

Determination of fatty acid composition content in the herbal antidiabetic collections

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Abstract

It was determined the qualitative composition and the quantitative content of fatty acids in the herbal antidiabetic collection № 3, № 4, № 7, № 13, № 19, which are used in folk medicine for prevention and treatment of diabetes mellitus type 2 in Ukraine by GC/MS method. According to the results it was identified 8 fatty acids in the herbal antidiabetic collection № 3, 13 fatty acids in the herbal antidiabetic collection № 4, 12 fatty acids in the herbal antidiabetic collection № 7, 13 fatty acids in the herbal antidiabetic collection № 13 and 11 fatty acids in the herbal antidiabetic collection № 19. The saturated fatty acids were found to be dominated in the raw plant material. The results of the quantitative research showed that the herbal antidiabetic collection № 3 contains 16.13 mg/g of fatty acids, the herbal antidiabetic collection № 4 – 27.17 mg/g, the herbal antidiabetic collection № 7 – 31.98 mg/g, the herbal antidiabetic collection № 13 – 27.37 mg/g, the herbal antidiabetic collection № 19 – 18.79 mg/g. The GC/MS analysis of the fatty acid composition in the herbal antidiabetic collections has shown that this raw material has a lot of fatty acids, which can have a positive effect for the treatment and prevention of diabetes mellitus type 2.

Keywords

diabetes mellitus, herbal antidiabetic collection, GC/MS, fatty acids

Introduction

Diabetes mellitus is a major medical and social problem of the 21st century, which is gaining global scope, as the number of patients grows every year in geometric progression, and with it disability and mortality from serious complications (Harding et al. 2019; American Diabetes Association 2020) According to official forecasts of International Diabetes Federation (2019) experts, the number of patients is expected to increase by 1.5 times by 2030, which will amount to half a billion. Therefore, the search of new drugs for the correction of metabolic disorders in this disease is a topical issue of pharmacy and medicine. One of these areas is the application of herbal reme-

dies, as they have a number of advantages over synthetic agents, namely, they are low-toxic, have a mild pharmacological effect and can be used for long periods without significant side effects, are well combined with synthetic drugs (Gothai et al 2016). Their phytochemical composition is quite diverse that has a multidirectional mechanism of influence on all links of the pathogenesis of diabetes mellitus (Oh and Jun 2014).

Special attention is paid to the collections of medicinal plants, because the combination of medicinal plant raw materials containing different groups of biologically active substances creates a phytocompositions that can cover all pathogenetic mechanisms of development of diabetes and its complications, namely to reduce insulin resistance,

stimulate the processes of regeneration in β -cells of the pancreas, increase insulin synthesis and prolong its action, activate the system of antioxidant protection, reduce lipid peroxidation, increase the protective functions of the organism, normalize secondary metabolic disorders (Oh and Jun 2014; Savych et al. 2019).

For this purpose, it is expedient to study the phytochemical composition, namely the fatty acids of the investigated herbal collections widely used in folk medicine for prevention and treatment of diabetes mellitus type 2 in Ukraine (Tovstuha 2010).

Fatty acids are very important active substances for the treatment and improving of the course of diabetes, because they prevent the oxidation of cell membrane lipids, contribute to the reduction of blood cholesterol, normalize lipid and protein metabolism, increase the liver's detoxification function, stimulate immune-protective function, increase the elasticity and reduce the permeability of the walls of blood vessels, improve microcirculation (Karpe et al. 2011; Sears and Perry 2015). Particularly important for the human body is linoleic acid, which is part of the omega-6 fatty acids, and linolenic acid – of omega-3 fatty acids, which normalize the function of cellular and sub-cellular membranes (Brown et al. 2019).

Aim of the research

The aim of this work was to identify and determine the quantitative content of fatty acids in the herbal antidiabetic collections № 3, № 4, № 7, № 13, № 19 with reliable hypoglycemic activity established during the screening study (Savych and Marchyshyn 2019a, 2019b, 2020), which used in folk medicine for prevention and treatment of diabetes mellitus type 2 in Ukraine (Tovstuha 2010) by gas chromatography/mass spectrometry method (GC/MS) (Ecker et al. 2012).

