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Research Article

Determination of inulin in the herbal mixtures by GC-MS method

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Abstract

The herbal mixtures due to the wide range of biologically active substances can influence on various links of the pathogenetic mechanism of development of diabetes mellitus and its complications. The carbohydrates, especially inulin, deserve the particular attention through their hypoglycemic, hypolipidemic, anticholesterolemic and detoxifying activities. The aim of the study was to investigate the content of inulin in the herbal mixtures No. 3, No. 4, No. 7, No. 13 and No. 19, which are used in folk medicine for the prevention and treatment of diabetes mellitus type 2 in Ukraine. The quantity content of inulin was defined by the difference between fructose as a product of enzymatic hydrolysis and fructose, a constituent of sucrose and free fructose, taking into account the empirical factor for the conversion of fructose from inulin. The carbohydrates were separated by gas chromatography-mass spectrometry after conversion into volatile derivatives as aldononitrile acetate. According to the results, the herbal mixture No. 3 contains 458.97 mg/g of inulin, the herbal mixture No. 4 – 99.21 mg/g, the herbal mixture No. 7 – 139.93 mg/g, the herbal mixture No. 13 – 203.84 mg/g, the herbal mixture No. 19 – 359.65 mg/g. The availability of inulin and its high content in the investigated herbal mixtures due to the presence of inulin-containing medicinal plants, such as *Cichorium intubus* roots (mixtures No. 3 and No. 13), *Taraxacum officinale* roots (mixtures No. 3, No. 7 and No. 19), *Arctium lappa* roots (mixture No. 4), *Inula helenium* rhizome with roots (mixture No. 7).

Keywords

diabetes mellitus, herbal mixture, inulin, GC-MS

Introduction

Diabetes mellitus is a major problem of World Health Organization, as the epidemiological situation is becoming alarming – the number of patients with diabetes is increasing every year, and with it the number of deaths and disabilities through the development of micro- and macroangiopathies (Harding et. al. 2019; American Diabetes Association 2020). According to the official information of International Diabetes Federation (2019) the number of diabetics will increase to 642 million by 2040. Therefore, the implementation of pharmacotherapy optimization, the search and study of new drugs for the prevention and treatment of this disease and its dangerous complications is a topical issue of pharmacy and medicine.

One such area is phytotherapy, as it has a number of advantages over traditional therapy with using oral synthetic agents, namely, it is low-toxic, has a mild pharmacological effect and can be used for long periods without significant side effects, is well combined with synthetic drugs (Gothai et. al. 2016; Governa et. al. 2018). Particular attention deserve the combinations of different medicinal plants, because such herbal mixtures will have more biologically active substances that will influence on the all links of the pathogenetic mechanism of development of diabetes mellitus and its complications (Oh and Jun 2014; Kooti et. al. 2016; Savych

et. al. 2020a, 2021). Biologically active substances of plant origin have a wide range of pharmacological action and a variety of mechanisms of influencing on the development of diabetes (the pathogenesis of which involves the development of insulin resistance; relative insulin deficiency, which becomes the cause of decrease the secretory activity of β-cells of the pancreatic gland) and diabetic angiopathies (the pathogenesis of which are activation of lipid peroxidation, inactivation of antioxidant protection system and development of oxidative stress) (Oh and Jun 2014; Kooti et. al. 2016; Skyler et. al. 2017; Savych et. al. 2019).

Thus, for this purpose, it is advisable to study the phytochemical compounds, namely the inulin from group of carbohydrates in the investigated herbal mixtures, which are used in folk medicine for the prevention and treatment of diabetes mellitus type 2 in Ukraine (Tovstuha 2010).

