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Chemotaxonomic Characteristics of *Satureja coerulea* (*Lamiaceae* family) based on Analysis of its Bioactive Compounds

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The genus Savory (Satureja L.) from the Lamiaceae Martinov family contains several dozen species of aromatic plants. The scientific study of the chemical compounds and bioactivities varies significantly among the species within this genus. Certain species, such as Satureja montana L. and Satureja hortensis L., are particularly renowned for their healing properties. They are famous medicinal plants of world medicine. This study aimed to analyze the chromatographic profile of polyphenols and essential oil in the herb of blue savory (Satureja coerulea Janca) within its cultivation in Ukraine. Based on the results obtained, it was crucial to analyze the chemotaxonomic features of this species and identify the therapeutic potential of its bioactive compounds. The conducted GC/MS analysis indicated that the major components of the Satureja coerulea essential oil decreased in the following order: thymol (33.18%) > o-cymene (14.42%) > terpinen-4-ol (8.30%) > ?-terpinene (7.25%). The HPLC study identified the presence of 10 flavonoids and 9 phenolic acids. Among these, rosmarinic acid was found to be the most dominant phenolic acid, with a concentration of 13227.10 mg/kg. Regarding flavonoids, the highest concentrations were observed for luteolin-7-O-glucoside (7518.97 mg/kg) and apigenin-7-O-glucoside (3513.28 mg/kg). The findings of this study highlight the the unique chemotaxonomic characteristics of the studied species and its considerable potential for further pharmacological research of the essential oil and extract derived from the aerial part of Satureja coerulea. These substances may provide a source of various bioactive molecules for the pharmaceutical industry.

Keywords: Blue savory; Chromatographic methods; Endemic species; Essential oil; Flavonoids; Medicinal plant of traditional medicine; Phenolic acids; Phytomedicine.

The primary bioactive constituents in essential oil-bearing plants of the Mint (*Lamiaceae* Martinov) family include terpenoids, phenolic acids, and flavonoids.[1-6] These classes of secondary metabolites exhibit a variety of biological activities.[7,8]

The genus Savory (*Satureja* L.) from this family contains several dozen species of herbs, semishrubs, and shrubs primarily cultivated in the Mediterranean region, Middle East, North Africa, and South America.[9] The levels of scientific study of the chemical compounds and bioactivities varies significantly among the species within this genus. Certain species, such as *Satureja montana* L. and *Satureja hortensis* L., are particularly renowned for their medicinal properties and their significant potential in the fields of aromatherapy, cosmetology, and nutrition.

A recent search in the PubMed database returned 638 articles related to the term '*Satureja*', most of which focused on the two abovementioned species, examining their chemical composition, biological activity, and traditional uses in healing. However, not all species of this genus have been studied to the same extent. For example, as of January 20, 2025, the PubMed database showed only 2 results for *'Satureja coerulea*', whereas there were 55 results for *'Satureja Khuzistanica*', 149 results for *'Satureja montana*' and 162 results for *'Satureja hortensis*'.

In the last decade, researchers have become interested in studying the chemical composition and pharmacological properties of endemic *Satureja* species. As previously reported by Fitsiou *et al*.,[10] the essential oils from two Greek species (*Satureja parnassica* Heldr. & Sartori ex Boiss. and *Satureja thymbra* L.) demonstrated the antioxidant and anticancer effects. As it was reported by Hickl *et al*.,[11] the extracts from 13 Mediterranean species of the *Lamiaceae* family, including *Satureja parnassica* and *Satureja thymbra*, effectively suppressed the growth of *Streptococcus mutans.* When applied at concentrations of 0.15 mg/mL or higher, these extracts demonstrated substantial antibiofilm activity. Jaradat *et al*.[12] studied the chemical composition and bioactive properties of essential oils from *Satureja thymbrifolia* **(**Hedge & Feinbrun) Bräuchler growing in Palestina. Shams *et al***.**[13] investigated the genes involved in carvacrol biosynthesis pathway in *Satureja Khuzistanica* Jamzad and *Satureja rechingeri*, two endemic Iranian species. The variations in phenolic content, antioxidant effect and acetylcholinesterase inhibitory capacities of *Satureja barceloi* (Willk) L. from Tunis was evaluated by Raadani *et al*.[14] It is very important to study the chemotaxonomic features of endemic species, as they can be a source of valuable bioactive compounds. Analyzing data from scientists worldwide, the study of the phytochemical peculiarities of endemic species in the genus Savory is a crucial task for pharmaceutical science and practicó.[10-15]

