**UDC 615.322: 582.916.21**
**DOI: 10.15587/1729-4061.2022.253541**

# CARBOXYLIC ACIDS IN THE FLOWERS OF *VERONICA SPICATA* L. AND *VERONICA INCANA* L.

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*In the Ukrainian flora, species of Veronica L. genus (Plantaginaceae Juss.) are classified into 8 sections. Among the representatives of Pseudolysimachion W. D. J. Koch section in the Kharkiv region, Veronica spicata L. (spike speedwell) and Veronica incana L. (Veronica spicata L. subsp. incana (L.) Walters, silver speedwell) are common. Plants are used for the treatment of upper respiratory tract diseases, malignant neoplasms, gastrointestinal tract and genitourinary system disorders, diabetes mellitus.*

***The aim*** *of the research was to study the carboxylic acids of flowers of Veronica spicata L. and Veronica incana L.*

***Materials and methods.*** *The objects of the research were flowers of Veronica spicata L. and Veronica incana L., collected in the flowering stage in the Botanical Garden of Karazin University (Kharkiv, Ukraine) in summer 2018. The study of carboxylic acid composition was performed by chromatography-mass spectrometry on a 6890N MSD/DS Agilent Technologies chromatograph with a 5973N mass spectrometric detector. Identification of methyl esters of acids was performed using data from the mass spectrum libraries NIST 05 and Willey 2007 in a combination with programs for the identification of AMDIS and NIST; also, their retention time and the retention times of standard compounds were compared.*

***Results.*** *In Veronica incana L. flowers, 37 carboxylic acids were identified and quantified, constituting 1.05 %. In Veronica spicata L. flowers, 32 carboxylic acids were identified and quantified, the total content of which was 2.75 %.*

***Conclusions.*** *A higher carboxylic acid content was established in the flowers of Veronica spicata L. The fatty acid composition of Veronica incana L. flowers is characterized by a comparable content of saturated and unsaturated acids, while in Veronica spicata L. flowers, unsaturated fatty acids prevail over saturated fatty acids. The content of aromatic acids in the flowers of studied species was comparable. The characteristic carboxylic acids in the flowers of Veronica incana L. are oxalic, 3-hydroxy-2-methylglutaric, pentadecanoic, heneicosanoic, tricosanic,4-hydroxybenzoic, 4-methoxybenzoic and 3,4-dimethoxybenzoic acids; in the flowers of Veronica spicata L. – 2-hydroxy-3-methylglutaric, α-furanic and homovanillic acids*

***Keywords:*** *carboxylic acids, GC-MS analysis, Veronica spicata L., Veronica incana L.*

**How to cite:**
Kovaleva, A., Raal, A., Ilina, T., Osmachko, A., Goryacha, O., Omelyanchik, L., Koshovyi, O. (2022). Carboxylic acids in the flowers of *Veronica spicata* l. and *Veronica incana* L.. ScienceRise: Pharmaceutical Science, 1 (35), 37–43. doi: http://doi.org/10.15587/1729-4061.2022.253541



## 1. Introduction

The genus *Veronica* L. (Plantaginaceae Juss.) [1] is represented by approximately 70 species in the Ukrainian flora [2]; according to other sources, the corresponding figure is 48 species classified into 8 sections [3, 4]. Among the species of *Pseudolysimachion* W. D. J. Koch section in the Kharkiv region, *Veronica spicata* L. (spike speedwell) and *Veronica incana* L. (by the International Plant Name Index *Veronica spicata* L. subsp. *incana* (L.) Walters, silver speedwell) are common [5].

The chemical profiles of most wild *Veronica* species of the Ukrainian flora are poorly characterized. It is known that the species of *Pseudolysimachion* W. D. J. Koch section accumulate cornoside, glycosides and derivatives of 6-hydroxyflavone acylated by phenolic acids [6, 7]; iridoids [8, 9]. In species of *Pseudolysimachion* W. D. J. Koch section, iridoid glucosides were reported [10, 11]. The major compounds in the essential oil extracted from the aerial parts of *Veronica spicata* were monoterpenoids and sesquiterpenoids. Phenolic compounds are represented by aromatic acids and flavonoids [12, 13]. In *V. incana* herb, flavonoids were reported [14, 15].

