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Research Article

Analysis of amino acids content in the plant components of the antidiabetic herbal mixture by GC-MS

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Abstract

Medicinal plants and their combinations due to the wide range of biologically active substances can influence on various links of the pathogenetic mechanism of development of DM type 2 and its complications. One of such combinations is an antidiabetic herbal mixture (*Urticae folia*, *Rosae fructus*, *Myrtilli folia*, *Menthae folia* and *Taraxaci radices*) with established hypoglycaemic, hypolipidemic, antioxidant, hepatoprotective, pancreatoprotective activity in previous pharmacological studies *in vivo* and *in vitro* and defined phytochemical composition. Thus, the aim of this study was to identify and establish the content of amino acids in the plant components of antidiabetic herbal mixture. The amino acids were separated by GC-MS method with pre-column derivatization. The calibration curves of twenty CRS of amino acids were linear ($R^2 > 0.98$) over the range of 1–100 μg/mL, the LODs and the LOQs were in the range of 0.01–0.07 μg/mL and 0.02–0.20 μg/mL, respectively. The results of analysis showed that the predominant essential amino acid was *L*-proline in *Taraxaci radices*, *Urticae folia*, *Rosae fructus* and *Menthae folia*, its total content was 101.46 mg/g, 25.31 mg/g, 23.04 mg/g and 19.30 mg/g, respectively. In addition, it was established total content of essential amino acid – *L*-leucine that can stimulate insulin secretion in β-cells of the pancreas. Its total content was 58.51 mg/g in *Taraxaci radices*, 9.58 mg/g in *Myrtilli folia*, 4.68 mg/g in *Rosae fructus*, 2.99 mg/g in *Urticae folia* and 0.79 mg/g in *Menthae folia*. Chromatographic examination also revealed *L*-phenylalanine, an essential amino acid important for antidiabetic therapy that can increase insulin secretion, stimulate proliferation and neogenesis of β-cells of the pancreas and reduce insulin resistance. Its total content was 13.42 mg/g in *Myrtilli folia*, 2.23 mg/g in *Rosae fructus*, 1.478 mg/g in *Urticae folia*, 1.46 mg/g in *Taraxaci radices* and 0.52 mg/g in *Menthae folia*. This phytochemical study shows, which plant material forms the amino acid composition and content in the finished herbal mixture and due to which biologically active substances the antidiabetic activity of this phytocomposition is manifested.

Keywords

antidiabetic herbal mixture, amino acids, gas chromatography-mass spectrometry, diabetes mellitus, *L*-proline, *L*-leucine, *L*-phenylalanine

Introduction

Diabetes mellitus (DM) is a global social problem in the field of health care, due to rapid spread of this disease and the development of serious complications such as micro- and macroangiopathies, which significantly reduce the quality and life expectancy of patients (Harding et al. 2019; American Diabetes Association 2020). According to the

official information of International Diabetes Federation (2019) the number of patients will increase to 642 million by 2040. Therefore, the optimization of pharmacotherapy, searching and study of new drugs for the prevention and treatment of DM and its dangerous complications is a top issue of pharmacy and medicine.

One of these areas is phytotherapy, as it has several advantages over traditional therapy, namely, it is low-toxic, has a mild pharmacological effect and possibility to be used for long periods of time without significant side effects, is well combined with synthetic drugs, has a complex activity through several biologically active compounds (Gothai et al. 2016; Governa et al. 2018; Budniak et al. 2021a, b, c, d; Feshchenko et al. 2021; Savych and Polonets 2021; Stechyshyn et al. 2021). The combinations of different medicinal plants deserve particular attention. Plant mixtures are expected to have several biologically active substances with a wide range of pharmacological actions and a variety of mechanisms for influencing the development of DM and its angiopathies (Kooti et al. 2016; Savych and Marchyshyn 2021a, 2021b; Savych et al. 2021g).

One of such combinations is an antidiabetic herbal mixture (*Urticae folia*, *Rosae fructus*, *Myrtilli folia*, *Menthae folia* and *Taraxaci radices*) with established hypoglycaemic, hypolipidemic, antioxidant, hepatoprotective, pancreatoprotective activity in pharmacological study *in vivo* (Savych et al. 2021a, d; Savych and Sinichenko 2021) and *in vitro* (Savych and Mazur 2021) and the defined phytochemical composition that determines such pharmacodynamics (Savych and Basaraba 2021; Savych and Milian 2021; Savych and Nakonechna 2021; Savych et al. 2021b, c, e, f).

