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CHEMICAL COMPOSITION OF ESSENTIAL OILS FROM FLOWERS OF *VERONICA LONGIFOLIA* L., *VERONICA INCANA* L. AND *VERONICA SPICATA* L.

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In the Ukrainian flora, species of Veronica L. genus (Plantaginaceae Juss.) are classified into 8 sections. The phytochemical research into secondary metabolites of Veronica L. genus most related to the study of phenolic compounds and iridoids, while terponoids of these species need further research. The chemical profiles of V. longifolia L., V. incana L. and V. spicata L. of Ukrainian flora are poorly studied. Phenolic acids, hydroxycinnamic acids, coumarins, flavonoids, tannins, iridoids, saponins, amino acids and organic acids have been reported for these species. Herbs harvested during the flowering stage are often used in the pharmaceutical industry, so the research into chemical composition of essential oils from Veronica species flowers are urgent.

The aim of this study *was a comparative GC/MS study of the chemical composition of essential oils from V. longifolia L., V. incana L. and V. spicata L. flowers of Ukrainian flora.*

Materials and methods. *The objects of the research were flowers of Veronica spp. of Pseudolysimachium W.D.J. Koch section, namely V. longifolia L., V. incana L. and V. spicata L., harvested in the Botanical Garden of V. N. Karazin Kharkiv National University. The study of the chemical composition of essential oils was carried out by chromatography mass spectrometry on a 6890N MSD/DS Agilent Technologies chromatograph (USA) with a 5973N mass spectrometric detector. The components of essential oils were identified by comparison of the retention indices and mass spectra of phytochemicals in the studied essential oils with the data of NIST02 mass spectral library. The quantification of substances in the raw materials was carried out in comparison with a standard sample of menthol.*

Results*. As a result, 72 compounds were detected and quantified. The total content of essential oil in V. longifolia L. flowers was 0.17 % (39 components), the following compounds dominated: benzoacetaldehyde – 8.05, squalene – 5.17, palmitic acid – 15.73, butyl phthalate – 7.18. The total content of essential oil in V. incana L. flowers was 0.15 % (43 components), the following compounds prevailed: squalene 20.47, fatty acids, namely palmitic – 26.88, palmitoleic – 17.15, oleic – 11.61. The total content of the essential oil in V. spicata L. flowers was 0.11 % (43 components), the following compounds dominated: squalene – 5.53, fatty acids: palmitic – 22.78, linoleic – 6.72, carbohydrates: heptacosan – 12.27, hexacosan – 7.45. Among the identified compounds, mono-, norsesqui-, sesqui-, di- and triterpenoids, their oxidation products (aromatic compounds, aldehydes and alcohols, ketones), fatty acids, hydrocarbons and their derivatives were detected.*

Conclusions*. The chemical composition of essential oils from flowers of V. longifolia L., V. incana L. and V. spicata L. from Ukrainian flora was first studied by means of chromatography mass spectrometry. The yield of essential oil from V. longifolia L. flowers is higher (0.17 %) compared to those from flowers of V. incana L. (0.15 %) and V. spicata L. (0.11 %). Among the identified compounds terpenoids, aromatic compounds, their oxidation products, fatty acids and their esters, hydrocarbons were detected.*

The study of biologically active substances in essential oils from Veronica species flowers expands the scientific data on the chemical composition of these species and gives background for the further development of medicinal products, their standardization and understanding of their pharmacological activity

Keywords*: essential oil, flowers, GC-MS analysis, V. longifolia L., V. incana L., V. spicata L.*

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1. Introduction

Veronica (*Veronica* L.) is the largest genus of the flowering plants in *Plantaginaceae* Juss. family [1] numbering about 500 species [2, 3]. Previously, *Veronica* L. genus was classified to *Scrophulariaceae* Juss. family, as well as *Veronicaceae* Durande. Family [4, 5]. In recent research articles, considering molecular phylogenetic data, the genus is classified to *Plantaginaceae* Juss. family [6, 7].

Up to 70 Veronica species grow on the territory of Ukraine [8, 9]. *Veronica spp.* are widely cultivated as fodder and ornamental plants, have many varieties and hybrids, which differ mainly in size of inflorescences and colour of flowers [10, 11].

Worldwide in folk medicine, tincture and juice from Veronica spp. herb were used in the treatment of the upper respiratory tract diseases as expectorant, emollient, and antibacterial agents [12, 13]. Decoctions from Veronica spp. herb and rhizomes were used in the treatment of gastrointestinal tract disorders, genitourinary system disorders, and diabetes mellitus [14, 15]. The herbal tincture shows analgesic, sedative, hemostatic and cytotoxic properties cytotoxic properties [16]. Biologically active substances of flowering tops were used as traps for free radicals [17,18].

Species of *Veronica* L. genus are of scientific and practical interest as available sources of biologically active substances with a wide range of pharmacological activities, namely antimicrobial, antistaphylococcal, anti-inflammatory, antitumor, cytotoxic, antiradical and antioxidant [19–21].