The species of *Pseudolysimachion* W. D. J. Koch section have been important in ethnomedicine as remedies for the treatment of upper respiratory tract diseases and malignant neoplasms; as well as gastrointestinal tract and genitourinary system disorders, diabetes mellitus, various dermatological diseases; they show analgesic, sedative, hemostatic and wound healing effects [6, 16]. *Veronica spicata* is used as an expectorant [17]. In experimental studies, antitumor [6, 11] antioxidant and antimicrobial [12, 18] and anti-inflammatory [19] activities of *Veronica* spp. were shown [20, 21].

Fatty acids [22, 23] and phenolic acids [24] are considered as potential chemotaxonomic markers [25]. Previously, we have established the carboxylic acid composition of the flowers and leaves of *V. longifolia* L., another representative of *Pseudolysimachion* W. D. J. Koch section [26]. As a continuation of our research into chemical composition of *Pseudolysimachion* W. D. J. Koch section species, the aim of the present research was to study the composition of carboxylic acids of the flowers of *Veronica spicata* L. and *Veronica incana* L. in order to establish characteristic carboxylic acids.

**2. Planning (methodology) of the research**

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

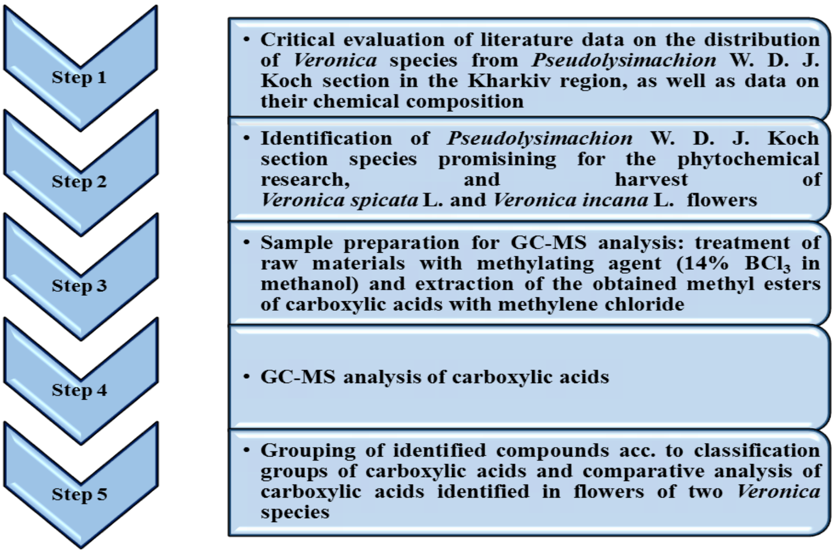


Fig. 1. Planning of the research

**3. Materials and Methods**

*Plant material*

The flowers of *Veronica spicata* L. and *Veronica incana* L. (about 50 grams of each) were collected in the flowering stage in the Botanical Garden of Karazin University (Kharkiv, Ukraine) in summer 2018 (North latitude 50°01′46″; East longitude 36°14′02″). Voucher specimens Nos. 26/06/2018 and 29/06/2018 are deposited at the Pharmacognosy Department (National University of Pharmacy, Kharkiv, Ukraine). The identity of *Veronica* L. species was established with the consulting assistance of Yurij G. Gamulya, PhD in Biology (Ecology), Associate Professor, Herbarium Curator of V. N. Karazin Kharkiv National University, and Tetiana M. Gontova, D.Sc. (Pharmacy), Professor of Pharmacognosy Department of the National University of Pharmacy [27]. Herbal materials were dried in the shade and at room temperature (25 °C) for 3 days and stored in paper bags protected from light.

