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DETERMINATION OF AMINO ACIDS CONTENT OF THE *MAHONIA AQUIFOLIUM* BY GC/MS

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Medicinal plants are unique sources of healing compounds that are used both for the prevention and treatment of various diseases of the human body. In this regard, one of the oldest medicinal plant families – Berberidaceae, is of great interest. The genus Mahonia Nuttall is the second largest genus in the Berberidaceae family and contains nearly 70 species which are used in traditional medicine. Mahonia aquifolium (Pursh) Nutt. (M. aquifolium) is one of the most abundant and cultivated medicinal plants of the genus Mahonia. There is insufficient information in the literature on the biologically active substances of Mahonia aquifolium. The studies of the Mahonia species have focused on alkaloids, such as berberine, jatrorrhizine, and palmatine, which are the main constituents of compounds. The Mahonia aquifolium, as an insufficiently studied plant, is a promising object of study, including amino acid composition.

The aim. *The aim of our study was to identify and determine the quantitative content of amino acids using the GC/MS method in Mahonia aquifolium fruits, flowers, and leaves.*

Materials and methods. *The determination of amino acids composition of Mahonia aquifolium was conducted using Agilent Technologies 6890 chromatograph with mass spectrometric detector 5973 (Agilent Technologies, USA).*

Results. T*he results of the study revealed that the raw material of Mahonia aquifolium contains more bound and less free amino acids. Bound L-leucine was present in all the analyzed samples in the greatest amount (30.885 mg/g in the flowers, 37.765 mg/g in the leaves, and 29.053 mg/g in the fruits). L-proline was among the free amino acids with a high content in flowers (73.304 mg/g) and leaves (32.031 mg/g) of Mahonia aquifolium. In addition, a high content of glycine in free form was found in the fruits (12.212 mg/g) of the study plant.*

Conclusions. *Using the GC/MS method determined, the amino acids in the herb of Mahonia aquifolium. High L-proline, L-leucine, and L-aspartic acid concentrations predominate among free and bound amino acids in all the analyzed samples. These amino acids are considered distinguishing markers of the Mahonia aquifolium. This research contributes to using this plant's raw material for new remedies that may be possible in the future*

Keywords: *Mahonia aquifolium, flowers, leaves, fruits, free amino acids, bound amino acids, GC/MS*

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1. Introduction

Phytochemical studies demonstrated that medicinal plants are unique sources of healing compounds used to prevent and treat various diseases of the human body [1]. In this regard, one of the oldest medicinal plant families, Berberidaceae, is of great interest.

The *Berberidaceae* family contains 9 genera and 590 species native to South America and the Northern Hemisphere. The genus *Mahonia Nuttall* is the second largest genus in the *Berberidaceae* family and contains nearly 70 species native to North America, Central America, and Eastern Asia [2]. *Mahonia* species have been widely used in traditional medicine and have many pharmacological activities like anti-inflammatory, antifungal, antimicrobial, antioxidant, and hepatoprotective [3–6].

Mahonia aquifolium (Pursh) Nutt. (*M. aquifolium*) is one of the most abundant and cultivated medicinal plants of the genus *Mahonia* [5]. The studies of the *Mahonia* species have focused on alkaloids, such as berberine, jatrorrhizine, and palmatine, which are the main constituents of compounds [7]. The alkaloids from *Mahonia aquifolium* are responsible for keratinocyte proliferation inhibition and anti-inflammatory, which explains why this plant has good efficacy in psoriasis and atopic dermatitis [7, 8]. Also, phytochemical analysis of *Mahonia aquifolium* evidenced the presence of secondary metabolites such as chlorogenic acid, *p*-coumaric acid, ferulic acid, rutin, isoquercitrin, and quercetin [9].

In America, *Mahonia aquifolium* has been used to treat fever, dyspepsia, diarrhoea, rheumatism, and diseases affecting the kidneys and liver [10]. Nowadays, extracts from *Mahonia aquifolium* have demonstrated antibacterial, antioxidant, anti-inflammatory, and antifungal properties [11].

