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ABOVEGROUND COMPONENTS OF *SALVIA SARAWSCHANICA*

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Abstract: Using the GC-MS method, 36 compounds were identified in the essential oil obtained by hydrodistillation from the air-dried above-ground part of *Salvia sarawschanica* by Regel et Schmalh, which constituted 95.1% of the total oil. The main components of the EO are 1,8-cineole, camphene, camphor, aromadendrene and limonene, the content of which was 33.8, 12.3, 6.8, 5.2 and 4.9%, respectively. Four individual phenolic compounds were isolated from various fractions of the 80% alcoholic extract of the roots of *S. sarawschanica*, which, based on the study of the ^{1}H, ^{13}C NMR, HSQC and HMBC spectra, were identified with acacetin, luteolin, cynaroside, luteolin-3'-O-β-D-glucuronide. Essential oil from the above-ground parts of the plant *S. sarawschanica* exhibits a noticeable antibacterial effect against the gram-positive strain of bacteria *B. subtilis*.

Keywords: *Salvia sarawschanica* Regel et Schmalh, essential oil, GC-MS analysis, flavonoids, antimicrobial activity.

Introduction. Salvia is one of the largest genera of the Lamiaceae family and is represented by more than 1000 species, widely distributed in various regions of the world [1]. There are 25 species of plants of this genus growing in Uzbekistan [2,3]. Many species of sage are known for their brightly colored flowers and some of them are used to create herbal remedies, as well as in folk and traditional medicine of various countries [4-8]. Sage species have long been known for their wide range of applications in folk medicine for pain relief, protection from oxidative stress, free radical damage, angiogenesis, inflammation, and bacterial and viral infection [5,6,7-9]. Sage is grown for its aromatic properties and the abundance of secondary metabolites, which are used to produce food additives and essential oils, pharmaceuticals, dyes, cosmetics, and biocides [6,9,10]. Several species of sage (*S. sclarea, S. officinalis* and *S. fruticosa*) and their preparations are included in the European Pharmacopoeia [9]. Species of sage are also known for a wide range of medicinal uses in folk medicine. The roots of *S. miltiorrhiza* are used in traditional Chinese medicine to treat circulatory disorders [5,7,8]. *S. sclarea* is used as a remedy for night sweats associated with menopause or tuberculosis, and is also used in perfumery as a flavoring agent [7]. Extensive studies of the chemical components of plants of this genus have revealed the presence of sesquiterpenoids, diterpenoids, sesterterpenoids,

triterpenoids, steroids, flavonoids, phenylpropanoids, phenolic acids and other classes of natural compounds [4-8,11-16]. Substances isolated from representatives of the genus *Salvia* are characterized by significant cytotoxic, anti-inflammatory, antimicrobial, antiviral, antiplasmodial, antiplatelet-aggregation, growth-inhibiting and repellent activity [6-8,11-13].

Salvia sarawschanica Regel et Schmalh - grows in dry riverbeds, rocks, stony, gravelly and fine-earth slopes of the lower and middle belts of the Pamir and Pamir-Alai mountains in Central Asia and Kazakhstan [2,3,11]. The infusion is used in traditional medicine of Tajikistan for heart diseases, the extract has antiportozoic and fungistatic properties. Previously, flavonoids, phenolic acids, tannins, saponins, alkaloids, coumarins and other natural compounds were isolated from the above-ground part of this plant [11,16,17].

In order to search for biologically active compounds from local plant materials, we studied the component composition of the EO and methanol extract of the above-ground part of *S. sarawschanica.*

Methodology & empirical analysis. The aboveground part of *S. sarawschanica* used in this work was harvested during the flowering period (July, 2024) in the Navoi region. The species was identified by Cand. Sci. (Biol.) O.M. Nigmatullaev in the Laboratory of Biology of Medicinal and Technical Plants, Institute of Plant Chemistry named after Academician S.Yu. Yunusov, Academy of Sciences of the Republic of Uzbekistan.

General experimental conditions. Silica gel of the KSK brand (100/200 μm, Tianjin Sinomed Pharmaceutical, China) was used for column chromatography (CC). Sephadex of the LH-20 brand (GE Healthcare Bio-Sciences AB, Sweden) was used to separate individual eluates. Fluka plates (Sigma-Aldrich, Germany) were used for thin-layer chromatography. The plates were examined in ultraviolet light in a UFS-254/365 chromatographic irradiator at 254 and 365 nm. UV spectra were recorded on EPS-3T "Hitachi", Specord UV-Vis and SF-26 spectrophotometers in ethanol. ^{1}H and ^{13}C NMR spectra were recorded on a JNM-ECZ600R spectrometer (Jeol, Japan) at an operating frequency of 600 MHz and 150 MHz. Internal standard TMS.

