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DETERMINATION OF CARBOHYDRATES IN *AGRIMONIA EUPATORIA* L. HERB

N. Huzio, A. Grytsyk, L. Slobodianiuk

*In recent years, methods of treatment using medicinal plants and biologically active substances based on them have become increasingly common. Today, the search for plants with a long history of use and minor side effects is of interest to our society. The most promising for procurement and use in medical practice is a common throughout Ukraine – common agrimony (*Agrimonia eupatoria* L.). The presence of tannins, which are represented by two groups: condensed and hydrolyzed tannins is indicated. There is insufficient information in the literature on the biologically active substances of *Agrimonia eupatoria* L. The common agrimony herb contains 1.2–1.6 % of flavonoids, which belong to different subgroups. Thus, from the group of flavones apigenin, luteolin, luteolin-7-glycoside have been identified. The herb of *Agrimonia eupatoria* L. as an insufficiently studied plant raw material is also a promising object of study, including carbohydrate composition.*

The aim. The aim of our study was to determine the content of carbohydrates in aerial parts of *Agrimonia eupatoria* L. collected from natural habitat.

Materials and methods. The determination of carbohydrates of *Agrimonia eupatoria* L. was carried out by gas chromatograph Agilent 6890N with a mass detector 5973 inert (Agilent Technologies, USA). The amounts of water-soluble polysaccharides and pectin substances were determined by gravimetric method.

Results. Three free carbohydrates were found in the studied grass of the *Agrimonia eupatoria* L., namely: D-glucose, D-galactose and D-fructose, the content of which was 15.02 mg/g, 0.28 mg/g and 12.90 mg/g, respectively. In the common agrimony, after acidic hydrolysis and derivatization with acetylated aldonitriles were identified 8 compounds. The predominant ones were D-glucose, D-galactose and D-xylose. The content of water-soluble polysaccharides and pectin substances in *Agrimonia eupatoria* L. was determined at 2.90 % and 8.54 % of total dry weight, respectively.

Conclusions. In completion, the present results of GC/MS indicated the presence of certain carbohydrates both in free and bound form in *Agrimonia eupatoria* L., that have important medicinal activity, which answered to the knowledge about the therapeutic activity of this analyzed plant. We established that in *Agrimonia eupatoria* L. herb the predominant monosaccharide compound was D-glucose: 23.88 mg/g and 15.02 mg/g among monosaccharides after hydrolysis and free monosaccharides respectively. Our findings propose that carbohydrates from the studied raw material can be used for creating drugs by the pharmaceutical industry, and could be used for the development of nutraceuticals

Keywords: *Agrimonia eupatoria* L., carbohydrates, GC/MS, water-soluble polysaccharides, pectin substances, gravimetric method

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1. Introduction

In recent years, methods of treatment using medicinal plants and biologically active substances based on them have become increasingly common. Today, the search for plants with a long history of use and minor side effects is of interest to our society [1]. Plants of the genus *Agrimonia* L. of the family *Rosaceae*, which have long been used in medicine in many countries as anti-inflammatory, hepatoprotective, choleric, diuretic, anthelmintic and antispasmodic agents, are promising objects for phytochemical study [2–5].

The most promising for procurement and use in medical practice is a common throughout Ukraine – common agrimony (*Agrimonia eupatoria* L.), which contains a large number of different biologically active substances. Ukrainian scientists H. S. Naprasnikova and others by the method of absorption spectrophotometry have determined the content of tannins, which are represented by two groups: condensed and hydrolysable tannins. Ellagic acid and agrimoniin are present among the hydrolyzed tannins [6]. *Agrimonia eupatoria* L. herb also

contains 1.2–1.6 % of flavonoids, which belong to different subgroups. Thus, from the group of flavones apigenin, luteolin, luteolin-7-glycoside have been identified [7]. Also Kato H. et al. have identified the following flavonoids in the herb of the common agrimony: luteolin 7-O-sophoroside, luteolin 7-O-(6"-acetylglucoside), acacetin 7-O-glucoside, luteolin 7-O-glucoside and apigenin 7-O-glucoside [8, 9]. Investigating the herb of the common agrimony, Kozak I. V. et al. showed that when quantifying the amount of flavonoids, the conversion should be carried out on luteolin or luteolin-7-O-glucoside and that the identification markers of the common agrimony herb selected rutin, hyperoside, isoquercitrin [10].