Polysaccharide complexes, including inulin, are very important active substances for the prevention and treatment of diabetes mellitus and diabetic angiopathies (Rao et. al. 2019). Inulin, which enters to gastrointestinal tract, stimulates the growth of beneficial bacteria in the colon, including *Bifidobacteria* and *Lactobacilli*, thereby modulating the composition of microflora. This creates an environment that protects against pathogens, toxins and free radicals resulting from lipid peroxidation (Shang et. al. 2018; Hoffman et. al. 2019). Inulin has the ability to regulate the lipid metabolism, a disorder of which occurs in diabetes and leads to the development of cardiovascular diseases and microcirculatory complications – diabetic nephropathy, neuropathy and retinopathy, the formation of diabetic foot. The effect of inulin on lipid metabolism is manifested by a decrease in triglycerides and cholesterol (Hiel et. al. 2018; Mistry et. al. 2018). Inulin has hypoglycemic activity due to its ability to increase glucagon-like peptide-1(GLP-1), which increases the secretion of insulin, inhibits the secretion of glucagon and somatostatin, causes the proliferation and neogenesis of β-cells and increases the response of β-cells to glucose (Kietsiriroje et. al. 2018; Paternoster and Falasca 2018).

Aim of the research

The aim of study was to determine the quantitative content of inulin in the herbal mixtures No. 3, No. 4, No. 7, No. 13 and No. 19 with reliable hypoglycemic activity established during the screening testing (Savych et. al. 2020b, c, d, e, f), which are used in folk medicine for the prevention and treatment of diabetes mellitus type 2 in Ukraine (Tovstuha 2010) by gas chromatography-mass spectrometry (GC-MS) method.

Materials and methods (experimental part)

Plant materials

It was used the herbal raw materials harvested in June – August 2019 in Ternopil region and Charpathians (*Vaccinium myrtillus* leaf) (Ukraine) during the study. After harvesting, the raw materials were dried, crushed and brought back to standard according to the general GACP requirements (WHO 2003). The plants were identified by Department of Pharmacognosy with Medical Botany, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine. Samples of herbal raw materials have been deposited in Departmental Herbarium for future record.

For the study were used the five different herbal mixtures with reliable hypoglycemic activity established during the screening testing (Savych et. al. 2020b, c, d, e, f), which are used in folk medicine for the prevention and treatment of diabetes mellitus type 2 in Ukraine (Tovstuha 2010). The compositions of the mixtures are given in Table 1.

Table 1. Composition of herbal mixtures.

The herbal mixtures	The herbals	Quantity of herbals in mixtures, g
No. 3	*Urtica dioica* leaf	26.32
	Cichorium intubus roots	26.32
	Rosa majalis fruits	21.05
	Elymys repens rhizome	15.79
	Taraxacum officinale roots	10.52
		Total: 100.0
No. 4	*Arctium lappa* roots	26.32
	Elymys repens rhizome	26.32
	Zea mays columns with stigmas	21.05
	Helichrysum arenarium flowers	15.79
	Rosa majalis fruits	10.52
		Total: 100.0
No. 7	*Inula helenium* rhizome with roots	10.0
	Helichrysi arenarium flowers	20.0
	Zea mays columns with stigmas	20.0
	Origanum vulgari herb	20.0
	Rosa majalis fruits	20.0
	Taraxacum officinale roots	10.0
		Total: 100.0
No. 13	*Cichorium intubus* roots	26.32
	Elymys repens rhizome	26.32
	Helichrysum arenarium flowers	21.05
	Rosa majalis fruits	15.79
	Zea mays columns with stigmas	10.52
		Total: 100.0
No. 19	*Urtica dioica* leaf	20.0
	Taraxacum officinale roots	20.0
	Vaccinium myrtillus leaf	20.0
	Rosa majalis fruits	20.0
	Mentha piperita herb	20.0
		Total: 100.0

Chemicals and standards

All applied reagents were of analytical grade (≥ 95% purity). Standard reagents including D-arabinose, D-glucose, D-fructose, saccharose were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The water used in the studies was produced by MilliQ Gradient water deionizaton system (Millipore, Bedford, MA, USA). Inulinase, acetate buffer**,** methanol, hydroxylamine hydrochloride, pyridine, dichloroethane, hydrochloride acid, heptanes, ethyl acetate were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Chromatographic condition

