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THE STUDY OF CARBOXYLIC ACIDS IN FLOWERS AND LEAVES OF *VERONICA CHAMAEDRYS* L. AND *VERONICA TEUCRIUM* L.

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The aim. *The genus Veronica (Veronica L.), family Plantaginaceae Juss. in the flora of Ukraine is represented by 64 species; Veronica chamaedrys L. and Veronica teucrium L. are widespread in the Kharkiv region. Plants are used in folk medicine as expectorants, anti-inflammatory, diaphoretic, anti-allergic, choleretic, antispasmodic, anticonvulsant, diuretic, sedative, wound healing and antibacterial agents. The aim of this work was to study the carboxylic acids of flowers and leaves of Veronica chamaedrys L. and flowers and leaves of Veronica teucrium L.*

Materials and methods. *The objects of the study were flowers and leaves of Veronica chamaedrys L. and Veronica teucrium L., harvested in the flowering phase in 2018 in Kharkiv region, Ukraine. The study of carboxylic acids was performed by chromatography-mass spectrometry on a 6890N MSD/DS Agilent Technologies chromatograph with a 5973N mass spectrometric detector. Identification of acid methyl esters was performed using data from the mass spectrum library NIST 05 and Willey 2007 in combination with programs for the identification of AMDIS and NIST; also compared the retention time with the retention time of standard compounds.*

Results. *In the flowers and leaves of Veronica teucrium L. 35 carboxylic acids were identified, the total content of which is 5.55 % and 2.93 %, respectively. 31 and 32 carboxylic acids were identified in the flowers and leaves of Veronica chamaedrys L., their total content is 5.39 % and 7.45 %, respectively.*

Conclusions. *It is established that the flowers and leaves of Veronica chamaedrys L. are characterized by a higher content of carboxylic acids compared to the flowers and leaves of Veronica teucrium L. As chemotaxonomic markers of flowers and leaves the following compounds can be used: α-furanic acid for Veronica chamaedrys L.; veratric, 4-hydroxybenzylacetic and syringic acids for Veronica teucrium L. The obtained results will be the basis for further chemotaxonomic studies*

Keywords. *Veronica chamaedrys L., Veronica teucrium L., carboxylic acids, chromatography-mass spectrometry*

1. Introduction

The genus Veronica (*Veronica* L.) of the family *Plantaginaceae* Juss. is widespread and represented in the world flora with more than 300 species [1, 2]. 64 wild species grow on the territory of Ukraine and are cultivated as fodder and ornamental plants, have many varieties and hybrids, which differ mainly in the size of inflorescences and colour of flowers [3]. *Veronica chamaedrys* L. and *Veronica teucrium* L. of *Chamaedrys* Griseb. section are widespread in the Kharkiv region.

In folk medicine, the herb is used, much less often the flowers or rhizomes of both *Veronica* species as expectorant, anti-inflammatory, diaphoretic, anti-allergic, choleretic, antispasmodic, anticonvulsant, diuretic, sedative, wound healing and antibacterial agents [4].

Experimental studies of the activity of substances from the herb *Veronica teucrium* L. were done. Thus, *in vitro* experiments have shown that water-acetone extract from this raw material inhibits the enzymes cyclooxygenase-1 and 12-lipoxygenase. Anti-inflammatory activity is positively correlated with the content of phenolic compounds, in particular baicalein, quercetin, hyperoside, isoquercetin, chlorogenic acid [5, 6]. It was found that antibacterial activity against *S. aureus* and *E. coli* is shown by aqueous extracts containing free polyphenols, hydroxycinnamic acids and extracts containing the sum of iridoids [7, 8].

It is known that species of the genus *Veronica* L. contain iridoids [9, 10], flavonoids and phenylpropanoids [11], carboxylic acids [12, 13].

In the raw material of *Veronica chamaedrys* L. iridoids [14], ascorbic acid, hydroxycinnamic acids: chlorogenic, rosemary, *p*-coumaric, caffeic and ferulic [15, 16]; fatty carboxylic acids: myristic, palmitic, stearic, linoleic, linolenic, arachidonic, lauric and palmitoleic [17]; flavonoids: apigenin, luteolin, chrysoeriol, pectolinarigenin and 3,4-dimethylluteolin [18, 19] were found.

Veronica teucrium L. herb contains malic, citric, tartaric, lactic, ascorbic acid; phenolic carboxylic acids: *p*-hydroxybenzoic, 2,5-dihydroxybenzoic (gentisin), phenylacetic, protocatechuic, gallic, vanillic, syringic [20, 21]; alicyclic acids: quinine, shikimic; hydroxycinnamic acids: *p*-coumaric, caffeic, rosemary, ferulic, chlorogenic, sinamic, isochlorogenic and neochlorogenic; flavonoids: apigenin, luteolin, baicalein, hyperoside, isoquercetin, methoxidized flavones: hispidulin and eupatorin; steroids: *β*-sitosterol, campesterol and stigmasterol [6, 7].

The aim of this work was to study the carboxylic acids of flowers and leaves of *Veronica chamaedrys* L. and flowers and leaves of *Veronica teucrium* L. to identify po-

tential chemotaxonomic markers of species and to establish the theoretical possibility of their use to correct the metabolic syndrome.

2. Planning (methodology) of the research

In Fig. 1 a graphical representation of the research planning process is shown.