Previous studies revealed that *Mahonia aquifolium* contained some major plant secondary metabolites, including polyphenols and alkaloids. However, it is interesting to note that limited information is available on the characteristics of primary metabolites. Thus, the purpose of this work was to evaluate and compare the content of the amino acids in the flowers, leaves, and fruits of *Mahonia aquifolium*.

2. Planning (methodology) of research

The planning of studies of medicinal plant material *Mahonia aquifolium* is shown in Fig. 1.

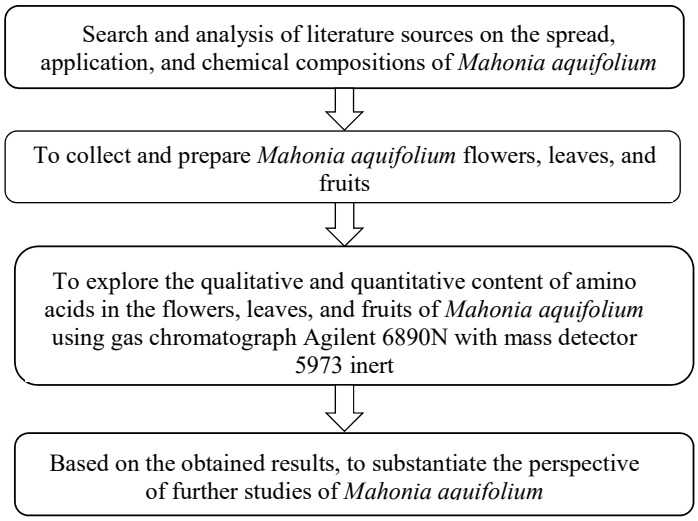

Fig. 1. Design of the experiment

3. Material and method

3. 1. Plant materials

Fruits, flowers, and leaves of the *Mahonia aquifolium* were collected at the experimental sites of M. M. Hryshko National Botanic Garden of the NAS of Ukraine in Kyiv. The raw material was authenticated by Senior Scientist Nadia Dzhurenko. A voucher specimen was deposited in the herbarium at the Department of Pharmacognosy and Medical Botany, TNMU, Ternopil, Ukraine.

3. 2. Sample preparation, GC/MS determination of amino acids

The amino acid composition of *Mahonia aquifolium* is 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 of 0.25 μm [12]. The evaporator temperature was 250 °C, and the interface temperature was 280 °C. The first set up oven temperature at 50 °C and held for 4 min, then raised to 300 °C at the rate of 5 °C/min and kept at this point 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.

The pre-column derivatization was conducted with the help of automatic programmable regulations. The dry samples of the plant were dissolved in 390 μL of 1 M sodium hydroxide, then 333 μL of methanol and 67 μL of pyridine were mixed thoroughly for 5 seconds. 80 μL of methyl chloroformate to the resulting mixtures was 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 [13].

For the extraction of free amino acids, the samples of the raw material were ground into a powder by laboratory mill, then about 0.1 g (accurately weighed) was selected and placed into a vial with 2.0 mL of 0.1 N 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 N an aqueous hydrochloric acid solution to 0.03 g (accurately weighed) of powdered raw materials. Hydrolysis was carried out for 24 hours in a thermostat at 110 °C [14].

The resulting extracts were centrifuged at 3,000 rpm, and the supernatants were evaporated to dryness on a rotary evaporator, washing three times with distilled water to remove hydrochloric acid.

Amino acids identification was performed by comparing the retention times 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 [15–17].

Table 1

The chromatographic conditions for the 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

3. 3. Validation of the method

The validation method and the analysis procedure of the amino acid content were performed according to validation guides for EURACHEM analytical methods. To evaluate the sensitivity and linearity of the signal in relation to the concentration, 5 linear calibrations were generated for each amino acid. The calibration curves of each amino acid were plotted in the 0.615–5 μmol/ml range, and the linearity range for which the correlation coefficient that characterizes the regression line R2 was obtained was examined visually. The performance parameters of the reference amino acid method, concentrations, limit of de-

tection (LOD), limit of quantification (LOQ), and calibration curves were statistically calculated using Statistica v 10.0 (StatSoft Inc.) program. All statistical tests were performed at a confidence level of 95 % and k=2.