The cultivation of promising plant species could be regarded as essential for preserving natural reserves, especially among endemic species with limited resources, while also ensuring the production of high-quality raw materials in controlled environments, as provided in the 'Guideline on good agricultural and collection practice' (GACP).[16]

A recent study highlighted the urgent need for new research strategies focused on the sustainable use of medicinal plants, particularly in light of global climate change.[17] One such strategy could involve cultivating various species of essential oil-bearing plants, which show promise for use in phytomedicine due to their therapeutic properties derived from mixtures of volatile compounds. In recent years, evidence-based medicine has been obtained on the effectiveness of aromatherapy as an alternative medicine therapy that benefits promoting general well-being and addressing mainly issues such as anxiety, depression, stress, insomnia, bronchitis and pain.[18]

In the last decades, several representatives of the *Satureja* genus have been successfully introduced to botanical gardens in Ukraine, with many of these species holding significant scientific interest.[6,19] One particularly intriguing species is blue savory (*Satureja coerulea* Janca), which is naturally found in specific regions of Eurasia where it is used in folk medicime. Its native range includes Romania, Bulgaria, and Turkey.[20] This subshrub grows primarily in the temperate biome.[21]

The study aimed to analyze the chromatographic profile of polyphenols and essential oil in in the herb of blue savory (*Satureja coerulea*), which is endemic to several southeastern European countries, within its cultivation in Ukraine. Another objective was to analyze the chemotaxonomic features of this species and to identify the therapeutic potential of its bioactive components.

MATERIALS AND METHODS

Plant raw material

The herb of *Satureja coerulea* was harvested in 2024 at the flowering stage from the plots in M.M. Gryshko National Botanical Garden located in Kyiv (Ukraine). (Fig. 1). Plants were grown in accordance with the requirements of the GACP.[16] The above-ground part of the plant was dried at 30-35°C.

Chromatographic analyses

The phytochemical analysis of polyphenols, including phenolic acids and flavonoids, in the studied raw material was conducted using high-performance liquid chromatography (HPLC). Additionally, gas chromatography-mass spectrometry (GC/MS) was employed to determine the qualitative composition and concentration of volatile compounds.

Table 1. Chemical compositions of volatile compounds isolated from the Satureja coerulea herb

Common name	Retention time, min	Relative quantity, %
α-Thujone	4.88	1.17
1-Octen-3-ol	5.74	4.48
(+)-2-Carene	6.52	2.10
o-Cymene	6.68	14.42
γ-Terpinene	7.35	7.25
Terpineol	7.52	1.54
Linalool	8.13	2.55
Borneol	9.54	1.06
Cis-4-thujanol	9.77	1.86
Terpinen-4-ol	11.23	8.30
Carvacrol	11.96	5.07
Thymol	12.18	33.18
β-Caryophyllene	14.52	4.87
Germacrene D	16.01	1.61
Caryophyllene oxide	17.40	1.01

For the HPLC analysis of polyphenols, the ground raw material (0.20 g) was extracted with 80% methanol for 2 hours at a temperature of 45°C using an ultrasonic bath. The resulting extracts were then filtered through membrane filters. The HPLC analysis was performed using an Agilent Technologies 1200 liquid chromatograph (Zorbax SB-C18 column:150 mm x 4.6 mm, 3.5 ìm).[22,23]