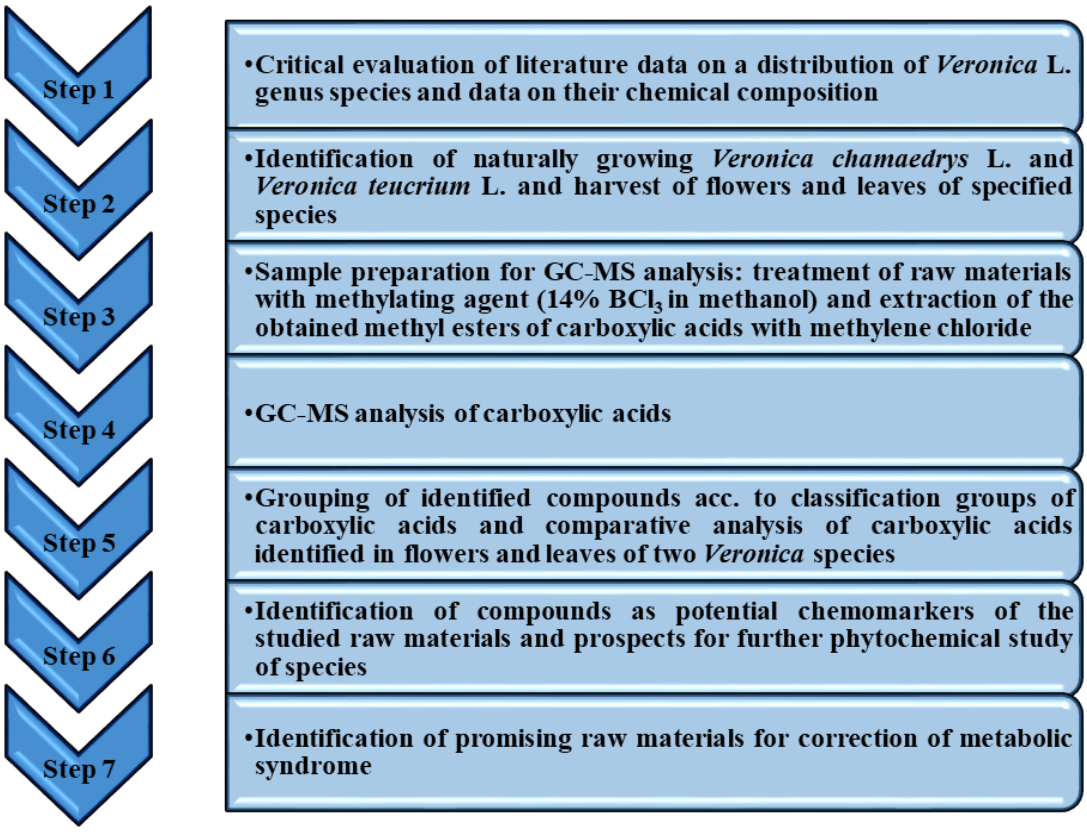

Fig. 1. Planning of the research

3. Materials and methods

The objects of the study were flowers and leaves of *Veronica chamaedrys* L. and *Veronica teucrium* L., harvested in the flowering phase in June 2018 (Kharkiv region, Ukraine). Herbarium specimens №№ 030602018-10062018 are stored at the Department of Pharmacognosy (National University of Pharmacy, Kharkiv, Ukraine). Identification of species was carried out with the advisory assistance of the Head of the Department of Botany of the National Pharmaceutical University, Doctor of Pharmaceutical Sciences, professor Gontova Tatyana Nikolaevna.

Studies of carboxylic acids of air-dry raw materials were performed by chromatography-mass spectrometry on a chromatograph 6890N MSD/DS Agilent Technologies (USA) with a mass spectrometric detector 5973N according to the previously described method [20, 21]. Identification of acid methyl esters was performed based on the calculation of the equivalent length of the aliphatic chain (ECL) using data from the mass spectrum library NIST 05 and Willey 2007 in combination with programs for the identification of AMDIS and NIST; also compared the retention time with the retention time of standard compounds (Sigma).

4. Research results

As a result of the study, 32 carboxylic acids in flowers and 31 carboxylic acids in the leaves of *Veronica chamaedrys* were identified and quantified; 35 carboxylic acids were identified and quantified in the flowers and leaves of *Veronica teucrium* (Fig. 2–5, Table 1).