Melting points of the isolated compounds were determined on an Electrothermal "MEL-TEMP®" device (Equipment, USA).

GC-MS analysis. The qualitative and quantitative composition of the essential oil was determined on an Agilent 5975C Inert MSD/7890A GC chromatograph-mass spectrometer. The components of the mixture were separated on an Agilent HP-INNOWax quartz capillary column (30 m × 250 μm × 0.25 μm) in the following temperature range: 60 °C (2 min) – 4 °C/min up to 220 °C (10 min) – 1 °C/min up to 240 °C (10 min). The volume of the introduced sample was 1.0 μm, the flow rate of the mobile phase (H2) was 1.1 ml/min. EI-MS spectra were obtained in the m/z range of 10–550 amu. The components were identified based on the comparison of mass spectral characteristics with data from electronic libraries (Wiley Registry of Mass Spectral Data-9th Ed. NIST Mass Spectral Library, 2017) and comparison of the retention indices (RI) of the compounds, determined in relation to the retention time of a mixture of n-alkanes (C9-

C39), as well as comparison of their mass spectral fragmentation with those described in the literature [18,19]. The quantitative content of EO components was calculated from the areas of chromatographic peaks.

Isolation of essential oil. EO was isolated from crushed air-dried aboveground part of *S. sarawschanica* (300 g) by hydrodistillation at atmospheric pressure for 2.5 hours. The resulting distillate was extracted with dichloromethane, the essential oil extract was dried with anhydrous sodium sulfate. EO was stored in the refrigerator until use.

Isolation of components of the aboveground part. In order to isolate the components, the crushed air-dried above-ground part of *S. sarawschanica,* harvested during the flowering period (9.4 kg), was extracted six times with methanol at room temperature. The combined extract was evaporated to dryness in a vacuum (yield 10.6% relative to the mass of the raw material), the residue was dissolved in alcohol and mixed with silica gel in a ratio of 1:1 and dried at room temperature, then in a drying cabinet at a temperature of 50-55 for 3 hours. The resulting mixture was fractionated on a column, washing successively with gasoline, chloroform, ethyl acetate, a mixture of ethyl acetate-methanol solvents (8:2). Distillation of the solvents yielded 190.0 g of gasoline, 235.0 g of chloroform, 84.5 g of ethyl acetate and 215.0 g of ethyl acetate-methanol fraction.

The chloroform fraction (250 g) was mixed with silica gel (500 g), dried and fractionated on a column, washing successively with gasoline and a mixture of gasoline-ethyl acetate solvents (9:1). The resulting subfraction in the amount of 51.4 g was chromatographed on a column with Sephadex LH-20 in the ethyl acetate-methanol (7:3) system. 20 mg of acacetin were isolated from the eluates. The ethyl acetate fraction was chromatographed on a column with silica gel (100 g) and washed with chloroform to obtain 38.25 g of subfraction 1, then washing with a mixture of chloroform-methanol solvents (9:1) - subfraction 2 in the amount of 44.64 g. Subfraction 2 in the amount of 44.64 g was also subjected to gel filtration on Sephadex LH-20 in methanol and 2.03 g of luteolin were isolated from the eluates. The ethyl acetate-methanol fraction (215.2 g) was mixed with silica gel (250 g), dried and chromatographed on a column, washing with a solvent mixture of ethyl acetate and methanol (9:1). The resulting subfraction (80.5 g) was subjected to gel filtration on Sephadex LH-20 in the ethyl acetate-methanol (7:3) system. 120 mg of cynaroside and 65 mg of luteolin-3'-O-β-D-glucuronide were isolated from the eluates.

Determination of antibacterial and antifungal activity. To determine the antibacterial and antifungal activity of EM, a modified disk diffusion method in agar was used [20]. The following microorganism strains were used as test cultures: gram-positive bacteria - *Bacillus subtilis* (RKMUz - 5), *Staphylococcus aureus* (ATCC 25923); gram-negative bacteria - *Pseudomonas aeruginosa* (ATCC 27879), *Escherichia coli* (RKMUz - 221) and the fungal strain *Candida albicans* (RKMUz - 247). The RKMUz strains were obtained from the collection of the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan. Ampicillin, ceftriaxone and fluconazole (Himedia Laboratories Pvt. Limited) were used as a positive control, and dichloromethane as a negative control.