The mechanism of anti-inflammatory action of biologically active substances of the common agrimony has been proved by scientists Correia H. S. et al. They have substantiated the association between the anti-inflammatory activity of *Agrimonia eupatoria* L. water-alcohol extracts and the presence of polyphenolic compounds in the plant [2].

It is also known that the herb of *Agrimonia eupatoria* L. accumulates substances of primary synthesis – carbohydrates [11]. The study of the content and qualitative composition of carbohydrates of the herb *Agrimonia eupatoria* L. is expedient due to the fact that carbohydrates, in addition to important functional significance and specific pharmacological activity, also influence the development of the total pharmacological effect of plant-derived drugs. The herb of *Agrimonia eupatoria* L. as an insufficiently studied plant

raw material is also a promising object of study, including carbohydrate composition.

Thus, the aim of our study was to determine the content and qualitative composition of carbohydrates in aerial parts of *Agrimonia eupatoria* L. collected from natural habitat.

2. Planning (methodology) of research.

To achieve this aim and get answers to all questions, the design of the experiment was done (Fig. 1).

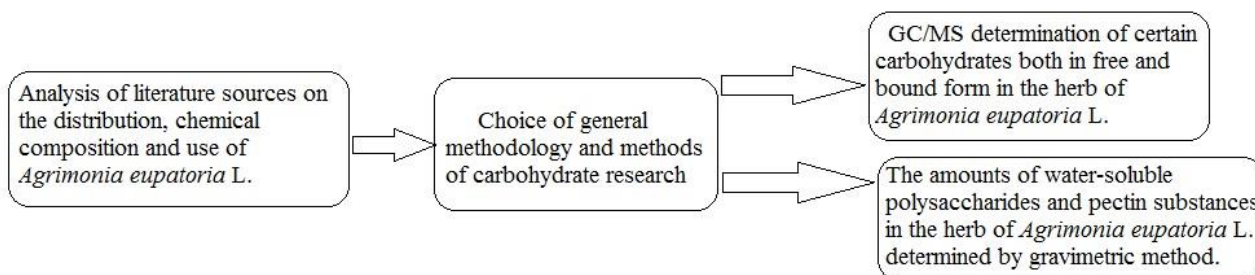


Fig. 1. Design of the experiment

3. Material and method

3.1. Plant materials

Agrimonia eupatoria L. herb was chosen as the object of study. The aboveground part of the studied plant was collected in Western Ukraine, at the territory of Vovchynets, Ivano-Frankivsk region (N 48°57'06"N 24°44'46"E) during the flowering period in 2017.

The raw material was authenticated by assoc. prof. Hanna Nedostup (IFNMU, Ivano-Frankivsk, Ukraine).

3.2. Chemicals and standards

The conventional methods for quantification of carbohydrates and determining their monomeric content were used: gas chromatography with mass spectrometry (GC/MS) and gravimetry. Standard reagents including L-rhamnose, D-mannose, D-ribose, D-xylose, D-arabinose, D-galactose, D-fucose, D-glucose, D-fructose, D-saccharose, and D-sorbitol were obtained from Sigma-Aldrich Chemical Co. (≥99 % purity) (Fig. 2). All solvents for metabolite extraction were of analytical grade (>95 % purity).