4. Results

The amino acid profiles of the fruits, flowers and leaves of *Mahonia aquifolium* were evaluated using the GC/MS method (Fig. 2–7, Table 2).

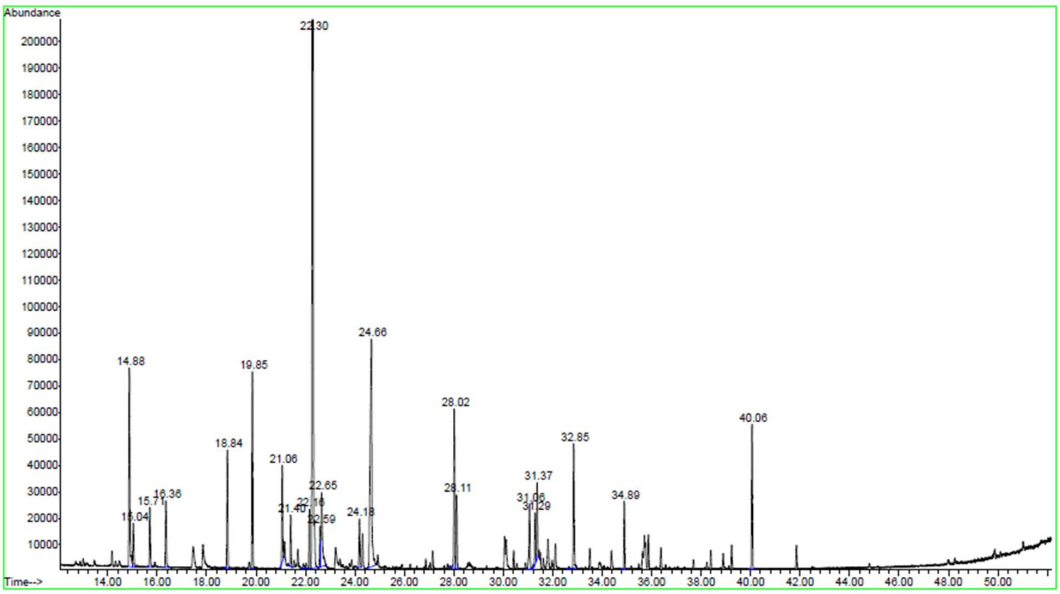

Fig. 2. GC/MS chromatogram of free amino acids of *Mahonia aquifolium* leaves

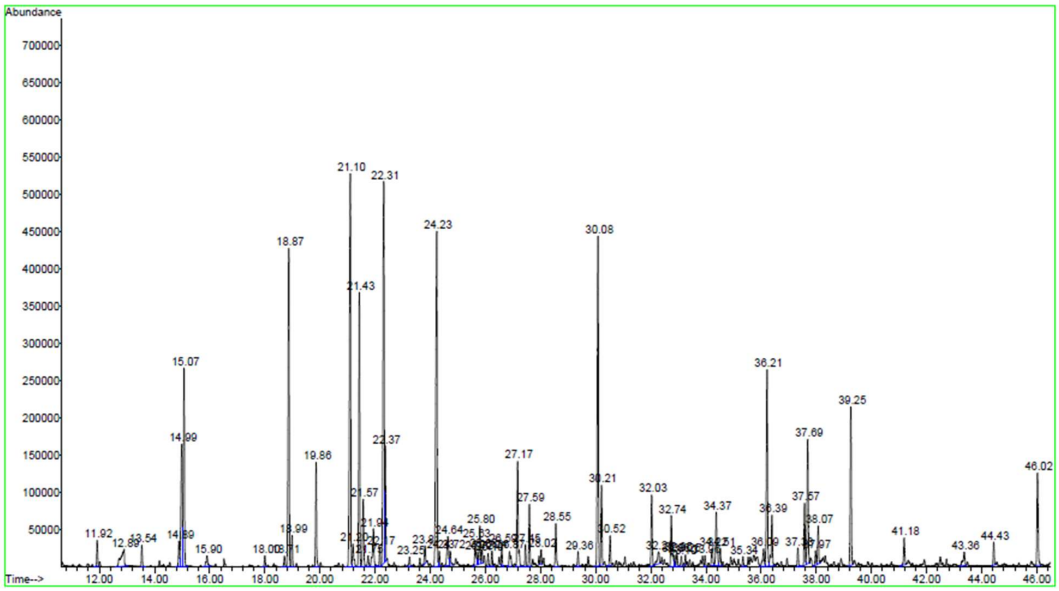

Fig. 3. GC/MS chromatogram of amino acids after hydrolysis of *Mahonia aquifolium* leaves

Table 2

The amino acid composition content of *Mahonia aquifolium*

Amino acid name	Amino acids content of *Mahonia aquifolium*, mg/g					
	Fruits		Flowers		Leaves	
	Free	Bound	Free	Bound	Free	Bound
1	2	3	4	5	6	7
Glycine	12.21±0.05	6.41±0.04	1.91±0.02	17.82±0.06	n/d	18.97±0.07
L-alanine	n/d	12.86±0.06	n/d	15.73±0.04	2.19±0.02	12.78±0.05
L-valine*	n/d	20.41±0.08	3.97±0.03	29.14±0.06	5.09±0.03	26.02±0.06
Nor-valine	Internal standart					
L-leucine*	n/d	29.05±0.06	8.45±0.05	30.89±0.07	4.08±0.02	37.77±0.07
L-serine	n/d	1.84±0.02	n/d	4.48±0.02	n/d	2.83±0.03
L-threonine*	n/d	4.51±0.02	n/d	7.67±0.04	n/d	6.95±0.04