The quantity content of inulin in the herbal mixtures was studied by GC-MS method. Chromatographic separation was performed on a gas chromato-mass spectrometric system model 6890N/5973inert (Agilent Technologies, USA) using a capillary column HP-5ms (30 m×0.25 mm×0.25 mkm, Agilent Technologies, USA). The evaporator temperature was 250 °C, the interface temperature – 280 °C. The separation was performed in the mode of temperature programming – the oven temperature was initially at 160 °C, held for 8 min, then ramped at the rate of 5 °C/min to 240 °C and finally held at this temperature for 6 min. The samples 1 μL were administered in a 1:50 flow divider mode. The detection was held in the SCAN mode in the range of (38–400 m/z). The carrier gas flow rate through the column was 1.2 mL/min.

Extraction

The samples of herbal raw materials were grinded into a powder by laboratory mill, then about 50–80 mg (accurately mass) was placed in a glass vial and 4 mL of 0.1 M acetate buffer (pH 4.5) was added. Extraction of inulin was performed in the ultrasonic bath at 80 °C for 3 hours. The resulting extracts were centrifuged at 3000 rpm and the supernatants were evaporated to dryness on a rotary evaporator. One part of the extract was used for enzymatic hydrolysis of inulin with 100 μL of inulinase at 60 °C for 30 min (Vendrell-Pascuas et. al. 2000). The rest of the extract was used for the determination of free fructose.

Derivatisation

To obtain the aldonitrile monosaccharide derivatives, an aliquots 0.6 mL of the extracts were taken and 0.3 mL of a derivatizing reagent (32 mg/mL of hydroxylamine hydrochloride in the mixture of pyridine/ methanol (4:1, v/v)) was added. Samples were incubated in a preheated water bath shaker at 75 °C for 25 min. After incubation, 1.0 mL of acetic anhydride was subsequently added to the samples and incubated at 75 °C for 15 min. 2 mL of dichloromethane was added to the mixture, the excess of the derivatization reagents was removed by the double extraction with 1 M hydrochloride acid solutions and water. Dichloromethane layer was dried and dissolved into 300 μL of the mixture of heptane/ethyl acetate (1:1, v/v) (Guerrant and Moss 1984, Chen et. al. 2009).

Identification and calculation

Identification of enzymatic hydrolysis products, free monosaccharides and disaccharide – sucrose was performed by comparing of the retention time of the mixture of standard and using the NIST 02 mass spectrum library. Quantitative analysis was performed by adding a solution of internal standard – arabinose 0.25 mg in the test samples. Under normal conditions of derivatization, the ketone carbohydrate (fructose) is converted into an aldo carbohydrate (glucose) (Agius et. al. 2018). According to this technique, fructose in derivatization gives 2 peaks, which are summed up during calculations.

The concentration of total fructose (C_1, mg/mL), free fructose (C_2, mg/mL) and sucrose (C_{sucr}, mg/mL) was determined by the method of internal standards according to the formula:

$$C = \frac{S_x \times m_{st} \times V_{sol}}{S_{st} \times m_x \times V_{extr}} \times 1000$$

where S_x – peak area of the studied substance;

m_{st} – mass of the internal standard injected into the sample, mg;

S_{st} – peak area of the internal standard;

m_x – mass of sample of raw materials, mg;

V_{sol} – volume of solvent for extraction, mL;

V_{extr} – volume of extract for derivatization, mL.

The concentration (C_3, mg/mL) of fructose released from sucrose was calculated by the formula:

$$C_3 = \frac{C_{\text{sucr}}}{B}$$

where C_{sucr} –concentration of sucrose, mg/mL;

B – empirical factor for the conversion of fructose from sucrose (2.13).

Quantitative content (X, mg/g) of inulin was determined as the subtraction from total content of fructose after enzymatic hydrolysis, free fructose and fructose released by decomposition of sucrose according to the formula:

$$X = \frac{A \times (C_1 - C_2 - C_3)}{m_1}$$

where C_1 – concentration of total fructose, mg/mL;

C_2 – concentration of free fructose, mg/mL;

C_3 – concentration of fructose released from sucrose, mg/mL,

A – empirical factor for the conversion of fructose from inulin (1.03);

m_1 – mass of raw materials on which was calculated, g.