The preparation of plant material for GC/MS analysis involved the following steps: A portion of plant material (1.50 g) was placed in a 20 mL vial, and tridecane was added as an internal standard at a rate of 30 ìg per portion. The concentration of the internal standard was then calculated for further use. Next, 10 mL of distilled water was added to the vial, and volatile compounds were hydrodistilled for 2 h. After the distillation, volatile substances that adsorbed on the inner surface of the reflux condenser were washed off by slowly adding 3 mL of ultrapure pentane into a 10 mL vial. This wash was concentrated to a residual extract volume of 100 ìL using a stream of nitrogen at a flow rate of 100 mL/min. Finally,

A B

Fig. 1. Illustrative photos of the *Satureja coerulea*: A - flowering stage on the experimental plots, B - dry raw material

the residue was completely collected using a chromatographic syringe.

The volatile compounds were analyzed by GC/MS on an Agilent Technologies 6890 chromatograph with a mass spectrometric detector and an HP-5ms capillary column (inner diameter – 0.25 mm, length – 30 m). Chromatographic conditions: carrier gas (helium) flow rate – 1.0 mL/min; sample introduction heater temperature – 250 °C. The temperature of the thermostat was programmed from 50 to 320 °C (rate of 4 °C/min). The obtained spectra were considered based on general patterns of fragmentation of molecules under the action of electron impact and by comparing the obtained results with the data of the NIST 08 mass spectrum libraries in combination with the AMDIS and NIST 08 identification programs. The contents of compounds was determined by the internal standard tridecane.

RESULTS

During the GC/MS analysis, more than 40 compounds were revealed in the essential oil of the studied plant. The primary components of the essential oil, each making up more than 1% of its content, are detailed in Table 1. These components collectively represent the distinct 'chromatographic pattern' of volatiles found in the studied plant. An example of the GC/MS chromatogram is provided in Fig. 2.

Our findings indicate that the content of the major compounds decreases in the following order: thymol (33.18%), *o*-cymene (14.42%),

Table 2. The quantities of flavonoids in the *Satureja coerulea* herb analyzed using the HPLC method

Common names and synonyms	Retention time, min	Content, mg/kg
Rutin (rutoside, quercetin-3-O-rutinoside)	22.731	237.78
Quercetin-3-glucoside (Isoquercitrin)	23.729	117.32
Kaempferol-3-glucoside (Astragalin)	25.202	123.21
Luteolin-7-O-glucoside (Cynaroside)	28.389	7518.97
Apigenin-7-O-glucoside (Cosmosiin)	30.290	3513.28
Quercetin (Sophoretin)	32.577	79.34