The phytochemical research into secondary metabolites of *Veronica* L. genus is often related with taxonomy and chemotaxonomy studies. Iridoids and flavonoids are the best characterized phytochemicals of *Veronica* L. genus [22, 23]. The chemical profiles of *V. longifolia* L., *V. incana* L. and *V. spicata* L. of Ukrainian flora are poorly studied; phenolic acids, hydroxycinnamic acids, coumarins, flavonoids, tannins, iridoids, saponins, amino acids and organic acids were reported [24–26]. In microelements addition to iridoids, 22 other terpenoids have been detected in *Veronica* genus. Eight abietane-type diterpenoids have been isolated from *V. sibirica* and tested for anticancer activity. In addition, 1 bis-sesquiterpene, 12 steroidal saponins and 4 other terpenoids have been isolated from *Veronica* [27]. Based on literature data regarding to the phytochemical study of the genus *Veronica*, it could be resulted that the most part of the research are devoted to the study of phenolic compounds (flavonoids, hydroxycinnamic acids, etc.) and iridoids. Terpenoids composition of essential oils of *Veronica* species raw materials has hardly been studied, while the pharmaceutical industry uses flowering herb. The content of essential oil in flowers is usually significant and have influence on the pharmacological effect of the raw material, namely in terms of antimicrobial, anti-inflammatory and anti-tumor activity. Therefore, the study of the chemical composition of *Veronica* species essential oils is an urgent task of pharmacognostic and pharmaceutical science.

The aim of this study was a comparative GC/MS study of the chemical composition of essential oils from *V. longifolia* L., *V. incana* L. and *V. spicata* L. flowers of Ukrainian flora.

2. Planning (methodology) of the research

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

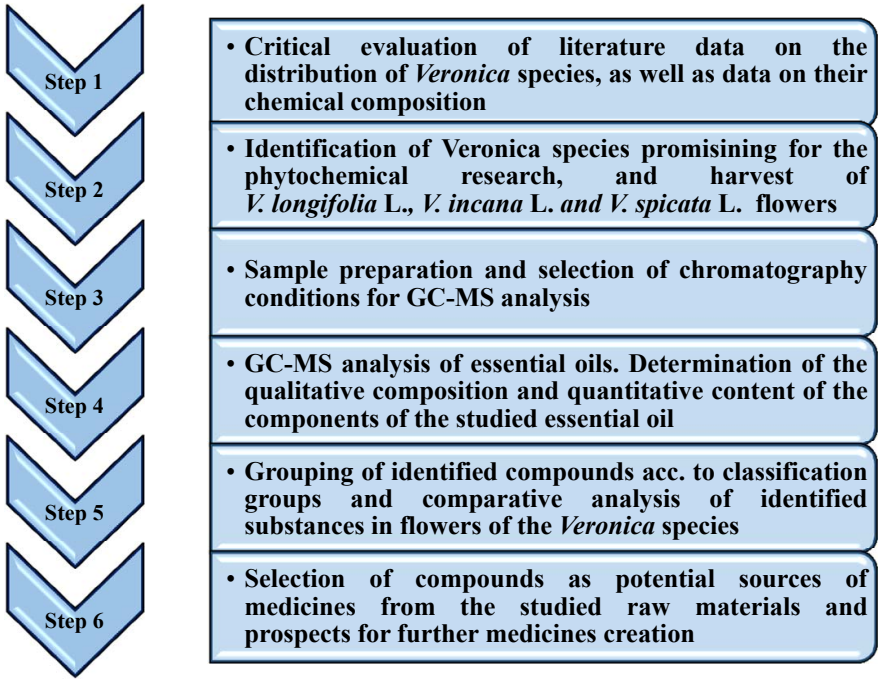

Fig. 1. Planning of the research

3. Materials and methods

Plant material.

The objects of the research were flowers of Veronica spp. of *Pseudolysimachium* W.D.J. Koch section, namely *V. longifolia L.*, *V. incana L.* and *V. spicata L.*, harvested in the Botanical Garden of V. N. Karazin Kharkiv National University (50°01'45.1"N, 36°13'50.3"E) in the flowering stage (mid-June) in 2019. Previously, we studied raw materials of *V. longifolia* "Blaubart" harvested in the flowering stage in Lyubotyn, Kharkiv region (49°56′54″ 35°55′46″) in June–July 2013, and in the Botanical Garden of V. N. Karazin Kharkiv National University in 2014. In contrast to *V. longifolia* (the species is 120–150 cm high, flowers blue-purple), *V. longifolia* "Blaubart" is 50 cm high, bearing 25 dark blue flowers on a dense inflorescence.

Analyses of the essential oil.

The study of the chemical composition of essential oils was carried out by chromatography mass spectrometry on a 6890N MSD/DS Agilent Technologies chromatograph (USA) with a 5973N mass spectrometric detector. The study of the chemical composition of essential oils was carried out according to the previously described technique used for the chemical characterization of many other essential oils [28, 29].

To obtain and study essential oils components from medicinal plant raw materials, Vinogradov's method was used, which enables to isolate volatile compounds using small amounts of raw materials, and fully extract their components for further qualitative and quantitative analysis, what is of great importance for preliminary chemical characterization of raw materials.

