**ESSENTIAL OIL COMPOSITION OF TWO SPECIES OF SCUTELLARIA AERIAL PARTS**

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The chemical composition of essential oils obtained by hydrodistillation method from two plants of the genus *Scutellaria*, grown in Uzbekistan and used in folk medicine were comparatively investigated by GC/MS and FID. Overall individually thirty three constituents were identified in both of aerial parts of *S. adenostegia* and *S. comosa* essential oils, representing 94.4 and 97.0% of the total, respectively. The main components were determined as acetophenone (24.2%), eugenol (12.3%), caryophyllene oxide (9.7%), and β-caryophyllene (7.0%) in the oil of *S. adenostegia*. β-Caryophyllene (12.5%), phytol (11.4%), linalool (11.1%), acetophenone (10.4%), caryophyllene oxide (6.6%), 1-hexanol (5.3%), and (E)-2-hexenal (5.1%) were found as major components in the *S. comosa* oil. The composition of the oils of *S. adenostegia* and *S. comosa* was being reported for the first time. The essential oils of *S. adenostegia* and *S. comosa* showed significant antimicrobial properties against *Bacillus subtilis*, moderate effect against *Salmonella enterica* and *Escherichia coli*.

**Keywords**: Scutellaria adenostegia; Scutellaria comosa; essential oil; GC/MS/FID; antibacterial activity.

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**Introduction**

The genus of *Scutellaria* L., skullcaps, "Ko’kamaron" (local name) which belongs to the family *Lamiaceae*, is represented by 360 species and is widely spread in mild, subtropical, and tropical regions of the world, including Europe, North America, and Eastern Asia [1]. Approximately 120 species and subspecies of the genus grow across the countries of Commonwealth of Independent States (CIS), mainly in the Caucasus Mountains and in Middle Asia [1, 2]. Skullcaps are perennial or, very rarely, annual grasses, rarely subshrubs or half-shrubs. Many of the skullcap species are decorative plants, some are medicinal herbs, but all belong to dyeing plants. In Uzbekistan, there are thirty eight species of the *Scutellaria* L. genus plants. It is used in Uzbekistan in treatment of inflammation, chorea, nervous tension and high-blood pressure [3]. Chemical composition of
plants of the *Scutellaria* L. genus is diverse. Earlier, flavonoids, phenylpropanoids, phenolic acids, iridoids, clerodane diterpenoids, steroids, triterpenes, lignans, alkaloids, phytosterols, polysaccharides, tannin substances, essential oils, and other classes of natural compounds have been isolated from different species [1, 2, 4]. *S. adenostegia* Briq. is a perennial native plant growing on rocky and clay mountain slopes, dried up riverbeds and streams, rocky placers and gravels along the banks of the rivers of Tian-Shan, Pamir-Alai Mountains (Central Asia). Previous phytochemical studies on this plant reported the isolation and identification of flavonoids [3]. *S. comosa* Juz. mainly occurring in the Tian Shan and Pamir-Alai Mountains, it is a perennial shrub species which endemic to the Central Asia. Flavonoids on this plant species have been extensively studied [3, 5]. Several authors reported on the study of essential oils of plants of *Scutellaria* genus [1, 6–10].

It was previously reported that essential oils isolated from plants of this genus possess antioxidant and antibacterial activities [10–14]. In the literature, there is a report on the study of the component composition and antioxidant activity of essential oils of three species of plants of the genus *Scutellaria*, growing in Uzbekistan [10]. But until now, there are no published reports concerning the phytochemistry and biological activities of the essential oils of *S. adenostegia* and *S. comosa*. We have reported here the isolation and characterization of essential oils, which to the best of our knowledge, is the first investigation on volatile compositions of the aerial parts of two *Scutellaria* species.

**Experimental**

The aerial parts (stems, leaves, flowers) of *S. adenostegia* and *S. comosa* employed in this investigation were collected in the flowering stage (May, 2019) from Chust (41°00′00″ N 71°13′59.88″ E) and Turukhansk District (41°00′00″ N 71°30′56.88″ E) Namangan Region of Uzbekistan respectively. The plants were identified at the Flora of Uzbekistan Department, Institute of Botany (Uzbekistan) by Dr. O.T. Turginov. The voucher specimens of *S. adenostegia* (accession number (A.N.) N20190550) and *S. comosa* (A.N. N20190551) have been deposited at the Flora of Uzbekistan Department. Isolation of the essential oil. The air-dried aerial parts (moisture content was 11–13% w.b.) of the *S. adenostegia* and *S. comosa* were hydrodistilled three times (3×100 g each) for 3 h, using a Clevenger-type apparatus. Further hydrodistillation of plant raw materials did not lead to an increase in the yield of essential oil. The obtained essential oils were then dried using anhydrous sodium sulfate and stored at 4 °C in the dark until use. The essential oil content was calculated as a relative percentage (v/w) of the dry plant material.