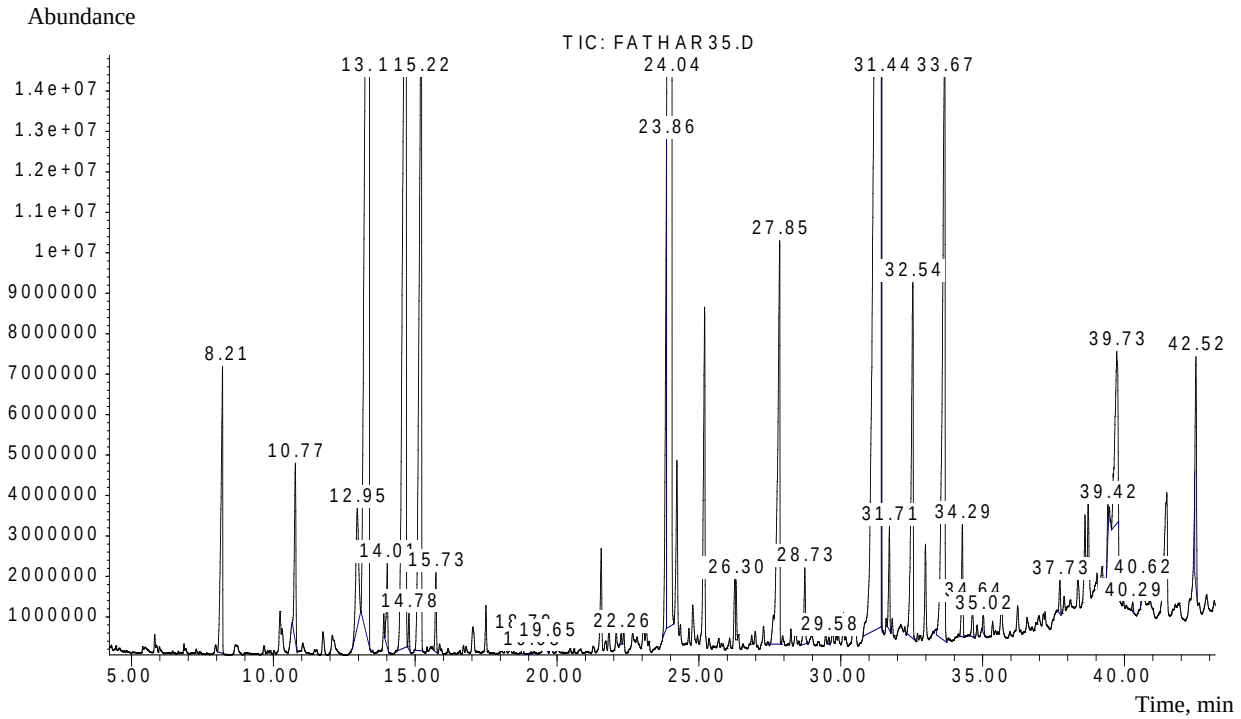

Fig. 2. Chromatographic profile of methyl esters of carboxylic acids of *Veronica chamaedrys* flowers

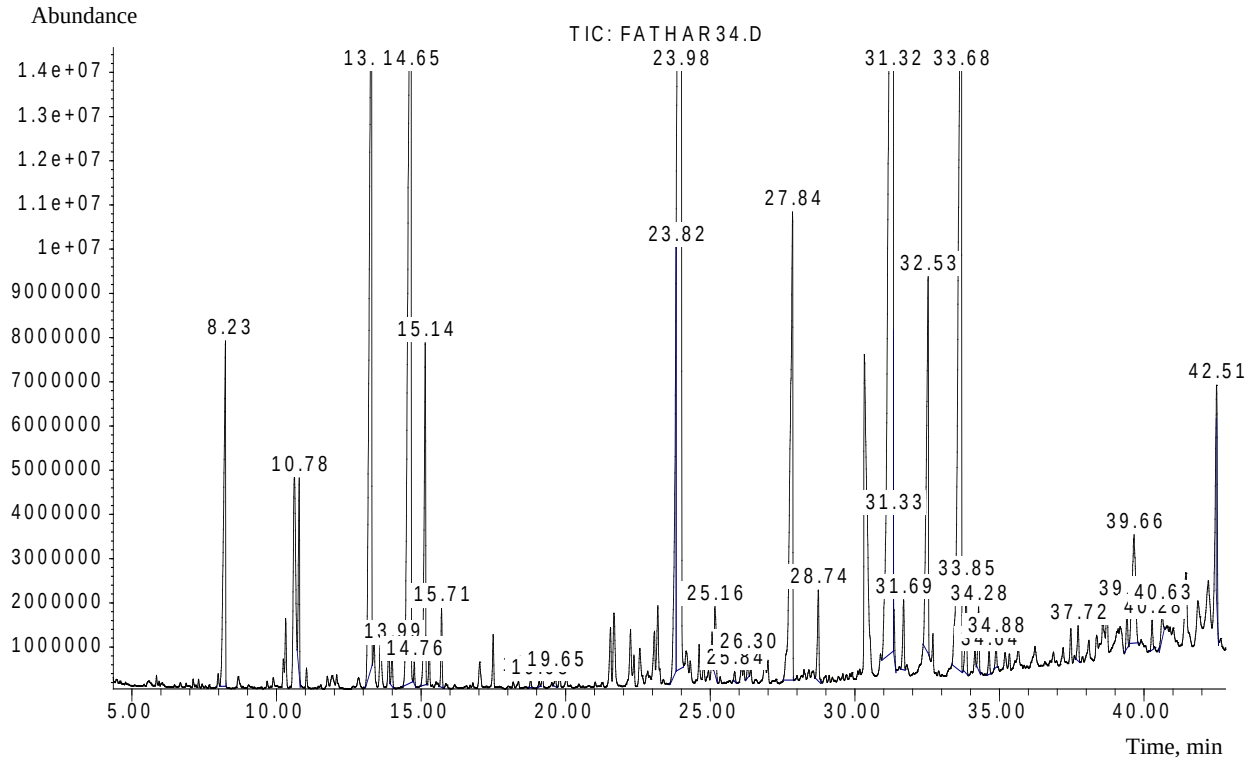

Fig. 3. Chromatographic profile of methyl esters of carboxylic acids of *Veronica chamaedrys* leaves