For the distillation of essential oils from raw materials, 22 mL Agilent vials (part number 5183-4536) with open lids, silicone sealant, equipped with a reflux condenser 50 cm long and 5–7 mm in diameter were used. 2.00 g of the raw materials (acc. weighed) were put in the vial, water was added to its' half level, screwed with the

lid with the reflux condenser, and placed in a small sand bath with variable temperature control. The degree of heating was calculated in advance, so that the vapours of boiling water did not evaporate from the reflux condenser. After boiling for one hour, the reflux condenser was removed. Its inner surface, which adsorbed the microquantities of essential oil, was twice washed with 1–2 ml of pentane. The rinses were collected in 22 mL vial. The obtained solutions of essential oils in pentane can be stored under ambient conditions before analysis. In laboratory conditions, 10–15 mg of sodium sulfate (for drying) were added to solutions, and solutions were evaporated to a volume of 50 μL in the flow of pure nitrogen, and chromatographed. This method enables complete extraction of essential oil components for further analysis, what is of great importance for preliminary chemical characterization of raw materials [30–32].

Chromatographic conditions: HP-5MS quartz capillary chromatographic column; column length: 30 m, inner diameter: 0.25 mm. The carrier gas: helium. The carrier gas velocity: 1 ml/min. Sample volume: 2 μL. The introduction of the sample with a flow separation of 1/50, the temperature of the thermostat: 50 °C with programming 4 °C/min to 220 °C, evaporator detector temperature 250 °C The sample was injected in a splitless mode. The suitability of the chromatographic system was determined according to the requirements of the SPhU [32].

The obtained spectra were considered both based on general regularities of fragmentation of molecules of organic compounds under the action of electron impact, and by comparing the obtained results with the data in NIST02 mass spectral database (more than 174,000 substances). For each chromatographic peak, the average mass spectrum was calculated from which the background spectrum was subtracted. The compounds were identified by comparing the obtained mass spectra of the chromatographic peak with the mass spectra of the reference compounds and based on comparison with the spectra of the database. Quantification of substances in the raw material was performed in comparison with a standard sample of menthol [30, 33]. According to literature data and preliminary analysis of the essential oils, menthol was not found in the studied essential oils. Therefore, it was used as internal standard for the preliminary calculation of the content of essential oil components. The choice of menthol as an internal standard due to the same class of determined substances, that allows to significantly reduce detection errors in the quantitative determination of substances.

Calculation of components content C (mg/kg) was carried out by the formula:

$$X=(P_1\times 50\times 1000)/(P_2\times m),$$

where P_1 – a peak area of tested compound; P_2 – a peak area of standard compound; 50 – mass of internal standard (μg), injected into the sample; m – sample mass (mg).

Statistical analysis.

Statistical properties of random variables with n-dimensional normal distribution are given by their correlation matrices, which can be calculated from the original matrices. Statistical assessment data are reported as mean ± SEM and were analyzed using STATISTICA 6 software. P values less than 0.05 were assumed to be statistically significant [34, 35].

4. Research results

The GC-MS technique used in the preliminary study enables to identify the main components of essential oils. The aim of the selection and definition of chromatographic conditions is to achieve a proper separation of the components of the oil, both for the qualitative analysis, as also for the proper quantification. To do so, well resolved peaks and not distorted ones, good relation signal-noise and horizontal base line with absence of drift, must be obtained for each one of the components. To accomplish this objective, a correct selection of the column is key. In general, for the development and selection of stationary phases, it must be considered, among other things, the thermal and chemical stability of the column, the selectivity in the separation of the components, the lining or coating surface, the diameter of the column, as well as the incorporation of more specific components to the stationary phase, or the use of different technologies to optimize the phase available to the specific regions of analyses that require better resolution. The variable that the analyst most frequently handles at the time of separation of the components of essential oils is perhaps the working temperature [36].

For the selection of chromatographic conditions some methods were analysed [28–30, 36–38]. The results of comparison are presented in Table 1.

The analyzed methods differ in the columns which were used for the analysis and the temperature interval of the thermostat. The used carrier gas in most methods was helium and its velocity is the same and amounts to 1 ml/min.

After analysing the given methods, the choice was made in favour to the method given in the first column, as experimental results have shown that it provides a good separation of essential oil components. HP-5ms is a medium polar column, widely used in laboratory analysis, a standard one with a standard sorbent. This makes it easy to reproduce the technique with its use in different laboratories. Previously, it was used for the analysis of many other essential oils [29, 30]. Split ratio 1/50 required to avoid column overloading. The analysis time of this technique is optimal and is 42.5 minutes. The technique used enables to compare components in essential oils, it separates them well, and enables to identify the main components, what is the basis for the further development of validated methods for the standardization of raw materials and essential oil. It meets the recommended requirements for essential oil analysis methods and can be used for routine analysis [39].

As a result, 72 compounds were detected and quantified, 4 of which were not identified. Among the identified compounds, mono-, norsesqui-, sesqui-, di- and triterpenoids, their oxidation products (aromatic compounds, aldehydes and alcohols, ketones), fatty acids, hydrocarbons and derivatives of compounds of these classes (Table 2, Fig. 2–4) were detected.

