

The phytochemical and chemotaxonomic study of *Salvia* spp. growing in Ukraine

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ABSTRACT

Salvia officinalis is a well-studied and popular medicinal plant containing toxic thujones, rather limited phytochemical and pharmacological studies have been performed on other *Salvia* spp. The aim of the study was to perform phytochemical and chemotaxonomic studies of *Salvia* genus species ($n = 16$) from Ukraine to identify the most promising species for the pharmaceutical industry. The content of essential oils from the leaves of 16 *Salvia* spp. has been studied by gas chromatography/mass spectrometry (MS) and polyphenols by high performance liquid chromatography/MS, and a chemotaxonomic study has been performed by calculation of coefficients of occurrence, originality, and pair affinity. The *Salvia* spp. is characterized by the presence of monoterpenoids, hydroxycinnamic acids – derivatives of caffeic acid, and flavonoids – derivatives of luteolin and apigenin. The highest content of essential oil is observed in the leaves of *S. officinalis*, *S. grandiflora*, and *S. scabiosifolia* (1.41%–1.85%). The highest amount of hydroxycinnamic acids is in the leaves of *S. pratensis*, *S. illuminata*, *S. austriaca*, and *S. verticillata*; the highest content of flavonoids and phenolic compounds is found in the leaves of *S. grandiflora*, *S. pratensis*, *S. illuminata*, *S. nemorosa*, *S. pendula*, *S. sylvestris*, *S. nutans*, *S. verticillata*, and *S. cernua*. The chemical profile of the genus has been determined; it is characterized by terpenoids such as α - and β -pinene, camphane derivatives (camphene, camphor, and borneol), *p*-cymene, 1,8-cineol, limonene, phenolic compounds (caffeic and rosmarinic acids), and flavonoids (cynaroside, cosmoiin, hispidulin, and cirsimaritin). A rather high concentration of toxic α - and β -thujone is typical only in the essential oil of *S. officinalis* (33.6% and 6.5%, respectively). The most promising species for use in the pharmaceutical industry are *S. grandiflora*, *S. cernua*, *S. pratensis*, *S. nemorosa*, and *S. verticillata*.

1. INTRODUCTION

The genus *Salvia* is one of the largest genera from the *Lamiaceae* family which comprises 700 species and is widely distributed in various regions of the world [1]. Of 151 pharmacopoeial species of medicinal plants grown on the territory of Ukraine, sufficient reserves are available only for 47 wild species, 32 species are cultivated; as a whole, it is 52%. There are 38 species (25%) that are in insufficient quantities in Ukraine, and it leads to a shortage of the raw material and the need to import it; moreover, 34 species (23%) should be imported for the needs of the Ukrainian pharmaceutical industry. Therefore, the relevance of the systematic scientific search for the medicinal plant raw material containing important

biologically active substances among plants of the Ukrainian flora and its introduction into the register of officinal medicinal plants is undeniable [2–5].

Over the past 10 years, a substantial literature dataset regarding the pharmacological properties of biologically active compounds contained in the representatives of the *Salvia* spp. that are presented, in particular, in the flora of Ukraine has been accumulated. For example, in recent works, it is shown that extracts of some representatives of the *Salvia* genus can inhibit hyperglycemia and lipid peroxidation, increase the activity of antioxidant enzymes, as well as reduce the frequency of hot flushes during menopause up to 100% [6,7]. Biologically active substances of *Salvia* improve memorization processes, and exhibit anxiolytic effect and a high level of cholinesterase inhibition [8–11]. It may have significant therapeutic potential in the treatment of central nervous system diseases, in particular, Alzheimer's disease [12,13].

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Previously, the content of amino acids and monosaccharides has been studied in *Salvia* spp. growing in Ukraine [14]. Also, terpenoids and phenolic compounds have been analyzed in *Salvia officinalis* and some other species [5,15,16]. The chemical composition of other species of *Salvia* growing in Ukraine and their use in pharmaceutical and medical practice have not been practically studied. Very limited mechanistic studies and very little *in vitro* tests have been performed on the biologically active substances in *Salvia* spp. [17].

Chemosystematic studies clarify the position of the species object in the genus system and are carried out with the aim of clarifying the systematics of individual species; search for promising species for using in medicine; and to identify correlative relationships between the morphological structure, environmental conditions of existence, and the chemical composition of plants [18–20]. The modern classification of plants is based on their botanical structure, primarily the signs of their generative organs, it is based on a subjective approach to the description of plants and does not always correspond to the true state of things. Chemosystematics is one of the sources of information for synthetic integral systematics, which is more likely to recreate the true picture of the evolutionary development of taxa (species) of plants than any other extraneous approach to solving this problem [21,22].