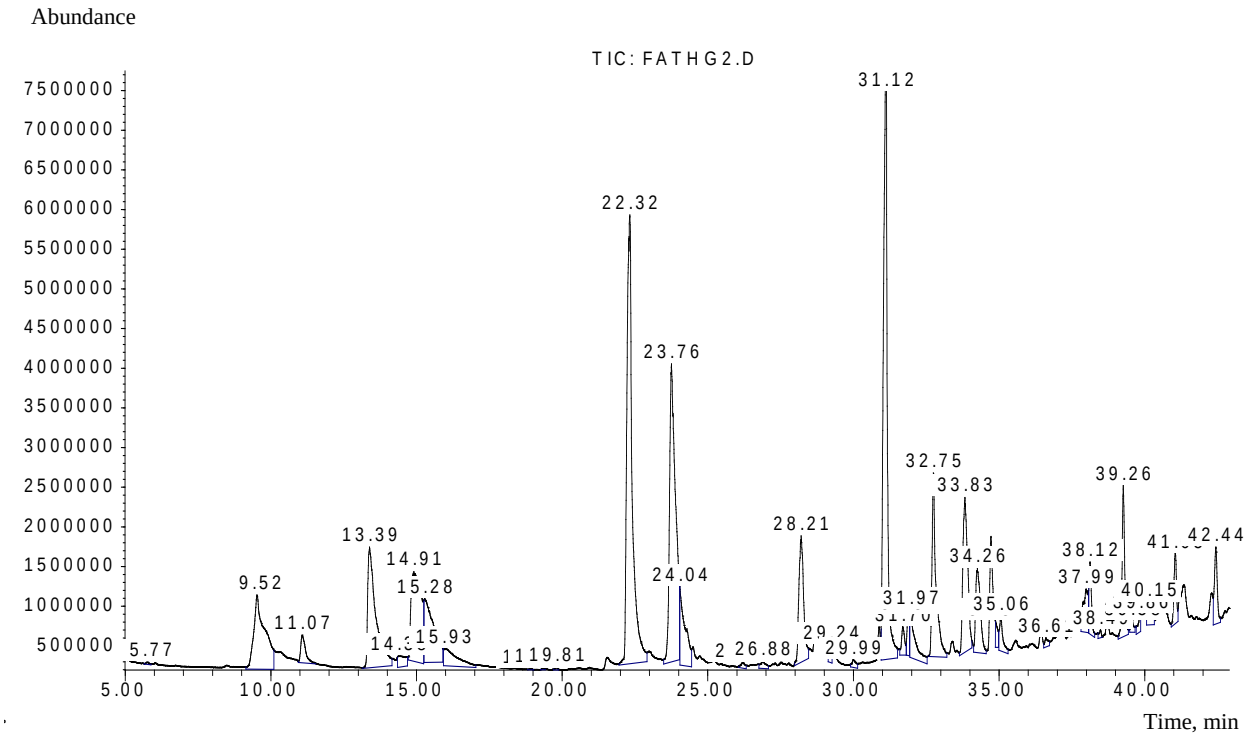

Fig. 4. Chromatographic profile of methyl esters of carboxylic acids of *Veronica teucrium* flowers

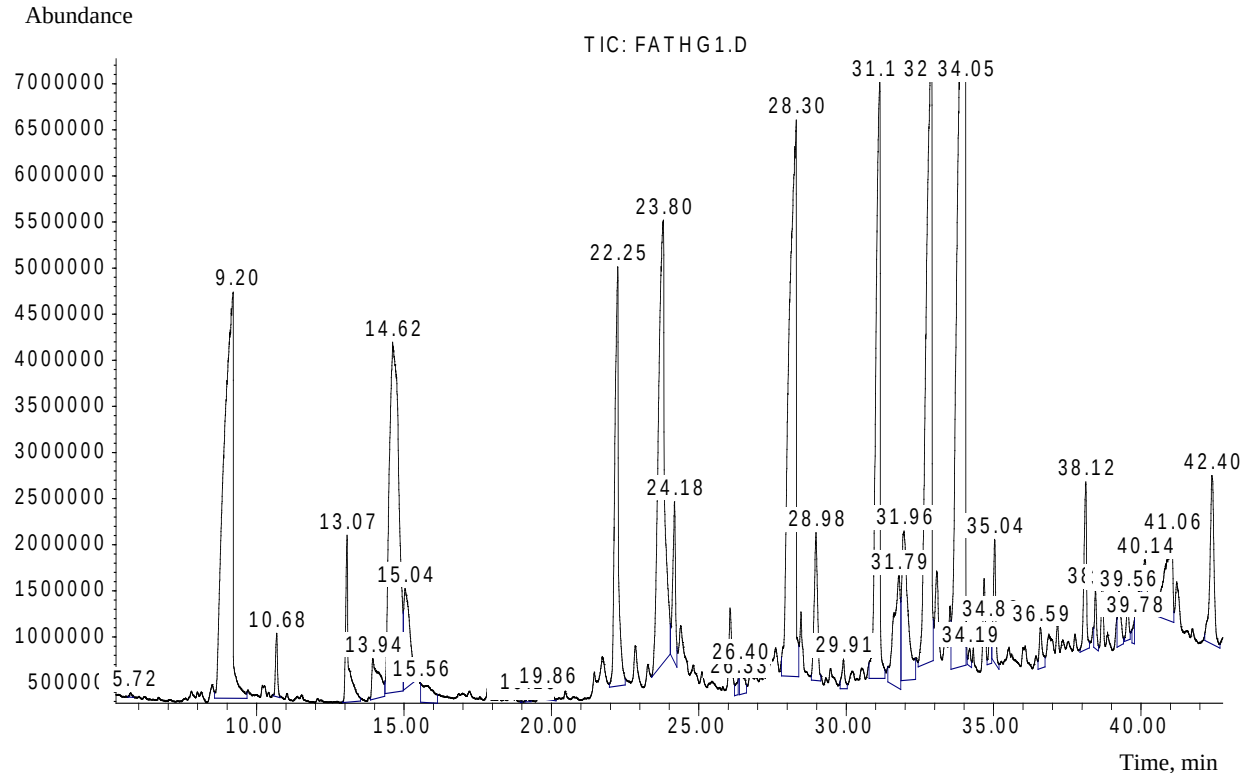

Fig. 5. Chromatographic profile of methyl esters of carboxylic acids of *Veronica teucrium* leaves