Table 1

The review of existing methods for essential oil analyses

Conditions	The methods				
	[29, 30]	[28]	[37]	[38]	[36]
Chromatograph	890N MSD/DS Agilent Technologies (USA) with a 5973N	Agilent Technologies 6890 with 5973 mass-spectrometric detector	GC 7890A with a flame ionization detector (FID)	Hewlett Packard GCD system	HIMADZU GC 14B
Chromatographic column	HP-5MS (30 m×0.25 mm, 0.50 μm)	DB-5 (30 m×0.25 mm, 0.50 μm)	HP-5MS (60 m×0.25 mm, 0.25 μm)	HP-Innowax FSC column (60 m×0.25mm, 0.25 μm)	Mega Bore DB-WAX P/N 125-7032 column (30 m×0.53 mm, 1 μm)
The carrier gas	helium	helium	helium	helium	nitrogen
The carrier gas velocity	1 ml/min	1.2 ml/min	1 ml/min	1 ml/min	–
Sample volume	2 μL	2 μL	–	–	–
Split ratio	1/50		1/100	1/50	–
The temperature of the thermostat	50 to 220 °C at a rate of 4 °C/min	50 to 320°C at a rate of 4 °C/min	60 to 240 °C at a rate of 4 °C/min	60 to 220 °C at a rate of 4 °C/min	60 to 200 °C at a rate of 5 °C/min
Evaporator detector temperature	250 °C	250 °C	250 °C	250 °C	220 °C

Table 2

Component composition of essential oils of *V. longifolia L.*, *V. incana L.* and *V. spicata L.* flowers

#	Compound/BAS group	RI	Content, mg/kg					
			V. longifolia L.		*V. incana L.*		*V. spicata L.*	
			mg/kg*	%**	mg/kg*	%**	mg/kg*	%**
1	2	3	4	5	6	7	8	9
Terpenoids								
1	*trans*-Linalooloxide	1160	27.97±0.51	2.60	0.17±0.01	0.01	–	–
2	*cis*-Linalooloxide	1068	10.69±0.23	1.00	0.23±0.01	0.02	–	–
3	Linalool	1093	24.98±0.36	2.33	0.51±0.02	0.03	–	–
4	*p*-Ment-8-en-1-ol	1131	33.23±0.65	3.09	0.30±0.01	0.02	–	–
5	Carvone	1220	–	–	–	–	7.9±0.28	0.73
6	Piperitone	1232	–	–	–	–	1.30±0.05	0.12
7	Safranal	1167	5.86±0.17	0.55	2.27±0.10	0.15	–	–
8	Terpinyl acetate	1351	–	–	–	–	1.51±0.05	0.14
9	Geraniol	1243	–	–	0.33±0.01	0.02	–	–
10	Dihydroedulane	1286	–	–	1.40±0.04	0.09	–	–
11	Eugenol	1341	21.21±0.44	1.97	0.96±0.02	0.06	–	–
12	β-Damascenon	1360	9.99±0.27	0.93	0.84±0.02	0.06	1.89±0.07	0.18
13	*trans*-Caryophyllene	1420	–	–	–	–	1.41±0.05	0.13
14	Geranil acetate	1751	3.91±0.12	0.36	1.41±0.05	0.09	8.59±0.31	0.8
15	Ionon-5,6-epoxide	1977	5.48±0.20	0.51	0.96±0.03	0.06	–	–
16	β-Ionone	1474	7.58±0.29	0.71	1.48±0.05	0.1	–	–
17	*trans*-β-Ionon	1927	–	–	–	–	6.21±0.19	0.58
18	*cis*-β-Ionon	1661	–	–	–	–	8.89±0.22	0.83
19	Dihydroactinidiolide	2331	–	–	–	–	2.98±0.13	0.28
20	Myristicin	1494	–	–	–	–	10.8±0.44	0.99
21	Leden oxide	1890	–	–	–	–	2.92±0.10	0.27
22	Caryophylene oxide	1560	–	–	–	–	3.09±0.11	0.29
23	Dihydroisocalamenediol	1745	–	–	–	–	24.15±1.04	2.24
24	*trans*-Methyldihydroasmonate	1657	–	–	–	–	14.45±0.53	1.34
25	Apiole	1682	–	–	–	–	8.56±0.38	0.8
26	1,4-*cis*-1,7-trans-acorenone	1694	–	–	–	–	42.01±1.65	3.9
27	Neophytadiene	1908	–	–	–	–	29.54±1.07	2.74
28	Phytol	2122	28.57±1.15	2.66	12.27±0.49	0.81	–	–
29	Squalene	2829	55.56±1.67	5.17	309.73±8.67	20.47	59.50±1.91	5.53
Sum:			235.03	21.88	332.86	21.99	235.69	21.89