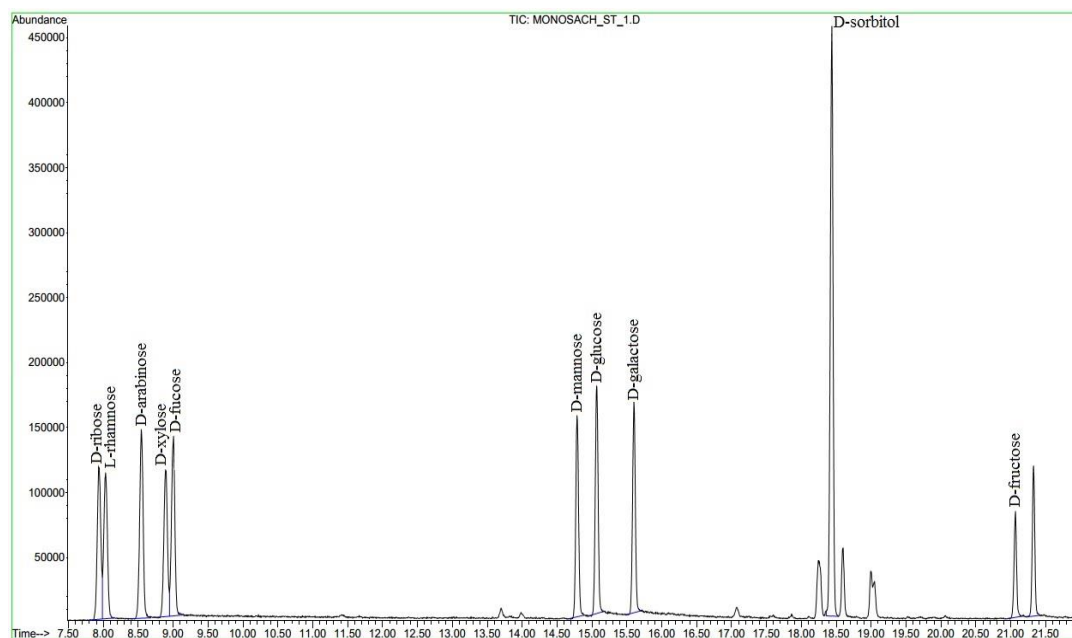


Fig. 2. GC/MS chromatogram of sugars standards

3.3. Determination of carbohydrates

The monosaccharides composition of *Agrimonia eupatoria* L. herb was determined by GC/MS method on gas chromatograph Agilent 6890N with 5973 inert mass detector (Agilent Technologies, USA). Samples were

analyzed on a capillary column HP-5MS of 30 m in length and an internal diameter of 0.25 mm, a thickness of the stationary phase is 0.25 μm. Firstly, we set up oven temperature at 160°C and held for 8 min, then raised to 240 °C at the rate of 5 °C/min and kept at this point for

6 min. At a constant flow rate of 1.2 sm³/min was used helium as the carrier gas. Detection was performed in the SCAN mode at the width range of 38–400 m/z.

Extraction of free monosaccharides. 0.5 mg of methanol solution with internal standard (sorbitol) was added to 500 mg of powdered raw materials. The extraction was performed at the ultrasonic water bath at 80 °C for 4 hours.

Extraction and hydrolysis of bonded monosaccharides. For the extraction of bonded monosaccharides or monosaccharides after hydrolysis 500 mg of powdered herb of the raw material was placed into the flask and added 5 ml of 2 M trifluoroacetic acid. Hydrolysis was performed under 100 °C for 6 hours. 2 mL of obtained hydrolysate was evaporated and was added 2 ml of an internal standard.

To obtain acetylated aldonitriles. 2 ml of the extract was evaporated to dryness and was added 0.3 ml of derivatization reagent (32 mg/ml of hydroxylamine hydrochloride in pyridine/methanol (4:1, v/v)). The extract was incubated at 75 °C for 25 min. To the samples was subsequently added 1 mL of acetic anhydride and incubated at 75 °C for 15 min. 2 ml of dichloroethane was added and the excess of the derivatization reagents was removed by the double extraction with water and 1 M hydrochloric acid. The dichloroethane layer was dried and dissolved in 300 µl of the mixture of ethyl acetate/heptane (1:1, v/v).

Identification of monosaccharides was based on comparing their retention times with retention times of standards of the mass spectral library NIST 02. Quantification was done by using sorbitol added to the sample [12–14].

The number of carbohydrates in mg/g was calculated according to the following equation:

$$X = \frac{Sx \times Minst \times 1000}{Sinst \times m}$$

where: Sx – is a peak area of each monosaccharide or disaccharide; $Minst$ – is a mass of the internal standard; $Sinst$ – is a peak area of the internal standard; m – is a mass of plant material [12, 15].

The amounts of water-soluble polysaccharides and pectin substances were determined by gravimetric method reported by Kyslychenko O. A. et al [16]. All samples were analyzed in triplicates and the results were averaged.

3.4. Statistical analysis

Statistical processing and data analysis were performed using program package Statistica v 7.0 (Statsoft, USA) for Microsoft Office for Windows – MS Exel 2007. The level of significance was set at * $p < 0.05$ for all statistical analyses.

4. Results

Gas chromatography coupled with mass spectrometry (GC/MS) was used to identify and measure the carbohydrates content in *Agrimonia eupatoria* L. herb. Three free sugars were detected in the studied herb of the *Agrimonia eupatoria* L. included D-glucose, D-galactose and D-fructose, the content of which was 15.02 mg/g, 0.28 mg/g and 12.90 mg/g, respectively (Fig. 3, Table 1).