Materials and methods (experimental part)

The objects of study were the herbal antidiabetic collections № 3 (*Urtica dioica* leaf, *Cichorium intubus* roots, *Rosa majalis* fruits, *Elymys repens* rhizome, *Taraxacum officinale* roots) № 4 (*Arctium lappa* roots, *Elymys repens* rhizome, *Zea mays* columns with stigmas, *Helichrysum arenarium* flowers, *Rosa majalis* fruits), № 7 (*Inula helenium* rhizome with roots, *Helichrysi arenarium* flowers, *Zea mays* columns with stigmas, *Origanum vulgare* herb, *Rosa majalis* fruits, *Taraxacum officinale* roots), № 13 (*Cichorium intubus* roots, *Elymys repens* rhizome, *Helichrysum arenarium* flowers, *Rosa majalis* fruits, *Zea mays* columns with stigmas), № 19 (*Urtica dioica* leaf, *Taraxacum officinale* roots, *Vaccinium myrtillus* leaf, *Rosa majalis* fruits, *Mentha piperita* herb) with reliable hypoglycemic activity established during the screening study (Savych and Marchyshyn 2019a, b, 2020), which used in folk medicine for

the treatment and prevention of diabetes mellitus type 2 (Tovstuha 2010).

The research used raw materials harvested in June – August 2019 in Ternopil region and Carpathians (*Vaccinium myrtillus* leaf) (Ukraine). 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.

All applied reagents were of the highest purity available and purchased from the Ltd. Sfera Sim, Lviv, Ukraine.

The sample of herbal raw material was grinded into a powder by laboratory mill, then about 0.5 g (accurately mass) was selected and placed into the glass vial and reacting mixture (methanol: toluene: sulfuric acid (44:20:2 v/v)) 3.3 mL and internal standard solution (undecanoic acid in heptane solution) 1.7 mL were added. The sample was kept at 80 °C for 2 hours, refrigerated and centrifuged for 10 minutes at 5000 rpm. It was taken 0.5 mL of the upper heptane phase, which containing methyl esters of fatty acids (Kucher and Galkevych 2011).

The fatty acid composition of the herbal antidiabetic collections was studied by gas chromatography-mass spectrometry using the Agilent Technologies 6890 gas chromatograph with mass spectrometry detector 5973 and capillary column. Chromatography conditions: chromatographic column – capillary HP- 5 ms (with internal diameter 0.25 mm, length 30 m), carrier gas velocity (helium) – 1.0 mL/min, temperature of the sample injection heater – 250 °C, temperature thermostat programmable from 60 to 320 °C at a speed of 7 deg/min (Ecker et al. 2012).

To identify the components, the obtained spectra were considered by comparing the results of retention time with the data of the mass spectra libraries NIST05 and WILEY 2007 with the total number of spectra more than 470000 in combination with programs for identification of AMDIS.

Quantitative content (X , mg/kg) was determined by the method of internal standards according to the formula:

$$X = \frac{S_x \times m_1 \times 1000}{S_1 \times m}$$

where S_1 – the peak area of the studied substance;

m_1 – the mass of the internal standard injected into the sample, mg;

S_x – peak area of the standard;

m – sample of raw materials, mg.

Results and discussion

The results of the study of fatty acid compositions in the herbal antidiabetic collections № 3, № 4, № 7, № 13, № 19 are shown in Figs 1–5 and Table 1.