*Isolation of carboxylic acids*

To 50 mg of the air-dried raw materials in a 2 ml vial, an internal standard (50 mg of tridecane in hexane) was added, as well as 1.0 mL of a methylation agent ($BCl_3$ in methanol, 14 % solution, Supelco 3-3033) [26]. The mixture was heated in a sealed vial for 8 h at 65 ºC in order to perform the extraction and hydrolysis of fats and other esters as well as simultaneous methylation of fatty acids and other organic acids. The reaction mixture was decanted, and the precipitate was diluted in 1 mL of distilled water; methyl esters of carboxylic acids were extracted with 0.2 mL methylene chloride. The mixture was gently shaken several times within an hour and then the obtained extract of the methyl esters was chromatographed [28, 29].

*Identification and comparative content determination of carboxylic acids*

The gas chromatography-mass spectrometry (GC-MS) analysis of carboxylic acids was performed on Agilent Technologies 5973N/6890N MSD/DS GC-MS (USA) according to the previously described procedure [28, 30]. The system was equipped with INNOWAX capillary column (30 m × 0.25 mm, i.d. of 0.25 µm). The column oven temperature was gradually increased from 50˚C to 250˚C at 4˚C/min. Helium was used as the carrier gas at 1.2 mL/min, and the sample (2 µL) was injected in the splitless ratio; the injector temperature was 250 ˚C. The MS conditions were as follows: the ionization voltage 70 eV; the acquisitions scan mass range of 40–500 amu; the ion source temperature 230 ˚C at a sampling rate of 1.0 scan/s. Identification of methyl esters of acids was performed based on the calculation of the equivalent length of the aliphatic chain (ECL) by 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) [31, 32].

*Statistical analysis*

Statistical processing of the results was performed using the Student's t-test. Statistical properties of random variables with n-dimensional normal distribution are given by their correlation matrices, which can be calculated from the original matrices. The reliability of the compared values was estimated using the Student, Wilcoxon, Mann-Whitney criteria with the probability level of ≤ 0.05 on a computer using Statistica 6.0 and Word Excel programs [33, 34]. The results were expressed as mean±standard deviation (SD).

**4. Research results.**

As the result of the present study, 37 carboxylic acids were identified and quantified in *V. incana* L. flowers, and 32 carboxylic acids were identified and quantified in *V. spicata* L. flowers (Fig. 2, 3, Table 1).

Table 1

Comparative content of Carboxylic Acids in *Veronica incana* L. and *Veronica spicata* L. flowers