The results of the study showed that the major components free carbohydrates of the herb of *Agrimonia eupatoria* L. were D-glucose and D-fructose (Table 1).

In this analyzed material, after acidic hydrolysis and derivatization with acetylated aldonitriles, D-rhamnose, L-arabinose, D-xylose, D-mannose, D-glucose, D-galactose, D-mannitol and D-Dulcitol were identified too (Fig. 4, Table 2). The predominant ones were D-glucose (23.88 mg/g), D-galactose (9.37 mg/g) and D-xylose (5.98 mg/g) (Table 2).

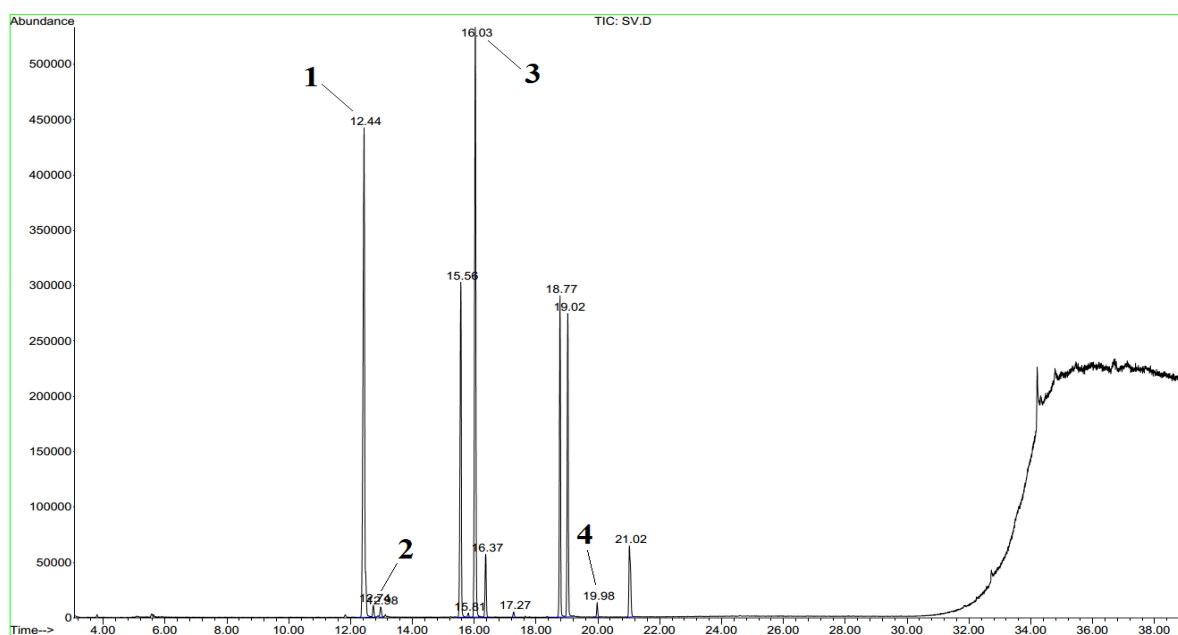


Fig. 3. GC/MS chromatogram of free carbohydrates in *Agrimonia eupatoria* L. herb

Table 1

The results of free carbohydrates determination in *Agrimonia eupatoria* L. herb.

Peak on chromatogram	RT, min	Content of carbohydrates, (mg/g) $\bar{x} \pm \Delta\bar{x}$, n=3, P<0.05	The name of the carbohydrate
1	12.387	15.02±0.03	D-glucose
2	12.9742	0.28±0.01	D-galactose
3	16.0342	internal standard	D-sorbitol
4	19.7711	12.90±0.02	D-fructose

