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Carboxylic acids of phenolic complexes from *Veronica teucrium* L.

Aim. To compare and study low molecular aliphatic, fatty and aromatic acids of phenolic complexes (PhC) obtained from *V. teucrium* L. flowers, leaves and rhizomes using chromatography-mass spectrometry.

Materials and methods. Phenolic complexes from flowers, leaves and rhizomes were obtained by the exhaustive circulating extraction method in a Soxhlet apparatus. The analysis of methyl esters of acids was performed on a 5973N/6890N MSD/DS Agilent Technologies (USA) chromatograph using the chromatography-mass spectrometry method. The sample injection in a HP-INNOWAX (0.25 mm × 30 m) chromatographic capillary column was performed by a *splitless* mode. Identification of methyl esters of acids was performed based on the calculation of the equivalent length of the aliphatic chain (ECL) using data from the mass spectra libraries NIST 05 and Willey 2007 in combination with programs for identifying AMDIS and NIST; the retention time of esters was also compared with the retention time of standard compounds (Sigma). The internal standard method was used for quantitative calculations.

Results and discussion. As the result of our study low molecular aliphatic, fatty and aromatic acids have been identified in phenolic complexes of *V. teucrium* L. flowers, leaves and rhizomes for the first time, their quantitative content is as follows: 2.34 % – in the complex from flowers, 2.78 % – in the complex from leaves, and 2.10 % – in the complex from rhizomes. In the phenolic complex from flowers low molecular aliphatic acids (malonic, levulinic, succinic, 3-hydroxy-2-methylglutaric); fatty acids (palmitic and linolenic); aromatic acids (vanillic, *p*-coumaric and *p*-hydroxybenzoic) prevail. The dominant compounds in the phenolic complex from leaves are low molecular aliphatic acids (malonic, levulinic, succinic, 3-hydroxy-2-methylglutaric, malic); fatty acids (palmitic, oleic, linoleic, linolenic); and aromatic acid (ferulic). In the phenolic complex from rhizomes low molecular aliphatic acids (levulinic, succinic, malic); fatty acids (palmitic, stearic, oleic, linoleic, linolenic); aromatic acids (veratric, vanillic, syringic, ferulic) dominate.

Conclusions. As the result of our study for the first time the following components have been identified in phenolic complexes: 40 low molecular aliphatic, fatty and aromatic acids – from flowers, 39 – from leaves, 38 – from rhizomes. The content of carboxylic acids in phenolic complexes is 2.34 % – from flowers, 2.78 % – from leaves, 2.10 % – from rhizomes. It has been found that the herbal drug of *V. teucrium* L. is a source of valuable biologically active acids with different pharmacological effect.

Key words: *Veronica teucrium L.; low molecular aliphatic acids; fatty acids; aromatic acids; phenolic complex from flowers; leaves and rhizomes*

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Карбонові кислоти фенольних комплексів *Veronica teucrium* L.

Мета роботи – порівняльне хромато-мас-спектрометричне дослідження низькомолекулярних аліфатичних, ароматичних і жирних кислот у фенольних комплексах, отриманих з квіток, листя і кореневищ вероніки широколистої (*V. teucrium* L.).

Матеріали та методи. Фенольні комплекси з квіток, листя і кореневищ отримували методом вичерпної циркуляційної екстракції в апараті Сокслета. Аналіз метилових естерів кислот проводили методом хромато-мас-спектрометрії на хроматографі 5973N/6890N MSD/DS Agilent Technologies (USA). Введення проби в хроматографічну капілярну колонку HP-INNOWAX (0,25 мм × 30 м) проводили в режимі *splitless*. Ідентифікацію метилових естерів кислот проводили на основі розрахунку еквівалентної довжини аліфатичного ланцюга (ECL) з використанням даних бібліотеки мас-спектрів NIST 05 і Willey 2007 в поєднанні з програмами для ідентифікації AMDIS і NIST; також порівнювали час утримання естерів з часом утримання стандартних сполук (Sigma). Для кількісних розрахунків використовували метод внутрішнього стандарту.

