

## Gas chromatographic-mass spectrometric studies of organic acids of *Veronica longifolia* L.

Alina Osmachko, Alla Kovalyova, Oleg Koshovyy, Olga Goryachaya

Pharmacognosy Department, National University of Pharmacy, Kharkiv, Ukraine

### Abstract

By means of chromatography-mass spectrometric method 24 fatty acids, 8 aromatic and 13 aliphatic acids in the leaves and flowers of *V. longifolia* L. has been identified and quantified.

**Keywords:** *Veronica longifolia* L., organic acids, fatty acids, gas chromatographic-mass spectrometric studies (GC/MS studies)

### 1. Introduction

*V. longifolia* L. belongs to the family *Plantaginaceae* and it could be a promising source of biological active substances (BAS) [1, 10], what had been confirmed by our previous studies of this herb drugs [6, 7]. Among the species of the genus *Veronica* L. plants with the highest content of BAS: *V. officinalis* L., *V. spicata* L., *V. incana* L., *V. chamaedrys* L., *V. longifolia* L. [2] have been identified, which have a large area of distribution in Ukraine [4, 8]. A critical analysis of scientific primary sources has shown that the chemical composition of *V. longifolia* L. is not completely studied for today. Iridoids and flavonoids are the most studied groups of BAS of *Veronica* L. genus [3, 5, 9]. The carboxylic acids composition of *V. longifolia* L. herb haven't been studied, and it is of scientific attention.

The objects of study were herb, leaves and flowers of *V. longifolia* L., that have been gathered in the flowering stage in Kharkiv region, Ukraine, in 2012.

The aim of our study was the GC/MS study of fatty and organic acids composition of *V. longifolia* L. leaves and flowers.

### 2. Materials and methods

The analysis of acid's methyl esters was performed using

chromatography-mass spectrometer 5973N/6890N MSD/DS Agilent Technologies. To 0.50 mg of herbal drugs in 2 ml vial an internal standard (50 mg of tridecane in hexane) was added and 1.0 ml of methylating agent – 14% BCl<sub>3</sub> in methanol. The mixture was kept in a sealed vial for 8 hours at 65 °C. The injection of 2 ml of sample in chromatographic column was performed in a mode *splitless* (without flow breakdown), the input samples rate – 1 ml/min, the period – 0.2 min. The identification of acid's methyl esters were performed by calculating the equivalent length of the aliphatic chain (ECL); using data from the mass spectra libraries NIST 05 and Willey 2007 in conjunction with programs for identifying – AMDIS and NIST; retention time was compared with the retention time of standard compounds (Sigma). Chromatographic profiles of organic acids of *Veronica longifolia* L. flowers and leaves are presented in figures 1 and 2.

### 3. Results and discussion

In the result of the study 24 fatty acids had been identified and quantified. Among the identified fatty acids 23 fatty acids had been found in flowers, and 12 – in leaves, of which 19 were saturated, 2 were monounsaturated and 3 were polyunsaturated fatty acids.

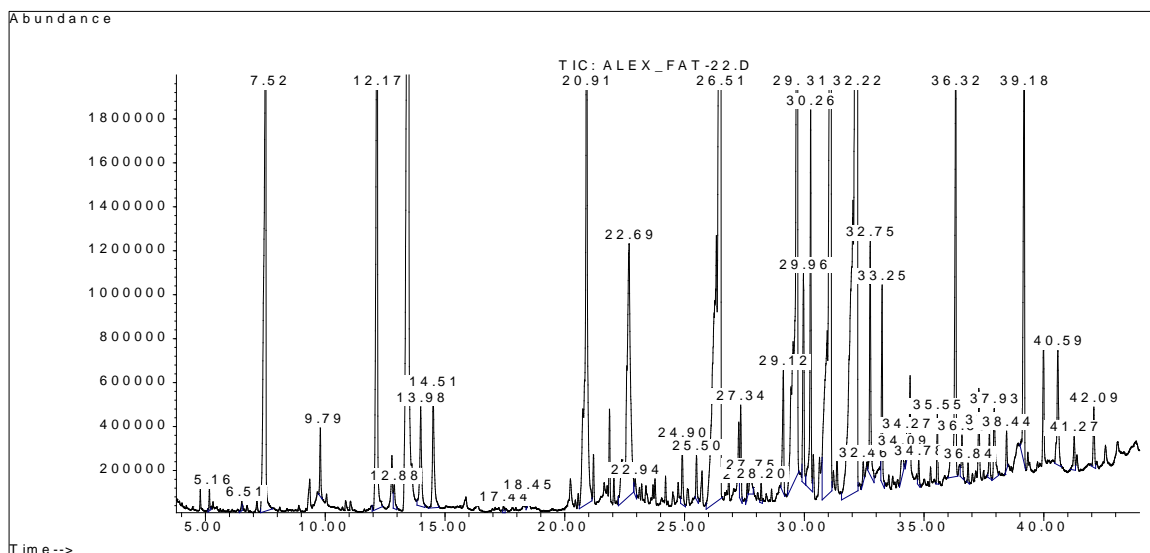


Fig 1: Chromatographic profile of organic acids of *Veronica longifolia* L. flowers.