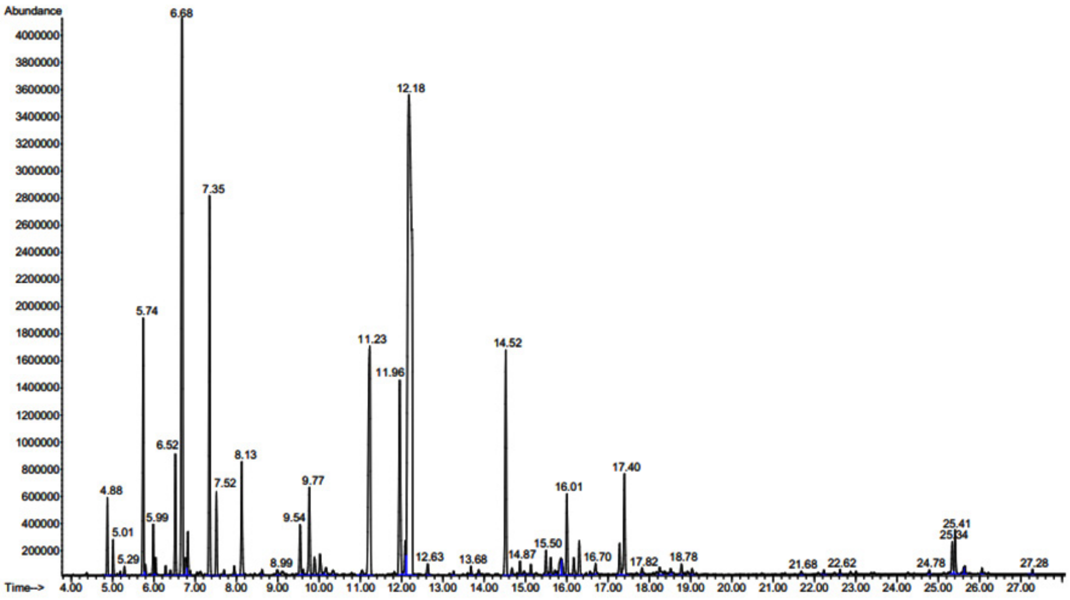

Fig. 2. The characteristic GC/MS chromatogram of the essential oil from *Satureja coerulea* herb

terpinen-4-ol (8.30%), and ã-terpinene (7.25%). The first two main components are aromatic terpenoids, while the other two are classified as monoterpenoids. Generally, the combination of major and minor components creates a unique chromatographic profile of volatile compounds of the *Satureja coerulea* herb.

As for the study of polyphenols, we determined the compositions and contents of flavonoids and phenolic acids in the herb of *Satureja coerulea* using HPLC. Ten flavonoids were identified in the analysis (Table 2, Fig. 3). Luteolin-7-O-glycoside was identified as the most concentrated compound, measuring 7518.97 mg/kg, followed by apigenin-7-O-glycoside at 3513.28 mg/kg.

The conducted HPLC analysis also detected 9 phenolic acids, with rosmarinic acid (13227.10 mg/kg) being the most abundant (Table 3, Fig. 4).

DISCUSSION

When compared to data from scientific literature, it is important to highlight that the major components of the essential oil extracted from the *Satureja coerulea,* which was collected in Turkey, were sesquiterpene compounds.[24] The most

Table 3. The quantities of phenolic acids in the studied *Satureja coerulea* herb analyzed using the HPLC method

Common names and synonyms	Retention time, min	Content, mg/kg
p-Hydroxyphenylacetic acid (HPA)	9.330	125.36
Chorogenic acid (3-O-Caffeoylquinic acid)	11.167	993.07
Caffeic acid (3,4-Dihydroxycinnamic acid)	12.208	289.65
Syringic acid (4-hydroxy-3,5-dimethoxybenzoic acid)	14.369	113.91
p-Coumaric acid (p-Hydroxycinnamic acid)	16.262	112.59
trans-Ferulic acid (4-Hydroxy-3-methoxycinnamic acid)	18.038	97.83
Rosmarinic acid (Labiatenic acid)	20.765	13227.10
trans-Cinnamic acid (3-Phenylpropenoic acid)	22.941	104.83
Quinic acid (L-Quinate)	27.085	176.85