Some species of *Salvia* genus were studied by chemosystematics in different countries, and using secondary metabolism substances as chemo attributes: terpenoids [23–25], as well as substances primary metabolism: fatty oils [26], although the latter is doubtful because of chemotaxonomic originality deprivation.

The aim of our work was to search for sources of biologically active substances among species of the genus *Salvia*, growing in Ukraine, using phytochemical and chemotaxonomic research methods. This helps to indicate the prospects of the in-depth study of these species for the needs of the pharmaceutical industry and to show the expediency of further research to create new drugs. According to the authors' hypothesis, not only *S. officinalis*, but also other *Salvia* spp. have promising pharmaceutical value.

2. MATERIALS AND METHODS

2.1. Plant Material

Of 21 species of the *Salvia* spp. presented in the flora of Ukraine, 16 species (*S. officinalis*, *S. sclarea*, *S. grandiflora*, *S. scabiosifolia*, *S. glutinosa*, *S. aethiopsis*, *S. pratensis*, *S. stepposa*, *S. Sibthorpii*, *S. illuminata*, *S. nemorosa*, *S. pendula*, *S. sylvestris*, *S. nutans*, *S. austriaca*, *S. verticillata*, and *S. cernua*) were studied. Data of *Salvia sclarea* for the chemotaxonomic study were taken from scientific literature [5,15,27,28]. The leaves of different *Salvia* spp. were harvested at the beginning of flowering in the Botanical Garden of V. N. Karazin Kharkiv National University (50°01'33.7"N 36°13'46.3"E). All collected plants were already planted as samples of different *Salvia* spp. in the botanical garden; they were not planted by the authors of the paper. The leaves have been collected from one plant of each studied species and were dried at room temperature in a well-ventilated area for ten days. The species *Salvia* genus studied were identified by prof. Yu. H. Hamulia from the V. N. Karazin Kharkiv National University using a special botanical catalog [29]. The voucher specimens are stored

at the Department of Pharmacognosy, The National University of Pharmacy, Kharkiv, Ukraine (No. 172–188).

2.2. Isolation of Essential Oils

Essential oil was isolated from the dried leaves of the *Salvia* spp. (10 g) by simultaneous distillation and extraction (SDE) with n-hexane (Fluka >99%) as a solvent (0.5 ml) via a Marcusson type micro-apparatus [30]. The SDE process was carried out for 2 hours. The oil amount (%) was determined using n-tetradecane (Reaching >99%) as an internal standard (2 µl) [31]. The reproducibility of three parallel SDE procedures with a single sample showed the variation coefficient to be below 20%.

2.3. Analysis of the Essential Oils

The qualitative composition and the quantitative content of terpenoids have been determined by gas chromatography using an *Agilent Technology* 6,890 chromatograph with a mass spectrometric detector 5,973 under standard conditions: HP-5 on fused silica capillary columns with bonded stationary phases SPB-5 (30 m × 0.25 mm, Supelco), film thickness of stationary phases was 0.25 µm. The temperature program was from 50°C to 250°C at 4°C/min, and the injector temperature was 250°C. Injection volume: 2.0 µl, two injections were carried out. Carrier gas He with split ratio 150:1, and the flow rate of 1 ml/min was applied. The temperature of the interface: 250°C; ion source temperature: 230°C. The mass spectrometry (MS) detector was operated in the electron ionization mode 70 eV at a scan rate of 2 scans/sec with an acquisition mass range of 29–450 a.m.u.

The identification of compounds was performed based on the comparison of the mass spectra obtained with the data from the *NIST05-WILEY* library (approximately 500,000 mass spectra) [30]. The retention indices of the components were calculated based on the results of control analyses of compounds when adding a mixture of normal alkanes (C₁₀–C₁₈). The yield of the essential oil was calculated by the sum of all peak areas on the chromatograms.

2.4. Sample Preparation, high performance liquid chromatography (HPLC)-diode array detector-MS/MS Analysis, and Quantification

About 500.0 mg (exact weavage) of *Salvia* spp. leaves were weighed in a 5.0 ml measuring tube and brought to the mark with 90% aqueous methanol. After 30 minutes in an ultrasonic bath, the sample was insisted at room temperature for 3–4 hours. The test tube was again placed on an ultrasound bath for 15 minutes, and then the solution was filtered through a Teflon filter with a pore size of 0.45 µm in a vial for analysis.