Результати та їх обговорення. В результаті дослідження вперше в фенольних комплексах квіток, листя і кореневищ вероніки широколистої були ідентифіковані низькомолекулярні аліфатичні, жирні і ароматичні кислоти і встановлено їх кількісний вміст: у комплексі квіток 2,34 %, у комплексі листя – 2,78 %, у комплексі кореневищ – 2,10 %. У фенольному комплексі квіток переважають низькомолекулярні аліфатичні кислоти (малонова, левулінова, бурштинова, 3-гідрокси-2-метілглутарова); жирні кислоти (пальмітинова і ліноленова); ароматичні кислоти (ванілінова, *п*-кумарова, *п*-гідроксибензойна). У фенольному комплексі листя домінуючими сполуками є низькомолекулярні аліфатичні кислоти (малонова, левулінова, бурштинова, 3-гідрокси-2-метилглутарова, яблучна); жирні кислоти (пальмітинова, олеїнова, лінолева, ліноленова); ароматична кислота (ферулова). У фенольному комплексі кореневищ домінуючими сполуками є низькомолекулярні аліфатичні кислоти (левулінова, бурштинова, яблучна); жирні кислоти (пальмітинова, стеаринова, олеїнова, лінолева, ліноленова); ароматичні кислоти (вератрова, ванілінова, бузкова, ферулова).

Висновки. В результаті дослідження вперше ідентифіковані в фенольних комплексах: з квіток 40 низькомолекулярних аліфатичних, жирних і ароматичних кислот, з листя 39, з кореневищ 38. Вміст карбонових кислот у фенольних комплексах становить: з квіток 2,34 %, з листя 2,78 %, з кореневищ 2,10 %. Встановлено, що сировина вероніки широколистої є джерелом важливих біологічно активних кислот з різною фармакологічною дією.

Ключові слова: *Вероніка широколиста; низькомолекулярні аліфатичні кислоти; жирні кислоти; ароматичні кислоти; фенольні комплекси з квіток; листя і кореневищ*

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Карбоновые кислоты фенольных комплексов *Veronica teucrium* L.

Цель работы – сравнительное хромато-масс-спектрометрическое исследование низкомолекулярных алифатических, ароматических и жирных кислот в фенольных комплексах, полученных из цветков, листьев и корневищ вероники широколистой (*V. teucrium* L.).

Материалы и методы. Фенольные комплексы из цветков, листьев и корневищ получали методом исчерпывающей циркуляционной экстракции в аппарате Сокслета. Анализ метиловых эфиров кислот проводили методом хромато-масс-спектрометрии на хроматографе 5973N/6890N MSD/DS Agilent Technologies (USA). Введение пробы в хроматографическую капиллярную колонку HP-INNOWAX (0,25 мм × 30 м) проводили в режиме *splitless*. Идентификацию метиловых эфиров кислот проводили на основе расчета эквивалентной длины алифатической цепи (ECL) с использованием данных библиотеки масс-спектров NIST 05 и Willey 2007 в сочетании с программами для идентификации AMDIS и NIST; также сравнивали время удержания эфиров со временем удержания стандартных соединений (Sigma). Для количественных расчетов использовали метод внутреннего стандарта.

Результаты и их обсуждение. В результате исследования впервые в фенольных комплексах цветков, листьев и корневищ вероники широколистной были идентифицированы низкомолекулярные алифатические, жирные и ароматические кислоты и установлено их количественное содержание: в комплексе цветков 2,34 %, в комплексе листьев – 2,78 %, в комплексе корневищ – 2,10 %. В фенольном комплексе цветков преобладают низкомолекулярные алифатические кислоты (малоновая, левулиновая, янтарная, 3-гидрокси-2-метилглутаровая); жирные кислоты (пальмитиновая и линоленовая); ароматические кислоты (ванилиновая, *п*-кумаровая, *п*-гидроксибензойная). В фенольном комплексе листьев доминирующими соединениями являются низкомолекулярные алифатические кислоты (малоновая, левулиновая, янтарная, 3-гидрокси-2-метилглутаровая, яблочная); жирные кислоты (пальмитиновая, олеиновая, линолевая, линоленовая); ароматическая кислота (феруловая). В фенольном комплексе корневищ доминирующими соединениями являются низкомолекулярные алифатические кислоты (левулиновая, янтарная, яблочная); жирные кислоты (пальмитиновая, стеариновая, олеиновая, линолевая, линоленовая); ароматические кислоты (вератровая, ванилиновая, сиреневая, феруловая).