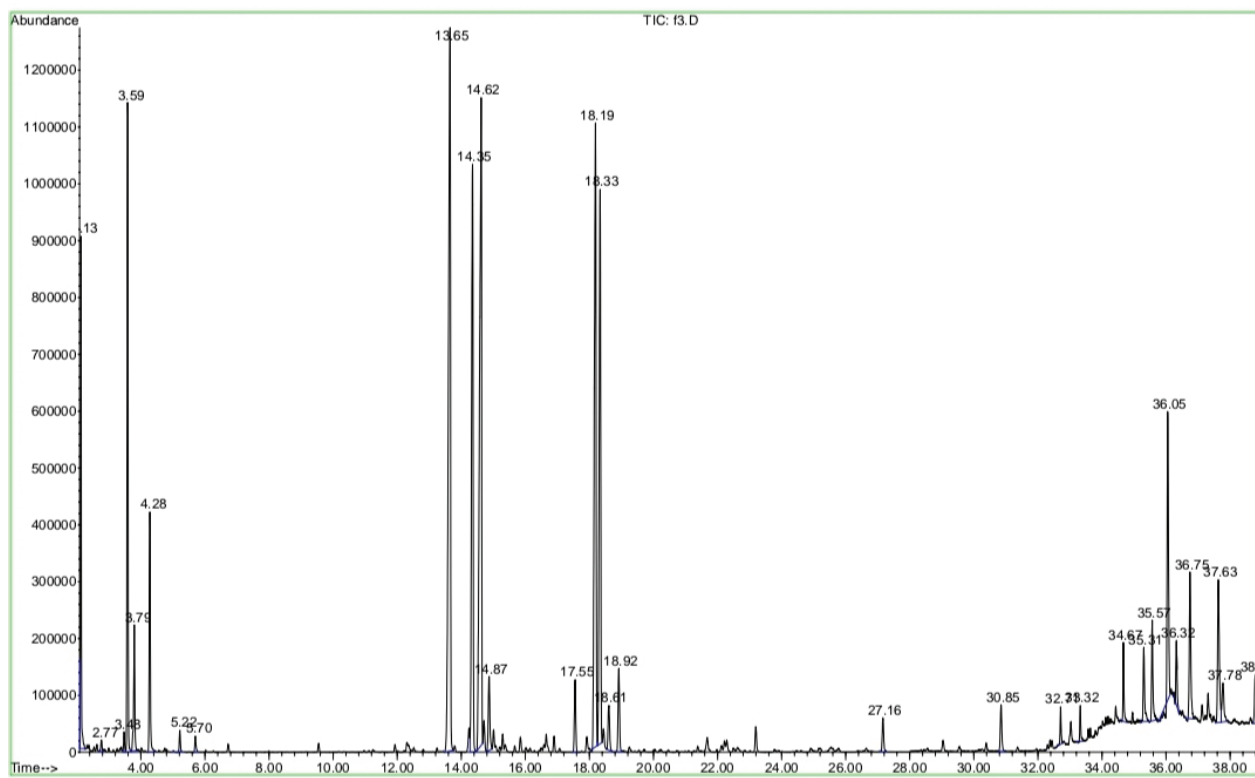


Figure 1. Chromatogram of fatty acids in the herbal antidiabetic collection № 3.