Biologically active substances of plant origin cause a large list of pharmacodynamics, including antidiabetic activity, which is manifested by different effects on the pathogenesis of DM type 2 and its complications (Kooti et al. 2016; Skyler et al. 2017; Budniak et al. 2020; Kritsak et al. 2021; Marchyshyn et al. 2021; Slobodianiuk et al. 2021c, d, e). One of the most important phytochemicals is amino acids that play a key role in many metabolic processes as they have a powerful secretolytic activity - stimulate the secretion of insulin, glucagon, cortisol, insulin-like growth factor-1 (IGF-1) (Comerford and Pasin 2016; Savych and Nakonechna 2021). Except this, literature sources indicate the regulatory role of amino acids in the transcription and translation of genes, as well as their important function in intracellular signalling (Comerford and Pasin 2016; Chen et al. 2010). The effectiveness of amino acids in the treatment and prevention of DM type 2 is primarily due to their ability to stimulate insulin secretion in pancreatic β-cells, as well as increase blood glucose utilization and reduce alimentary hyperglycaemia. The greatest insulinotropic effect is inherent in arginine, leucine, isoleucine, alanine, and phenylalanine (Chen et al. 2010; Birech et. al. 2017; Stechyshyn et al. 2020). In addition, amino acids can reduce muscle proteolysis and/or stimulate protein synthesis, which leads to improved protein balance in skeletal muscle and, as a result, increases the process of glucose utilization. This is an important component of antidiabetic therapy because such patients often have a deficiency of skeletal muscle mass, which, in turn, contributes to the development of insulin resistance and progression of this disease (Comerford and Pasin 2016).

Aim of the research

Thus, the aim of this study was to identify and establish the content of amino acids in *Urticae folia*, *Rosae fructus*, *Myrtilli folia*, *Menthae folia*, *Taraxaci radices* as the plant components of antidiabetic herbal mixture.

Materials and methods (experimental part)

Plant materials

It was used the herbal raw materials of *Urticae folia*, *Rosae fructus*, *Myrtilli folia*, *Menthae folia* and *Taraxaci radices* harvested from June to October 2020 in Ternopil region and Charpathians (*Myrtilli folia*) (Ukraine) during the study. The raw materials were then dried, crushed and stored according to the general GACP requirements (WHO 2003). Plants were identified at the Department of Pharmacognosy with Medical Botany, Ivan Horbachevsky Ternopil National Medical University, Ternopil, Ukraine. A vouchers specimens of *Urticae folia* No. 279, *Rosae fructus* No. 168, *Myrtilli folia* No. 254, *Menthae folia* No. 312 and *Taraxaci radices* No. 357 are kept in departmental herbarium for future record.

Chemicals and standards

All applied reagents were of analytical grade (≥ 99% purity). Chemical reference substances (CRS) of amino acids including glycine, *L*-alanine, *L*-valine, *L*-leucine, *L*-serine, *L*-threonine, *L*-isoleucine, *L*-proline, *L*-asparagine, *L*-aspartic acid, *L*-glutamic acid, *L*-methionine, *L*-cysteine, *L*-phenylalanine, *L*-glutamine, *L*-lysine, *L*-histidine, *L*-tyrosine, *L*-tryptophan were purchased from Sigma-Aldrich Chemical Co. (USA), as well as hydrochloric acid, sodium hydroxide, methanol, pyridine, methyl chloroformate, chloroform, sodium bicarbonate. Water used in the studies was produced by MilliQ Gradient water deionizaton system (USA).

Extraction of amino acids

For the extraction of free amino acids the samples of the herbal raw material were grinded into a powder by laboratory mill, then about 0.1 g (accurately weighed) was selected and placed into flask with 2.0 mL of 0.1 M aqueous solution of hydrochloric acid. The extractions were carried out in the ultrasonic water bath at 50 °C for 3 hours. Extraction of bound amino acids was carried out by adding 2 mL of 6 M an aqueous solution of hydrochloric acid to 0.03 g (accurately weighed) of powdered herbal raw materials. Hydrolysis was carried out for 24 hours in a thermostat at 110 °C.

The resulting extracts were centrifuged at 3000 rpm and the supernatants were evaporated to dryness on a rotary evaporator washing three times with distilled water to remove hydrochloric acid.