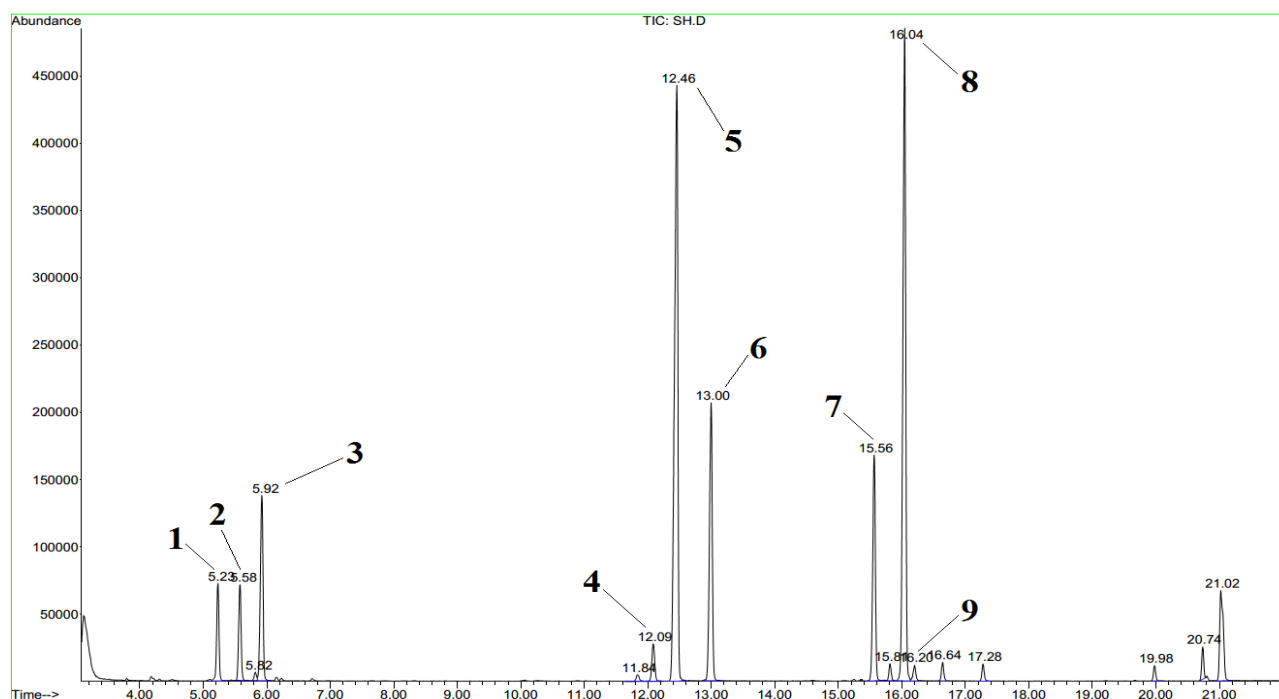
Fig. 4. GC/MS chromatogram of monosaccharides and their derivatives after hydrolysis in *Agrimonia eupatoria* L. herb

Table 2

The results of monosaccharides and their derivatives after hydrolysis in *Agrimonia eupatoria* L. herb.

Peak on chromatogram	RT, min	Content of carbohydrates, (mg/g) $\bar{x} \pm \Delta\bar{x}$, n=3, P<0.05	The name of the carbohydrate
1	5.2308	2.72±0.03	D-rhamnose
2	5.5793	2.78±0.02	L-arabinose
3	5.9236	5.98±0.02	D-xylose
4	12.086	1.32±0.01	D-mannose
5	12.4558	23.88±0.02	D-glucose
6	12.9965	9.37±0.03	D-galactose
7	15.8133	0.49±0.01	D-mannitol
8	16.0428	internal standard	D-sorbitol
9	16.2000	0.50±0.01	D-Dulcitol

The content of water-soluble polysaccharides and pectin substances in *Agrimonia eupatoria* L. was deter-

mined at 2.90±0.02 % and 8.54±0.04 % of total dry weight, respectively (Fig. 5).

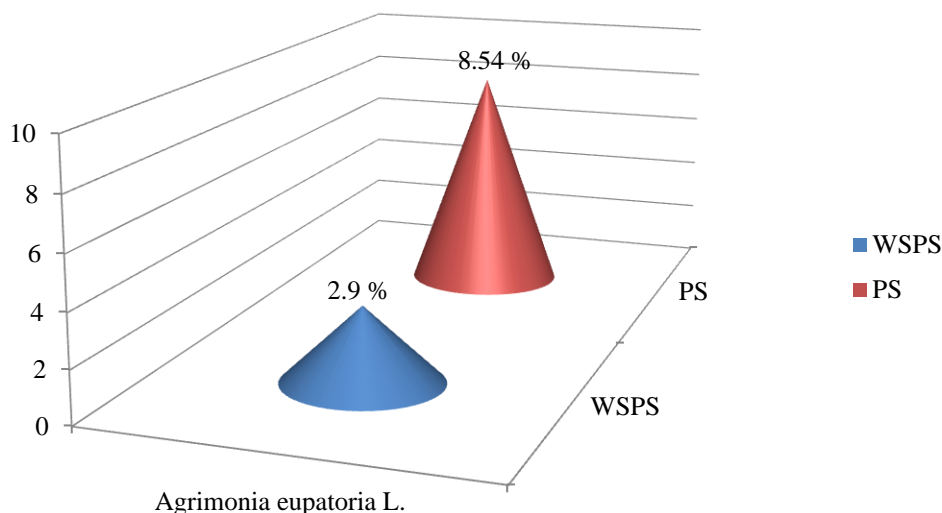


Fig. 5. Water-soluble polysaccharides (WSPS) and pectin substances (PS) in *Agrimonia eupatoria* L.