Выводы. В результате исследования впервые идентифицированы в фенольных комплексах: из цветков – 40 низкомолекулярных алифатических, жирных и ароматических кислот, из листьев – 39, из корневищ – 38. Содержание карбоновых кислот в фенольных комплексах составляет: из цветков 2,34 %, из листьев – 2,78 %, из корневищ – 2,10 %. Установлено, что сырье вероники широколистой является источником важных биологически активных кислот с разным фармакологическим действием.

Ключевые слова: *Вероника широколистная; низкомолекулярные алифатические кислоты; жирные кислоты; ароматические кислоты; фенольные комплексы из цветков; листьев и корневищ*

The genus of *Veronica* L. belongs to *Plantaginaceae* Juss. family and includes more than 350 species of the world flora, which are common in the northern and northwest areas of the forest-steppe (optimal zone) and separate areas of the steppe zone. In the flora of Ukraine the genus is represented by more than 50 species [1]. One of the common species of *Veronica* L. on the Ukrainian territory is unofficial species of *V. teucrium* L. (Veronica broadleaf) – a herbaceous perennial plant growing on meadows, edges of the forest, forest lawns, slopes, at forests, in shrubs, at banks of rivers and streams, sometimes as weeds.

It should be noted that the chemical composition of most wild species of *Veronica* genus of the Ukrainian flora has not been completely studied. It is known that *V. teucrium* L. herb contains phenylethanoid, phenolcarboxylic and hydroxycinnamic acids, hydroxycoumarins, flavonoids, iridoids, saponins, but the chemical composition of rhizomes has been studied poorly, only the presence of ascorbic acid is known [2, 3, 4, 5, 6].

Previously, the main groups of biologically active substances (BAS) of *V. teucrium* L. herb and V. *longifolia* L. herb were studied, and the extracts having a pronounced antibacterial activity against Gram (-) bacteria and a moderate activity in relation to Gram (+) bacteria were obtained from them [4, 7]. It has been found that polysaccharide complexes of *V. teucrium* L. leaves, flowers and rhizomes reveal the immunomodulatory activity [8].

V. teucrium L. is used only in folk medicine as an anti-inflammatory, expectorant, antiseptic, hemostatic, detoxification, choleretic remedy [9]. A wide range of biological effects creates the preconditions for a complex phytochemical study of *V. teucrium* L. and the extracts obtained from it.

The **aim** of the study was the chromatography-mass spectrometric determination of the content of low molecular aliphatic, fatty and aromatic acids in phenolic complexes (PhC) obtained from *V. teucrium* L. flowers, leaves and rhizomes.

Materials and methods

The objects of the study were *V. teucrium* L. flowers, leaves and rhizomes collected in the flowering stage in the Kharkiv region in summer 2015.