Continuation ot Table 2

1	2	3	4	5	6	7	8	9
Aromatic compounds								
30	Benzacetaldehyde	1016	133.08±5.21	8.05	0.68±0.02	0.04	–	–
31	Benzofuranone	1480	15.74±0.57	0.95	0.25±0.01	0.02	–	–
32	4-Vinyl-2-methoxyphenol	1250	34.62±1.19	2.09	1.53±0.06	0.1	–	–
33	2,5,8-Trimethyl-1,2-dihydronaphthalene	1990	3.98±0.17	0.24	0.77±0.02	0.05	–	–
34	Vinyl cyclohexacarboxylate	823	–	–	–	–	6.8±0.28	0.63
35	2,4-Di-tert-butylphenol	1539	–	–	0.92±0.03	0.07	–	–
36	Butyl phthalate	1970	118.68±4.67	7.18	–	–	–	–
37	1-Allyl-2,3,4,5-tetra methoxybenzene	1531	–	–	–	–	7.51±0.31	0.7
38	Benzophenone		14.62±0.48	0.88	4.25±0.17	0.28	18.51±0.72	1.72
39	2-(Phenylmethylene) octanal	1728	–	–	–	–	13.47±0.54	1.25
40	(1-Methylundecyl) benzene	1692	–	–	–	–	4.41±0.16	0.41
41	Hexyl benzoate	1550	–	–	–	–	2.48±0.15	0.23
Sum:			320.72	29.86	8.4	0.56	53.18	4.94
Alcohols, aldehydes and ketones								
43	Nonanal	1805	–	–	0.99±0.03	0.07	2.28±0.08	0.22
44	Decanal	1183	14.93±0.58	0.9	1.41±0.05	0.09	3.56±0.12	0.33
45	Dodecanal	1386	–	–	–	–	1.96±0.07	0.18
46	Octadec-3,15-diene-1-ol	2075	–	–	–	–	3.92±0.15	0.36
47	4- (2,6,6-Trimethylcyclohexa-1,3-dienyl) -butan-2-one	1460	17.05±0.76	1.03	–	–	–	–
48	7,9-Di-tert-butyl-1-oxaspiro- [4,5] -deca-6,9-diene-2,8-dione	1916	–	–	–	–	7.78±0.29	0.72
Sum:			31.98	2.98	2.4	0.16	19.5	1.81
Carboxylic acids								
49	Caprylic acid	1154	50.15±2.32	4.67	0.78±0.02	0.05	–	–
50	1- (2-Hydroxy-1-methylethyl) -2,2-dimethylpropyl-2-methylpropionate	1389	–	-	0.55±0.02	0.04	–	–
51	3-Hydroxy-2,4,4-trimethylpentyl 2-methylpropionate	1331	–	–	0.34±0.01	0.02	–	–
52	Capric acid	1345	8.55±0.35	0.80	2.35±0.17	0.16	–	–
53	Lauric acid	1344	8.32±0.32	0.77	13.32±0.51	0.88	–	–
54	Tridecanoic acid	1668	–	–	7.48±0.35	0.49	–	–
55	Myristic acid	1748	18.17±0.81	1.69	84.45±3.42	5.58	22.26±0.92	2.07
56	Isopropyl myristate	1836	–	–	–	–	11.89±0.49	1.1
57	Pentadecanoic acid	1840	26.32±1.03	2.45	62.26±2.76	4.11	–	–
58	Methylpentadecanate	1598	–	–	–	–	26.49±1.24	2.46
59	Palmitoleic acid	2223	18.88±0.91	1.76	259.67±10.53	17.15	13.12±0.51	1.22
60	Palmitic acid	2204	168.99±7.72	15.73	406.85±19.34	26.88	245.25±10.78	22.78
61	Ethyl palmitate	1979	–	–	–	–	9.61±0.46	0.89
62	Heptadecanoic acid	2042	20.38±0.90	1.90	7.86±0.23	0.52	–	–
63	Linolenic acid	2443	10.02±0.47	0.93	32.54±1.34	2.15	6.05±0.27	0.56
64	Linoleic acid	2490	18.06±0.79	1.68	56.84±2.67	3.75	72.3±3.05	6.72
65	Oleic acid	2040	21.69±0.92	2.02	175.71±6.78	11.61	5.12±0.22	0.48
66	Stearic acid	2188	17.72±0.75	1.65	30.28±1.31	2	3.74±0.15	0.35
Sum:			387.25	36.05	1141.28	75.39	415.83	38.63
Higher hydrocarbons								
67	Tricosan	369	6.49±0.25	0.4	3.04±0.12	0.2	9.77±0.41	0.91
68	Tetracosan	402	–	–	–	–	2.05±0.07	0.19
69	Pentacosane	396	13.92±0.62	0.84	3.15±0.12	0.21	3.60±0.12	0.33
70	Hexacosan	415	23.78±1.06	1.44	4.84±0.18	0.32	80.08±3.65	7.45
71	Heptacosan	426	26.01±1.26	1.57	9.44±0.42	0.62	132.09±5.11	12.27
72	Nonakozan	454	20.02±0.92	1.21	8.19±0.38	0.54	111.57±5.01	10.36
Sum:			90.22	8.40	28.66	1.89	339.16	31.51
Sum of unidentified compounds:			8.94	0.83	0.18	0.01	13.13	1.22
Total:			1074.14	100	1513.78	100	1076.49	100

*Note: *– mg per 1 kg of raw material; ** – from the sum of the identified compounds; «–» – Compound not found*