L-isoleucine*	n/d	17.99±0.08	1.42±0.02	23.70±0.05	2.13±0.03	22.46±0.09

Continuation of Table 2

1	2	3	4	5	6	7
L-proline	6.47±0.04	10.22±0.04	73.30±0.06	11.02±0.04	32.03±0.07	11.11±0.05
L-asparagine	n/d	n/d	n/d	n/d	0.30±0.01	1.49±0.02
L-aspartic acid	6.89±0.05	13.98±0.04	3.95±0.03	64.27±0.07	2.33±0.02	36.50±0.06
L-glutamic acid	n/d	7.63±0.03	n/d	18.08±0.04	n/d	10.79±0.03
L-methionine*	n/d	n/d	n/d	2.33±0.03	n/d	1.86±0.01
L-cysteine	n/d	25.47±0.07	n/d	0.65±0.01	n/d	n/d
L-phenylalanine*	3.68±0.03	17.75±0.08	3.82±0.03	28.82±0.06	n/d	32.58±0.07
L-glutamine	n/d	n/d	n/d	n/d	n/d	2.13±0.01
L-lysine*	n/d	13.97±0.05	n/d	27.29±0.07	n/d	19.68±0.05
L-histidine*	n/d	n/d	n/d	1.28±0.01	n/d	1.79±0.02
L-tyrosin	n/d	11.21±0.04	n/d	19.61±0.05	6.52±0.04	8.98±0.03
L-tryptophan*	n/d	n/d	n/d	0.65±0.01	n/d	n/d
Total essential amino acids	3.68	103.68	17.66	151.77	11.3	149.11
Total nonessential amino acids	25.57	89.68	79.16	151.66	43.37	105.58
Total amino acids	29.25	193.36	96.82	303.43	54.67	254.69