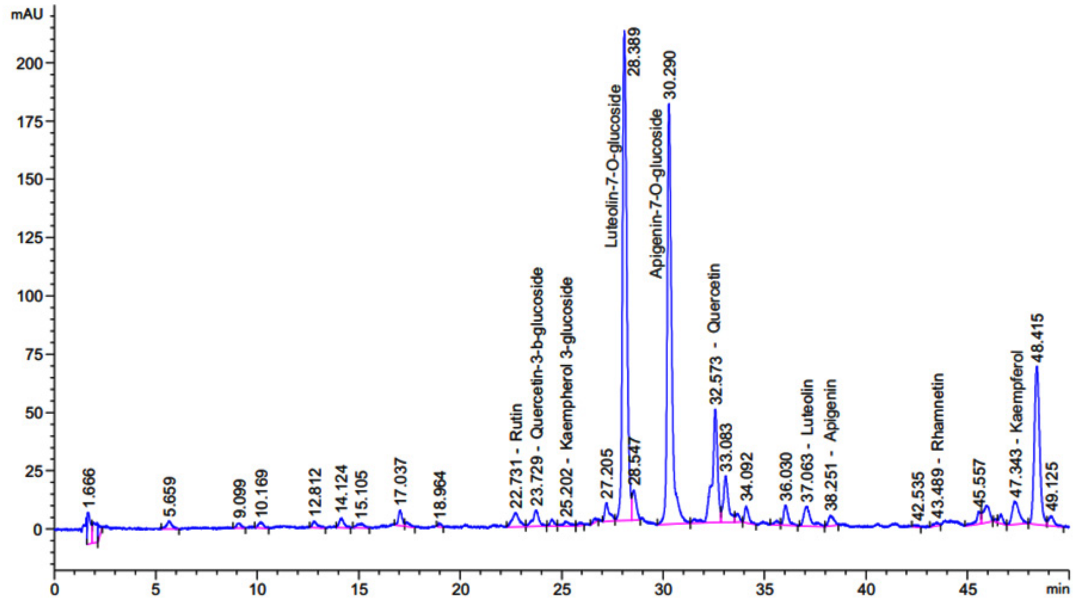

Fig. 3. HPLC chromatogram of flavonoids detected in the *Satureja coerulea* herb

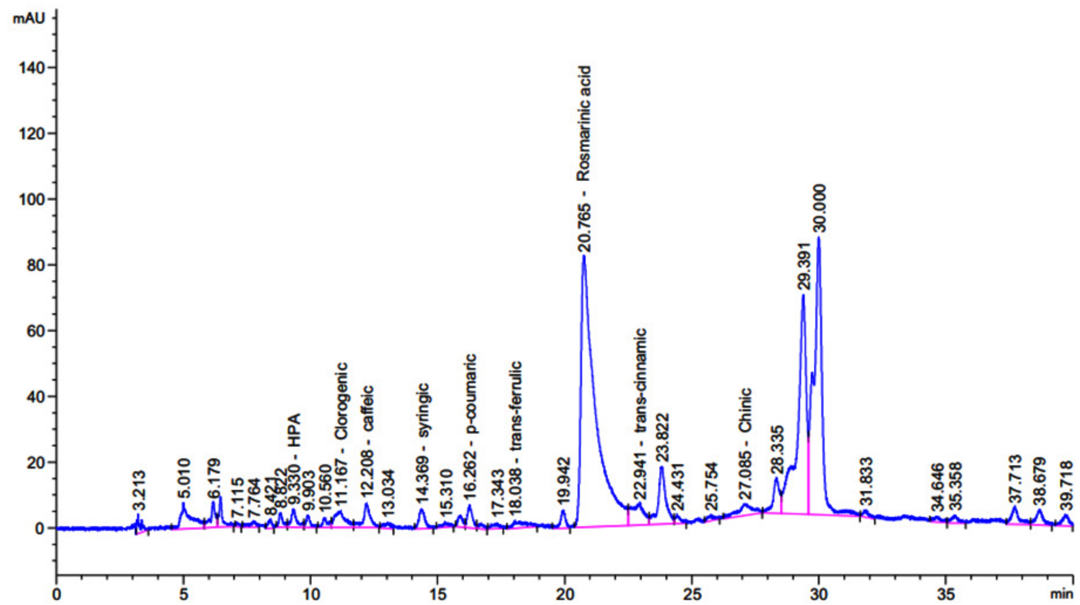

Fig. 4. HPLC chromatogram of phenolic acids detected in the *Satureja coerulea* herb

abundant of these are *â*-caryophyllene, making up 10.6%, and caryophyllene oxide, which accounts for 8.0%.