Table 1

Carboxylic acids of flowers and leaves of *Veronica chamaedrys* and *Veronica teucrium*

No.	Acid	RI	Species and raw materials							
			Veronica chamaedrys, flowers		*Veronica chamaedrys*, leaves		*Veronica teucrium*, flowers		*Veronica teucrium*, leaves	
			Content							
			mg/kg *	%**	mg/kg *	%**	mg/kg *	%**	mg/kg *	%**
1	2	3	4	5	6	7	8	9	10	11
Low molecular weight aliphatic acids										
1	2-Hydroxy-3-methylpentanoic	1064	1078.83±31.94	1.45	–	–	9598.09± ±238.72	17.29	1475.19± ±33.12	5.03
2	α-Furoic (furan-2-carboxylic)	1086	120.36±3.12	0.16	55.75± ±1.38	0.10	–	–	–	–
3	Oxalic (ethanedioic)	1359	854.75±23.45	1.15	594.46± ±17.46	1.10	530.22± ±16.72	0.95	118.23± ±3.10	0.40
4	Malonic (propanedioic)	1477	9177.97±240.42	12.32	4883.63± ±136.75	9.06	3858.87± ±115.37	6.95	462.99± ±13.88	1.58
5	Levulinic (4-oxopentanoic)	1501	7047.00±197.31	9.46	6764.58± ±162.06	12.55	3542.82± ±111.21	6.38	3188.84± ±95.64	10.87
6	Fumaric (*trans*-butenedioic)	1516	352.17±9.22	0.47	131.38± ±3.81	0.24	347.21± ±8.78	0.63	276.26± ±8.28	0.94
7	Succinic (butanedioic)	1575	5426.62±152.95	7.28	1395.15± ±39.06	2.59	2411.81± ±69.92	4.34	570.76± ±16.12	1.94
8	Malic (2-hydroxybutanedioic)	2008	11937.78±358.13	16.03	11641.35± ±325.56	21.60	7072.67± ±198.01	12.74	2656.96± ±77.10	9.05
9	Azelaic (nonanedioic)	2114	203.29±6.10	0.27	78.65± ±2.20	0.15	151.79± ±4.25	0.27	104.38± ±3.13	0.36
10	Citric (2-Hydroxypropane-1,2,3-tricarboxylic acid)	2367	21928.81±592.06	29.44	11891.37± ±332.95	22.06	9184.74± ±257.17	16.54	2382.37± ±64.32	8.12
Sum:			58127.58±161.47	78.03	58127.58± ±161.47	78.03	37436.32± ±113.47	69.45	36698.22± ±113.35	66.09
Higher fatty acids										
11	Caproic (hexanoic)	1120	–	–	–	–	29.19± ±1.42	0.05	9.05± ±0.43	0.03
12	Lauric (dodecanoic)	1793	25.34±0.91	0.03	46.67±1.57	0.09	25.81± ±0.94	0.05	55.78± ±2,02	0.19
13	Myristic (tetradecanoic)	1994	826.8±29.77	1.11	451.42±16.25	0.84	1556.96± ±56.05	2.80	418,49± ±15.07	1.43
14	Pentadecylic (pentadecanoic)	2101	33.87±0.94	0.05	–	–	95.47± ±3.65	0.17	34.79± ±1,32	0.12
15	Palmitic (hexadecanoic)	2204	3068.63±110.71	4.12	3251.11± ±116.69	6.03	2349.43± ±84.58	4.23	3706,63± ±140.85	12.63
16	Palmitoleic (9-*cis*-hexadecenoic)	2223	378.23±13.62	0.51	380.86±13.71	0.71	152.40± ±5.48	0.27	472.73± ±17,96	1.61
17	Margaric (heptadecanoic)	2292	29.81±0.54	0.04	–	–	128.66± ±0.56	0.23	82.63± ±0.68	0.28
18	Stearic (octadecanoic)	2384	530.92±19.24	0.71	184.38±6.63	0.34	351.11± ±12.64	0.63	685.73± ±26.06	2.34
19	Oleic (*cis*-9-octadecenoic)	2402	389.15±20.17	0.52	275.73±10.48	0.51	954.19± ±34.35	1.72	852.16± ±32.38	2.90
20	Linoleic (*cis*-9,12-octadecadienoic)	2443	2273.30±102.39	3.05	2028.94± ±73.04	3.76	2887.37± ±103.95	5.20	3307.20± ±125.67	11.27
21	Linolenic (*cis*, *cis*, *cis* - 6,9,12-octadecadienoic)	2490	5208.58±197.90	6.99	6462.77± ±245.59	11.99	2945.01± ±111.91	5.30	5518.60± ±209.70	18.80
22	2-Hydroxypalmitic (2-hydroxyhexadecanoic)	2542	69.48±2.64	0.09	93.10±3.35	0.17	209.43± ±7.96	0.38	90.72± ±3.45	0.31
23	Arachidonic (eicosatetraenoic)	2543	146.54±5.58	0.20	87.93±3.17	0.16	452.54± ±1.15	0.81	276.98± ±9.19	0.94
24	Heneicosanoic	2597	–	–	–	–	120.13± ±1.64	0.22	122.63± ±4.65	0.42
25	Behenic (docosanoic)	2698	138.81±5.27	0.19	142.10± ±5.11	0.26	671.92± ±24.19	1.21	406.77± ±14.64	1.39
26	Tricosanoic	2743	–	–	–	–	130.02± ±4.68	0.23	102.74± ±3.12	0.35
27	Lignoceric (tetracosanoic)	2843	67.71±2.57	0.09	159.84± ±5.75	0.30	–	–	619.32± ±19.82	2.11
Sum:			13187.17±22.08	17.70	13564.85± ±41.73	25.16	13059.64± ±37.84	23.50	16762.95± ±36.88	57.12
Aromatic acids										
Derivatives of benzoic acid										
28	Veratric (3,4-dimethoxybenzoic)	1172	–	–	–	–	340.69±9.54	0.61	–	–
29	Benzoic	1600	310.43±8.69	0.42	249.98±6.92	0.46	938.30± ±28.05	1.69	192.84± ±6.17	0.66
30	*p*-Hydroxybenzoic (4-hydroxybenzoic)	2780	125.06±3.75	0.17	158.56±4.44	0.29	1475.15± ±5.30	2.66	159.64±4.47	0.54

