-MS)

1. Introduction
Carbohydrates form the bulk of the plant organism and are an important class of natural compounds with a diverse range of biological effects on the human body. Carbohydrates are biologically active substances of primary synthesis, are widely distributed in nature and are part of the tissues of all living organisms. Plants are the main sources of their production. During the last decade, scientific interest in this class of compounds has been significantly cut off. It has been established that polysaccharides have a wide range of biological activity, used as expectorants, enveloping, softening, anti-inflammatory, anti-ulcer, wound healing, antitumor, anabolic drugs, involved in the creation of immunity, increase the body's resistance, reduce the side effects of antibiotics, cytostatics, glucocorticoids [1-3].
One of the priority tasks of modern pharmacy is the search for promising plants that could become a raw material base for the creation on their basis of drugs that could compete with synthetic drugs worthily. To such interesting and promising in practical terms, plants belong to the genus *Agastache J. Clayton ex Gronov.* (adopted synonymous name – Lophantus).
Representatives of this genus have valuable beneficial properties, they are not only scientific, but also practical value - are considered decorative, medicinal, honey plants. Plants of the genus are widely used in folk medicine, in particular, it is known that the agastache herb infusion restores the body after nerve shocks, strokes, is considered as a strong biostimulant, an immunomodulator, and exhibits antibacterial activity in treating inflammatory diseases of the gastrointestinal tract [4, 5].
However, there is no information in available literature on the content of carbohydrates in this raw material, therefore, the purpose of our research was to determine the qualitative composition and quantitative content of free sugars and to quantify the polysaccharide fractions by gravimetric method in the *A. foeniculum* and *A. urticifolia* raw material.

2. Materials and methods
The object of investigation was the herb of *Agastache foeniculum* (Pursch) O.Kuntze and *Agastache urticifolia* (Fisch. et Mey) O.Kuntze. It was harvested on the experimental sites of the acad. O.V. Fomin Botanical Garden (Kyiv) in July 2016. The raw material was dried by air-shade method, crushed, sieved through a sieve with a diameter of holes of 3 mm.
The isolation of polysaccharide fractions from the raw materials was carried out in accordance with the method of N.K. Kochetkov [6]. The gravimetric method was used in accordance with State Pharmacopoeia of Ukraine 1.3 to determine the content of polysaccharide fractions [7].
1 g of powdered air-dry raw material was poured into 50 ml of 80% ethanol and heated to reflux in a water bath for 1 hour for isolation of polysaccharide fractions.

The extract was filtered through the filter "red tape", cooled to 21 °C, and brought to volume of 50 ml with 80% ethyl alcohol; the resulting extract was used to determine the free sugars (extract A).

The residue of raw material was poured into 50 ml of water and heated to reflux in a water bath for 1 hour, the extract was filtered through the paper filter "red tape", cooled to 21 °C, and brought to volume of 50 ml with water; it was used to determine the water soluble polysaccharides (extract B).

The remaining raw material was poured into 50 ml of a mixture of 0.5% oxalic acid solution and 0.7% ammonium oxalate solution (1:1) and heated to reflux in a water bath for 1 hour, the extract was filtered through the filter "red tape", cooled to temperature 21 °C and brought to volume of 50 ml with suitable mixture of extractants; it was used to determine pectin substances (extract C).

The residue was poured into 50 ml of 5% potassium hydroxide solution and heated to reflux in a water bath for 1 hour, the extract was filtered through the "red tape" filter, cooled to 21 °C and brought to volume of 50 ml with 5% solution of potassium hydroxide, it was used to determine hemicellulose (extract D).

Threefold volume of absolute ethyl alcohol was added to the extracts B, C, and D. As a result, within 24 hours, polysaccharides were precipitated. The precipitation was filtered through the paper filter "red tape" (weighed previously). The precipitation was dried on the filter and weighed together with the filter [8].

The content of polysaccharides (water-soluble polysaccharides, pectin substances and hemicelluloses) in terms of air-dry raw materials in percentages was calculated gravimetrically by the formula:

$$\% = \frac{(m_2 - m_1) * 100}{m}$$

where:

m_2 – mass of filter with sediment, g;

m_1 – mass of filter, g;

m – mass of raw materials, 1,000 g;

Further investigation of sugars was carried out by the method of gas chromatography–mass spectrometry (GC-MS). The method is based on the extraction of free monosaccharides and full acid hydrolysis of raw materials for determination of the total monosaccharide composition and the production of the acetates of their aldonitrile derivatives, followed by analysis GC-MS [9, 10].