Separation, identification, and quantification of phenolic compounds were determined by HPLC using an *Agilent Technologies* chromatograph (model 1100) equipped with a vacuum degasser G1379A, four-channel low-pressure gradient pump G13111A, automatic injector G1313A, G13116A column thermostat, and G1316A diode-matrix detector. Columns, 2.1 mm × 150 mm, were filled with octadecylsilyl sorbent grains of 3.5 µm “ZORBAX-SB C-18.” The analysis of methanol extracts from *Salvia* spp. leaves was carried out under the following conditions: thermostat temperature: 35°C; flow rate of the mobile phase:

0.25 ml/min; as a mobile phase: solution A (0.1% H₃PO₄, 180 µl/l triethylamine, 3 ml/l tetrahydrofuran in water) and solution B (MeOH) in the ratio of 90:10 (first 8 minutes), 70:30 (from 8 for 24 minutes), and from 24 minutes only solution B was used; and working pressure of the eluent: 240–300 kPa. In the analysis, the following detection parameters were set: scale of measurement: 1,0; scan time: 0.5 second; the parameters for removing the spectrum: each peak is 190–600 nm. The identification of phenolic compounds was performed by the retention time of standards and ultraviolet spectral characteristics.

2.5. Chemotaxonomic Study

For the chemotaxonomic study, the data from the scientific literature of *S. sclarea* and other species were used [5,15,27,28]; 5719 states of signs being characteristic of terpenoids and phenolic compounds were analyzed. The coefficients of originality, the informational content of the sign, the sums of positive normal and qualified states of signs used for further calculations in taxonomy, as well as the coefficients of pair and group affinity were calculated [32–34].

The coefficient of occurrence (Coc) traits was calculated by the formula:

$$\text{Coc} = \frac{M}{N} \times 100$$

where M - the total number of occurrence of this trait;

N = S is the sum of the positive and negative states of the feature, which is equal to the S-population of the object being studied or the number of species (taxons).

The coefficient of originality (Cor) traits was calculated by the formula:

$$\text{Cor} = \left(1 - \frac{M}{N} \right) \times 100 = 100 - \text{Coc}$$

Informative character (sign weight) was calculated by the formula:

$$\text{JPi} = \frac{N}{2} - n$$

where n is the sum of positive states of signs.

The peculiarity (A), or informational, of taxons were determined only for positive values of JPi:

$$A = \sum \text{JPi}$$

The calculation of coefficients of pair affinity (CPA) was carried out according to the formula: coefficient of pair affinity

$$\text{CPA} = \left(\frac{P}{P} + d \right) \times 100$$

where P is the number of positive signs and d is the sum of states of attributes that are different.

In turn

$$P = P1 + P2$$

where P1 is the sum of the positive normal states of signs and P2 is the sum of positive qualifying states of attributes.

The value of the coefficients of the pair affinity characterizes the interspecific relationship or the taxonomic distance between the species. The calculations were carried out using the program Microsoft Excel 2010.

The signs and taxons were given numbering accordingly to it they were placed in the matrix table. The presence of signs was marked with the number “1”, while in the absence of a sign the cell was left empty, i.e., the continuum of the values was in the range from 0 to 1. The table served as the basis for the numerical quantitative taxonomic analysis: calculations of the information weight of the signs, frequency indices, originality of signs, and distinctness of taxons [33,34]. Using the signs of the morphological structure and the chemical composition, the coefficients of pair and group affinity of the species of the *Salvia* genus were calculated. The values of the CPA characterize the interspecific relationship or the taxonomic distance between the species. Taking into account the CPA and the coefficients of group affinity, the dendrograms characterizing the taxonomic distances between species of the genus by chemical signs were constructed.

2.6. Statistics

The leaves have been collected from one plant of each studied species of *Salvia* spp., therefore no statistics were performed. The standard deviation of used methods does not exceed 5%.

3. RESULTS AND DISCUSSION

3.1. Composition of Essential Oils

The content of the main components of the essential oils in the leaves of *Salvia* spp. is shown in Table 1. In general, 185 substances were found in the objects studied; among them, 114 substances were identified. The highest content of essential oil (0.32%–1.85%) was observed in the leaves of *S. officinalis*, *S. grandiflora*, and *S. scabiosifolia*. It indicates the expediency of further study of these species in order to create new drugs.

In the leaves of *S. officinalis* 28 compounds were found; among them, 1,8-cineol, α-pinene, α- and β-thujone, camphene, camphor, borneol, bornylacetate, caryophyllene, caryophyllene oxide, viridiflorol, humulene, humulene oxide, and manool dominated. Similar results have been shown in the study of essential oils of *S. officinalis* from different countries [35] where the level of toxic - and -thujone was 3.0%–26.6% and 1.5%–12.9%, respectively. Other *Salvia* spp. studied by us do not contain thujones or their concentration was very low (0.05%–0.2%), and they are safe. Thujone as a modulator of GABA_A receptor can act as a convulsant, it may have benefits within the safety limit, such as anxiolytic and antipsychotic actions, also in suppressing dementia symptoms [36].