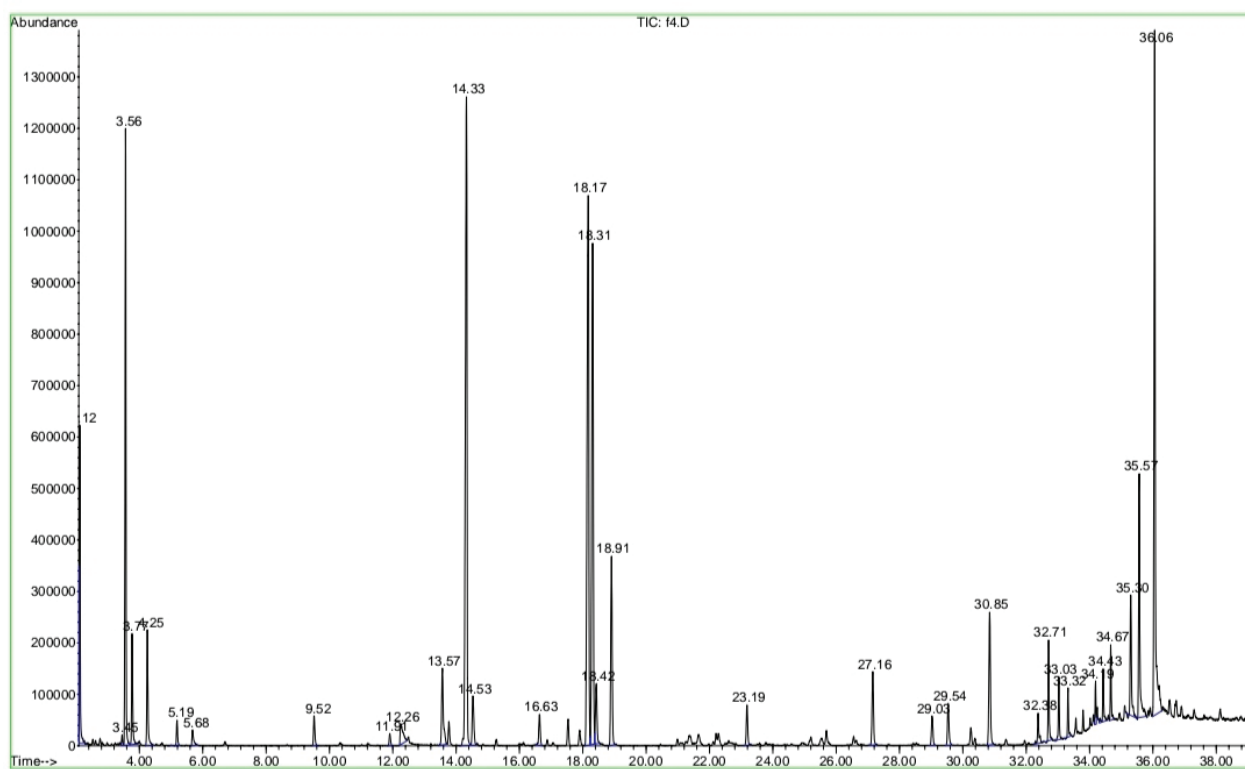


Figure 2. Chromatogram of fatty acids in the herbal antidiabetic collection № 4.

According to the results of the gas chromatographic study, it was identified 8 fatty acids (6 saturated and 2 polyunsaturated) in the herbal antidiabetic collection № 3;

13 fatty acids (11 saturated and 2 polyunsaturated) in the herbal antidiabetic collection № 4; 12 fatty acids (10 saturated and 2 polyunsaturated) in the herbal antidiabetic