**GC and GC/MS analysis of essential oils.** The qualitative and quantitative composition of the essential oils were determined on an Agilent 5977B MSD/8890A GC (Agilent Technologies, USA) gas chromatography-mass spectrometer equipped with flame ionization detector (FID) and an Agilent 7693A ALS autoinjector. The components of the mixture were separated on an Agilent DB-Wax quartz capillary column (30 m × 250 μm × 0.25 μm film thickness) in the following temperature mode: 50 °C (1 min) – 4 °C/min to 200 °C (6 min) – 15 °C/min to 250 °C (35 min). The samples were prepared in dichloromethane and 1.0 μL injected in splitting mode (50 : 1). The flow rate of the mobile phase (H₂) was 1.1 mL/min. The injector temperature was 220 °C. MS conditions were as follows: ionization energy 70 eV, source temperature 230 °C, quadrupole temperature 150 °C. EI-MS spectra were obtained in the m/z range of 10–550 a.m.u.

FID was used for the quantification of the volatile compounds. The chromatographic conditions and the column were identical to those used for the GC/MS analysis. The injector temperature was 250 °C and the carrier gas was H₂ at 1.1 mL/min.

Components of essential oils were identified by comparison of the chromatographic peaks retention times with those of authentic compounds analyzed under the same conditions, and by comparison of retention indices (as Kovats indices) with literature data [15].

Comparisons of MS fragmentation patterns with mass spectrum database search were performed using the Wiley Registry of Mass Spectral Data-9th Ed., NIST Mass Spectral Library (2011) and the Automated Mass Spectral Deconvolution and Identification System (AMDIS, Version 2.72) containing NIST14 Library. A C₉–C₃₂ n-alkane standard solution (Agilent Technologies, USA) was used for the determination of chromatographic retention indices (RI). Percent composition was obtained for each constituent on the basis of flame ionization detection analyses of the essential oils.

**Antimicrobial activity.** The essential oils antimicrobial activities were assessed against four pathogenic bacterial strains, two Gram-positive *Staphylococcus aureus* (MTCC 737) and *Bacillus subtilis* (NK-1, isolated from...
Natto) and two Gram-negative *Salmonella enterica* (ATCC 14028) and *Escherichia coli* (MTCC 1302). The above microorganisms were obtained from the Department of Microbiology of the Medical Institute of Surgut State University. The assay was performed using a 96-well microtiter plate-based method with resazurin as a cell growth indicator following a previously used method with minor adjustments [16].

**Statistical analysis.** Results were expressed as mean ± standard deviation. Statistical comparisons were performed with Student’s *t*-test using GraphPad Prism version 7.00. Differences were considered significant at *p* < 0.05.

**Results and discussion**

**Chemical composition of essential oils.** The average yields of essential oils obtained from three independent determinations by the hydrodistillation method were 0.19% (v/w; *S. adenostegia*; light yellow), and 0.17% (v/w; *S. comosa*; yellow) on a dry weight. The chemical compositions of the essential oils isolated from the air-dried aerial parts of plants were investigated by GC/MS/FID. Chromatographic profile of the volatiles from two *Scutellaria* species on the DB-Wax column is presented on Figure and Table 1 show the composition of compounds identified from the studied oil samples. Totally thirty three compounds representing 94.4% of the total oil were characterized in essential oil of *S. adenostegia*. The classes of compounds present in *S. adenostegia* were aldehydes and ketones (35.2%), phenols (16.0%), alcohols (12.4%), sesquiterpene hydrocarbons (12.3%) and oxygenated sesquiterpenes (11.0%).

The main compounds of *S. adenostegia* were acetophenone (24.2%), eugenol (12.3%), caryophyllene oxide (8.9%), and β-caryophyllene (7.0%). Moreover, high amount of 1-hexanol (3.8%), furfural (3.3%) and γ-himachalene (2.7%) were also observed in essential oil. It should be noted that acetophenone was also the main component of the essential oils of *S. immaculate* and *S. schachristanica* [10]. The total phenolic compounds content in essential oil of this plant was 13.3%, of which 11.8% was eugenol. *S. adenostegia* essential oil were rather poor in content of oxygenated monoterpenes (3.0%).
Chromatographic profile of the volatiles from essential oils of *Scutellaria adenostegia* Briq. (upper) and *Scutellaria comosa* Juz. (lower)