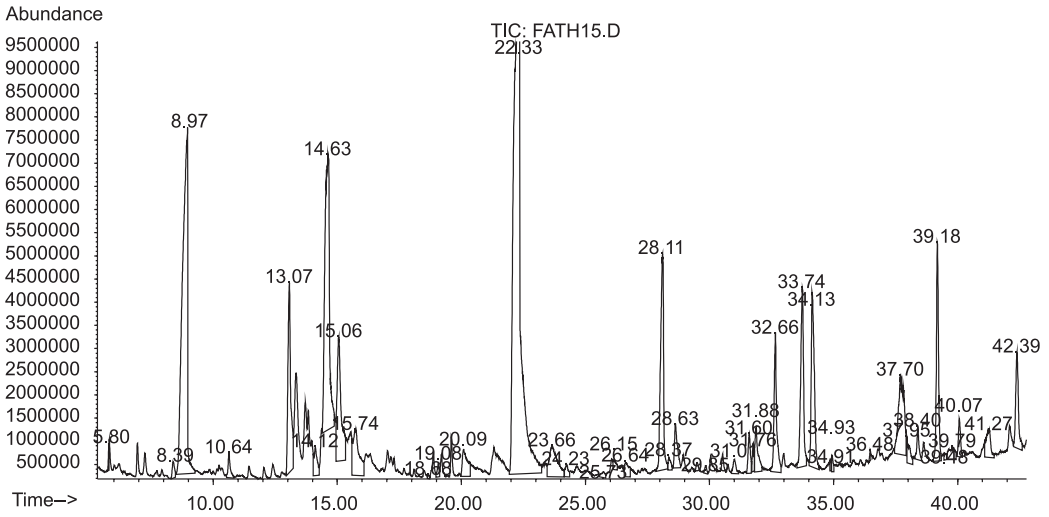

Fig. 1. The chromatogram of methyl esters of carboxylic acids of PhC from *V. teucrium* L. flowers (PhC-fl)

After obtaining the lipophilic complexes from flowers and leaves with chloroform as an extractant, and the complexes from rhizomes with hexane and chloroform the extraction cakes in filter bags were dried in air before removing extractants from them and placed in filter bags in a Soxhlet apparatus, then ethyl acetate was added and extracted while heating on a water bath (60 °C) for 40 h. The completeness of the extraction was assessed by the absence of the color of the extract in the drain pipe and by the chromatographic method. The extracts obtained were evaporated under vacuum to remove extractants.

The analysis of carboxylic acids of PhC was performed on a 6890N MSD/DS Agilent Technologies (USA) chromatograph with a 5973N mass spectrometric detector using the chromatography-mass spectrometry method [10, 11, 12]. To do this, their methylation was preliminarily carried out: an internal standard (50 μg of tridecane in hexane) and 1.0 ml of a methylating agent – 14% BCl_3 in methanol (Supelco 3-3033) were added in a vial with 50 mg of PhC. The mixture was kept in a sealed vial for 8 h at 65 °C, after that 0.2 ml of methylene chloride was added with shaking for one hour, and then was chromatographed. The chromatographic parameters were as follows: the sample injection – 2 μl by a *splitless* mode; the flow rate – 1.2 ml/min; the period – 0.2 min; the stationary phase – chromatographic capillary column HP-INNOWAX (0.25 mm × 30 m); the mobile phase – helium; the flow rate of a carrier gas – 1 ml/min; the temperature of the sample injection evaporator – 250 °C. Identification of methyl esters of acids was performed based on the calculation of the equivalent length of the aliphatic chain (ECL) using data from the mass spectra libraries NIST 05 and Willey 2007 in combination with programs for identifying AMDIS and NIST; the retention time of esters was also compared with the retention time of standard compounds (Sigma) (Fig. 1, 2, 3).

The internal standard method was used for quantitative calculations. The relative content of acids was also calculated as a percentage of their amount (Table).

Results and discussion

The yields of PhC calculated with reference to the absolutely dry raw material were: from flowers (PhC-fl) – 24.02 %, from leaves (PhC-l) – 19.77 %, from rhizomes (PhC-rh) – 8.71 %. PhC-fl is a dark brown viscous mass with a fragrant balsamic odor; PhC-l is a dark green