Pre-column derivatization

The dry samples of plant mixtures were dissolved in 390 µL of 1 M sodium hydroxide, and then 333 µL of methanol and 67 µL of pyridine were added and mixed thoroughly for 5 seconds. To the resulting mixtures was added 80 µL of methyl chloroformate, stirred thoroughly for 60 seconds. The amino acid derivatives were extracted with 400 µL of chloroform followed by the addition of 400 µL of 50 mM sodium bicarbonate. The chloroform phase was used for future analysis (Vancompernolle et al. 2016; Slobodianiuk et al. 2021a, b).

Instrumentation and conditions of gas chromatography-mass spectrometry

The amino acids composition in the samples of the herbal raw materials was studied by gas chromatography-mass spectrometry (GC-MS) method using the Agilent Technologies (USA) system, model 6890N/5973inert (6890 gas chromatography with mass spectrometry detector 5973) and capillary column HP-5ms 5% Phenyl Methyl Siloxane (30 m × 0.25 mm × 0.25 mm, Agilent Technologies) (Chen et. al. 2010; Savych and Nakonechna 2021). 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 set to 50 °C, held for 4 min, then ramped at the rate of 5 °C/min to 300 °C and finally held at this temperature for 5 min. Injections of 1 µL were made in the split mode 1:50. The carrier gas flow rate through the column was 1.0 mL/min.

Identification and calculation by GC-MS

Amino acid identification was performed by comparing the retention times (t_R) of amino acid standards and the presence of representative molecular and fragment ions (Table 1). The content of bound amino acids was determined by subtracting the content of free amino acids from their total content (Chen et. al. 2010).

Method validation

The method was validated for linearity, limit of detection (LOD), limit of quantitation (LOQ) and precision. A standard calibration solution containing 200 µg/mL for each CRS of amino acids was prepared (0.5 g of each CRS of amino acid was dissolved in 250 mL of 0.1 M aqueous solution of hydrochloric acid). From this solution, six decreasing dilutions were made (100, 50, 25, 10, 5, 1 µg/mL). A stock solution of internal standard (Nor-valine) at 5 µg/mL was prepared in 0.1 M aqueous solution of hydrochloric acid. Linearity was performed by injecting a series of standard solutions with a threefold derivatization procedure and a single injection for each CRS of amino acids. The mean value and standard deviation, as well as regression analysis were calculated using Microsoft Excel software package 2016 (USA). The values for LOD and LOQ were calculated based on the data obtained during linearity testing in the low concentration range of the working in the test solution, using the following formulas: LOD = 3.3 * s / Slope; LOQ = 10 * s / Slope. Linearity testing was repeated with the same samples after a complete restart of the system with removal and re-installation of the column. Repeatability precision was determined by six-fold injection of the same sample in a row. For the resulting relative peak area of the quantifier ions the relative standard deviation (RSD) was calculated. To determine intra-day precision, six standard preparations of each CRS of amino acids with the same concentration were single injected and the resulting relative peak areas were used to calculate the RSD. Inter-day precision for the day of sample preparation and the two following days was specified by injecting six standard sample of each reference standard preparations once each on all three days. The RSD of the samples on that day together with the previous samples were calculated as above (Wang et. al 2020).

Table 1. Conditions for chromatographic identification of amino acids.

Amino acids	t_R, min	Molecular ion, m/z	Main fragmentary ions, m/z
Glycine	14.77	147	88
L-alanine	14.85	161	102, 88
L-valine	18.56	189	146, 130, 115, 98
L-leucine	19.57	203	144, 115, 102, 88
L-serine	20.77	191	176, 144, 114, 100, 88
L-threonine	21.11	205	147, 115, 100, 88
L-isoleucine	21.31	203	144, 115, 101, 88
L-proline	21.87	187	128, 84
L-asparagine	21.97	262	146, 127, 95
L-aspartic acid	23.90	219	160, 128, 118, 101
L-glutamic acid	24.02	233	201, 174, 142, 114
L-methionine	26.86	221	147, 128, 115
L-cysteine	27.14	192	192, 176, 158, 146, 132
L-phenylalanine	29.18	237	178, 162, 146, 131, 103, 91
L-glutamine	29.74	276	141, 109, 82
L-lysine	31.90	276	244, 212, 142, 88
L-histidine	35.91	285	254, 226, 210, 194, 140, 81
L-tyrosine	37.24	296	252, 236, 220, 192, 165, 146, 121
L-tryptophan	38.91	276	130