Continuation of Table 1

1	2	3	4	5	6	7	8	9	10	11
Phenyl and phenolcarboxylic acids										
31	4-Hydroxybenzyl acetatic (2-(4-hydroxyphenylacetic)	1545	–	–	–	–	70.40±1.98	0.13	114.52± ±3.20	0.39
32	Phenylacetic (α-toluic)	1746	72.80±0.51	0.10	29.74±0.83	0.06	9.26±0.24	0.02	1.57±0.04	0.01
33	Salicylic (2-hydroxybenzoic)	1757	6.88±0.24	0.01	18.78±0.49	0.03	15.01±0.36	0.03	12.29±0.32	0.04
34	Vanillic (4-hydroxy-3-methoxybenzoic)	2522	479.00± ±13.41	0.64	226.51± ±0.66	0.42	1590.56± ±39.80	2.86	39.85±1.10	0.14
35	Syringic (4-hydroxy-3,5-dimethoxybenzoic)	2793	–	–	-	-	162.24± ±4.05	0.29	36.48±1.02	0.12
36	Gentisic (2,5-dihydroxybenzoic)	2805	34.25±0.86	0.05	114.20± ±3.20	0.21	331.60± ±9.29	0.60	133.37± ±3.73	0.45
Hydroxycinnamic acids										
37	*trans*-Cinnamic ((E)-3-phenyl-2-propenoic)	1450	–	–	316.55± ±8.22	0.59	–	–	–	–
38	p-Methoxycinnamic (E) -3-(4-methoxyphenyl-2-propenoic)	1733	–	–	374.50± ±10.42	0.69	–	–	–	–
39	p-Coumaric (3- (4-hydroxyphenol) -2propenoic)	2801	1740.70± ±48.64	2.34	963.92± ±24.57	1.79	835.37± ±23.38	1.50	–	–
40	Ferulic (3-methoxy-4-hydroxycinnamic)	2919	408.48± ±11.23	0.55	449.99± ±11.34	0.83	–	–	657.88± ±17.76	2.24
Sum:			3177.60± ±10.96	4,27	2902.73± ±5.67	5.39	5768.58± ±12.20	10.39	1348.44± ±3.78	4.59
Total:			74492.35± ±64.17	100	53903.90± ±53.62	100	55526.44± ±54.46	100	29347.37± ±25.36	100
Total in raw materials, %			7.45±0.09		5.39±0.10		5.55±0.10		2.93±0.09	

*Note: * – mg/kg in the raw material, ** – % percent of the sum of the detected components, "–" – the compound is not identified*

5. Discussion of the results

The study found that total carboxylic acid content in the flowers of *Veronica chamaedrys* is 74492.35 mg/kg (7.45 %), including 58127.58 mg/kg of low molecular weight aliphatic acids, which is 78.03 % of the total carboxylic acids, 13187.17 mg/kg acids – 17.70 % of the total amount and 3177.60 mg/kg of aromatic acids – 4.26 % of the total amount (Fig. 6). Among the fatty acids, unsaturated ones predominate, the content of which is 8249.26 mg/kg, almost twice less is the content of saturated acids – 4937.91 mg/kg. Among aromatic acids, the content of hydroxycinnamic acids is 2149.18 mg/kg – 2.88 % of the total amount of carboxylic acids; phenyl and phenolic acids – 592.93 mg/kg – 0.80 % of the amount; benzoic acid and its derivative 435.49 mg/kg – 0.58 % of the total amount (Fig. 7).