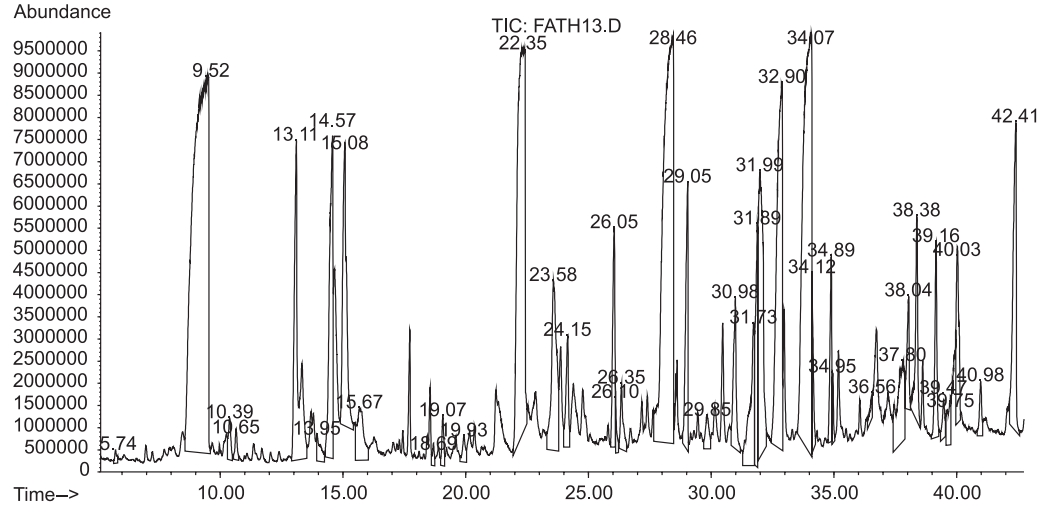

Fig. 2. The chromatogram of methyl esters of carboxylic acids of PhC from *V. teucrium* L. leaves (PhC-l)

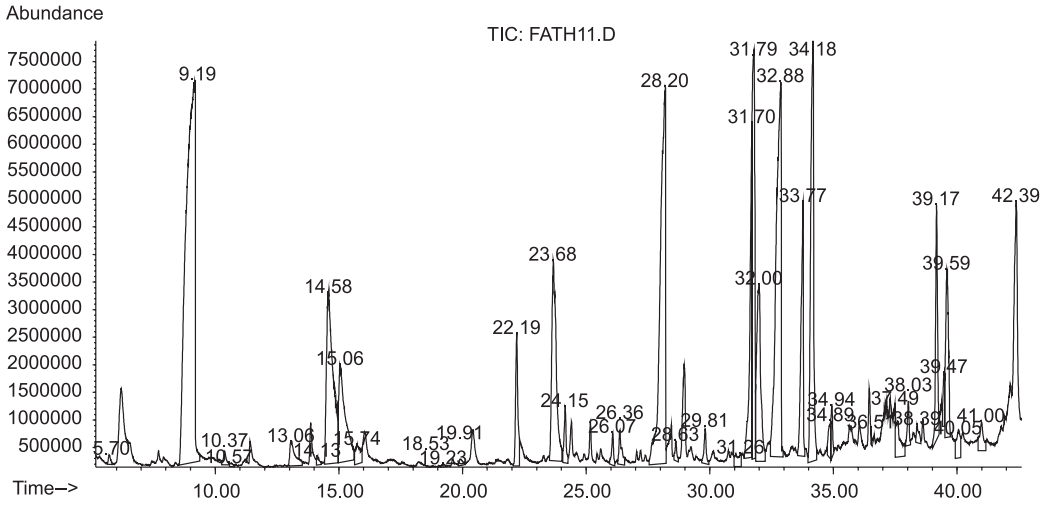

Fig. 3. The chromatogram of methyl esters of carboxylic acids of PhC from *V. teucrium* L. rhizomes (PhC-rh)

powder with a characteristic odor; PhC-rh is a dark brown powder with a characteristic odor. The PhC obtained are soluble in ethyl acetate, in 96º-50° ethanol, slightly soluble in water.

As the result of our study 40 carboxylic acids in PhC-fl; 39 acids – in PhC-l; 38 acids – in PhC-rh have been identified in phenolic complexes of *V. teucrium* L. flowers, leaves and rhizomes for the first time and their quantitative content has been determined.

In PhC-fl 10 low molecular aliphatic, 16 fatty and 14 aromatic acids have been identified; in PhC-l – 10 low molecular aliphatic, 17 fatty and 12 aromatic acids; in PhC-rh – 10 low molecular aliphatic, 17 fatty and 11 aromatic acids.