Results and discussion

The analytical procedure has been validated to confirm its reliability. All the peaks of CRS of amino acids showed good linearity ($R^2 > 0.98$) in a wide concentration range (1–100 µg/mL). The results showed that the LODs and the LOQs of amino acids were in the range of 0.01–0.07 µg/mL and 0.02–0.20 µg/mL, respectively, indicating that the sensitivity of the method was satisfactory (Table 2.). The repeatability of the subsequent derivatization and GC-measurement of six standard samples of each reference standard with the same concentration resulted in precision values for the derivatization procedure. For intra- and inter-day precision, the RSD was in a range of 1.24% to 8.10%, which is acceptable.

Table 2. Results of linearity data obtained for CRS of amino acids after GC-MS analysis.

CRS of amino acids	Regression equations	R^2	LOD, µg/mL	LOQ, µg/mL
Glycine	y = 95.25x + 4.308	0.992	0.01	0.03
L-alanine	y = 81.03x + 2.372	0.996	0.01	0.04
L-valine	y = 108.40x – 1.502	0.996	0.02	0.06
L-leucine	y = 44.24x + 2.285	0.984	0.01	0.03
L-serine	y = 110.90x – 0.241	0.998	0.01	0.03
L-threonine	y = 77.24x + 3.222	0.990	0.01	0.04
L-isoleucine	y = 44.24x + 2.285	0.984	0.01	0.03
L-proline	y = 124.50x + 0.359	0.998	0.01	0.02
L-asparagine	y = 80.84x + 2.885	0.990	0.01	0.03
L-aspartic acid	y = 154.40x + 2.375	0.999	0.01	0.03
L-glutamic acid	y = 65.30x + 3.934	0.992	0.06	0.20
L-methionine	y = 198.80x + 0.203	0.999	0.01	0.03
L-cysteine	y = 189.40x + 2.673	0.994	0.01	0.03
L-phenylalanine	y = 149.50x + 9.568	0.990	0.01	0.04
L-glutamine	y = 44.24x + 2.285	0.984	0.06	0.20
L-lysine	y = 127.80x + 5.598	0.984	0.07	0.20
L-histidine	y = 69.28x + 1.579	0.992	0.03	0.10
L-tyrosine	y = 124.90x + 2.897	0.995	0.01	0.05
L-tryptophan	y = 189.40x + 2.673	0.994	0.01	0.04

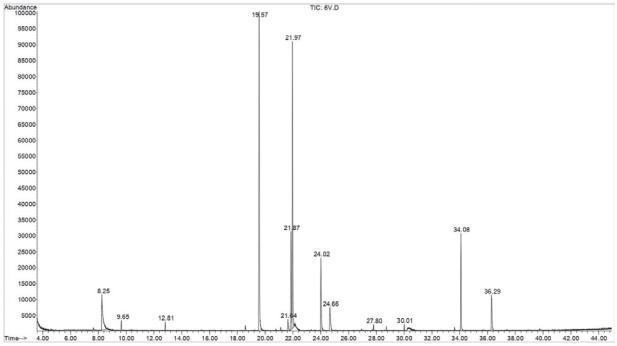

Figure 1. GC-MS chromatogram of derivatives of free amino acids in *Urticae folia*.

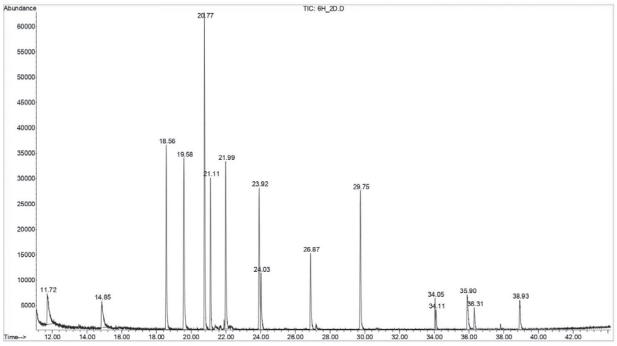

Figure 2. GC-MS chromatogram of derivatives of amino acids after hydrolysis in *Urticae folia*.