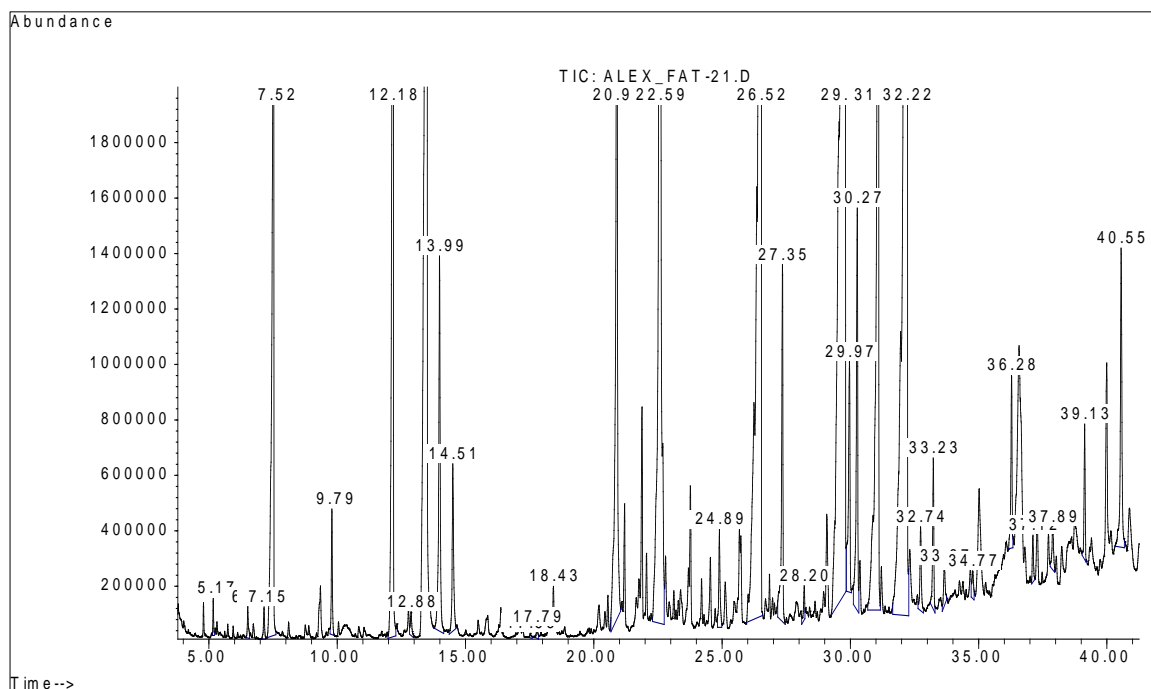


Fig 2: Chromatographic profile of organic acids of *Veronica longifolia* L. leaves.

The relative content of fatty acids has been calculated in a percent of their total content. The results were subjected to statistical analysis (tables 1, 2, 3).

Table 1: Fatty acids of *Veronica longifolia* L.

№	Formula IUPAC	The retention time, min	The name of acid	Content			
				Flowers		Leaves	
				mg/kg	%	mg/kg	%
1	16:0	25.49	14-Methylpentadecanoic	59,0	0,4		
2	16:0	26.52	Palmitic	4027,9	26,9	3370,1	31,7
3	16:1Δ9	27.35	Palmitoleic	125,6	0,8	354,5	3,3
4	17:0	27.61	15-Methylpalmitic	16,4	0,1		
5	17:0	28.2	Heptadecanoic	23,9	0,2	28,7	0,3
6	18:0	29.12	16-Methylheptadecanoic	189,3	1,3		
7	18:0	29.96	Stearic	306,3	2,0	266,5	2,5
8	18:1Δ9	30.27	Oleic	516,8	3,4	405,0	3,8
9	18:2Δ9,12	31.09	Linoleic	2719,3	18,1	1661,6	15,7
10	18:3Δ9,12,15	32.21	Linolenic	482,7	32,2	4065,7	38,4
11	18:0	32.46	17-Methylheptadecanoic	27,2	0,2		
12	20:0	33.23	Arachidic (Eicosanoic)	240,0	1,6	126,1	1,2
13	16:0	33.67	2-Hydroxypalmitic	66,4	0,4	46,1	0,4
14	20:2Δ11,13	34.26	11,13-Eicosadienoic	42,6	0,3		
15	21:0	34.77	Heneicosanoic			31,7	0,3
16	21:0	34.77	19-Methylarachidic	32,3	0,2		
17	21:0	35.55	18-Methylarachidic	79,2	0,5		
18	22:0	36.28	Begenic	727,1	4,9	143,2	1,4
19	18:0	36.83	Hexadecadicarboxylic	22,9	0,2		
20	23:0	37.72	Tricosanoic	65,9	0,4		
21	23:0	38.44	22-Methyltricosanoic	43,2	0,3		
22	24:0	39.13	Tetracosanoic	682,4	4,6	101,3	1,0
23	25:0	41.26	24-Methylpenta	56,1	0,4		
24	26:0	42.08	Hexacosanoic	91,8	0,6		
Total amount:				14988,3	100	10600,5	100

The total content of fatty acids in flowers was 14988.3 mg/kg. The total content of fatty acids in leaves was 10600.5 mg/kg. The percent of unsaturated fatty acids in their total content in flowers was 54.8%, and 61.2% – in leaves.