The leaves of *S. grandiflora* and *S. scabiosifolia* contain 1,8-cineol, α- and β-pinene which have antibacterial activities. The antibacterial activity has been shown for extracts of many different *Salvia* spp. [17,37–39]. The leaves of essential oils from other *Salvia* spp. typically contain heptacosane, nonacosane, hentriacontane, and tritriacontane as principal compounds.

Table 1: Composition (%) of essential oils of *Salvia* spp. leaves from Ukraine.

Compounds	<i>S. officinalis</i>	<i>S. grandiflora</i>	<i>S. scabrosifolia</i>	<i>S. glutinosa</i>	<i>S. aethiopsis</i>	<i>S. pratensis</i>	<i>S. stepposa</i>	<i>S. Sibthorpii</i>	<i>S. illuminata</i>	<i>S. nemorosa</i>	<i>S. pendula</i>	<i>S. sylvestris</i>	<i>S. nutans</i>	<i>S. austriaca</i>	<i>S. verticillata</i>	<i>S. cernua</i>
α -Pinene	1.5	22.5	1.1	0	0	0.10	<0.1	0.1	0	2.8	0	0.1	0	0.6	0	0
Camphene	2.6	5.1	3.9	0	0	0	0	0.2	0	0.4	0	<0.1	0	0.2	0	0
Sabinene	0	0	2.7	0	0	0	0	0	0	0	0	0	0	0	0	0
β -Pinene	0	12.3	2.7	0	0	0	0	<0.1	0	1.2	0	0	0	<0.1	0	0
<i>p</i> -Cymene	0.2	3.6	2.3	1.1	0	0	0	2.2	0	42.1	0	0	0	0	0.1	0
1,8-Cineol	8.5	3.5	4.0	0	0	0.3	<0.1	<0.1	0	0.4	0	<0.1	0	<0.1	<0.1	0.2
Limonene	0	2.1	0.7	0.1	0	0	0.1	<0.1	0	0.1	0	<0.1	0	<0.1	<0.1	<0.1
<i>cis</i> -Sabinene hydrate	0	0	2.3	0	0	0	0	0	0	0	0	0	0	0	0	0
Dehydro- <i>p</i> -cymene	0	0	0	0	0	0	0	0.1	0	4.1	0	0	0	0	0	0
β -Thujone	6.5	0	0	0	0	0	0	<0.1	0	1.1	0	0	0	0	0	0
α -Thujone	33.6	0	0.2	0	0	0	0	0	0	0	0	0	0	0	0	0
Camphor	19.8	10.1	22.2	0	0	0	0	0	0	1.2	0	0	0	<0.1	0	0
Pinocarvone	0	2.9	0	0	0	0	0	0	0	0.8	0	0	0	0	0	0
Borneol	4.4	5.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Menthol	0	0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0
<i>trans</i> -2-Decenal	0	0	0	0	0.6	0.3	1.2	0.7	0	2.2	0	0	0	0	0	0
Bornylacetate	2.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
α -Copaene	0	4.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
β -Burbonene	0	0.5	6.9	0	0	0	0	0	0	0	0	0	0	0	0	0
Caryophyllene	1.2	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tetradecane	0	0	0	2.8	1.4	1.5	3.0	0.9	1.7	7.1	0.5	0	0.6	2.1	1.4	2.4
α -Amorfen	0	6.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dihydroactinidiolide	0	0	0	0	0	0	0	0	0	0	0.6	0	0	0	0.6	0
1,5-Epoxysalvial-4(14)-ene	0	0	0	0	0	0	0	0	0	0	0	0	1.0	0	0	0
Caryophyllene oxide	1.4	0.8	0	0	0	0	0	0	0	0	0	0	0	0	1.6	0
Spathulenol	0	0	3.9	0	0.3	0	0	0	0	0	0	0	0	0	0	0
Viridiflorol	7.7	0.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Humulene	2.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hexahydrofarnesyl acetone	0	0	0	0	0.4	2.3	2.2	1.6	2.1	6.3	1.0	0	1.8	0	2.1	0.8
<i>cis</i> -Neofitadiene	0	0	0	0	0	0.6	1.0	2.0	0.6	16.9	1.1	1.1	1.0	0	0.9	1.3
Humulene oxide	1.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>cis</i> - and <i>trans</i> -Neofitadiene	0	0	0	0	0	0	0	0	0	3.6	0.2	0	0	0	0	0.2
Manool	3.0	0.9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heneicosane	0	0	0.6	0	1.4	1.2	1.4	0	0	6.5	0.7	1.3	1.2	1.2	1.9	1.2
Docosane	0	0	0.9	0	1.1	1.4	0.9	0.5	0.5	6.6	1.9	0.8	1.1	1.3	2.4	1.4
Pentacosane	0	0	1.4	0.7	1.7	1.3	1.0	0.6	0.5	7.7	0.6	0.5	0.7	3.6	2.0	0.7
Heptacosane	0	0	0.6	4.1	2.4	3.5	2.4	1.3	1.2	11.4	1.4	1	2.5	9.2	2.3	1.2
Nonacosane	0	0	2.0	13.0	3.7	8.7	5.3	4.5	5.3	42.4	7.0	4.4	5.4	16.4	11.5	4.8
Hentriacontane	0	0	5.3	24.0	11.9	10.4	15.3	10.5	18.6	63.2	20.8	14.2	19.0	11.1	0	3.0
Dotriacontane	0	0	0.9	2.6	4.5	4.4	3.6	2.9	5.0	35.6	4.1	4.8	2.7	1.9	1.1	1.6
γ -Sitosterol	0	0	0	0	0	3.1	4.0	0	3.1	11.7	0	5.3	0	5.3	2.9	2.5
Tritriacontane	0	0	8.7	16.3	28.8	16.9	12.9	11.7	23.0	11.6	25	33.4	12.3	7.6	15.6	10.9
Tetatriacontane	0	0	0	0	7.4	0	0	0	0	0	0	3.1	0	0	0	0
Content of essential oil (%)	1.85	1.45	1.41	0.43	1.35	0.44	0.32	0.38	0.58	0.62	0.92	0.28	0.61	0.69	0.43	0.47
Number of compounds in essential oils	28	34	35	29	47	47	47	57	31	57	37	33	32	30	35	37