According to the results of the GC-MS analysis, it was identified eleven amino acids in free form in *Taraxaci radices* (Fig. 7), ten amino acids in *Myrtilli folia* (Fig. 3), five amino acids in *Urticae folia* (Fig. 1) and *Menthae folia* (Fig. 9), four amino acids in *Rosae fructus* (Fig. 5). GC-MS analysis of amino acids after hydrolysis showed that the largest number of species of these compounds was contained in *Taraxaci radices* (Fig. 8) and it was sixteen, as for other plant components of antidiabetic herbal mixtures, *Myrtilli folia* contained fourteen amino acids (Fig. 4), *Rosae fructus* – twelve amino acids (Fig. 6), *Urticae folia* – eleven amino acids (Fig. 2) and *Menthae folia* – ten amino acids (Fig. 10).

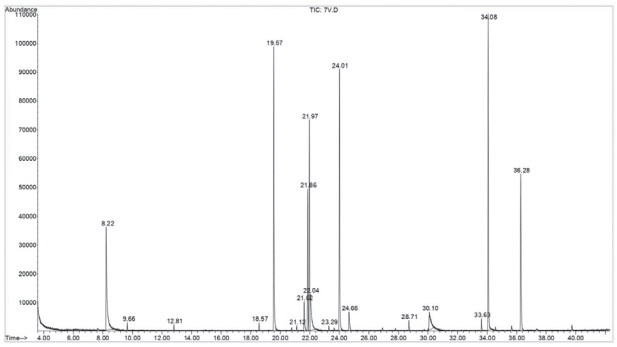

Figure 3. GC-MS chromatogram of derivatives of free amino acids in *Myrtilli folia*.

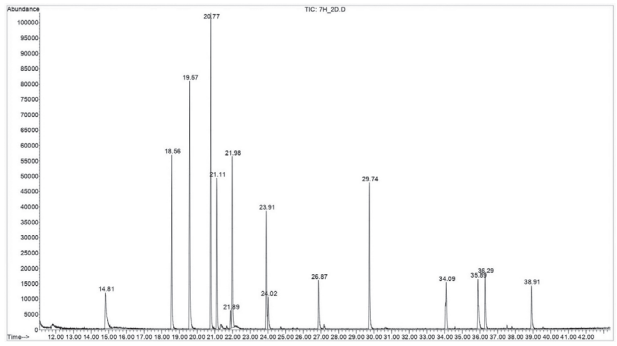

Figure 4. GC-MS chromatogram of derivatives of amino acids after hydrolysis in *Myrtilli folia*.

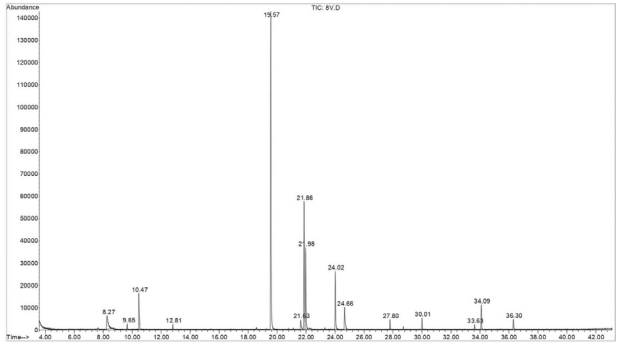

Figure 5. GC-MS chromatogram of derivatives of free amino acids in *Rosae fructus*.

The results of the quantitative study showed that the predominant amino acid in free form was *L*-proline in *Taraxaci radices* (17.33±0.13 mg/g), in *Menthae folia* (7.35±0.15 mg/g), in *Urticae folia* (6.49±0.07 mg/g), in *Rosae fructus* (1.96±0.13 mg/g) and *L*-isoleucine in *Myrtilli folia* (3.46±0.17 mg/g). As for amino acids after hydrolysis, the predominant compound was *L*-proline in four plant components of antidiabetic herbal mixture. Its content was 84.13±0.23 mg/g in *Taraxaci radices*, 21.08±0.19 mg/g in *Rosae fructus*, 18.82±0.21 mg/g in *Urticae folia* and 11.95±0.17 mg/g in *Menthae folia*. However, *Myrtilli folia* contained the largest amount of *L*-isoleucine, its content was 28.55±0.22 mg/g (Table 3). Proline – essential amino acids that exhibits significant hypoglycaemic activity, which is due to a decrease in hepatic glucose production owing to inhibition of glycogenolysis, gluconeogenesis and glucose-6-phosphatase activity (Chen et al. 2010).