Table 1. Essential oil composition of two *Scutellaria* species

| No | Compounds                                      | RT, min | LRI*  | *S. adenostegia*, (Conc., %) | *S. comosa*, (Conc., %) |
|----|-----------------------------------------------|---------|-------|-----------------------------|--------------------------|
| 1  | 1,8-Cineol                                     | 9.231   | 1192  | –                           | 0.9                      |
| 2  | (E)-2-Hexenal                                  | 9.495   | 1202  | 1.6                         | 5.1                      |
| 3  | 1-Hexanol                                      | 13.466  | 1341  | 3.8                         | 5.3                      |
| 4  | (Z)-3-Hexen-1-ol                               | 14.364  | 1369  | –                           | 2.0                      |
| 5  | 1-Octen-3-ol                                   | 16.315  | 1433  | –                           | 3.9                      |
| 6  | Furfural                                       | 16.584  | 1442  | 3.3                         | –                       |
| 7  | α-Cubenene                                     | 17.465  | 1472  | 0.7                         | 0.8                      |
| 8  | Pentadecane                                    | 17.637  | 1500  | –                           | 1.4                      |
| 9  | Camphor                                        | 18.158  | 1516  | –                           | 1.7                      |
| 10 | Benzaldehyde                                   | 18.301  | 1520  | 1.8                         | 1.1                      |
| 11 | Linalool                                       | 19.142  | 1549  | 1.1                         | 11.1                     |
| 12 | 1-Octanol                                      | 19.491  | 1560  | 1.9                         | 1.5                      |
| 13 | (E)-β-Caryophyllene                            | 20.475  | 1593  | 7.0                         | 12.5                     |
| 14 | Acetophenone                                   | 21.923  | 1624  | 24.2                        | 10.4                     |
| 15 | α-Caryophyllene                                | 22.461  | 1644  | 1.1                         | 1.7                      |
| 16 | α-Terpin-ol                                    | 23.319  | 1676  | 0.6                         | 1.2                      |
| 17 | γ-Himachalene                                  | 23.886  | 1697  | 2.7                         | 2.4                      |
| 18 | δ-Cadinene                                     | 24.841  | 1733  | 0.8                         | 0.5                      |
| 19 | Grandulere II                                  | 27.290  | 1829  | –                           | 0.7                      |
| 20 | (Z)-Geranylacetone                             | 27.387  | 1833  | 0.5                         | –                       |
| 21 | Benzyl alcohol                                 | 27.960  | 1856  | 2.1                         | 1.3                      |
| 22 | 2-Phenylethanol                                | 28.835  | 1892  | 1.2                         | 0.7                      |
| 23 | (E)-β-Ionone                                   | 29.476  | 1918  | 0.4                         | 0.5                      |
| 24 | Caryophyllene oxide                            | 30.546  | 1961  | 8.9                         | 6.6                      |
| 25 | Alloaromadendrene oxide-(1)                    | 30.866  | 1974  | –                           | 0.5                      |
| 26 | α-Cresol                                       | 31.038  | 1981  | 1.6                         | –                       |
| 27 | (Z)-Nerolidol                                  | 31.845  | 2015  | 0.6                         | –                       |
| 28 | 4-Phenyl-3-butene-2-one                        | 33.573  | 2092  | 0.4                         | –                       |
| 29 | Hexahydrofarnesyl acetone                      | 33.882  | 2120  | 3.0                         | 2.1                      |
| 30 | Eugenol                                        | 34.769  | 2145  | 12.3                        | 1.3                      |
| 31 | α-Muurol                                       | 35.101  | 2159  | 0.6                         | 0.9                      |
| 32 | 9β-Acetoxy-3,4,8-trimethyltricyclo[6.3.1.0(1,5)]dodec-3-ene | 35.272 | 2167  | –                           | 0.8                      |
| 33 | p-Vinylguaiacil                                | 35.393  | 2172  | 1.3                         | 0.6                      |
| 34 | Caryophylla-(4,12),(8,13)-dien-5α-ol            | 37.647  | 2272  | 1.5                         | 1.2                      |
| 35 | Dihydroactinidiolide                           | 38.471  | 2309  | 1.3                         | 1.8                      |
| 36 | 8-Cedren-13-ol                                 | 39.335  | 2347  | –                           | 0.9                      |
| 37 | 2,3-Dihydro-benzofuran                         | 39.650  | 2361  | 2.1                         | –                       |
| 38 | Coumarin                                       | 40.863  | 2416  | 0.7                         | –                       |
| 39 | Pentacosane                                    | 42.528  | 2500  | 0.8                         | 1.1                      |
| 40 | Phytol                                         | 45.601  | 2643  | 2.8                         | 11.4                     |
| 41 | Acetovanillone                                 | 46.253  | 2675  | 0.8                         | –                       |
| 42 | Methoxyacetic acid 2-pentadecyl ester          | 47.260  | 2723  | 0.9                         | 1.1                      |