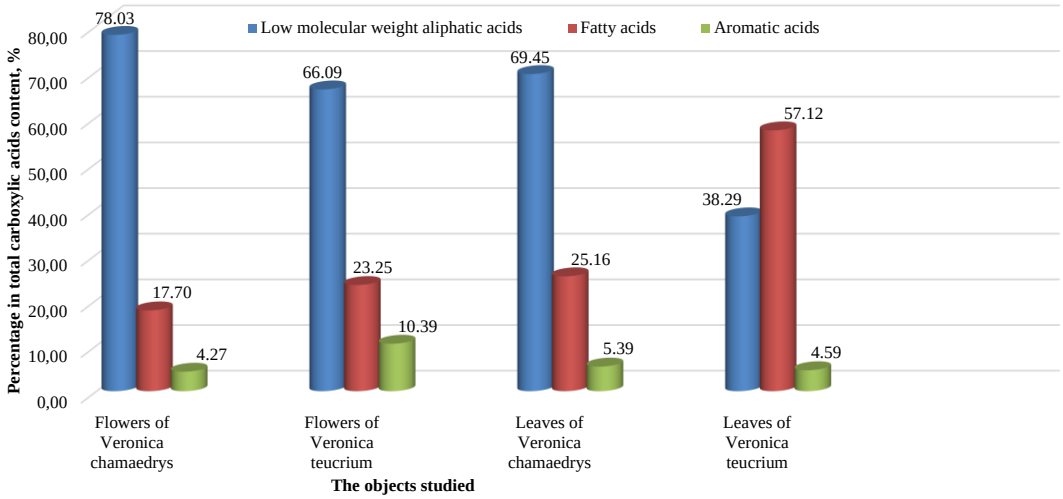

Fig. 6. The composition of carboxylic acids of flowers and leaves of *Veronica chamaedrys* and flowers and leaves of *Veronica teucrium*

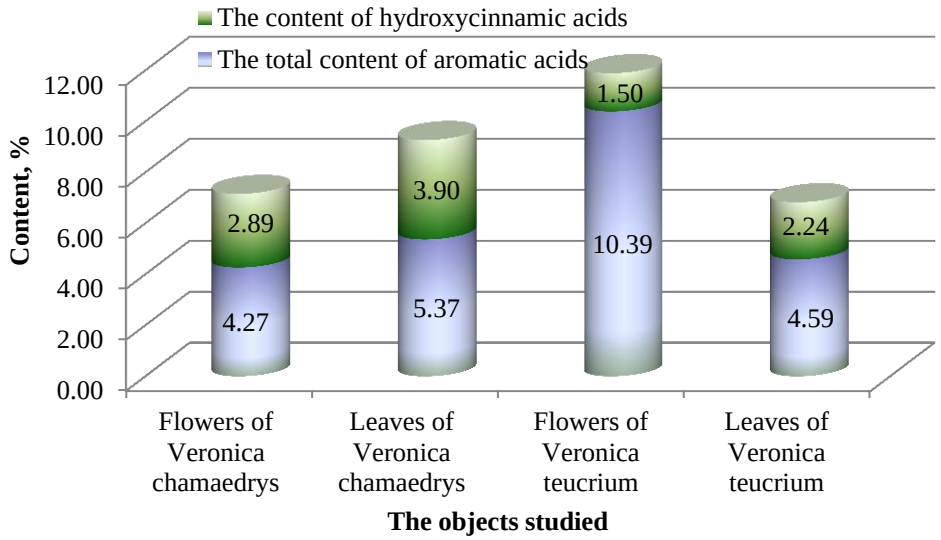

Fig. 7. The content of hydroxycinnamic acids in the composition of aromatic acids in the flowers and leaves of *Veronica chamaedrys* and in the flowers and leaves of *Veronica teucrium*

In the leaves of *Veronica chamaedrys* the total content of carboxylic acids is 53903.90 mg/kg (5.39 %), including 37436.32 mg/kg of low molecular weight aliphatic acids, which is 69.45 % of the total amount of carboxylic acids, 13564.85 mg/kg of fatty acids – 25.16 % of the amount and 2902.73 mg/kg of aromatic acids – 5.36 % of the amount. Among the fatty acids unsaturated acids dominate, the content of which is 9148.30 mg/kg, and they prevail over saturated acids (4416.55 mg/kg). Among aromatic acids the main part constitute hydroxycinnamic acids, their content is 2104.96 mg/kg – 3.90 % of the total sum, almost equivalent content of benzoic acid and its derivative and phenyl- and phenolic carboxylic – 408.54 mg/kg – 0.76 % of the amount and 389.23 mg/kg – 0.72 % of the total amount, respectively.

In the flowers and leaves of *Veronica chamaedrys* the dominant acids are (mg/kg): among low molecular weight aliphatic acids – citric (21928.81 and 11891.37, respectively) and malic (11937.78 and 11641.35, respectively); among higher fatty acids – linolenic (5208.58 and 6462.77, respectively); among aromatic – *p*-coumaric (1740.70 and 963.92, respectively).

In the flowers of *Veronica teucrium* the total content of carboxylic acids is 55526.44 mg/kg (5.55 %), including 36698.22 mg/kg of low molecular weight aliphatic acids, which is 66.09 % of the total carboxylic acids; 13059.64 mg/kg of fatty acids – 23.50 % of the amount, 5768.58 mg/kg of aromatic acids – 10.39 % of the amount. Among the fatty acids, unsaturated ones predominate, the content of which is 6938.97 mg/kg, slightly lower is the content of saturated acids - 6091.48 mg/kg. Aromatic acids are dominated by benzoic acid and its derivatives, the content of which is 2754.14 mg/kg, which is 4.96 % of the sum of carboxylic acids; slightly lower is the content of phenyl- and phenolcarboxylic acids – 2179.07 mg/kg – 3.92 % of the total amount; the lowest is the content of hydroxycinnamic acid – 835.37 mg/kg – 1.50 % of the total sum.