| No | Carboxylic acid | RI | *Veronica* species | |
|---|---|---|---|---|
| | | | *V. incana* | *V. spicata* |
| | | | Content, mg/kg* | |
| 1 | 3-Hydroxy-2-methylglutaric acid (3-hydroxy-2-methylpentanedioic) | 1064 | 137.24±3.57 | - |
| 2 | α-Furanoic (furan-2-carboxylic, pyromucic) | 1086 | – | 36.22±0.98 |
| 3 | Caproic (hexanoic) | 1120 | 5.63±0.14 | 5.65±0.16 |
| 4 | Veratric (3,4-dimethoxybenzoic) | 1172 | 265.56±7.97 | - |
| 5 | Oxalic (ethanedioic) | 1359 | 85.57±2.24 | - |
| 6 | p-Hydroxybenzoic (4-hydroxybenzoic) | 1451 | 486.57±14.35 | - |
| 7 | Malonic (propanedioic) | 1477 | 265.73±7.86 | 920.54±27.61 |
| 8 | Levulinic (4-oxopentanoic) | 1501 | 449.85±13.18 | 7700.14±244.86 |
| 9 | Fumaric (trans-butenedioic) | 1516 | 25.69±0.69 | 21.56±0.58 |
| 10 | Succinic (butanedioic) | 1575 | 345.10±10.35 | 594.19±17.82 |
| 11 | Benzoic (benzoic) | 1600 | 566.22±17.27 | 498.46±15.05 |
| 12 | Homovanillic (2-(4-hydroxy-3-methoxyphenyl)acetic) | 1657 | – | 22.79±0.62 |
| 13 | Phenylacetic (α-toluic) | 1746 | 14.59±0.37 | 7.99±0.20 |
| 14 | Salicylic (2-hydroxybenzoic) | 1757 | 20.05±0.50 | 3.7±0.09 |
| 15 | Lauric (dodecanoic) | 1793 | 19.68±0.48 | 11.11±0.27 |
| 16 | 3-Hydroxy-2-methylglutaric (3-hydroxy-2- methylpentanedioic) | 1917 | – | 16.12±0.40 |
| 17 | Myristic (tetradecanoic) | 1994 | 121.02±3.63 | 46.39±1.25 |
| 18 | Malic (2-hydroxybutanedioic) | 2008 | 536.27±16.08 | 2879.71±89.36 |
| 19 | Pentadecanoic (pentadecilic) | 2101 | 29.69±0.74 | - |
| 20 | Azelaic (nonanedioic) | 2114 | 68.15±1.92 | 141.05±3.92 |
| 21 | Palmitic (hexadecanoic) | 2204 | 1137.67±35.95 | 2682.47±73.49 |
| 22 | Palmitoleic (cis-9-hexadecanoic) | 2223 | 69.82±2.04 | 109.52±3.29 |
| 23 | Margarinic (heptadecanoic) | 2292 | 34.54±0.96 | 57.42±1.60 |
| 24 | Citric (2-hydroxy-1,2,3-propanetricarboxylic) | 2367 | 592.59±17.36 | 4747.51±145.27 |
| 25 | Stearic (octadecanoic) | 2384 | 279.15±7.82 | 157.43±4.57 |
| 26 | Oleic (cis-9-octadecenoic) | 2402 | 372.64±10.43 | 390.22±11.36 |
| 27 | Linoleic (9,12-octadecadienoic) | 2443 | 1163.90±36.31 | 2304.41±71.67 |
| 28 | Linolenic (cis,cis,cis-6,9,12-octadecatrienoic) | 2490 | 901.67±28.87 | 1452.55±43.43 |
| 29 | Vanillic (4-hydroxy-3-methoxybenzoic) | 2522 | 649.78±18.45 | 1868.65 ±55.69 |
| 30 | 2-Hydroxypalmitic (2-hydroxyhexadecanoic) | 2542 | 133.24±3.67 | 63.32±1.77 |
| 31 | Arachidic (eicosanoic) | 2543 | 121.05±3.53 | 78.19±2.17 |
| 32 | Heneicosanoic | 2597 | 15.59±0.42 | - |
| 33 | Begenic (docosanoic) | 2698 | 249.78±7.27 | 175.36±5.20 |
| 34 | Tricosanoic | 2743 | 28.31±0.76 | - |
| 35 | p-Methoxybenzoic (4-methoxybenzoic) | 2780 | 94.86±2.68 | - |
| 36 | Syringic (4-hydroxy-3,5-dimethoxybenzoic) | 2793 | 103.63±2.90 | 13.24±0.36 |
| 37 | p-Coumaric (3-(4-hydroxyphenyl)-propenoic) | 2801 | 195.11±5.85 | 11.24±0.30 |
| 38 | Gentisic (2,5-dihydroxybenzoic) | 2805 | 57.25±1.57 | 25.21±0.69 |
| 39 | Lignoceric (tetracosanoic) | 2843 | 380.36±11.30 | 208.65±6.18 |
| 40 | Ferulic (3-methoxy-4-hydroxycinnamic) | 2919 | 441.13±13.23 | 274.11±7.98 |
| Total | | | 10464.68±339.05 | 27525.12±872.55 |

*Note: * mg/kg in the air-dried herbal materials, «–» the compound was not identified*