*Note: n/d – not detected, * – essential amino acids.*

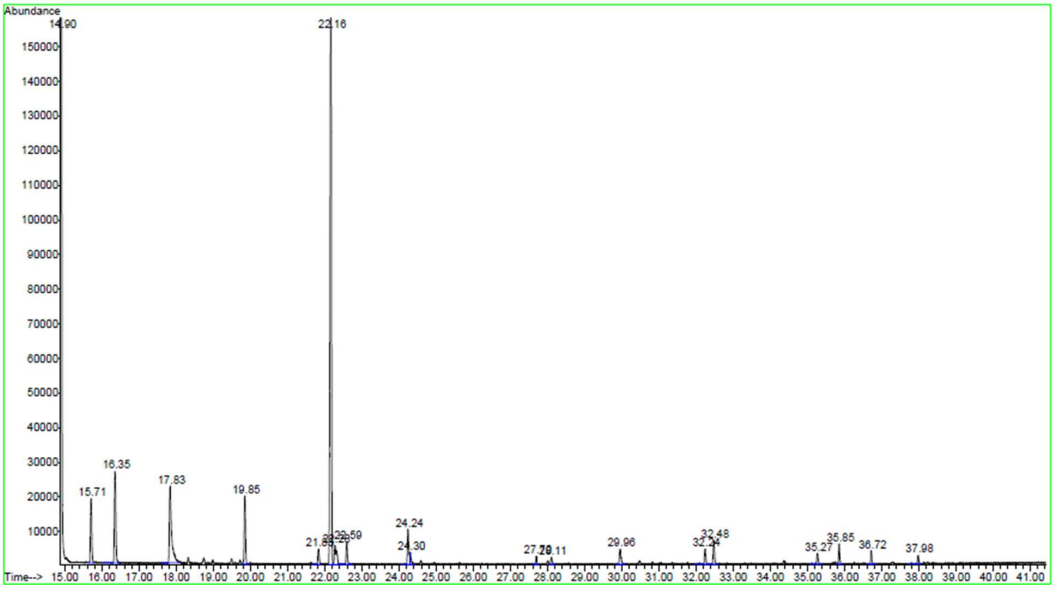

Fig. 4. GC/MS chromatogram of free amino acids of *Mahonia aquifolium* fruits

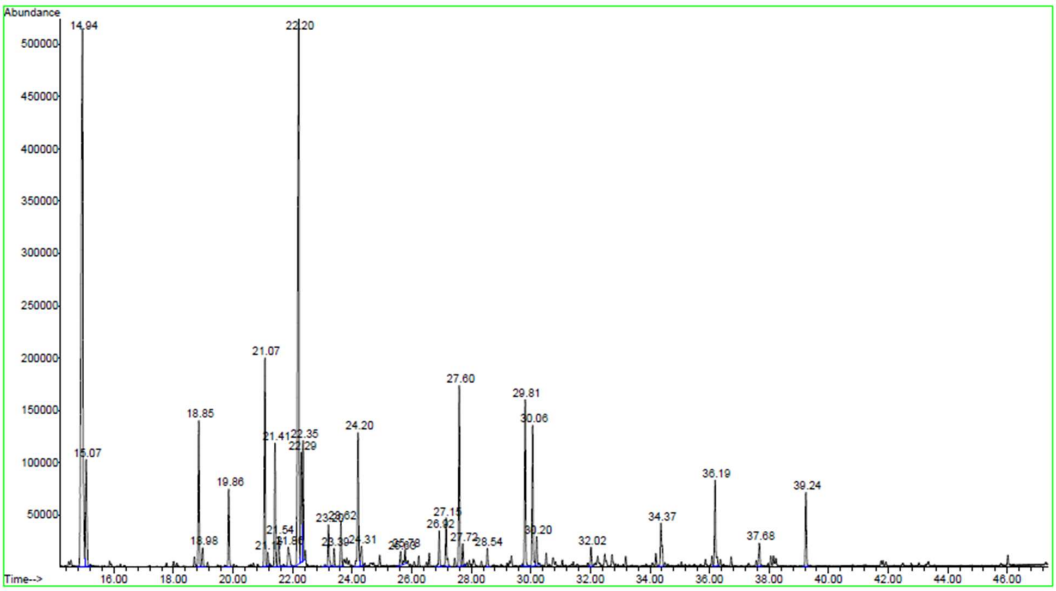

Fig. 5. GC/MS chromatogram of amino acids after hydrolysis of *Mahonia aquifolium* fruits