In PhC-fl low molecular aliphatic acids (malonic, levulinic, succinic, 3-hydroxy-2-methylglutaric); fatty acids (palmitic and linolenic); aromatic acids (vanillic, *p*-coumaric and *p*-hydroxybenzoic) prevail. The dominant compounds in PhC-l are low molecular aliphatic acids (malonic, levulinic, succinic, 3-hydroxy-2-methylglutaric, malic); fatty acids (palmitic, oleic, linoleic, linolenic); and aromatic acid (ferulic). In PhC-rh low molecular aliphatic acids (levulinic, succinic, malic); fatty acids (palmitic, stearic, oleic, linoleic, linolenic); aromatic acids (veratric, vanillic, syringic, ferulic) dominate (Table).

The content of low molecular aliphatic acids in PhC-fl is 1333.25 mg/%, the content of fatty acids – 488.78 mg/%, and the content of aromatic acids – 518.89 mg/%. In the total amount of the components identified the content of low molecular aliphatic acids is 56.95 %, fatty acids – 20.88 %, aromatic acids– 22.17 %.

The dominant low molecular aliphatic acids in PhC-fl are (mg/%) malonic (110.10), levulinic (271.94), succinic (105.32) and 3-hydroxy-2-methylglutaric (740.35); fatty acids – palmitic (138.04) and linolenic (107.32); aromatic acids – vanillic (113.21), *p*-coumaric (95.29) and *p*-hydroxybenzoic (98.19).

The content of low molecular aliphatic acids in PhC-l is 840.49 mg/%, the content of fatty acids – 1439.49 mg/%, and the content of aromatic acids – 499.81 mg/%. In the total amount the content of low molecular aliphatic acids is 30.24 %, fatty acids – 51.78 %, and aromatic acids – 17.98 %.

In PhC-l the dominant acids are (mg/%) among low molecular aliphatic acids – malonic (129.32), levulinic (115.99), succinic (113.47), 3-hydroxy-2-methylglutaric (312.28), malic (92.76); fatty acids – palmitic (421.65); oleic (138.30), linoleic (208.31) and linolenic (369.76); aromatic acid – ferulic.

The content of low molecular aliphatic acids in PhC-rh is 478.49 mg/%, the content of fatty acids – 969.90 mg/%, and the content of aromatic acids – 649.18 mg/%. In the total amount of the components identified the content of low molecular aliphatic acids is 22.81 %, fatty acids – 46.24 %, and aromatic acids – 30.95 %.

In PhC-rh the dominant acids are (mg/%) among low molecular aliphatic acids – levulinic (167.55), succinic (93.15), malic (131.50); fatty acids – palmitic (290.20), stearic (90.84), oleic (106.85), linoleic (270.77), linolenic (84.67); aromatic acids – veratric (156.76), vanillic (186.51), syringic (70.81) and ferulic (67.18).

The total content of carboxylic acids identified in PhC-fl is 2340.92 mg/% or 2.34 %; in PhC-l – 2779.80 mg/% or 2.78 %; in PhC-rh – 2097.57 mg/% or 2.10 %.

Lactic and phenylpropanoic acids are inherent only to PhC-fl.

The results of the study show that the highest content of low molecular aliphatic acids of the total amount of carboxylic acids identified (1333.25 mg/% or 56.95 %) is found in PhC-fl.

Fatty acids are the largest part of the total amount of carboxylic acids of PhC-l (13707.97 mg/kg or 51.78 %); moreover, unsaturated fatty acids prevail (7774.70 mg/% or 27.97 %).

It should be noted that in PhC-rh aromatic acids are 649.18 mg/%, i.e. they are 30.95% of the total content of acids (Fig. 4).