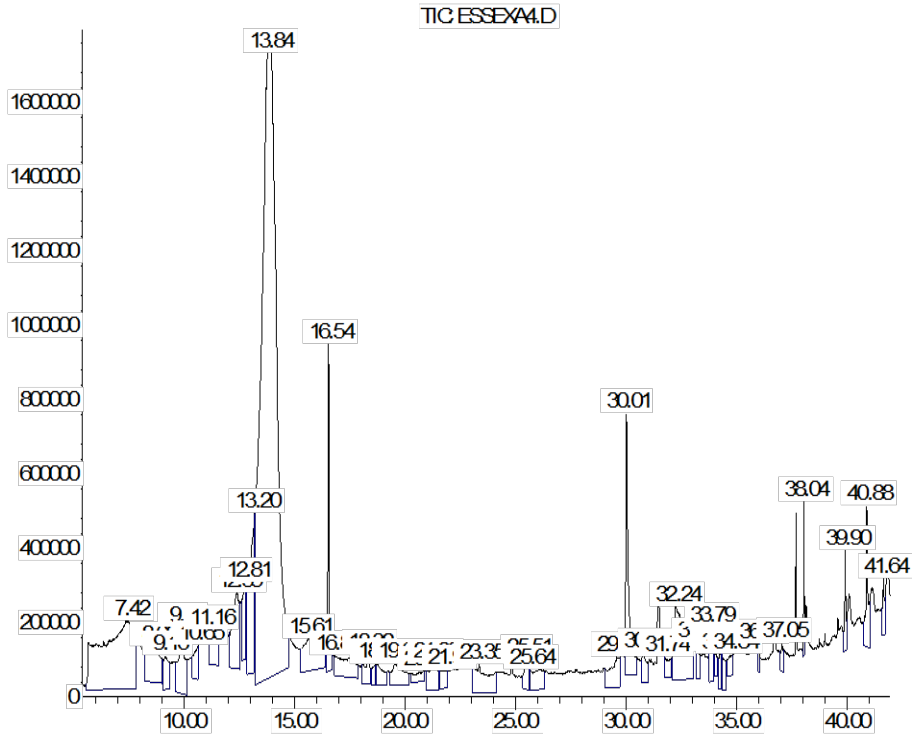

Fig. 2. Chromatographic profile of essential oil components from *V. longifolia L.* flowers

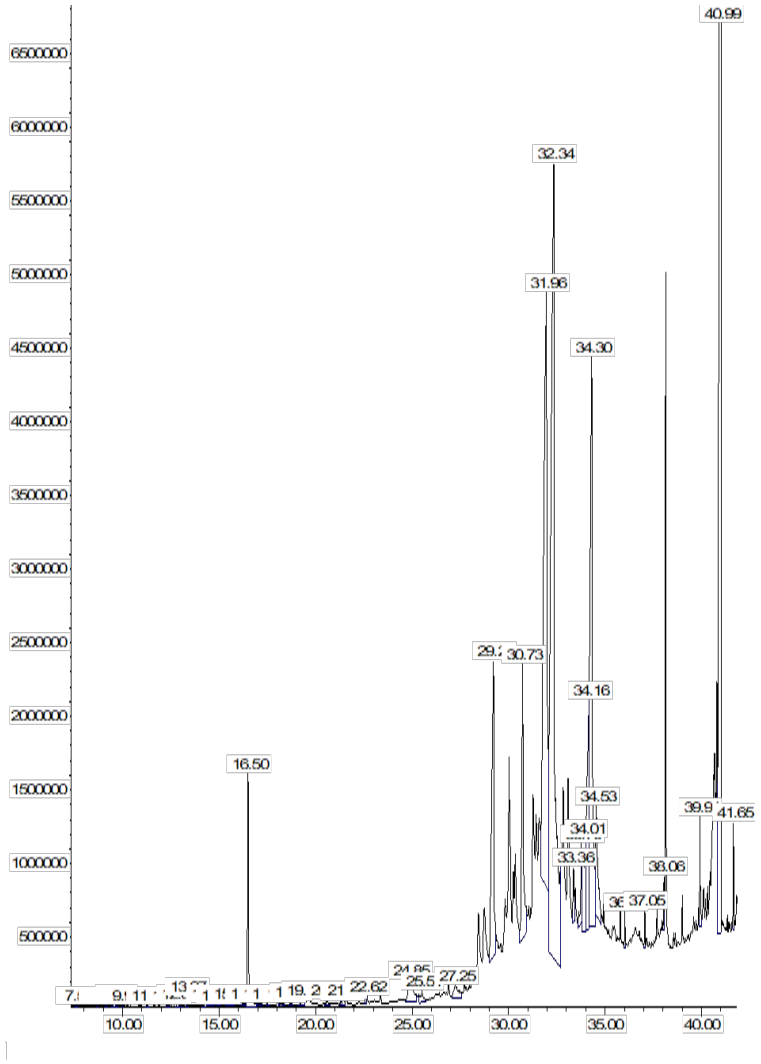

Fig. 3. Chromatographic profile of essential oil components from *V. incana L.* flowers