11, 13-Eicosadienoic acid haven't been found in leaves. The dominant fatty acids in flowers were saturated acids – palmitic, begenic, tetracosanoic, and polyunsaturated acids – linoleic and linolenic.

The dominant fatty acids in leaves were saturated palmitic acid, unsaturated oleic acid, and polyunsaturated acids such as linoleic and linolenic.

The content of low molecular organic (aliphatic) and aromatic acids had been calculated similarly to fatty acids (tables 2 and 3).

In the results of the study 8 aromatic acids had been identified and quantified, 5 of which were found in flowers, 7- in leaves.

Aromatic acids such as benzoic, phenylacetic, salicylic, 3-phenyllactic, vanillic, 4-hydroxyphenylacetic, 4-hydroxybenzoic and ferulic had been identified.

**Table 2:** Aromatic acids of *Veronica longifolia* L.

S. no	The retention time, min	The name of acid	Content			
			Flowers		Leaves	
			mg/kg	%	mg/kg	%
1	14.5	Benzoic	243,8	24,2	168,3	24,5
2	17.53	Phenylacetic			4,5	0,7
3	17.78	Salicylic			11,6	1,7
4	27.75	3-Phenyllactic	58,8	5,8		
5	32.73	Vanillic	344,0	34,1	83,5	12,2
6	37.11	4-Hydroxyphenylacetic			37,0	5,4
7	37.88	4-Hydroxybenzoic	141,8	14,1	48,7	7,1
8	40.55	Ferulic	219,6	21,8	332,1	48,4
Total amount			1008,0	100	685,7	100

Phenylacetic, hydroxyphenylacetic and salicylic acids had been found in leaves, which hadn't been found in flowers. The dominant aromatic acids in leaves were (mg/kg): benzoic (168.3), ferulic (332.1) acids; in flowers – benzoic (243.8), ferulic (219.6), 4-hydroxybenzoic (141.8), vanillic (344.0) acids, 3-phenyllactic acid was the original component in

flowers. The total content of aromatic acids in leaves was 685.7 mg/kg, in flowers – 1008.0 mg/kg.

More aromatic acids were identified in leaves than in flowers, but the content of aromatic acids in flowers was approximately two-fold larger than in leaves.

**Table 3:** Aliphatic acids of *Veronica longifolia* L.

S. no	The retention time, min	The name of acid	Content			
			Flowers		Leaves	
			mg/kg	%	mg/kg	%
1	5.16	Caproic (hexanoic)	21,2	0,3	17,7	0,2
2	6.51	3-Hexenoic	11,2	0,2	22,9	0,2
3	7.14	2-Hexenoic			25,0	0,2
4	9.79	Oxalic	79,9	1,2	89,0	0,8
5	12.17	Malonic	1325,4	19,9	1234,4	10,8
6	12.88	Fumaric	43,0	0,6	24,7	0,2
7	13.99	Succinic	205,7	3,1	392,9	3,4
8	17.43	10-Methylundecanoic	10,7	0,2		
9	20.9	3-Hydroxy-2-methylglytaric	1441,9	21,7	1084,3	9,5
10	22.59	Malic	1139,7	17,1	2211,3	19,4
11	22.93	Suberic	29,2	0,4		
12	24.89	Azelaic	89,6	1,3	90,3	0,8
13	29.71	Citric	2259,9	33,9	6235,3	54,6
Total amount:			6657,4	100	11427,4	100

In the results of the study 13 mono-, dicarboxylic and hydroxycarboxylic acids had been identified and quantified, 12 of which were found in flowers, 11 of which in leaves. Among them: caproic (hexanoic), 3-hexenoic, 2-hexenoic, oxalic, malonic, fumaric, succinic, 10-methylundecanoic, suberic, azelaic and 3-hydroxy-2-methylglytaric, malic and citric.

The dominant acids (mg/kg) in flowers and leaves were: malonic 1325.4 and 1234.4; 3-hydroxy-2-methylglutaric 1441.9 and 1084.3; malic 1139.7 and 2211.3; citric acids 2259.9 and 6235.3, respectively.

The total content of aliphatic acids in flowers was 6657.4 mg/kg, in leaves – 11427.4 mg/kg.

#### 4. Conclusions

1. By means of chromatography-mass spectrometric method 24 fatty acids, 8 aromatic and 13 aliphatic acids

in the leaves and flowers of *V. longifolia* L. had been identified and quantified

2. Considering the important contribution of studied BAS (especially polyunsaturated fatty acids and some aromatic acids) in the total activity of herbal preparations, the further in-depth study of obtained extracts from *Veronica longifolia* L. can be considered promising.

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