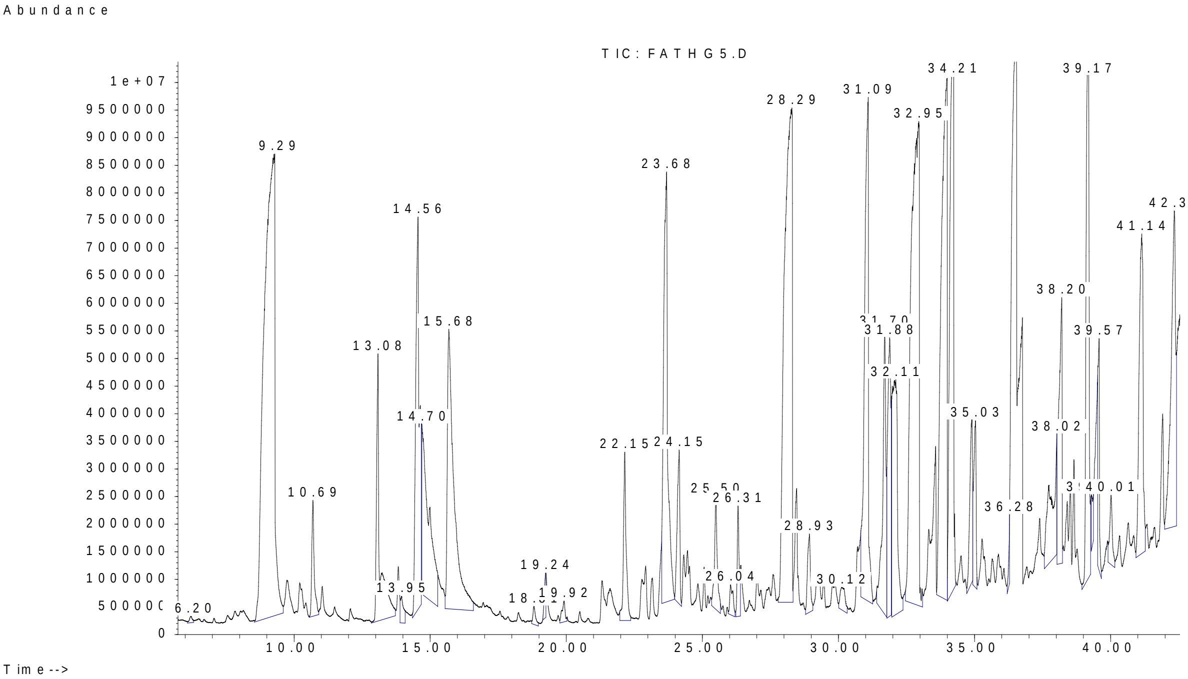


Fig. 2. The typical GC-MS chromatogram of methyl esters of carboxylic acids of *Veronica incana* L. flowers