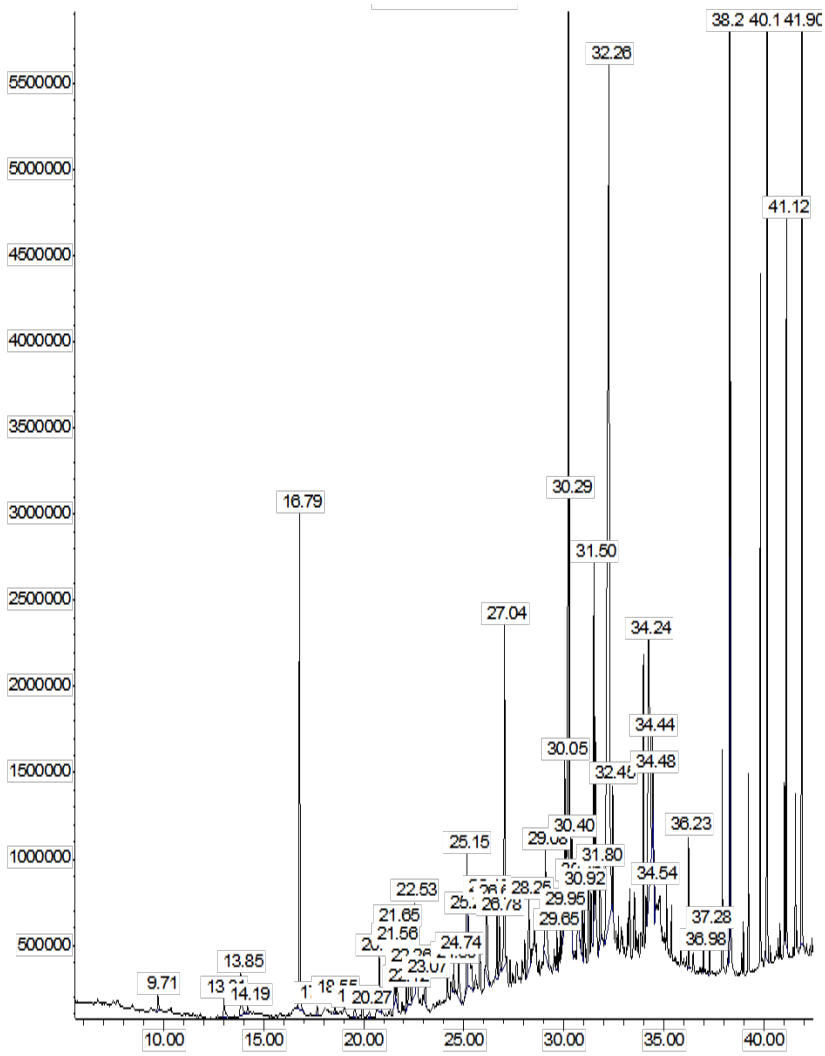

Fig. 4. Chromatographic profile of essential oil components from *V. spicata L.* flowers

5. Discussion of the results

The total content of the essential oil in *V. longifolia* L. flowers was 0.17 %, previously in "Blaubart" variety, the yield of essential oil was reported as 0.82 %; in the flowers of *V. incana* L. and *V. spicata* L. the content of the essential oil was 0.15 %, and 0.11 %, respectively. As a result of the research, in the essential oil from *V. longifolia* L. flowers 39 constituents were quantified (in the variety "Blaubart" 38), 38 were identified; 12 of which were terpenoids [31, 40].

The phytochemicals of the essential oil from *V. longifolia* L. flowers were represented by acyclic monoterpenoids: linalool, its derivatives trans-linalooloxide and cis-linalooloxide, geranyl acetone; monocyclic monoterpenoid p-ment-1-ene-8-ol; norterpenoids β-ionone and its oxidized form ionone-5,6-epoxide, β-damascenone; monocyclic aromatic terpenoid eugenol; acyclic diterpenoid alcohol phytol and triterpenoids squalene, safranal; aromatic compounds, aldehydes and alcohols, ketones. The stearoptene fraction was represented by aliphatic hydrocarbons and higher fatty acids. The content of terpenoids in the sum of the detected components in the essential oil from *V. longifolia* L. flowers was 21.88 %, aromatic compounds constituted 29.86 %, aldehydes, ketones and alcohols accounted for 2.98 %, fatty acids and their esters accounted for 36.05 %, hydrocarbons – 8.40 %, unidentified compounds constituted 0.83 %.

In the essential oil from *V. longifolia* L. flower, the following compounds dominated (constituted more than 5 %), %: benzoacetaldehyde – 8.05, squalene [41] – 5.17, palmitic acid – 15.73), butyl phthalate – 7.18.

In the essential oil from *V. incana* L. flowers 43 constituents were detected and quantified, 42 were identified; 14 of which belong to terpenoids. The content (%) of terpenoids in the sum of the detected compounds was 21.99, aromatic compounds constituted 0.56, aldehydes and alcohols accounted for 0.16, fatty acids and their esters constituted 75.39, hydrocarbons accounted for 1.89, unidentified compounds constituted 0.01. The chemical composition of the essential oil from *V. incana* L. flowers is like that from *V. longifolia* L. flowers. The characteristic components of *V. incana* L. essential oil distinguishing it from both *V. longifolia* L. and *V. spicata* L. are geraniol, dihydroedulane, 2,4-ditert-butylphenol, 1-(2-hydroxy-1-methylethyl)-2,2-dimethylpropyl-2-methylpropionate and 3-hydroxy-2,4,4-trimethylpentyl 2-methylpropionate. In the flowers of *V. incana* L. the following compounds dominated (%): squalene 20.47, fatty acids [42], namely palmitic – 26.88, palmitoleic – 17.15, oleic – 11.61.