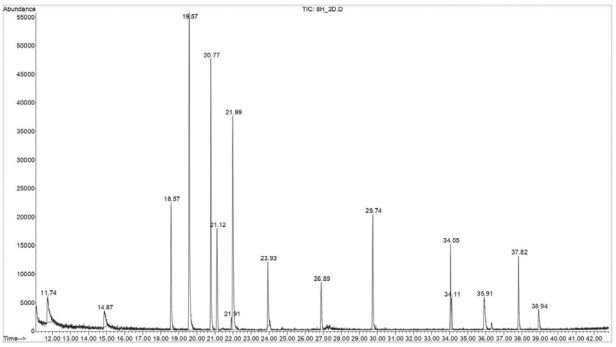

Figure 6. GC-MS chromatogram of derivatives of amino acids after hydrolysis in *Rosae fructus*.

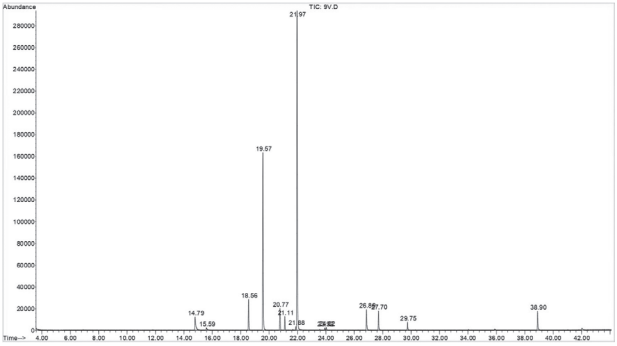

Figure 7. GC-MS chromatogram of derivatives of free amino acids in *Taraxaci radices*.

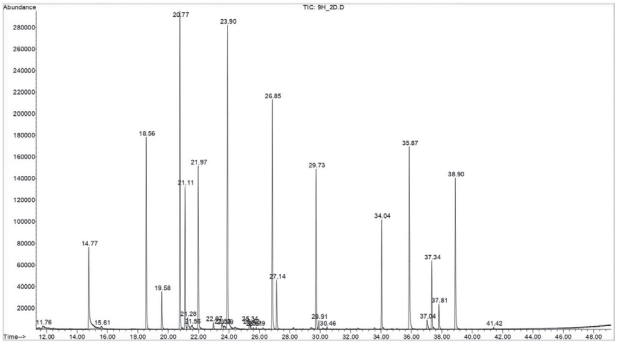

Figure 8. GC-MS chromatogram of derivatives of amino acids after hydrolysis in *Taraxaci radices*.

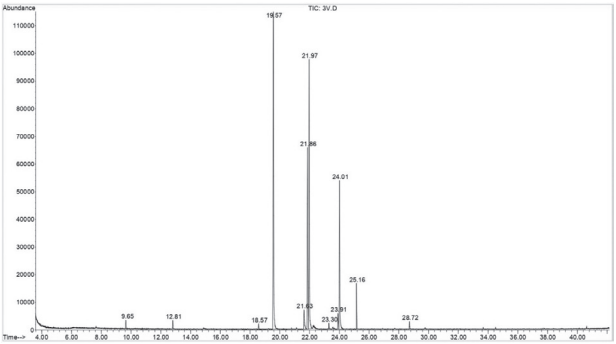

Figure 9. GC-MS chromatogram of derivatives of free amino acids in *Menthae folia*.

In addition, some plant components of antidiabetic herbal mixture contained an important essential amino acid – *L*-leucine. During chromatographic analysis, it was detected 57.34±0.22 mg/g and 1.17±0.14 mg/g of *L*-leucine in bound and free form, respectively in *Taraxaci radices*, 9.57±0.21 mg/g of *L*-leucine in bound form in *Myrtilli folia*, 4.678±0.16 mg/g – in *Rosae fructus*, 2.99±0.15 mg/g – in *Urticae folia* and 0.79±0.04 mg/g – in *Menthae folia* (Table 3.) Leucine is a branched-chain amino acid that plays an important role in controlling protein synthesis and regulating cell metabolism. One of the most important functions of L-leucine in DM type 2 is that it can stimulate insulin secretion in β-cells of the pancreas and acts as a source of energy for metabolic processes and an allosteric activator of glutamate dehydrogenase to enhance glutaminolysis (Birech et al. 2017). Isoleucine, which is an isomer of leucine, does not have itself the ability to stimulate insulin synthesis, but in combination with leucine, their secretolytic activity increases significantly, causing a more pronounced hypoglycaemic effect (Comerford and Pasin 2016; Birech et al. 2017).