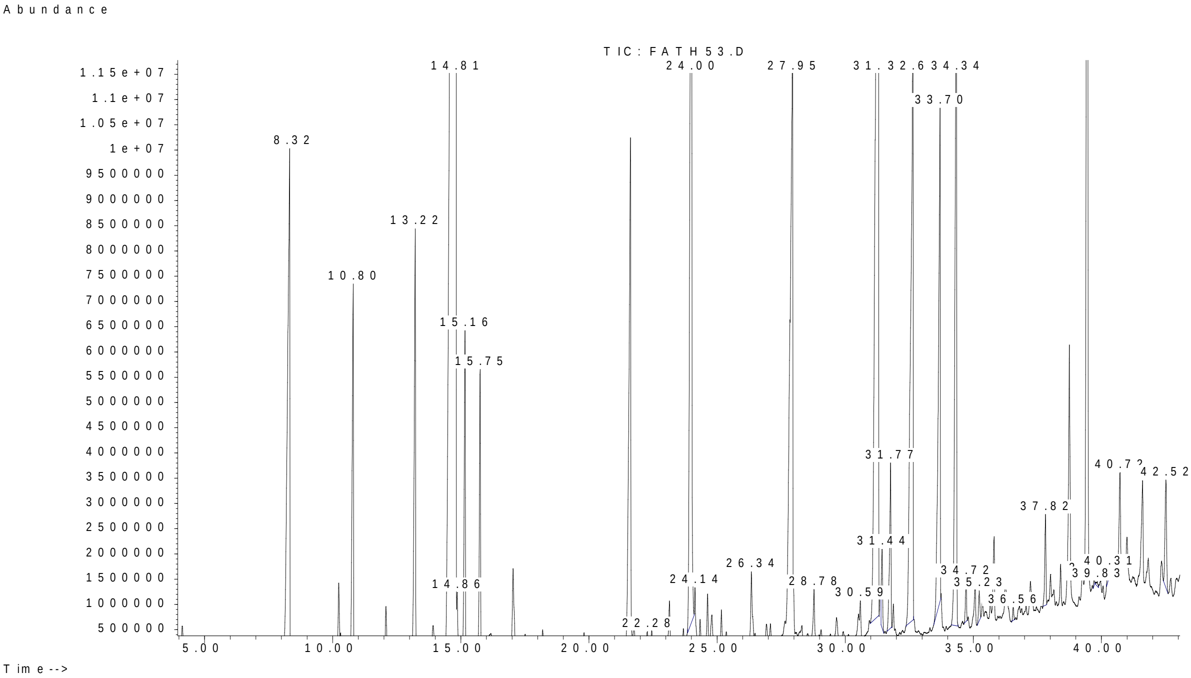


Fig. 3. The typical GC-MS chromatogram of methyl esters of carboxylic acids *ofVeronica spicata* L. flowers

## 5. Discussion

Only a few studies have been found on the chemical composition of *V. spicata* and *V. incana*. Naimushina and Zykova [20] reported the presence of 40 components in *V. spicata* essential oil, which were mainly oxygenated terpenoids and hydrocarbons. According to [12], the main compounds of the essential oil of *V. spicata* were phytol, heptacosane and pentacosane. Also, 10 phenolic compounds (chrysin, rutin, quercitrin and cichoric, ferulic, protocatehuic, rosmarinic, syringic and tannic acids) were identified and quantified in *V. spicata*. Previously, five 6-hydroxyluteolin glycosides acylated with phenolic acids, called spicosides B–F were detected in *V. spicata*, and the survey of other species of *Veronica* has shown that this type of flavonoid is common in species belonging to the subgenus *Pseudolysimachium*, which may be a possible chemosystematic marker [25].

In the herb of *V. incana* growing in Yakutia, tannins, flavonoids, coumarins and iridoids were identified, and the content of total phenolics, tannins and flavonoids was established [21].

In *V. incana* flowers, the total carboxylic acid content was 10464.68 mg/kg. In the total content of carboxylic acids, low-molecular aliphatic acids account for 23.95 % (2506.19 mg/kg), fatty acids make up 48.39 % (5063.74 mg/kg), and aromatic acids constitute 27.66 % (2894.75 mg/kg). The fatty acid composition of *V. incana* flowers is characterized by the comparable content of saturated and unsaturated acids (2555.71 and 2508.03 mg/kg, respectively). Benzoic acid and its derivatives are the dominant aromatic acids (1413.21 mg/kg or 13.50 % of the total content); the content of phenyl and phenolic acids is significantly lower (845.3 mg/kg or 8.08 % of the total content); hydroxycinnamic acids are a minor group of carboxylic acids (636.24 mg/kg or 6.08 % of the total carboxylic acid content).

In *V. incana* flowers, the dominant low-molecular aliphatic acids are citric acid and malic acid (592.59 and 536.27 mg/kg, respectively); linoleic acid and palmitic acid are the major fatty acids (1163.90 and 1137.67 mg/kg, respectively); among aromatic acids, vanillic acid and benzoic acid prevail (649.78 and 566.22 mg/kg, respectively).

The total content of the carboxylic acids in *V. spicata* flowers is 27525.12 mg/kg, of which the low-molecular aliphatic acids constitute 61.96 % (17057.04 mg/kg), the fatty acids account for 28.14 % (7742.69 mg/kg), and the aromatic acids make up 9.90 % of the total carboxylic acid content (2725.39 mg/kg). Unsaturated fatty acids (4256.70 mg/kg) were detected to prevail in the total amount of the fatty acids, while saturated fatty acids constituted 3485.99 mg/kg. In the total amount of aromatic acids, phenyl- and phenolcarboxylic acids were dominant (1941.58 mg/kg or 7.05 % of the total carboxylic acid content); a significantly lower content of benzoic acid was established (498.46 mg/kg or 1.81 % of the total content); hydroxycinnamic acids were the minor group (285.25 mg/kg or 1.04 %). The following carboxylic acids are dominant in *V. spicata* flowers: levulinic acid (the content of which reaches up to 7700.14 mg/kg) prevails in the total amount of the low-molecular-weight aliphatic acids; palmitic and linoleic acids (2682.47 and 2304.41 mg/kg, respectively) are the dominant higher fatty acids; vanillic acid is the major aromatic acid (1868.65 mg/kg).