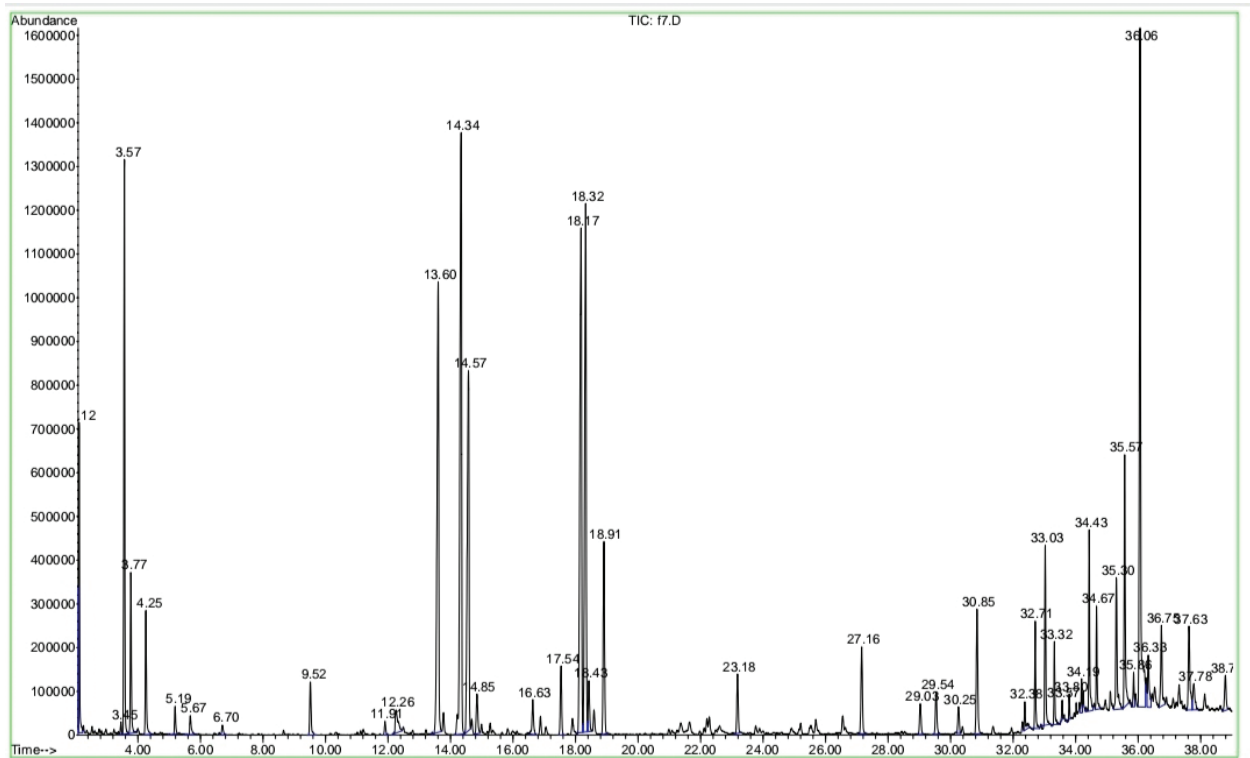


Figure 3. Chromatogram of fatty acids in the herbal antidiabetic collection № 7.