3.2. Polyphenols

Wu et al. [38] classified the polyphenols in *Salvia* spp. into two subgroups: phenolic acids and flavonoids, which play an important role in the prevention of human diseases. In the leaves of *S. officinalis*, 13 substances of phenolic nature were found; among them, caffeic, 3,4-dicaffeoylquinic and rosmarinic acids, apigenin, luteolin, 3-methoxyluteolin, and cosmosiin were identified (Table 2). For the first time in 16 species of *Salvia* in the flora of Ukraine, 60 substances of phenolic nature were found; among them, 14 substances were identified. In the leaves of the species studied, there are mainly derivatives of flavones: luteolin and apigenin. In addition, the leaves of *S. stepposa*, *S. Sibthorpii*, and *S. cernua* also contain derivatives of kaempferol and quercetin. The highest content of hydroxycinnamic acids is observed in the leaves of *S. pratensis*, *S. illuminata*, *S. austriaca*, and *S. verticillata*. The highest content of flavonoids and phenolic compounds is in the leaves of *S. grandiflora*, *S. pratensis*, *S. illuminata*, *S. nemorosa*, *S. pendula*, *S. sylvestris*, *S. nutans*, *S. verticillata*, and *S. cernua*; it indicates the prospects of the in-depth study of these species for the needs of the pharmaceutical industry.

Thus, the highest quantitative content of phenolic compounds is typical for the leaves of *S. grandiflora*, *S. pratensis*, *S. illuminata*, *S. nemorosa*, *S. pendula*, *S. sylvestris*, *S. nutans*, *S. verticillata*, and *S. cernua*; it indicates the prospects of using these species as sources of phenolic biologically active substances.

In the study [6], a close correlation was shown between the total phenol contents and the antioxidant activities of the *Salvia* extracts. The plants of the genus *Salvia* are typically rich in antioxidant polyphenols and abietane diterpenoids such as rosmarinic acid, which showed considerable anticancer and antimicrobial activity

[7]. *S. officinalis* contains less rosmarinic acid (596 mg/kg) than ten other *Salvia* spp. studied (1,123–12,310 mg/kg), the difference between minimum and maximum content is more than 20 times. Rosmarinic acid and carnosol were the main compounds in all the antioxidant phenolic extracts isolated from *S. officinalis* [38]. It also has antihepatitis, inhibition of HIV-1, and hepatoprotection effects [40]. *Salvia* spp. and their individual active constituents may also impact on neurological and cognitive function [41]. Especially, caffeic acid derivatives play an important role as potent antiviral agents in *Salvia* spp. [17].

In addition to terpenoids in essential oils and polyphenols, diterpenes and triterpenoids are important constituents in *Salvia* spp., more than 200 triterpenoids of different classes were isolated from 113 species of the genus [1]. *Salvia* spp. have been reported as showing antioxidant, antimicrobial, antiinflammatory, antidiabetic, anti-HIV, anti-Alzheimer's disease, antileishmanial, antimalarial, antiprotozoal, and cytotoxicity activities [17,42], which may be also characteristic to the *Salvia* spp. studied by us in addition to *S. officinalis*. From these objects, only some species passed the studies of their activities, for example, the methanol extract of *S. aethiopsis* has been shown antioxidant activity [38].