The results obtained and their discussions. Using the GC-MS method, 36 compounds were identified in the EO from the air-dried above-ground part of *S. sarawschanica,* which constituted 95.1% of the total amount of essential oil (Table 1). The EO is dominated by oxidized monoterpenes (41.5%) and monoterpenes (32.7%). Sesquiterpenes make up 10.3% of the essential oil, oxidized sesquiterpenes were not detected. The main components of the essential oil are 1,8-cineole, camphene, camphor, aromadendrene and limonene, the content of which was 33.8, 12.3, 6.8, 5.2 and 4.9%, respectively. 1,8-Cineole is used for respiratory diseases such as bronchitis or colds of the respiratory tract, chronic and inflammatory respiratory diseases, asthma and hay fever [21]. The aboveground part of *S. sarawschanica* can serve as a rich source of 1,8-cineole.

Table 1. Component composition of essential oil *Salvia sarawschanica*

Constituent	RT*	RI*	Content, %
1,8-Cineol	2.833	1172	33.8
D-Camphene	2.994	1189	12.3
2-Methyl-5-isopropenylfuran	3.240	1202	0.1
м-Cymene	3.615	1211	0.6
Furfural	7.257	1298	0.7
5- Methylfurfural	7.943	1423	0.6
Camphor	8.422	1441	6.8
L- Camphene	10.220	1510	6.5
2,5-Dihydro-3-methylfuran	10.718	1528	0.3
p-Mentha-3,8-diene	11.003	1538	3.3
2-Isopropylidene-3-methylhexa-3,5-dienal	11.281	1548	0.6
Alloaromadendrene	12.154	1580	2.4
α-Pinene	12.762	1602	3.2
Cosmene	13.124	1616	0.3
Limonene	13.512	1631	4.9
Ethylidenecyclopropane	14.424	1666	0.5
3,4-Epoxy-2-methyl-1-butene	14.509	1669	0.3
1,5,8-p-Mentatriene	15.544	1708	0.3
Dehydro-p-cymene	16.986	1765	0.1
1-Allyl-2-methylbenzene	17.090	1769	0.7
Aromadendrene	20.175	1847	5.2
Isolongifolene	23.707	2045	0.3
Eugenol	24.140	2065	1.8
Carvacrol	25.240	2114	0.2
2-Methoxy-4-vinylphenol	25.525	2127	3.3
β-Selinen	25.867	2143	0.8
(3aR,7aR)-7a-Methyl-2,3,3a,4,6,7-hexahydro-1H-inden-5-one	26.864	2189	0.9
γ-Neolovene	27.109	2200	0.5
α-Longipinene	27.271	2207	0.5
Geranyl-p-cymene	27.556	2221	0.3

4,4-Dimethyl-3-(3-methylbut-3-enylidene)-2-methylidene-bicyclo[4.1.0]heptane	28.843	2282	0.6
2,3-Dihydrobenzofuran	29.095	2294	0.1
1-Decene	31.327	2403	0.8
Benzylmethyl ether	34.296	2560	0.3
(1E,3Z,6E,10Z,14S)-3,7,11-Trimethyl-14-propan-2-yl-cyclotetradeca-1,3,6,10-tetraene	34.484	2570	0.4
Z-3-Tetradecene	39.161	2826	0.8
Monoterpenes			**32.7**
Oxidized monoterpenes			**41.5**
Sesquiterpenes			**10.3**
Others			**10.6**
Total			**95.1**

From different fractions of the 80% methanol extract of the aerial part of *S. sarawschanica,* six individual compounds were isolated, which, based on the study of ^{1}H, ^{13}C NMR, HSQC and HMBC spectra, were identified with acacetin, luteolin, cynaroside, and luteolin-3'-O-β-D-glucuronide.

Acacetin (5,7-dihydroxy-4'-methoxyflavone, 1). УФ-спектр (λ_{max}, EtOH, nm): 270, 329. ^{1}H NMR (600 MHz, C_5D_5N, ppm, δ, J/Hz): 3.79 (3H, s, 4'-OCH_3), 6.64 (1H, d, *J*=2.0, H-6), 6.73 (1H, d, *J*=2.0, H-8), 6.96 (1H, s, H-3), 7.29 (2H, d, *J*=8.8, H-3', 5'), 7.96 (2H, d, *J*=8.8, H-2', 6'). ^{13}C NMR spectrum (150 MHz, C_5D_5N, δ, ppm): 165.31 (C-2), 104.53 (C-3), 183.32 (C-4), 163.23 (C-5), 99.06 (C-6), 166.33 (C-7), 93.34 (C-8), 158.58 (C-9), 106.37 (C-10), 122.62 (C-1'), 129.48 (C-2',6'), 117.40 (C-3',5'), 163.28 (C-4') 56.38 (4`-OCH_3) [22].