As for data on other species of the genus *Satureja*, it was found that the essential oils of *Satureja rechingeri*, an endemic species from Iran, collected at various phenological stages, primarily contained carvacrol (83.6%-90.4%), *ã*-terpinene (0.6%-2.4%) and *p*-cymene (0.8%-2.9%).[25] The essential oil of *Satureja montana* subsp. *variegata*, collected in Italy, contained a significant amount of carvacrol (22.5%), followed by *p*-cymene (17.6%) as well as thymol (17.4%). In contrast, *Satureja montana* subsp. *montana* was characterized by a very high carvacrol content (61.9%), but had much lower levels of *p*-cymene (9.9%) and thymol (0.2%).[26]

In the essential oil of *Satureja metastasiantha* from Turkish flora, the major compounds were *p*-cymene (22.3%) and thymol (21.0%) followed by carvacrol (18.4%) and *ã*-terpinene (12.1%).[15] *p*-Thymol constituted approximately 81.79% of the essential oil from *Satureja montana* aerial part collected in Ukraine.[19] Other predominant components included linalool (2.09%), *ã*-terpinene (1.65%), *o*-cymene (1.26%), etc. Thus, in the essential oils of most species of the genus *Satureja* there is a dominance of aromatic monoterpenoids. Besides it, it was found that essential oils derived from *Satureja hortensis* (thymol 1.7%; carvacrol 46.6%) as well as from *Origanum vulgare* (thymol 0.4%; carvacrol 66.2%) and *Thymus vulgaris* (thymol 4%; carvacrol 33.9%) contained high levels of aromatic compounds.[27] These essential oils demonstrated promising antioxidant activities, making them increasingly attractive as alternatives to synthetic antioxidants.

As is well known, bioactive compounds of secondary synthesis, especially terpenoids, have much greater chemotaxonomic significance compared to primary plant metabolites from the *Lamiaceae* representatives.[28-30] Thus, the study conducted by Mertzanidis *et al.*[29] in Greece found the distinct chemotaxonomic differences in the essential oil composition of two subspecies of *Origanum vulgare*: *Origanum vulgare* subsp. *hirtum*, which has white flowers, and *Origanum vulgare* subsp. *vulgare* with purple flowers. The first subspecies was noted for its high carvacrol content, reaching up to 92.6%. In contrast, the second subspecies was characterized by a dominance of sesquiterpenes, including *â*-caryophyllene, *ä*-cadinene, D-germacrene, and *â*-bisabolene. Fourteen Croatian populations of *Satureja montana* and *Satureja subspicata* (seven for each species) were examined to determine the chemical composition of their essential oils, as it was reported by Dunkiæ et co-authors.[30] In all populations of *Satureja montana*, the main constituents of the essential oil were carvacrol and thymol. In contrast, *Satureja subspicata* primarily contained sesquiterpenoids such as spathulenol, á-eudesmol, and â-eudesmol. Additionally, three distinct chemotypes of *Satureja subspicata* were identified. It was waund that carvacrol was the main volatile compound in all four studied cultivars of *Satureja montana* cultivated in Southern Ukraine (58.3-87.0%). *p*-Cymene was the second most prevalent component (5.0-8.8%) in the essential oil across all cultivars.[31]

It has been determined that the aromatic terpenoids are responsible for the high level of antimicrobial effect observed in some species of the *Lamiaceae* family. Thus, the results of the *in vitro* studies of antimicrobial effect of *Satureja khuzestanica* and the main essential oil components thymol, carvacrol, and eugenol suggested their potency against *Trichomonas vaginalis*.[32] The essential oil of *Satureja thymbra*, along with its main constituents carvacrol, thymol, and *ã*-terpinene, demonstrated excellent antimicrobial activity against the tested microorganisms, especially against fungi.[33] *In vitro* research conducted by Harmati *et al*.[34] demonstrated that a binary mixture of volatile fractions from the *Satureja hortensis* and *Origanum vulgare* herbs was effective in reducing *Helicobacter pylori* colonization. Generally, the noticeable antifungal and antimicrobial activities of thymol are unquestionable.[35]

Recent study demonstrated that essential oil *Satureja hortensis* protected rats' vital organs against deleterious effects of lead acetate[1]. Ebadollahi *et al*.[36] concluded that essential oils from various *Satureja* species have significant potential for managing detrimental ticks, insects, and nematodes. Recent conclusions by Hassanpour *et al*.,[37] based on a conducted meta-analysis, indicate that within the *Lamiaceae* family, the herbal substances from the species of genera *Satureja* and *Coleus* showed significant effects in reducing triglyceride and low-density lipoprotein/cholesterol levels.