The chromatographic separation was made on a gas chromatographic mass spectrometric system of Agilent 6890N/5973inert (Agilent technologies, USA). Column HP-5ms capillary (30m×0.25mm×0.25mkm, Agilent technologies, USA). The temperature of the evaporator was 250°C, the temperature of the interface was 280°C. The separation was carried out in the programming mode of temperature – the initial temperature of 160 °C was held for 8 minutes, raised with a gradient of 5°C/min to 240 °C. The final temperature was held for 6 minutes. A sample of 1 µl was injected in 1:50 split mode. Detection was made in SCAN mode in the range (38-400 m/z). The gas flow rate of the carrier through the column was 1.2 ml/min. Preparation of samples for analysis consisted in the fact that the raw material was triturated to the powder-like state in a glass mortar. The weight of the drug was placed in vial, and 80% ethanol solution was added. Extraction of free monosaccharides was carried out in an ultrasound bath at 80 °C for 4 hours. The extract was selected, evaporated to dryness, and the internal standard solution was added to it.

0.3 ml of extract/hydrolyzate was collected, evaporated to dryness on a rotary evaporator, and 0.3 ml of a derivatizing reagent (32 mg/ml hydroxylamine hydrochloride in a mixture of pyridine / methanol (4:1)) was added for preparation of aldonitrile monosaccharides. The dissolved extract was kept for 25 minutes at 75 °C. 0.5 ml of acetic anhydride was added and held for 15 minutes at 75 °C for acetylation of aldonitrile derivatives of monosaccharides. 1 ml of dichloroethane was added to the reaction mixture, the excess derivatization reagents were removed by double extraction this 1N hydrochloric acid and water. The layer of dichloriethane was dried to dryness and dissolved in 300 ml mixture of heptane/ethyl acetate (1:1) [11, 12].

The identification was carried out according to the time of keeping of monosaccharide standards and the use of the NIST 02 mass-spectrometer library. Quantitative analysis was performed by adding solution of the internal standard – solution of sorbitol in the test samples.

The content of monosaccharides (X, in mg per 1 kg of raw material) was calculated by the formula:

$$X = \frac{S_x \times m_{вист} \times V_{роз} \times 1000}{S_{вист} \times m \times V_{екстр}},$$

where: S_x – area of the peak of the monosaccharide or disaccharide studied;

$S_{вист}$ – area of the peak of the internal standard;

$m_{вист}$ – mass of internal standard for a sample;

m – mass of raw material sample;

$V_{роз}$ – volume of solvent for extraction;

$V_{екстр}$ – volume of extract for derivatization.

3. Results and Discussion

Based on the conducted studies, the content of polysaccharide fractions in the studied *Agastache* species herb was determined, the results of which are shown in Table 1.

Table 1: *Quantitative content of individual polysaccharide fractions in herb of Agastache foeniculum and Agastache urticifolia, %*

The fraction of polysaccharides	Content of polysaccharide fractions,% by mass of air-dry raw materials	
	A. foeniculum herb	*A. urticifolia* herb
Water soluble polysaccharides	15,08 ± 0,12	14,89 ± 0,11
Pectin substances	8,89 ± 0,06	9,10 ± 0,07
Hemicellulose A	1,35 ± 0,08	1,31 ± 0,12
Hemicellulose B	5,29 ± 0,06	4,68 ± 0,05

According to the table, the content of polysaccharides in the herb of both species of *Agastache* is quite similar. The predominant fractions of the polysaccharide complex were water soluble polysaccharides, the content of pectin substances and hemicellulose B was rather lower, and the hemicellulose A had the smallest contents.

5 free monosaccharides – glucose, galactose, sorbose, fructose, ribose, 2 disaccharides – sucrose, melibiose, and 1 polyhydric alcohol – inositol were identified in herb of *A. foeniculum* under conditions of introduction in Acad. O.V. Fomin Botanical Garden using chromato-mass-spectrometric method (Fig.1). 5 free monosaccharides – mannose, glucose, galactose, sorbose, fructose, 4 disaccharides – sophorose, sucrose, melibiose, cellobiose and 1 polyhydric alcohol – inositol were identified in herb of *A. urticifolia* (Fig.2). The

results of the quantitative content of free sugars in the *Agastache* herb are given in Table 2.

Table 2: *Quantitative content of free sugars in the Agastache foeniculum and Agastache urticifolia herb*

Substance	Free sugars content, mg/kg of air-dry raw material	
	A. foeniculum	***A. urticifolia***
Mannose	–*	660
Glucose	10050	5210
Galactose	450	380
Sorbose	260	340
Fructose	1360	670
Melibiose	310	130
Cellobiose	–	440
Sophorose	–	210
Sucrose	48220	62870
Ribose	220	–
Inositol	5280	3690

** Note: "-" – the substance is absent in the sample.*

As a result of the research, it was found that quantitatively in *A. foeniculum* and *A. urticifolia* herb sucrose is significantly dominated (62870 mg/kg and 48220 mg/kg by weight of air dry matter, respectively). As for other compounds, it should be noted the high content of glucose and inositol in the raw material of *A. foeniculum*, which is 10050 mg/kg and 5280 mg/kg, respectively, and *A. urticifolia* has 5210 mg/kg and 3690 mg/kg, respectively.