Oxygenated monoterpenes – 1, 9, 11, 16, 19, 35.
Sesquiterpene hydrocarbons – 7, 13, 15, 17, 18.
Oxygenated sesquiterpenes – 24, 25, 31, 32, 34, 36.
Aldehides and ketones – 2, 6, 10, 14, 20, 23, 28, 29.
Alcohols – 3, 4, 5, 12, 21, 22, 27, 40.
Phenols – 26, 30, 33, 41.
Others – 8, 37, 38, 39, 42.
Total
Identified compounds

*LRI* – Linear retention indices on DB-Wax column; Conc., % calculated from FID data.

In the essential oil of *S. comosa* thirty five components, representing 97.0% of the total ones were characterized (Tab. 1). In the studied essential oil alcohols (26.1%) dominated. Aldehydes and ketones (19.2%), sesquiterpene
hydrocarbons (17.9%), oxygenated monoterpenes (17.4%) and oxygenated sesquiterpenes (10.9%) were the remaining groups of components (Tab. 1).

β-Caryophyllene (12.5%), phytol (11.4%), linalool (11.1%), acetophenone (10.4%), caryophyllene oxide (6.6%), 1-hexanol (5.3%), and (E)-2-hexenal (5.1%) were found as the main constituents. Literature information indicated that β-caryophyllene was the main component of the essential oils of S. brevibracteata [6], S. sibthorpii [7], S. luteo-caerulea [9], S. albida [12] and other species. Acetophenone was the main component of S. schachristanica and S. immaculata essential oil [10], while linalool was determined as a major component for S. cypria var. elatior [7]. In total, forty four volatile compounds were identified in two Scutellaria species from Uzbekistan. Aldehydes and ketones, alcohols, phenols and sesquiterpene hydrocarbons are the dominant components of the essential oil of the plant S. adenostegia. Sesquiterpene hydrocarbons and oxygenated monoterpenes were the major group of terpenes found in the essential oil of S. comosa. On the contrary, monoterpene hydrocarbons were not detected in both oil of S. adenostegia and S. comosa.

Evaluation of antibacterial activity. The literature contains data on the antimicrobial activity of essential oils of some plants of the Scutellaria genus [7, 11–14]. That is, we have studied the antibacterial properties of the isolated essential oils. The essential oils of S. adenostegia and S. comosa showed significant antimicrobial properties against Bacillus subtilis (318.0±8.62 and 401.1±14.49 µg/mL), moderate effect against Salmonella enterica (519.4±16.29 and 803.1±31.62 µg/mL) and Escherichia coli (528.3±14.63 and 802.4±32.57 µg/mL), and weak effect against Staphylococcus aureus (1297.6±34.78 and 1676.3±52.94 µg/mL) respectively (Table 2). We are inclined to believe that the antibacterial activity of essential oils is due to the presence of β-caryophyllene and eugenol in their composition [17, 18]. Pure β-caryophyllene showed more pronounced antibacterial activity against Gram-positive bacteria than Gram-negative bacteria [17]. Overall, the antibacterial activity of the essential oils can be related to the content of many of the compounds identified in the oils, including eugenol and β-caryophyllene.

Table 2. Minimum inhibition concentration (MIC) of essential oils of S. adenostegia and S. comosa against four bacterial strains

| Samples          | MIC (µg/mL)          |
|------------------|----------------------|
|                  | E. coli | S. aureus | B. subtilis | S. enterica |
| S. comosa.       | 25.8±1.23 | 3.1±0.12 | 25.8±1.07 | 25.8±1.28 |
| S. comosa.       | 528.3±14.63 | 1297.6±34.78 | 318.0±8.62 | 519.4±16.29 |
| S. comosa.       | 802.4±32.57 | 1676.3±52.94 | 401.1±14.49 | 803.1±31.62 |

All results are presented as mean±standard deviations for triplicate assays. Reference.

Conclusions

For the first time, the chemical composition of the essential oils grown in Uzbekistan of two Scutellaria species was studied. Acetophenone and β-caryophyllene are the dominant terpenes of the essential oils of S. adenostegia and S. comosa. On the other hand the high amount of eugenol and caryophyllene oxide in essential oil of S. adenostegia and linalool, phytol and caryophyllene oxide in essential oil of S. comosa were revealed. The essential oils of these plants showed significant antibacterial properties against Bacillus subtilis, moderate effect against Salmonella enterica and Escherichia coli.

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