The empirical factor for the conversion of fructose from inulin and sucrose (the factor of conversion of inulin to fructose and sucrose to fructose) was determined by sequential processing of samples with different amounts of inulinase using arabinose as the internal standard and determining the amount of fructose released (Vendrell-Pascuas et. al. 2000).

Results and discussion

During the study it was detected the inulin in all investigated herbal mixtures No. 3, No. 4, No. 7, No. 13 and No. 19 by the products of its enzymatic hydrolysis after conversion into volatile derivatives as aldononitrile acetate (Figs 1, 3, 5, 7, 9). The results of quantitative study of inulin, as an important substance of natural origin with hypoglycemic, hypolipidemic, anticholesterolemic and

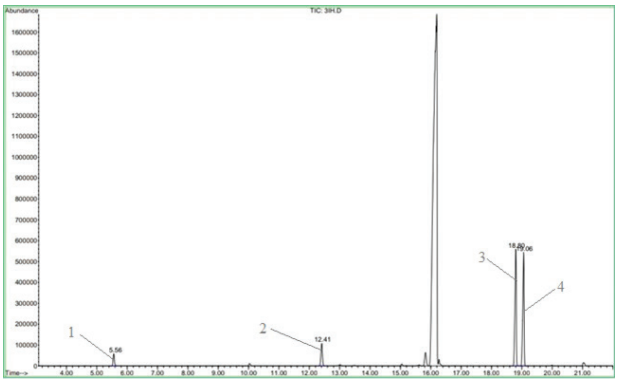

Figure 1. Chromatogram of carbohydrates formed as a result of enzymatic hydrolysis in the herbal mixture No. 3 (1 – arabinose, internal standard; 2 – glucose; 3–4 – fructose).

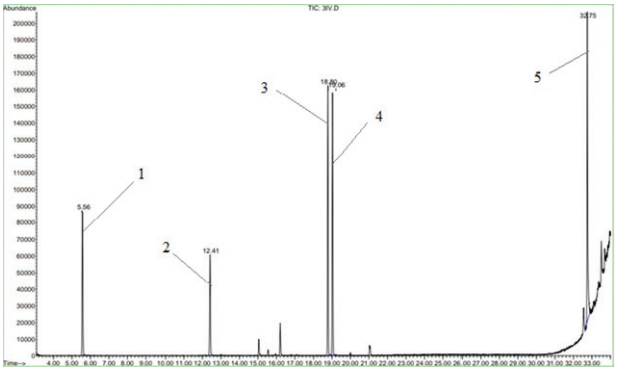

Figure 2. Chromatogram of free carbohydrates in the herbal mixture No. 3 (1 – arabinose, internal standard; 2 – glucose; 3–4 – fructose; 5 – sucrose).

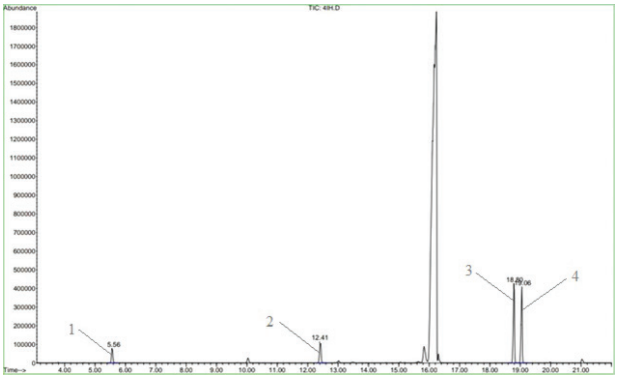

Figure 3. Chromatogram of carbohydrates formed as a result of enzymatic hydrolysis in the herbal mixture No. 4 (1 – arabinose, internal standard; 2 – glucose; 3–4 – fructose).