It is noteworthy that percentages of benzoic acid and its derivatives (13.50 %), and hydroxycinnamic acids (6.08 %) in the total carboxylic acid content in *V. incana* flowers are significantly higher than those in *V. spicata* flowers (1.81 % and 1.04 %, respectively). Whereas the percentage of phenyl- and phenolcarboxylic acids in the total carboxylic acid content in the flowers of *V. incana* and *V. spicata* is comparable (8.08 % and 7.05 %, respectively).

Considering our previously reported data on the chemical composition of the flowers of *V. longifolia* [26], some features of aromatic acids profile of flowers of *Pseudolysimachion* W. D. J. Koch section *Veronica* species of Ukrainian flora are seen. The common acids are benzoic, vanillic and ferulic acids. Phenylacetic, salicylic, syringic, *p*-coumaric and gentisic acids are characteristic only for flowers of *V. spicata* and *V. incana*.

Also, based on data obtained, we could state that the highest content of carboxylic acids among the three studied species of the section *Pseudolysimachion* W. D. J. Koch is observed in *V. spicata* flowers, with the highest content of low-molecular-weight aliphatic acids – 1.70 %. The highest content of fatty acids was detected in the flowers of *V. longifolia* – 1.50 %. The content of aromatic acids in the flowers of *V. incana* and *V. spicata* is comparable (0.29 % and 0.27 %, respectively), and these values are almost 3-folds higher than the content of aromatic acids in *V. longifolia* flowers (0.10 %).

The qualitative composition of carboxylic acids in *V. incana* is similar to that of *V. longifolia* flowers. Unlike *V. spicata*, both species produce oxalic, 3-hydroxy-2-methylglutaric, heneicosanoic, tricosanic, 4-hydroxybenzoic acids, whereas the flowers of *V. spicata* differ in the presence of 2-hydroxy-3-methylglutaric, *α*-furanic and homovanillic acids.

Raw materials containing aromatic acids are considered as a potential source of drugs for the treatment of metabolic syndrome, diabetes and heart disease [35, 36] and correction of immunity [37, 38]. Hypolipidemic and antidiabetic activity of ferulic acid was reported [39].

**Study limitations.** During the GC-MS study, several compounds were not identified due to the absence of their characteristics in NIST 05 and Willey 2007 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 carboxylic acids composition on plant development, season, and growth conditions. Also, since studied herbal materials contain phytochemicals with hypolipidemic and antidiabetic activities, it is reasonable to obtain corresponding substances and study their hypoglycemic and hypolipidemic potential.

## 6. Conclusions

By means of GC-MS analysis, carboxylic acids were first studied in the flowers of *Veronica spicata* L. and *Veronica incana* L. The content of carboxylic acids in the flowers of *Veronica spicata* is two times higher than in *Veronica incana* flowers. The characteristic carboxylic acids in *Veronica spicata* flowers are 2-hydroxy-3-methylglutaric, α-furanic and homovanillic acids. *Veronica incana* flowers are notable for the presence of oxalic, 3-hydroxy-2-methylglutaric, pentadecanoic, heneicosanoic, tricosanic, 4-hydroxybenzoic, 4-methoxybenzoic and 3,4-dimethoxybenzoic acids. The data obtained can be employed as chemical tools for species identification.

## Conflicts of interest

The authors declare that they have no conflicts of interest.


## Funding

This work was supported by the Ministry of Health of Ukraine from the State Budget in the framework [grant number 2301020] “Scientific and scientific-technical activity in the field of health protection” on the topic “Modern approaches to the creation of new medicines for a correction of metabolic syndrome”.


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*Received date 21.12.2021*
*Accepted date 26.01.2022*
*Published date 28.02.2022*

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