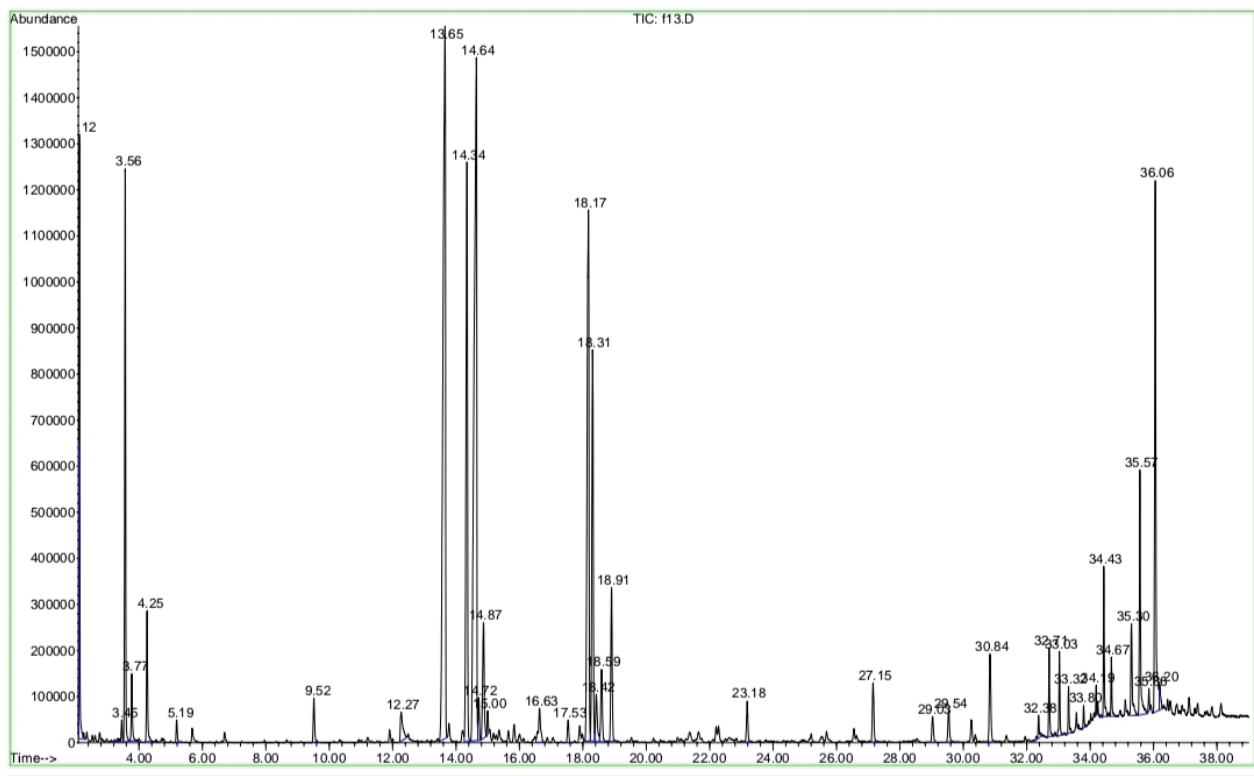


Figure 4. Chromatogram of fatty acids in the herbal antidiabetic collection № 13.

collection № 7; 13 fatty acids (11 saturated and 2 polyunsaturated) in the herbal antidiabetic collection № 13 and 11 fatty acids (9 saturated and 2 polyunsaturated) in the herbal antidiabetic collection № 19 (Figs 1–5).

The results of the quantitative study showed that the herbal antidiabetic collection № 3 contains 12.91 mg/g of saturated fatty acids, of which the largest amount of behenic acid 3.34 mg/g and stearic acid 3.22 mg/g and