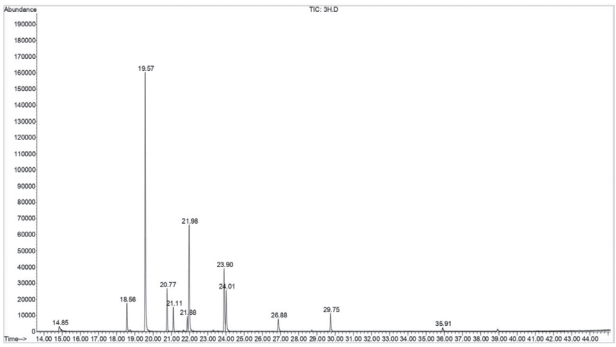

Figure 10. GC-MS chromatogram of derivatives of amino acids after hydrolysis in *Menthae folia*.

Another extremely important essential amino acid for antidiabetic therapy is *L*-phenylalanine. Chromatographic examination revealed this amino acid in the studied objects, it was established that *Myrtilli folia* contains 1.52±0.14 mg/g and 11.89±0.16 mg/g of *L*-phenylalanine in free and bound form, respectively, *Rosae fructus* – 2.23±0.14 mg/g of *L*-phenylalanine in bound form, *Urticae folia* – 0.10±0.12 mg/g and 1.49±0.11 mg/g of *L*-phenylalanine in free and bound form, respectively, *Taraxaci radices* – 0.32±0.03 mg/g and 1.13±0.11 mg/g of *L*-phenylalanine in free and bound form, respectively and *Menthae folia* – 0.52±0.03 mg/g of *L*-phenylalanine in bound form (Table 3). Phenylalanine, an aromatic amino acid that has a direct effect on the course of DM type 2 due to its ability to regulate carbohydrate metabolism by stimulating the release of glucan-like peptide-1 (GLP-1), which in turn enhances insulin secretion, stimulates proliferation and neogenesis of β-cells of the pancreas, reduces insulin resistance (Chen et al. 2010; Green and Lamming 2019; Alqudah et al. 2021).

The results show that all plant components, such as *Urticae folia*, *Rosae fructus Myrtilli folia*, *Menthae folia* and *Taraxaci radices*, of the antidiabetic herbal mixture have a high content of amino acids, which provide numerous pharmacological properties of this phytomixture. Amino acids obtained from plants are very important active substances for the prevention and treatment of DM type

Table 3. The results of the GC-MS analysis of amino acids in the plant components of antidiabetic herbal mixture.