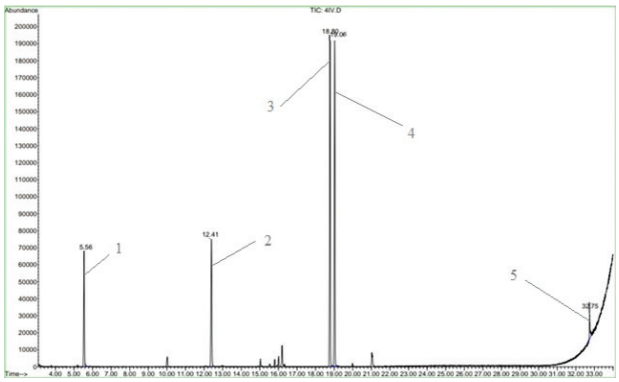

Figure 4. Chromatogram of free carbohydrates in the herbal mixture No. 4 (1 – arabinose, internal standard; 2 – glucose; 3–4 – fructose; 5 – sucrose).

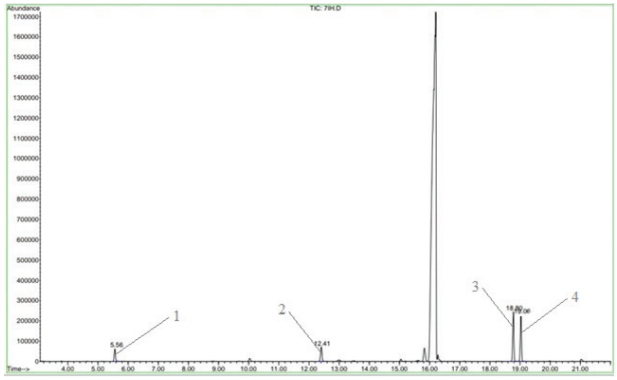

Figure 5. Chromatogram of carbohydrates formed as a result of enzymatic hydrolysis in the herbal mixture No. 7 (1 – arabinose, internal standard; 2 – glucose; 3–4 – fructose).

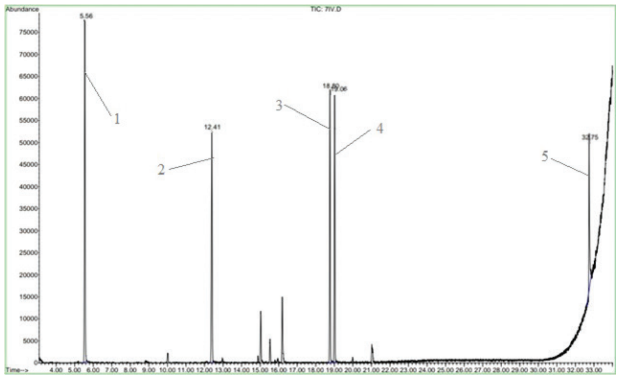

Figure 6. Chromatogram of free carbohydrates in the herbal mixture No. 7 (1 – arabinose, internal standard; 2 – glucose; 3–4 – fructose; 5 – sucrose).

detoxifying activities in the herbal mixtures No. 3, No. 4, No. 7, No. 13 and No. 19 are shown in Table 2.

During the chromatographic analysis of the herbal mixtures extracts to which inulinase was added, the total content of D-fructose as a product of enzymatic hydrolysis and free D-fructose was determined (Figs 1, 3, 5, 7, 9, Table 2). In the second phase of the study, it was established the quantitative content of free D-fructose and sucrose in the herbal mixtures extracts (Figs 2, 4, 6, 8, 10, Table 2). Since during enzymatic hydrolysis, it was received fructose in the amount corresponding to the content of the product of enzymatic hydrolysis of inulin and sucrose and D-fructose contained in the free state, so the calculation of the content of inulin was carried out by subtracting free D-fructose and D-fructose, which is part of sucrose, given the empirical factor for the conversion of fructose from inulin.

The results of the quantitative study showed that the herbal mixture No. 3 contains 458.97 mg/g of inulin, the herbal mixture No. 4 – 99.21 mg/g, the herbal mixture No.