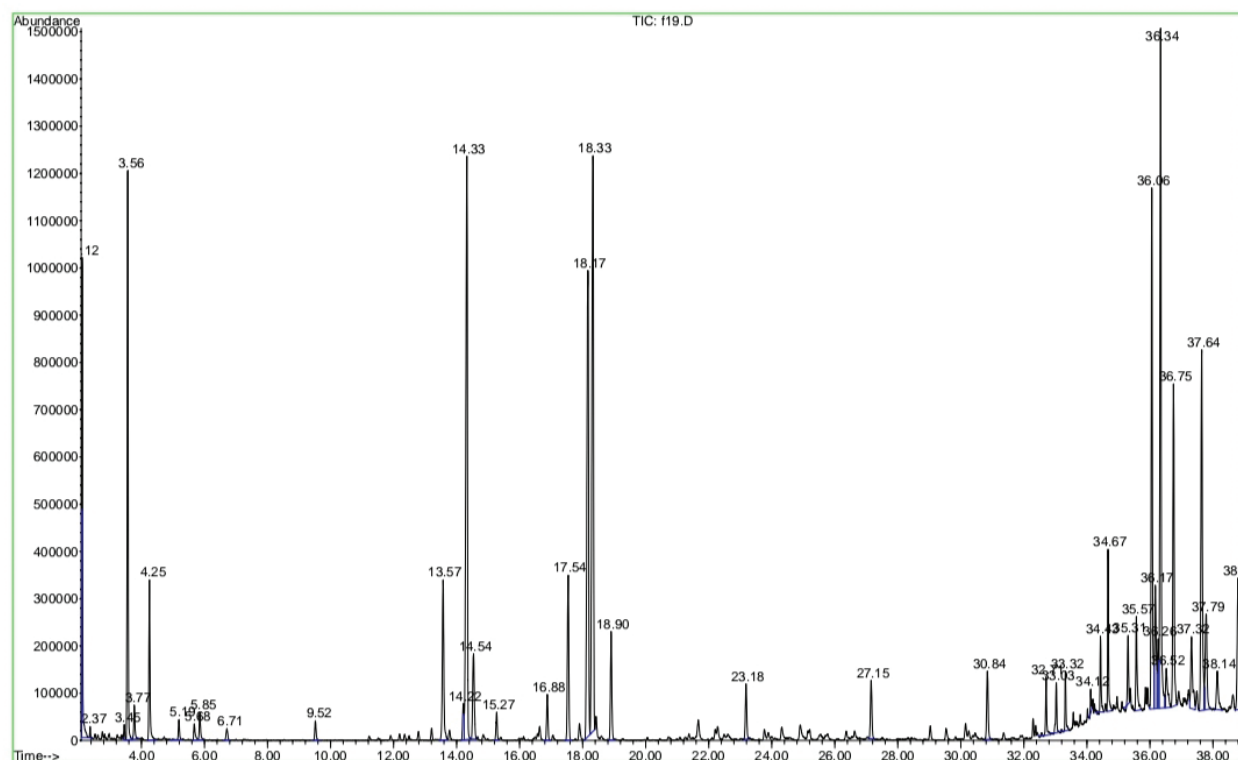


Figure 5. Chromatogram of fatty acids in the herbal antidiabetic collection № 19.

Table 1. The results of the comparative analysis of fatty acids in the herbal antidiabetic collections.

№	Retention time	Common name of fatty acid	Chemical nomenclature	Quantitative content of methyl esters of fatty acids in the herbal antidiabetic collections, mg/g				
				№ 3	№ 4	№ 7	№ 13	№ 19
Saturated acids								
1.	5.19	Lauric (dodecanoic)	C 12:0	1.53	1.64	–	1.59	1.67
2.	9.52	Myristic (tetradecanoic)	C 14:0	–	1.64	1.66	1.66	1.67
3.	14.33	Pentadecylic (pentadecanoic)	C 15:0	–	3.42	3.88	3.34	1.74
4.	14.54	Palmitic (hexadecanoic)	C 16:0	1.6	–	–	–	1.6
5.	16.63	Margaric (heptadecanoic)	C 17:0	–	1.69	1.7	1.64	–
6.	18.91	Stearic (octadecanoic)	C 18:0	3.22	5.19	6.88	4.14	1.74
7.	23.18	Arachidic (eicosanoic)	C 20:0	–	1.73	1.72	1.7	1.74
8.	27.15	Behenic (docosanoic)	C 22:0	3.34	1.5	3.33	3.26	1.74
9.	29.03	Tricosylic (tricosanoic)	C 23:0	–	1.73	2.62	1.7	–
10.	30.84	Lignoceric (tetracosanoic)	C 24:0	1.61	1.73	1.72	1.7	1.74
11.	32.38	Pentacosylic (pentacosanoic)	C 25:0	–	1.71	1.66	1.68	–
12.	33.32	Cerotic (hexacosanoic)	C 26:0	1.61	1.73	3.37	1.56	1.67
Polyunsaturated acids (ω-3 and ω-6)								
13.	18.17	Linoleic (octadecadienic, ω -6)	C 18:2	1.61	1.73	1.72	1.7	1.74
14.	18.33	Linolenic (octadecatrienic, ω -3)	C 18:3	1.61	1.73	1.72	1.7	1.74
The amount of saturated fatty acids				12.91	23.71	28.54	23.97	15.31
The amount of unsaturated fatty acids				3.22	3.46	3.44	3.4	3.48
Total				16.13	27.17	31.98	27.37	18.79

it contains 1.61 mg/g of linoleic and linolenic acids in each from group of polyunsaturated fatty acids. The herbal antidiabetic collection № 4 contains 23.71 mg/g of saturated fatty acids with the greatest content of stearic acid 5.19 mg/g and pentadecylic acid 3.42 mg/g and it contains 1.73 mg/g of linoleic and linolenic polyunsaturated fatty acids in each. It was established the content of saturated fatty acids 28.54 mg/g with the greatest content of stearic acid 6.88 mg/g, pentadecylic acid 3.88