3.3. Determination of Promising Species of the *Salvia* Genus as Sources of Biologically Active Substances

As a result of the study of the chemical composition of *Salvia* in the flora of Ukraine, it has been found that the genus is characterized by the presence of monoterpenoids, hydroxycinnamic acids – derivatives of caffeic acid and flavonoids – derivatives of luteolin and apigenin.

For the chemotaxonomic study (Figs. 1–2) of the *Salvia* genus, 5,719 states of 301 chemo signs being characteristic of terpenoids

Table 2: Content of individual phenolic compounds (mg/kg) and their groups (%) in *Salvia* spp. leaves from Ukraine.

Compounds	<i>S. officinalis</i>	<i>S. grandiflora</i>	<i>S. scabiosifolia</i>	<i>S. glutinosa</i>	<i>S. aethiopsis</i>	<i>S. pratensis</i>	<i>S. stepposa</i>	<i>S. Sibthorpii</i>	<i>S. illuminata</i>	<i>S. nemorosa</i>	<i>S. pendula</i>	<i>S. sylvestris</i>	<i>S. nutans</i>	<i>S. austriaca</i>	<i>S. verticillata</i>	<i>S. cernua</i>
Caffeic acid	129	188	108	44	39	118	26	87	258	138	37	191	89	245	294	30
Vicenin-2	0	964	0	0	0	0	0	0	0	0	0	0	1428	0	0	375
3,4-Dicaffeoylquinic acid	86	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cynaroside	1955	6209	1255	203	198	11493	245	673	1931	5494	224	1395	2349	435	6886	3992
Rutin	0	0	0	0	0	0	358	409	0	0	0	0	0	0	0	614
Rosmarinic acids	596	4601	4625	53	552	6899	1223	1123	6811	3100	92	3489	112	4147	12310	521
Cosmosiin	0	1651	1107	0	218	0	0	209	0	0	0	1015	512	0	4070	1887
Kaempferol-3-O-glucoside	0	0	0	0	0	0	207	436	0	0	0	0	0	0	0	0
3-Methoxyluteolin	296	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6-Hydroxyluteolin-5-glucoside	0	0	0	0	0	0	0	0	0	0	0	0	893	0	835	1830
Luteolin	292	0	0	0	0	0	0	0	0	0	0	0	4369	0	0	3239
Apigenin	404	0	0	234	201	0	0	0	0	0	0	212	0	0	0	419
Hispidulin	0	102	0	0	461	88	70	195	931	579	1256	1198	711	0	0	2227
Cirsimaritin	0	452	946	0	1378	468	110	373	1559	2104	1269	1748	3258	0	172	5082
The content of hydroxycinnamic acids	0.09	0.51	0.49	0.01	0.07	0.98	0.16	0.14	0.87	0.52	0.01	0.52	0.02	0.88	1.40	0.09
The content of flavonoids	0.45	2.96	0.38	0.12	0.40	1.68	0.12	0.27	0.92	1.61	0.34	0.82	4.29	0.04	1.20	3.20
Content of the sum of phenolic compounds	0.54	3.47	0.87	0.13	0.46	2.66	0.28	0.41	1.80	2.13	0.35	1.34	4.29	0.92	2.59	3.28

and phenolic compounds were analyzed in 17 species of *Salvia* from different morpho-taxonomic groups. The coefficients of originality, the informational content of the sign, the sums of positive normal and qualified states of signs used for further calculations in taxonomy, as well as the coefficients of pair and group affinity were calculated. According to the results of the chemotaxonomic analysis conducted, the dendrogram with *S. nemorosa* in its base was constructed by chemical signs (Fig. 1).

The results of our chemotaxonomic studies of 17 species of the *Salvia* genus allowed us to solve a number of discussion questions regarding to the identity of the species that were identified: *S. cernua* and *S. pendula*; *S. sylvestris* and *S. nemorosa*; and *S. illuminata* and *S. nemorosa*. All these species turned out to be independent, showing a relation degree at the level of 13%—*S. cernua* and *S. pendula*; 20%—*S. sylvestris* and *S. nemorosa*; 31%—*S. illuminata* and *S. nemorosa*.

Four main clusters of species, which contain 1,8-cineole, rosmarinic acid, isovitexin-8-C-beta-glucoside (vicenin 2); apigenin and hispidulin; quercetin and rutin; *p*-cimen, kaempferol, hispidulin, and apigenine derivatives, were established. The first one includes *S. officinalis* L., *S. grandiflora* Etl., *S. verbenata* L., which contain *p*-cymen, β -pinene, 1,8-cineole, limonene, *trans*-linalool oxide, camphor, borneol, linalool, linalyl acetate, myrtenol, caryophyllene, caryophyllene oxide, viridiflorol, β -caryophyllene, luteolin, apigenin, and diosmetin-luteol-7-O-glucoside. The second one has *S. pratensis* L., *S. stepposa* Schost., *S. Sibthorpii* Sm., *S. illuminata*, *S. nemorosa* L., *S. nutans* L., which characterized by α -pinene, 1,8-cineole, limonene, *cis*-neophytadiene, caffeic acid, 8 derivatives of caffeic acid, rosmarinic acid, hispidulin and cirsimaritin. The third cluster (*S. glutinosa* L., *S. nemorosa* L., *S. austriaca* Jacq., and *S. verticillata* L.) distinguishes with *p*-cimen,