5. Discussion

Carbohydrates are energy-rich molecules providing energy for life processes as well as building parts of the cell walls of plants and animals. In animals, they play crucial role in the immune system, pathogenesis, and blood clotting responses. Polysaccharides have sorbent, expectorant, antiulcer, anti-inflammatory, and detoxifying actions [15].

We established that in *Agrimonia eupatoria* L. herb the predominant monosaccharide compound was D-glucose: 23.88 mg/g and 15.02 mg/g among monosaccharides after hydrolysis and free monosaccharides respectively. The basic appointment of this aldohexose is to delivery energy for physiological processes [17]. Of the all mammalian organs and tissues, the brain is highly requiring in terms of energy metabolism. Its functions, such as memory, thinking and cognitive learning, are inter-linked to the effective utilization of glucose [18, 19]. If the concentration this monosaccharide lowery, the processes demanding mental effort are deteriorated [13, 17].

In the herb of the *Agrimonia eupatoria* L. also predominant ones were D-galactose 9.37 mg/g and D-xylose 5.98 mg/g. D-galactose is a physiological nutrient that can be combining with glucose and form the disaccharide lactose [20, 21]. D-xylose has antifungal and antibacterial properties, namely is affecting with *Candida species* and gram-negative organisms. In contradistinction to saccharose, xylose promotes the rise of “friendly flora” in the intestines, thus increasing the manufacture and absorption of all foods and strengthening the immune system to help fight off any type of disease [22].

Study limitations. The research needs additional study of water-soluble polysaccharides and pectin substances of the *Agrimonia eupatoria* L. herb in various phases of vegetation. For the statistical importance of the

study, it would be expedient to investigate wild samples of raw materials from different regions of Ukraine.

Prospects for further research. According to the results of the studies, further screening of pharmacological studies, and development of parameters for standardization of *Agrimonia eupatoria* L. herb are planned.

6. Conclusion

In completion, the present results of GC/MS indicated the presence of certain carbohydrates both in free and bound form in *Agrimonia eupatoria* L., that have important medicinal activity, which answered to the knowledge about the therapeutic activity of this analyzed plant. Three free carbohydrates as D-glucose (15.02 mg/g), D-galactose (0.28 mg/g) and D-fructose (12.90 mg/g) were determined in the herb of raw material as the result of the experiment. D-rhamnose, L-arabinose, D-xylose, D-mannose, D-glucose, D-galactose, D-mannitol and D-Dulcitol, after acidic hydrolysis and derivatization with acetylated aldonitriles, were identified in the herb of *Agrimonia eupatoria* L. Among monosaccharides, after hydrolysis and free monosaccharides D-glucose was the predominant monosaccharide compound in the analyzed raw material. The amounts of water-soluble polysaccharides and pectin substances in the herb of *Agrimonia eupatoria* L. were determined by gravimetric method. The content of which was 2.90 % and 8.54 %, respectively. Our findings propose that carbohydrates from the studied raw material can be used for creating medicine of the pharmaceutical industry, and could be used for the development of nutraceuticals.

Conflict of interests

The authors declare that they have no conflicts of interest.

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Natalia Huzio, Assistant, Department of Pharmacy, Ivano-Frankivsk National Medical University, Halytska str., 2, Ivano-Frankivsk, Ukraine, 76018,
E-mail: nataliihuzo@gmail.com

Andriy Hrytsyk, Doctor of Pharmaceutical Sciences, Professor, Department of Pharmacy, Ivano-Frankivsk National Medical University, Halytska str., 2, Ivano-Frankivsk, Ukraine, 76018
E-mail: grycyk@ukr.net

Liudmyla Slobodianiuk, PhD, Assistant Professor, Department of Pharmacognosy and Medical Botany, I. Horbachevsky Ternopil National Medical University of Ministry of Health of Ukraine, Voli ave., 1, Ternopil, Ukraine, 46001
E-mail: lydahusak29@gmail.com