Inositol (vitamin B8) activates lipid metabolism, stimulates the activity of the brain, improves the concentration of attention, stimulates mental activity, reduces fatigue of the brain, and increases its ability to memorize information.

It should be noted the content of disaccharides, such as sophorose, melibiose, cellobiose in *A. urticifolia* herb, and melibiose in *A. foeniculum* herb. These compounds are rarely represented in the composition of the BAS of medicinal plant material, which will allow for the standardization of raw materials of *A. urticifolia* and *A. foeniculum.*

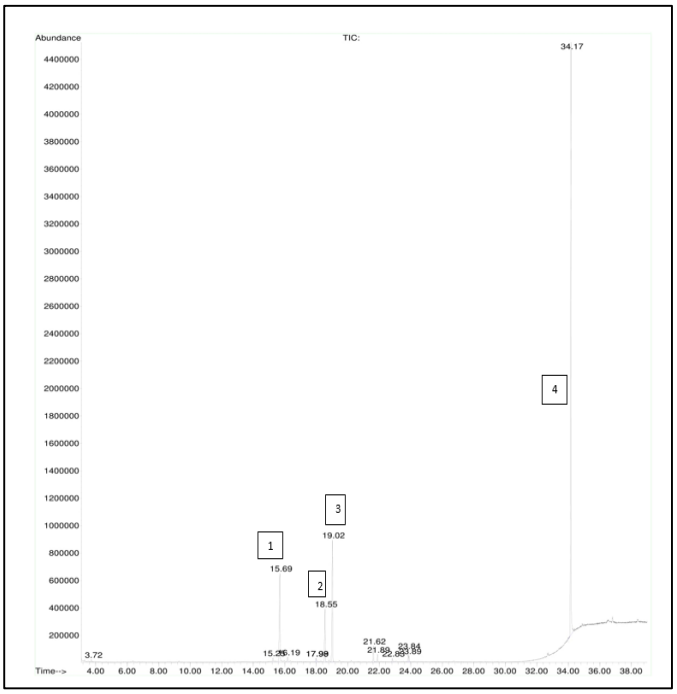

Fig 1: Chromatogram of free sugars of *Agastache foeniculum* herb; 1 – glucose, 2 – inositol, 3 – internal standard (sorbitol), 4 – sucrose

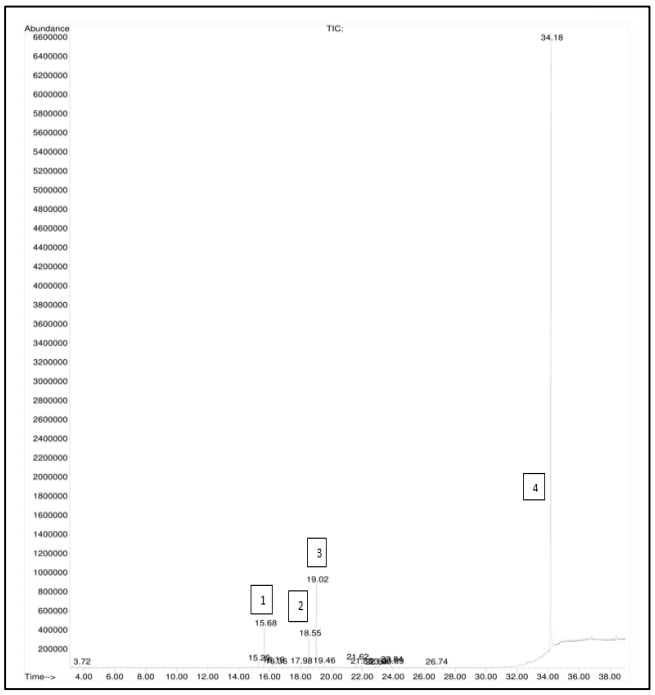

Fig 2: Chromatogram of free sugars of *Agastache urticifolia* herb; 1 – glucose, 2 – inositol, 3 – internal standard (sorbitol), 4 – sucrose.

4. Conclusions

1. The content of polysaccharides and their individual fractions in *A. foeniculum* and *A. urticifolia* herb was determined.
2. It was found out that the predominant fractions of the polysaccharide complex were water soluble polysaccharides, the contents of pectin substances and hemicellulose B was rather lower, and the hemicellulose A had the smallest content.
3. For the first time, the content of mono- and disaccharides in *A. foeniculum* and *A. urticifolia* herb was determined by gas chromatography–mass spectrometry (GC-MS).
4. As a result of the conducted research it was ascertained that sucrose, glucose, inositol and fructose are dominated in quantitative content of *A. foeniculum* herb, and sucrose, glucose, inositol are dominated in *A. urticifolia* herb.
5. Obtained results on qualitative composition and quantitative content research of free mono- and disaccharides in *A. foeniculum* and *A. urticifolia* herb are of practical importance for further in-depth study, namely the determining of pharmacological effects, in particular immunostimulating, anti-inflammatory and antioxidant effects.

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