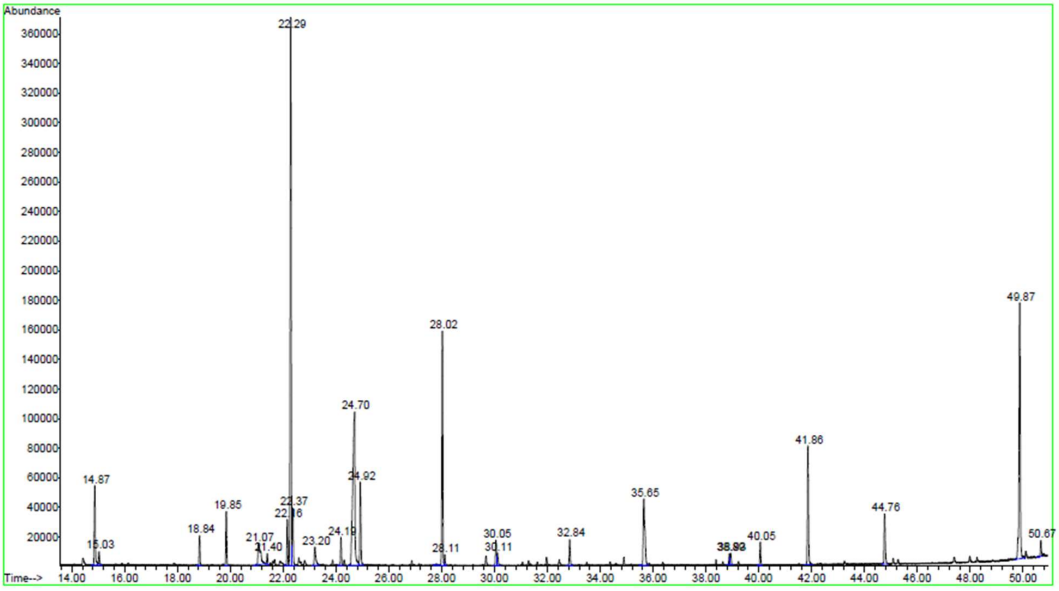

Fig. 6. GC/MS chromatogram of free amino acids of *Mahonia aquifolium* flowers

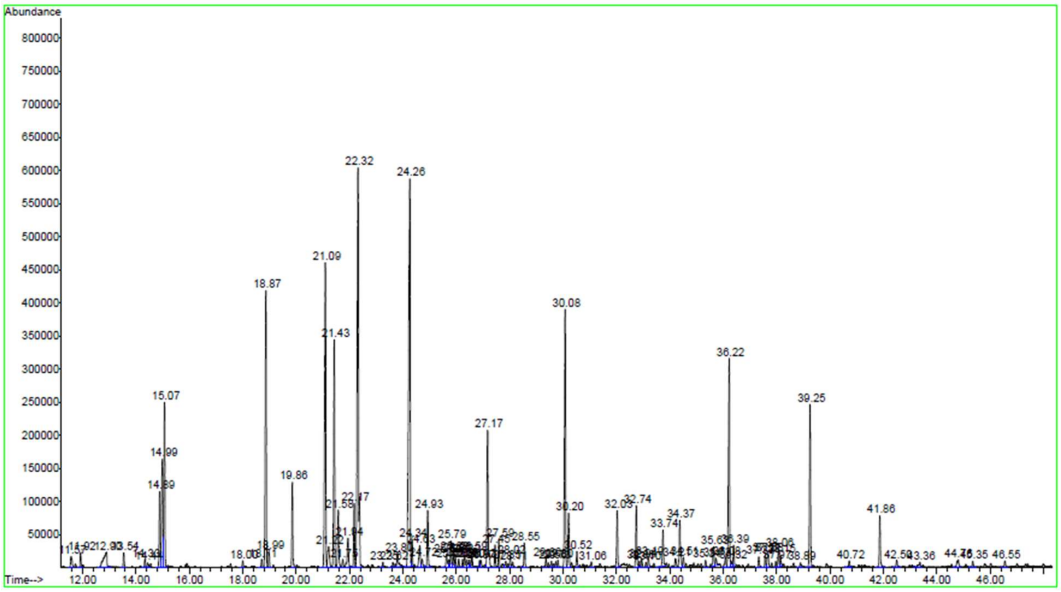

Fig. 7. GC/MS chromatogram of amino acids after hydrolysis of *Mahonia aquifolium* flowers

The GC/MS method were identified eight, seven, and four free amino acids in the leaves, flowers, and fruits of *Mahonia aquifolium*, respectively (Fig. 2, 4, 6).