Of the pharmacological value are acids that reveal the following actions: antibacterial (ferulic, vanillic, *p*-hydroxybenzoic, syringic and p-methoxybenzoic acid);

Table

Carboxylic acids of phenolic complexes obtained from *V. teucrium* L. flowers, leaves and rhizomes

The name of acid	The content of acids in phenolic complexes					
	PhC-fl		PhC-l		PhC-rh	
	mg/kg*	%**	mg/kg*	%**	mg/kg*	%**
Caproic (hexanoic)	31.05	0.13	29.91	0.11	31.51	0.15
Lactic (2-hydroxypropanoic)	79.00	0.34	–	–	–	–
Internal standard	3676.47	–	6097.56	–	9.19	–
Caprylic (octanoic)	–	–	110.08	0.40	56.56	0.27
Oxalic (ethanedioic)	117.38	0.50	69.36	0.25	16.44	0.08
Malonic (propanedioic)	1100.99	4.70	1293.15	4.65	195.95	0.93
Fumaric (trans-butenedioic)	198.24	0.85	107.12	0.39	31.43	0.15
Levulinic (4-oxopentanoic)	2719.37	11.62	1159.92	4.17	1675.54	7.99
Succinic (butanedioic)	1053.17	4.50	1134.73	4.08	931.48	4.44
Benzoic (benzoic)	612.33	2.62	385.21	1.39	145.93	0.70
Phenylacetic (α-toluic)	13.51	0.06	44.22	0.16	4.47	0.02
Salicylic (2-hydroxybenzoic)	57.9	0.25	102.13	0.37	13.2	0.06
Lauric (dodecanoic)	292.96	1.25	82.22	0.30	35.7	0.17
3-Hydroxy-2-methylglutaric (3-hydroxy-2-methylpentanedioic)	7403.49	31.63	3122.82	11.23	435.39	2.08
Malic (2-hydroxybutanedioic)	539.83	2.31	927.62	3.34	1314.99	6.27
Myristic (tetradecanoic)	78.53	0.34	301.49	1.08	169.2	0.81
p-Methoxybenzoic (4-methoxybenzoic)	50.12	0.21	501.72	1.80	–	–
Pentadecanoic (pentadecanoic)	57.76	0.25	60.09	0.22	94.18	0.45
Azelaic (nonanedioic)	131.98	0.56	188.38	0.68	138.68	0.66
Palmitic (hexadecanoic)	1380.44	5.90	4216.45	15.17	2902.04	13.84
Palmitoleic (cis-9-hexadecanoic)	68.05	0.29	611.04	2.20	54.71	0.26
Phenylpropionic (hydroxyphenylpropionic)	267.86	1.14	–	–	–	–
Margarinic (Heptadecanoic)	33.61	0.14	129.46	0.47	116.91	0.56
Citric (2-hydroxy-1,2,3-propanetricarboxylic)	90.01	0.38	450.23	1.62	95.63	0.46
Stearic (octadecanoic)	185.71	0.79	638.55	2.30	908.37	4.33
Veratric (3,4-dimethoxybenzyl alcohol)	103.65	0.44	548.4	1.97	1567.63	7.47
Oleic (cis-9-octadecenoic)	280.25	1.20	1383.04	4.98	1068.54	5.09
Linoleic (9,12-octadecadienoic)	749.91	3.20	2083.05	7.49	2707.7	12.91
Linolenic (cis,cis,cis-6,9,12-octadecatrienoic)	1073.15	4.58	3697.61	13.30	846.69	4.04
Vanillic (4-hydroxy-3-methoxybenzoic)	1132.11	4.84	215.78	0.78	1865.05	8.89
2-Hydroxypalmitic (2-hydroxyhexadecanoic)	27.93	0.12	340.12	1.22	122.36	0.58
Arachidic (eicosanoic)	54.82	0.23	95.96	0.35	125.67	0.60
Heneicosanoic (heneicosanoic)	25.86	0.11	68.69	0.25	15.51	0.07
p-Coumaric (3-(4-hydroxyphenyl)-propenoic)	952.89	4.07	623.67	2.24	276.16	1.32
Begenic (docosanoic)	149.77	0.64	268.34	0.97	120.18	0.57
p-Acetylsalicylic (2-(Acetyloxy)benzoic)	146.42	0.63	494.09	1.78	67.9	0.32
p-Hydroxybenzoic (4-hydroxybenzoic)	981.93	4.19	428.08	1.54	671.76	3.20
Tricosanoic (tricosanoic)	26.39	0.11	102.38	0.37	109.63	0.52
Syringic (4-hydroxy-3,5-dimethoxybenzoic)	151.34	0.65	163.8	0.59	708.14	3.38
Gentisic (2,5-dihydroxybenzoic)	167.74	0.72	465.36	1.67	167.05	0.80
Lignoceric (Tetracosanoic)	270.64	1.16	128.04	0.46	162.89	0.78
Ferulic (3-methoxy-4-hydroxycinnamic)	551.06	2.35	1025.68	3.69	1004.51	4.79
	23409,15	100	27797.99	100	20975.68	100