In the essential oil from *V. spicata* L. flowers, 47 constituents were detected and quantified, 45 were identified; 18 of which were classified as terpenoids. The content (%) of terpenoids in the sum of the detected components was 21.89, aromatic compounds constituted 4.94, aldehydes and alcohols accounted for 1.09, ketones constituted 0.72, fatty acids and their esters accounted for 38.63, hydrocarbons constituted 31.51, unidentified compounds accounted for 1.22. The following compounds dominated (%): squalene – 5.53, fatty acids: palmitic [43] – 22.78, linoleic – 6.72, carbohydrates: heptacosan – 12.27, hexacosan – 7.45.

At the same time, it should be noted that the used column (HP-5ms) is not a specialized column for the analysis of chiral compounds. In the practice of pharmaceutical analysis, specialized columns are used for this purpose (for example, columns of the HP-Chiral β Columns series or similar containing specialized sorbents that are sharpened specifically for the analysis of enantiomers [44]. However, in this case, the efficiency of the chromatographic system (choice of the column, correctly selected chromatographic conditions, sensitivity of the detector) allows not only to effectively separate the various components of the mixture, but also to separate some enantiomers (for example, trans-linalooloxide and cis-linalooloxide, trans-β-Ionone, cis-β-Ionone, etc.). Which testifies to the ineffectively increasing quality of modern universal sorbents for gas chromatography, because of which using the standard for the laboratory pharm analysis of the column makes it possible to solve separations that are unique in their selectivity.

It is noteworthy that the content of squalene [45, 46] in the essential oil from *V. incana* L. flowers was 5.57 and 5.20 times higher than in *V. longifolia* L. and *V. spicata* L., respectively. The chemical composition of the essential oil from *V. spicata* L. flowers significantly differs from those from *V. incana* L. and *V. longifolia* L. flowers. In the essential oil from *V. spicata* L. flowers, carvone [47], piperitone, terpenyl acetate, *trans*-caryophyllene, *trans*-β-ionone, *cis*--ionone, dihydroactinidiolide, myristicin, caryophylene oxide, dihydroisocalamenediol, *trans*-methylsidine, 1,4-methyldihydro,7-*trans*-acorenone, neophytadiene, vinylcyclohexacarboxylate, 1-allyl-2,3,4,5-tetramethoxybenzene, 2-(phenylmethylene) octanal, (1-methylundecyl) benzene, hexylbenzoate, dodecanal, octadec-3,15-act-1-ol, 9-di-tert-butyl-1-oxaspiro-[4,5]-deca-6,9-diene-2,8-dione, isopropyl myristate, methylpentadecanate, ethyl palmitate and tetracosan were detected, and these compounds were not detected in the essential oils from *V. incana* L. and *V. longifolia* L. flowers.

Common components of essential oils of all the studied species are geranyl acetone and β-damaskenone, benzophenone, decanal, fatty acids, namely myristic, palmitoleic, palmitic, linolenic, linoleic, oleic, and stearic; tricosan, pentacosan, hexacosan, heptacosan and nonacosan.

Terpenoids, aldehydes and ketones, as well as phenolic compounds present in the essential oil are of particular importance for understanding the pharmacological activity of the essential oil. Linalool [48], geraniol [49] and damascenon [50] and their derivatives show a sedative effect [51, 52]. The triterpenoid squalene shows anticancer, antioxidant, hypoglycemic and immunomodulatory properties [45, 46]. The raw materials contain lipophilic substances with pronounced antimicrobial activity – terpenoids, aromatic compounds, carboxylic acids, what gives background for the development of technologies for obtaining biologically active complexes.

Study limitations. During the GC-MS study, several compounds were not identified due to the absence of their characteristics in NIST05 mass spectra libraries, as well as in AMDIS and NIST programs.

The prospects for the further research. In future, it is reasonable to study a dependence of essential oils composition on plant development, season, and growth conditions. Also, since studied herbal materials contain phytochemicals with different activities, it is reasonable to obtain corresponding substances and study their potential activities.

6. Conclusions

The chemical composition of the essential oils from flowers of *V. longifolia* L., *V. incana* L. and *V. spicata* L. from Ukrainian flora was first studied by means of chromatography mass spectrometry. The yield of essential oil from the *V. longifolia* L. flowers is higher (0.17 %) compared to those from flowers of *V. incana* L. (0.15 %) and *V. spicata* L. (0.11 %).

In the essential oil from *V. longifolia* L. flowers, 39 compounds were detected and quantified; in the essential oil from *V. incana* L. flowers – 43 compounds; 47 compounds were detected and quantified in the essential oil from *V. spicata* L. flowers. Among the identified compounds terpenoids, aromatic compounds, their oxidation products, fatty acids and their esters, hydrocarbons were detected.

29 Terpenoids and their derivatives were identified. The content of terpenoids in the sum of the detected compounds was: 21.88 % in the flowers of *V. longifolia* L., 21.99 % in the flowers of *V. incana* L., 21.89 % in the flowers of *V. spicata* L.

The study of biologically active substances in essential oils from Veronica species flowers expands the scientific data on the chemical composition of these species and gives background for the further development of medicinal products, their standardization and understanding of their pharmacological activity.

Conflicts of interest

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 paper.

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