It is worth noting that predominant flavonoids that were revealed by us, like other flavone derivatives, have promising antioxidant, anti-inflammatory, antitumor and other valuable properties.[38] According to Abdelshafeek *et al*.,[39] the alcoholic extract of *Satureja montana* which was rich in polyphenols exhibited promising antioxidant, and anti-apoptotic potential. Molecular docking studies of the isolated phenolic and terpenic compounds demonstrated significant binding affinity of rhis herbal medicine against several examined enzymes.

Notably, the prevalence of rosmarinic acid and certain flavone derivatives is characteristic of other members of the *Nepetoideae* Burnett. subfamily within the *Lamiaceae* family.[3,4,6] As it was found previously[6] by using the HPTLC method, rosmarinic acid dominated among hydroxycinnamic acids in all 5 studied species (*Dracocephalum moldavica* L., *Monarda fistulosa* L., *Ocimum americanum* L., *Lophanthus anisatus* (Nutt.) Benth., and *Satureja hortensis*) harvested in Ukraine. Its content was quite high in the herb of *Satureja hortensis* (1.877 %). Two flavone glycosides and eight oligomers of caffeic acid were isolated from the methanolic extract of *Satureja biflora* leaves, with rosmarinic acid identified as the principal compound.[40]

It is worth noting that rosmarinic acid and other phenolic acids found in *Satureja coerulea* herb can exhibit significant immunomodulatory, antioxidant, hepatoprotective and chemopreventive properties, among other therapeutic benefits.[41,42] The study of Haouat *et al*.[43] found a lot of phenolic compounds in the herb of endemic species *Satureja hispidula* (Boiss. & Reut.) Govaerts, which grows in Algeria. It was demonstrated that both ethanolic and aqueous extracts exhibit high antioxidant activity and effectively inhibit *á*-glucosidase. The aqueous extract contained 67.13 µg/mg of caffeoylquinic acids while ethanolic 27.57 µg/mg. These findings explained the use of *Satureja hispidula* in Algerian traditional medicine for treating hyperglycemic conditions.

CONCLUSIONS

The conducted GC/MS analysis indicated that the main components of the *Satureja coerulea* essential oil cultivated in Ukraine decreased in the following order: thymol (33.18%) > *o*-cymene (14.42%) > terpinen-4-ol (8.30%) > *ã*-terpinene (7.25%). The HPLC analysis identified the presence of nine phenolic acids, with rosmarinic acid being the most dominant compound (13227.10 mg/kg). Additionally, ten flavonoids were revealed, with the predominance of luteolin-7-O-glucoside (7518.97 mg/kg) and apigenin-7-O-glucoside (3513.28 mg/kg).

The findings of this study highlight the the unique chemotaxonomic characteristics of the studied species and its considerable potential for further pharmacological research of the essential oil and extract derived from the aerial part of *Satureja coerulea*. These substances may serve as a valuable source of various bioactive compounds for developing new phytomedicines in the pharmaceutical industry.

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Conflict of interest

The authors declare no conflict of interest.

Data Availability

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Authors' Contribution

Mariia Shanaida: Conceptualization, Methodology, Analyses, Writing – Original Draft. Olha Korablova: Cultivation and gathering raw material, Writing – Review & Editing. Daryna Bakalets: Visualization, Reference search and interpretation of the data. Nataliya Potikha: Writing – Review & Editing. Dzhamal Rakhmetov: Supervision, Project Administration.

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