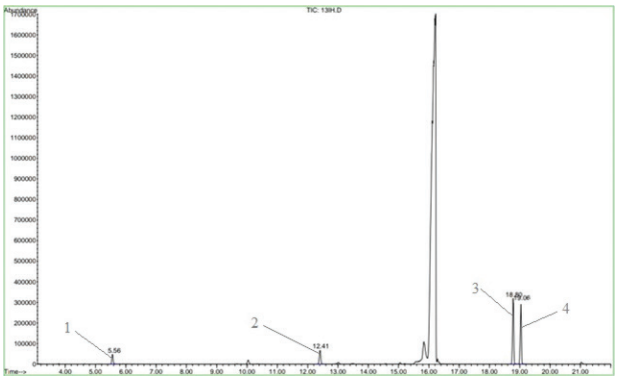

Figure 7. Chromatogram of carbohydrates formed as a result of enzymatic hydrolysis in the herbal mixture No. 13 (1 – arabinose, internal standard; 2 – glucose; 3–4 – fructose).

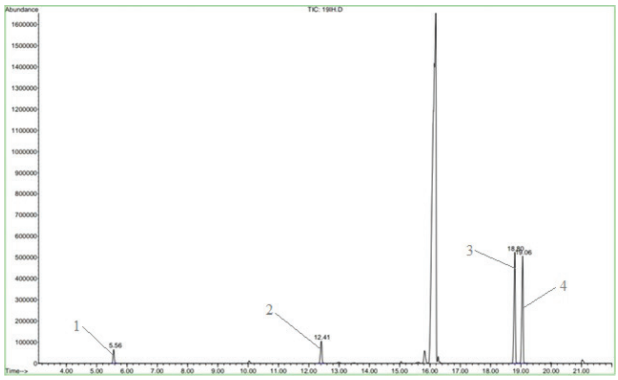

Figure 9. Chromatogram of carbohydrates formed as a result of enzymatic hydrolysis in the herbal mixture No. 19 (1 – arabinose, internal standard; 2 – glucose; 3–4 – fructose).

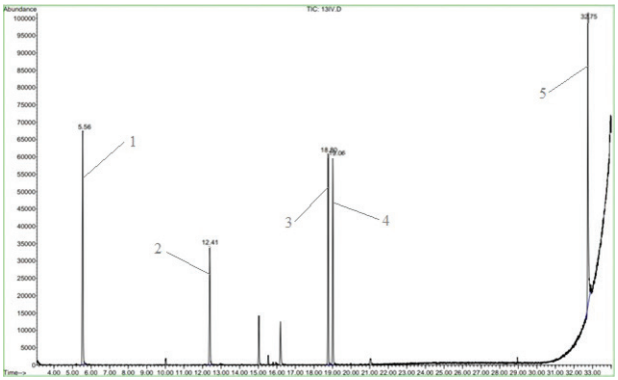

Figure 8. Chromatogram of free carbohydrates in the herbal mixture No. 13 (1 – arabinose, internal standard; 2 – glucose; 3–4 – fructose; 5 – sucrose).

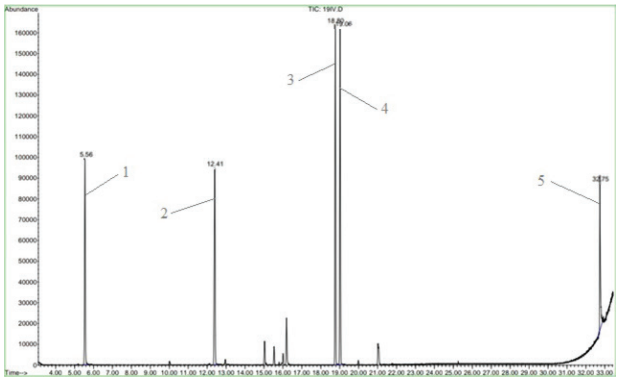

Figure 10. Chromatogram of free carbohydrates in the herbal mixture No. 19 (1 – arabinose, internal standard; 2 – glucose; 3–4 – fructose; 5 – sucrose).