Veronica teucrium leaves contain 29347.37 mg/kg (2.93 %) of carboxylic acids, including 11235.98 mg/kg of low molecular weight aliphatic acids, which is 38.29 % of the total carboxylic acids; 16762.95 mg/kg of fatty acids – 57.12 % of the total amount, 1348.44 mg/kg of aromatic acids – 4.59 % of the total sum. Among the fatty acids unsaturated ones dominate, the content of which is 10150.69 mg/kg, the content of saturated acids is 6603.1 mg/kg. Aromatic acids are dominated by hydroxycinnamic acis – ferulic acid, the content of which is 657.88 mg/kg – 2.24 % of the amount; almost twice less content of benzoic acid and its derivative – 352.48 mg/kg – 1.20 % of the amount; the content of phenyl- and phenolcarboxylic acids is comparable – 338.08 mg/kg – 1.15 % of the amount.

In the flowers of *Veronica teucrium* prevail (mg/kg): among low molecular weight aliphatic acids – 2-hydroxy-3-methylglutaric (9598.09) and citric (9184.74); among higher fatty acids – palmitic (2349.43); among aromatic acids – vanillic (1590.56) and p-hydroxybenzoic (1475.15).

In the leaves of *Veronica teucrium* following substances are dominated (mg/kg): among low molecular weight aliphatic acids – levulinic (3188.84) and malic (2656.96); among higher fatty acids – linolenic (5518.6) and palmitic (3706.63); among aromatic acids – ferulic (657.88). Only the flowers and leaves of *Veronica chamaedrys* contain α-furanic acid. Caproic, tricosanic, heneicosanic, 4-hydroxybenzylacetic and syringic acids were found only in the flowers and leaves of *Veronica teucrium*.

Within the species, the qualitative composition of carboxylic acids of different raw materials also has its differences. Thus, for *Veronica chamaedrys*, 2-hydroxy-3-methylglutaric, pentadecanoic and margaric acids are original for flowers; for the leaves of *Veronica chamaedrys* – cinnamic and *p*-methoxycinnamic acids. For *Veronica teucrium*, veratric and *p*-coumaric acids are original for flowers; for leaves – lignoceric and ferulic acids. These differences can be used as chemotaxonomic markers in the chemical identification of species.

The obtained data on the presence of *p*-coumaric

and ferulic, linolenic, linoleic and palmitic acids in the leaves and flowers of *Veronica chamaedrys* are consistent with the results of other studies of this species herb [16, 17]. Carboxylic acids of flowers and leaves of *Veronica teucrium* of Ukrainian flora were first studied. For the first time in the flowers and leaves of *Veronica chamaedrys* of domestic flora aromatic acids were identified: benzoic, *p*-hydroxybenzoic, 4-hydroxybenzyloacetic, salicylic, vanillic and gentisic; in the leaves - *trans*-cinnamic and methoxycinnamic acids.

For the treatment of metabolic syndrome, diabetes and heart disease, raw materials containing polyphenolic compounds, including aromatic acids, are considered as a potential source of drugs [22, 23]. Literature data indicate hypolipidemic and antidiabetic activity of ferulic acid found in the studied raw material [24, 25]. Numerous studies confirm the hypolipidemic effect of unsaturated fatty acids [26].

Thus, the obtained results allow us to consider the leaves of *Veronica teucrium* as a more promising raw material for the development of substances and study of their effects on the metabolic syndrome.

Study limitations. During the study of raw materials by GC-MS, a number of compounds could not be identified because their characteristics are missing in the library of mass spectra NIST 05 and Willey 2007 and programs for the identification of AMDIS and NIST.

The prospects for the further research. The obtained results indicate the feasibility of developing substances based on flowers and leaves of *Veronica chamaedrys* and *Veronica teucrium* and can be used for further chemotaxonomic studies.

For further medical use it is necessary to develop and validate methods of quality control of raw materials from the species of *Veronica*, to develop technological schemes for obtaining substances.

Given the results of phytochemical studies, it was found that the leaves of *Veronica chamaedrys* and flowers of *Veronica teucrium* are promising for the study of hypoglycemic and hypolipidemic effects in the correction of metabolic syndrome.

6. Conclusions

The composition of carboxylic acids of flowers and leaves of *Veronica chamaedrys* and *Veronica teucrium* was first studied by chromatophy-mass spectrometry. As a result of the study, 32 carboxylic acids in flowers and 31 carboxylic acids in the leaves of *Veronica chamaedrys*, 35 carboxylic acids in flowers and leaves of *Veronica teucrium* were identified and quantified. The flowers and leaves of *Veronica chamaedrys* have a higher content of carboxylic acids (7.45 % and 5.39 %, respectively) compared to the flowers and leaves of *Veronica teucrium* (5.55 % and 2.93 %, respectively). The flowers and leaves of *Veronica chamaedrys* are distinguished by the presence of α-furanic acid. Flowers and leaves of *Veronica teucrium* differ in the presence of caproic, tricosan, heneicosan, veratric, 4-hydroxybenzylacetic and syringic acids. These differences can be used as chemotaxonomic markers in the chemical identification of species. The obtained results allow us to consider the leaves of *Veronica teucrium* as a more promising raw material for the development of substances for the treatment of metabolic syndrome.

Conflict of interests

The authors declare that there is no conflict of interests.

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