1,8-cineole, limonene, *cis*-2-hexen-1-ol acetate, β -thujone, camphor, borneol, para-cimen-8-ol, γ -sitosterol, luteolin-7-O-glycoside, caffeic acid, 12 derivatives of caffeic acid and rosmarinic acid. The fourth group (*S. aethiopis* L., *S. sylvestris* L., *S. pendula* Besser) designates with α -pinene, α -pinene oxide, α -thujone, *p*-cimen-8-ol, carvacrol, nerolidol, hexahydropharnesylacetone, viridiflorol, isocaryophyll-3,8(13)-diene-5-ol, luteolin-7-O-glycoside, apigenin-7-O-glycoside, apigenin aglycone, hispidulin, cirsimaritin, caffeic and rosmarinic acid.

The dendrogram (Fig. 1) shows the division of the taxons studied into several groups that formed branches. *S. nemorosa* branches off into *S. austriaca* and *S. sibthorpii* containing 1,8-cineol, rosmarinic acid, as well as vicenin-2 in a considerable amount.

The branch with *S. aethiopis* in its base is represented by species that do not contain 1,8-cineole or contains it in an insignificant amount and characterized by the presence of apigenin and hispidulin. Unlike other species, the species with *S. stepposa* in their base are characterized by the presence of quercetin and rutin. For species of the group with *S. austriaca* in its base, the presence of terpenoids—camphane derivatives and *p*-cymene unlike other species, flavonoids—hispidulin and derivatives of apigenin is typical.

According to the results of the chemotaxonomic study conducted for the first time, the chemical profile of the *Salvia* genus has been determined; it is characterized by terpenoids (α - and β -pinene), camphane derivatives (camphene, camphor, and borneol), *p*-cymene, 1,8-cineole, limonene; phenolic compounds (caffeic and rosmarinic acids); and flavonoids (cynarside, cosmoiin, hispidulin, and cirsimaritin), which are markers of the genus.

The novelty of the studies is that for the first time on the basis of morphological and chemical characteristics of 17 *Salvia* species,

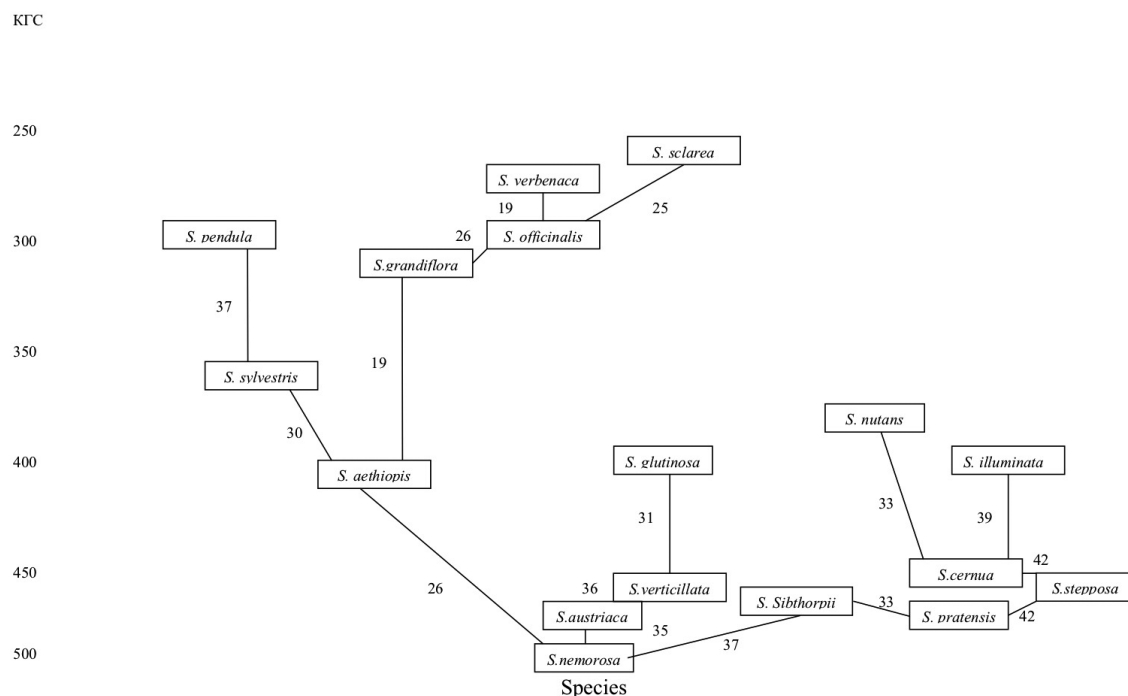


Figure 1: The dendrogram of taxonomic distances for sage in the flora of Ukraine by the chemical signs.