Table 2. The results of the GC-MC analysis of carbohydrates in the herbal mixtures.

No. of peak on chromatograms	Retention time, min	Identified substance	Derivatization products	Content in the herbal mixtures, mg/g				
				No. 3	No. 4	No. 7	No. 13	No. 19
			FREE CARBOHYDRATES					
1.	5.56	arabinose	2,3,4,5-tetra-O-acetyl-D-arabinononitrile	internal standard				
2.	12.41	glucose	2,3,4,5,6-penta-O-acetyl-D-gluconitrile	23.01	37.80	17.67	11.09	26.99
3.	18.80	fructose	naphthalene-1-carboxylic acid, 4-butylamino-6,7-dimethoxy-2-methyl-ethyl ester	51.56	87.08	17.70	16.54	38.29
4.	19.06	fructose	1-nitro-4-phenoxyanthraquinone	49.88	82.48	16.66	15.98	37.55
5.	32.75	sucrose	sucrose octaacetate	58.74	5.84	9.25	24.27	17.14
			CARBOHYDRATES AFTER HYDROLISIS					
1.	5.56	arabinose	2,3,4,5-tetra- O-acetyl- D-arabinononitrile	internal standard				
2.	12.41	glucose	2,3,4,5,6-penta-O-acetyl-D-gluconitrile	60.29	44.98	32.94	33.08	48.23
3.	18.80	fructose	naphthalene-1-carboxylic acid, 4-butylamino-6,7-dimethoxy-2-methyl-ethyl ester	297.68	140.75	90.79	128.62	224.46
4.	19.06	fructose	1-nitro-4-phenoxyanthraquinone	276.95	127.87	83.77	113.19	208.61

7 – 139.93 mg/g, the herbal mixture No. 13 – 203.84 mg/g, the herbal mixture No. 19 – 359.65 mg/g (Fig. 11).

The detection of inulin and establishment of its high content in the investigated herbal mixtures is a predictable result,because these phytomixtures include the medicinal inulin-containing plants: *Cichorium intubus* roots (herbal mixtures No. 3 and No. 13), *Taraxacum officinale* roots (herbal mixtures No. 3, No. 7 and No. 19), *Arctium lappa* roots (herbal mixture No. 4), *Inula helenium* rhizome with roots (herbal mixture No. 7) (Tovstuha 2010).

The chromatographic analysis of inulin as an important biologically active substance with hypoglycemic,

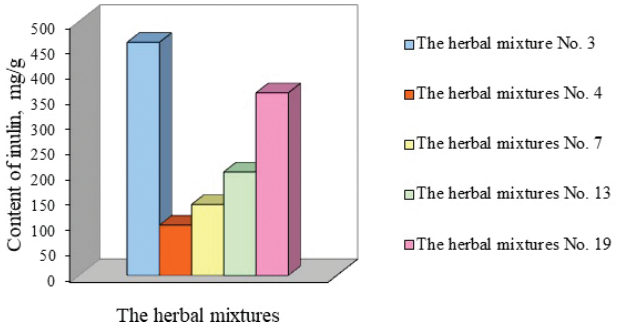

Figure 11. The diagram of the comparative analysis of inulin in the herbal mixtures.

hypolipidemic, anticholesterolemic and detoxifying activities in the herbal mixtures indicate the advisability of the further pharmacological and phytochemical research of these phytomixtures as promising herbal medicines for the prevention and treatment of diabetes mellitus and its complications.

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The authors have declared that no competing interests exist.

The authors have no support to report.

Conclusion

We established for the first time, the quantity content of inulin in herbal mixtures No. 3, No. 4, No. 7, No. 13 and No. 19 after enzymatic hydrolysis by GC-MS method. The obtained results make these phytomixtures perspective for the future medical application against diabetes, its complications and metabolic disorders. However, in the future studies phytochemical and pharmacological investigations should be undertaken to better assess their potential.

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