5. Discussion

Free L-proline was present in *Mahonia aquifolium* in the greatest amount (73.304 mg/g in the flowers and 32.031 mg/g in the leaves) (Table 2). Proline stabilizes subcellular structures (e.g., membranes and proteins), scavenges free radicals, and buffers cellular redox potential under stress conditions. It can also act as a protein-compatible hydrotrope, alleviating cytoplasmic acidosis and maintaining appropriate NADP+/NADPH ratios compatible with metabolism [18, 19].

Notably, glycine (12.212 mg/g) was the dominant free amino acid in the fruits of *Mahonia aquifolium* (Table 2). Glycine plays an important role in the regulation of gene expression, protein configuration and activity, and several other biological functions [20]. There are other beneficial activities in glycine as an antacid, modulator of growth through the regulation of growth hormone synthesis, improvement of muscle tone, collagen synthesis, and delaying muscular degeneration [21, 22].

Among the contents of bound amino acids, the predominant component was L-leucine in the fruits (29.053 mg/g), flowers (30.885 mg/g), and leaves (37.765 mg/g) of the raw material (Table 2). Leucine is an essential amino acid for protein synthesis. In addition, like other amino acids, the carbon skeleton of leucine can be used to generate ATP [23]. Also, leucine can regulate several cellular processes, such as protein synthesis, tissue regeneration, and metabolism. Thus, leucine supplementation has been studied in various conditions such as ageing, muscle damage, obesity, and diabetes [24–26].

During chromatographic analysis, it was found that flowers and leaves contain the largest amount of bound amino acid L-aspartic acid, 64.266 mg/g and 36.503 mg/g, respectively. L-aspartic acid is an amino acid present in nervous tissues and endocrine glands. It is also used as a stock in the food and pharmaceutical industries [27, 28]. Aspartic acid is used to bolster immune function and as a natural combatant to depression [29, 30].

The studied amino acid content of various parts of *Mahonia aquifolium* contained the same components, which differed in their quantitative content. However, only in the flowers of the *Mahonia aquifolium* was the presence of L-tryptophan (0.651 mg/g) among the bound amino acids detected, while in the leaves and fruits, it was not detected.

Practical relevance. New data have been obtained on the amino acids composition in the flowers, leaves, and fruits of *Mahonia aquifolium*. The data we obtained can be used for further study of these raw materials and the development of new plant medicinal products.

Study limitations. The limitation of the research is the comparative study of the component composition of only amino acids. It is promising to study the comparative component composition of primary metabolites and biological activity of plant raw materials, which are harvested from geographically wider regions and different years, possibly in comparison with foreign samples.

Prospects for further research. The obtained results might be used in the standardization and quality assurance of new remedies containing *Mahonia aquifolium*. Further research can be directed to the study of the pharmacological activity of obtained amino acids.

6. Conclusion

As a result of this work, the comparative analyses of amino acids content in the leaves, flowers, and fruits of *Mahonia aquifolium* using a sensitive GC/MS method were carried out for the first time. It should be noted that the highest content of amino acids was in *Mahonia aquifolium* flowers. High concentrations of the free and bound amino acids, such as L-proline, L-leucine, and L-aspartic acid, predominate in all the analyzed samples. Thus, *Mahonia aquifolium* displayed a particular composition of amino acids, which could be of interest for pharmaceutical manufacturing, and this plant's raw material can be used as a source for new medicines in the future.

Conflict of interests

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

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Data availability

The manuscript has no associated data.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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