mg/g, cerotic acid 3.37 mg/g and behenic acid 3.33 mg/g and content of polyunsaturated fatty acids 3.44 mg/g (1.74 mg/g of linoleic and linolenic acids in each) in the herbal antidiabetic collection № 7. As the results of the study was visible that the herbal antidiabetic collection № 13 contains 23.97 mg/g of saturated fatty acids among which prevail stearic acid 4.14 mg/g, pentadecylic acid 3.34 mg/g and behenic acid 3.26 mg/g and it contains 3.4 mg/g of polyunsaturated fatty acids (1.7 mg/g of li-

noleic and linolenic acids in each). The chromatographic research showed that the herbal antidiabetic collection № 19 contains 15.31 mg/g of saturated fatty acids with the largest content of pentadecylic, stearic, arachidic, behenic and lignoceric acid 1.74 mg/g in each and it contains 1.74 mg/g of linoleic and linolenic acids in each from group of polyunsaturated fatty acids (Table 1).

The total content of polyunsaturated fatty acids was 19.96 % in the herbal antidiabetic collection № 3, was 12.75 % in the herbal antidiabetic collection № 4, was 10.76 % in the herbal antidiabetic collection № 7, was 14.42 % in the herbal antidiabetic collection № 13 and was 18.52 % in the herbal antidiabetic collection № 19, which are part of omega-3 and omega-6 and play an important role in the human body, namely provide functions of cell membranes, exhibit anticholesterolemic activity, by converting cholesterol into cholic acids and eliminating them from the body, participate in the metabolism of fats, exhibit anti-inflammatory action, as they participate in the synthesis of prostaglandins, exhibit immunomodulatory activity, improve blood circulation and nervous system function and it is important for the treatment of patients with diabetes and to prevent its complications in the form of micro- and macroangiopathies (Brown et al. 2019).

The chromatographic analysis of the fatty acid composition in the herbal antidiabetic collections indicate the advisability of the further pharmacological and phytochemical research of these data collections as a perspective herbal medicines for the treatment and prevention of diabetes mellitus.

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Conclusion

1. The fatty acid qualitative composition and quantitative content of the herbal antidiabetic collections № 3, № 4, № 7, № 13, № 19, which are used in folk medicine for prevention and treatment of diabetes mellitus type 2 in Ukraine, was determined by the GC/MS method.
2. It was identified 8 fatty acids in the herbal antidiabetic collection № 3, 13 fatty acids in the herbal antidiabetic collection № 4, 12 fatty acids in the herbal antidiabetic collection № 7, 13 fatty acids in the herbal antidiabetic collection № 13 and 11 fatty acids in the herbal antidiabetic collection № 19.
3. It was established that the herbal antidiabetic collection № 3 contains 16.13 mg/g of fatty acids, the herbal antidiabetic collection № 4 – 27.17 mg/g, the herbal antidiabetic collection № 7 – 31.98 mg/g, the herbal antidiabetic collection № 13 – 27.37 mg/g, the herbal antidiabetic collection № 19 – 18.79 mg/g.
4. The amount of saturated fatty acids was 12.91 mg/g in the herbal antidiabetic collection № 3, was 23.71 mg/g in the herbal antidiabetic collection № 4, was 28.54 mg/g in the herbal antidiabetic collection № 7, was 23.97 mg/g in the herbal antidiabetic collection № 13 and was 15.31 mg/g in the herbal antidiabetic collection № 19.
5. The total content of polyunsaturated fatty acids was 3.22 mg/g in the herbal antidiabetic collection № 3, was 3.46 mg/g in the herbal antidiabetic collection № 4, was 3.44 mg/g in the herbal antidiabetic collection № 7, was 3.4 mg/g in the herbal antidiabetic collection № 13 and was 3.48 mg/g in the herbal antidiabetic collection № 19, which are part of omega-3 and omega-6.
6. The conducted researches allow to predict the use of the investigated raw materials for the treatment and prevention of diabetes mellitus type 2.

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