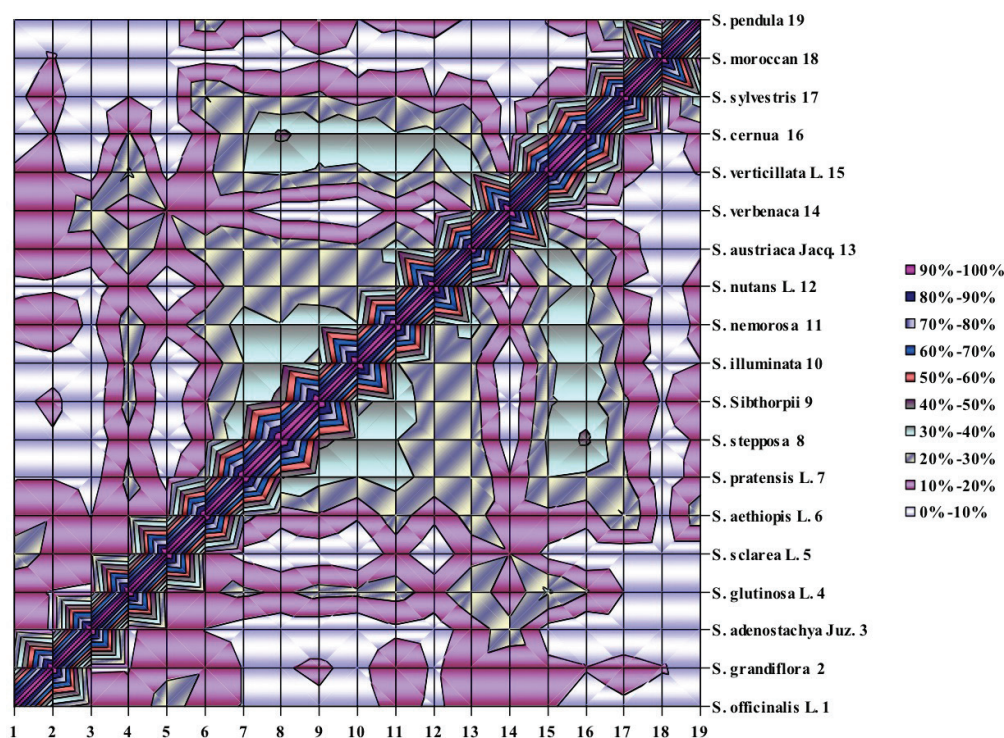


Figure 2: Diagram of taxonomic distances of species of the *Salvia* spp. based on the study of the terpenoid and phenolic composition of the sage in the flora of Ukraine.

which are present in the flora of Ukraine, chemotaxonomic studies were carried out and four main clusters of species were established, which made it possible to solve a number of discussion questions regarding the identity of species, to show some aspects of evolutionary development of *Salvia* species in Ukrainian flora and created the prerequisites for a targeted search for the biologically active substances among representatives of this genus.

4. CONCLUSION

The *Salvia* spp. from Ukraine were characterized by the presence of monoterpenoids such as α - and β -pinene, camphane derivatives (camphene, camphor, borneol), *p*-cymene, 1,8-cineol, limonene; and hydroxycinnamic acids – derivatives of caffeic acid (caffeic and rosmarinic acids) and flavonoids – derivatives of luteolin and apigenin (cynaroside, cosmosiin, hispidulin, and cirsimaritin). The highest content of terpenoids has been observed in the leaves of *S. officinalis*, *S. grandiflora*, and *S. scabiosifolia*; the highest content of hydroxycinnamic acids in the leaves of *S. pratensis*, *S. illuminata*, *S. austriaca*, and *S. verticillata*; the highest content of flavonoids and phenolic compounds was found in the leaves of *S. grandiflora*, *S. pratensis*, *S. illuminata*, *S. nemorosa*, *S. pendula*, *S. sylvestris*, *S. nutans*, *S. verticillata*, and *S. cernua*. The most promising species for use in the pharmaceutical industry are *S. grandiflora*, *S. cernua*, *S. pratensis*, *S. nemorosa*, and *S. verticillata*, which are mostly related to *S. officinalis* and contain a significant amount of phenolic compounds and terpenoids.

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CONFLICTS OF INTEREST

Authors declare that they do not have any conflicts of interest.

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