t_R, min (SD±0.02)	Identified substance	Content in the herbal raw materials, mg/g									
		Urticae folia		*Myrtilli folia*		*Rosae fructus*		*Taraxaci radices*		*Menthae folia*	
		Free	Bound	Free	Bound	Free	Bound	Free	Bound	Free	Bound
14.77	Glycine	n/d	n/d	n/d	n/d	n/d	n/d	1.34±0.12	21.45±0.19	n/d	n/d
14.85	*L*-alanine	n/d	0.65±0.12	n/d	2.83±0.16	n/d	0.76±0.08	n/d	n/d	n/d	0.217±0.05
18.56	*L*-valine*	n/d	1.95±0.17	1.19±0.16	5.76±0.19	n/d	2.45±0.12	1.63±0.11	33.45±0.21	0.16±0.08	0.381±0.03
19.57	Nor-valine	Internal standard									
20.77	*L*-leucine*	n/d	2.99±0.15	n/d	9.57±0.21	n/d	4.68±0.16	1.17±0.14	57.34±0.22	n/d	0.785±0.04
21.11	*L*-serine	n/d	1.49±0.17	0.10±0.04	4.79±0.19	n/d	n/d	n/d	23.15±0.15	n/d	n/d
21.31	*L*-threonine*	n/d	n/d	0.43±0.11	3.19±0.16	0.28±0.09	1.39±0.12	0.63±0.03	2.86±0.12	0.85±0.07	0.437±0.02
21.87	*L*-isoleucine*	0.26±0.08	0.22±0.04	3.46±0.17	28.54±0.22	0.93±0.11	18.95±0.17	0.15±0.02	1.86±0.11	4.76±0.13	2.915±0.12
21.97	*L*-proline*	6.49±0.07	18.82±0.21	2.73±0.18	18.92±0.23	1.96±0.13	21.07±0.19	17.33±0.13	84.13±0.23	7.35±0.15	11.951±0.17
23.90	*L*-asparagine	n/d	n/d	0.09±0.02	0.65±0.03	n/d	n/d	n/d	n/d	n/d	n/d
24.02	*L*-aspartic acid	0.86±0.11	2.58±0.12	0.74±0.04	3.79±0.12	1.37±0.08	20.44±0.18	0.13±0.05	53.64±0.22	4.16±0.13	5.849±0.15
26.86	*L*-glutamic acid	n/d	0.96±0.11	n/d	2.07±0.13	n/d	0.99±0.05	1.24±0.12	31.75±0.17	n/d	0.451±0.05
27.14	*L*-methionine*	n/d	n/d	n/d	n/d	n/d	n/d	1.01±0.11	6.85±0.15	n/d	n/d
29.18	*L*-cysteine	n/d	n/d	0.13±0.06	0.18±0.08	n/d	n/d	n/d	19.32±0.14	n/d	n/d
29.74	*L*-phenylalanine*	0.10±0.12	1.49±0.11	1.52±0.14	11.89±0.16	n/d	2.23±0.14	0.32±0.03	1.13±0.11	n/d	0.519±0.03
31.90	*L*-glutamine	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
35.91	*L*-lysine*	1.02±0.13	1.38±0.14	0.45±0.03	6.04±0.15	n/d	1.12±0.12	n/d	21.01±0.11	n/d	0.167±0.02
37.24	*L*-histidine*	n/d	n/d	n/d	n/d	n/d	1.36±0.11	n/d	1.13±0.08	n/d	n/d
38.91	*L*-tyrosine	n/d	0.41±0.05	n/d	1.97±0.11	n/d	0.49±0.06	1.23±0.12	16.74±0.16	n/d	n/d
42.01	*L*-tryptophan	n/d	n/d	n/d	n/d	n/d	n/d	n/d	0.13±0.04	n/d	n/d

1.*– essential amino acid;
2. n/d – not detected;
3. Values are expressed as mean ± SD (n = 6).

2 and diabetic angiopathies as they have hypoglycaemic effects by different pathogenic mechanisms.

This phytochemical study of amino acid content in *Urticae folia*, *Rosae fructus*, *Myrtilli folia*, *Menthae folia* and *Taraxaci radices* shows, which plant material forms the amino acid composition and content in the finished herbal mixture that was established in previous studies and shows due to which biologically active substances is manifested the antidiabetic activity of this phytocomposition. The results of GC-MS analysis indicate the need to include each plant component in the antidiabetic herbal mixture to form the hypoglycaemic activity required for the treatment of DM type 2 (Green and Lamming 2019; Alqudah et al. 2021).

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Conclusion

We identified and established the quantity content of amino acids in free and bound form in *Urticae folia*, *Rosae fructus*, *Myrtilli folia*, *Menthae folia* and *Taraxaci radices*, which are plant components of antidiabetic herbal mixture with hypoglycaemic, hypolipidemic, antioxidant, hepatoprotective, pancreatoprotective activity and defined phytochemical composition. The results of GC-MS analysis showed that the predominant essential amino acid was *L*-proline in *Taraxaci radices*, *Urticae folia*, *Rosae fructus* and *Menthae folia*, its total content was 101.46 mg/g, 25.31 mg/g, 23.04 mg/g and 19.30 mg/g, respectively and *L*-isoleucine in *Myrtilli folia*, its total content was 32.01 mg/g. In addition, it was established total content of essential amino acids, as *L*-leucine that can stimulate insulin secretion in β-cells of the pancreas and *L*-phenylalanine that can increase insulin secretion, stimulate proliferation and neogenesis of β-cells of the pancreas and reduce insulin resistance, which are important factors in the treatment of DM type 2. This phytochemical study shows, which plant material forms the amino acid composition and content in the finished herbal mixture and due to which biologically active substances is manifested the antidiabetic activity of this phytocomposition.

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