Note: * – mg/kg in the complex; ** – % of the amount of components identified; "–" – the compound is not identified.

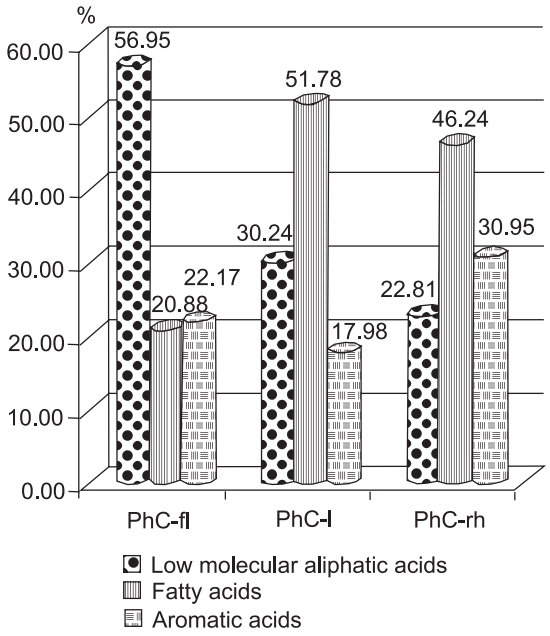

Fig. 4. The content of carboxylic acids of phenolic complexes of *V. teucrium* L.

antioxidant (ferulic, *p*-coumaric and syringic acid); anti-inflammatory (benzoic, ferulic, vanillic, salicylic, gentisic, veratric and *p*-acetylsalicylic acid); antipyretic (salicylic, gentisic and p-acetylsalicylic acid); antitumor (ferulic, *p*-coumaric and gentisic acid); hepatoprotective, antiviral and antiarrhythmic (ferulic acid); hypoglycemic (syringic acid) and analgesic (gentisic acid) [13, 14].

Therefore, the plant raw material of *V. teucrium* L. is a source of pharmacologically valuable acids.

CONCLUSIONS

The comparative study of the acid composition of phenolic complexes obtained from *V. teucrium* L. flowers, leaves and rhizomes has been carried out by the chromatography-mass spectrometry method.

In phenolic complexes the content of 41 carboxylic acids has been identified, among them there are 11 low molecular aliphatic, 18 fatty and 12 aromatic acids. The total content of carboxylic acids identified in PhC from *V. teucrium* L. flowers is 2.34 %; in PhC from leaves – 2.78 %; and in PhC from rhizomes – 2.10 %.

All phenolic complexes from flowers, leaves and rhizomes contain the highest content (%) of the following components: low molecular aliphatic acids (levulinic –11.62, 4.17, 7.99; 3-hydroxy-2-methylglutaric – 31,.63, 11.23, 2.08); fatty acids (palmitic – 5.90, 15.17, 13.84; oleic – 1.20, 4.98, 5.09; linoleic – 3.20, 7.49, 12.91, linolenic – 4.58, 13.30, 4.04); aromatic acids (ferulic – 2.35, 3.69, 4.79; *p*-coumaric – 4.07, 2.24, 1.32; *p*-hydroxybenzoic – 4.19, 1.54, 3.20, respectively). In phenolic complexes from flowers and rhizomes vanillic acid is 4.84 % and 8.89 %.

The results obtained have shown the feasibility of further pharmacological studies of these substances.

Conflict of Interests: authors have no conflict of interests to declare.

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