Luteolin (5,7,3',4'-tetrahydroxyflavone, 2). Mp 227-229 °C, UV spectrum (λ_{max}, EtOH, nm): 257, 265, 356; ^{1}H NMR (600 MHz, ДМСО-d_6+CCl_4, ppm, δ, J/Hz): 6.11 (1H, d, *J*=2.0, H-6), 6.34 (1H, d, *J*=2.0, H-8), 6.47 (1H, s, H-3), 6.85 (1H, d, *J*=8.3, H-5'), 7.29 (1H, dd, *J*=8.3, 2.1, H-6'), 7.32 (1H, d, 2.1, H-2'), 9.13 (1H, s, 3'-OH), 9.48 (1H, s, 4'-OH), 10.41 (1H, s, 7-OH), 12.81 (1H, s, 5-OH). ^{13}C NMR spectrum (150 MHz, ДМСО-d_6+CCl_4, δ, ppm): 163.66 (C-2), 102.67 (C-3), 181.34 (C-4), 161.61 (C-5), 98.65 (C-6), 163.89 (C-7), 93.43 (C-8), 157.18 (C-9), 103.73 (C-10), 121.59 (C-1'), 113.05 (C-2'), 145.49 (C-3'), 149.33 (C-4'), 115.67 (C-5'), 118.27 (C-6') [23].

Cynaroside (luteolin-7-O-β-D-glucopyranoside, 3). Yellow crystals with mp 256–259°C. ^{1}H NMR (600 MHz, DMSO-d_6+CCl_4, ppm, δ, J/Hz): 3.22 (1H, m, 4''), 3.30 (1H, m, H-2''), 3.31 (1H, m, H-3''), 3.41 (1H, ddd, J = 9.7, 5.7, 2.3, H-5''), 3.53 (1H, dd, J = 11.6, 5.6, H-6''a), 3.75 (1H, dd, J = 11.6, 2.3, H-6''b), 4.46 (1H, br.s, OH), 4.97 (1H, d, J =7.4, H-1''), 5.26 (1H, br.s, OH), 6.39 (1H, d, J =2.2, H-6), 6.58 (1H, s, H-3), 6.72 (1H, d, J =2.2, H-8), 6.86 (1H, d, J =8.4, H-5'), 7.34 (1H, dd, J =8.4, 2.3, H-6'), 7.36 (1H, d, J =2.3, H-2'), 9.29 (1H, br.s, OH), 9.60 (1H, br.s, OH), 12.88 (1H, s, 5-OH). ^{13}C NMR spectrum (150 MHz, DMSO-d_6+CCl_4, δ, ppm): 164.35 (C-2), 102.90 (C-3), 181.60 (C-4), 161.24 (C-5), 99.53 (C-6), 162.88 (C-7), 94.49 (C-8), 156.84 (C-9), 105.38 (C-10), 121.28 (C-1'), 113.19 (C-2'), 145.59 (C-3'), 149.69 (C-4'),

115.76 (C-5'), 118.60 (C-6'), 100.16 (C-1''), 72.88 (C-2''), 73.33 (C-3''), 69.48 (C-4''), 77.02 (C-5''), 60.70 (C-6'') [23].

Luteolin-3'-O-β-D-glucuronide (4). ^{1}H NMR (600 MHz, DMSO-d_6+CCl_4, ppm, δ, J/Hz): 3.33 (2H, m, H-3'', 4''), 3.36 (1H, m, H-2''), 3.54 (1H, m, H-5''), 4,79 (1H, d, J=7.7, H-1''), 6.08 (1H, d, J=2.1, H-6), 6.48 (1H, d, J=2.1, H-8), 6.65 (1H, s, H-3), 6.93 (1H, d, J=8.4, H-5'), 7.54 (1H, dd, J=8.4, 2.2, H-6'), 7.92 (1H, d, J=2.2, H-2'), 12.78 (1H, s, 5-OH). Спектр ЯМР ^{13}C (150 MHz, DMSO-d_6+CCl_4, δ, ppm): 163.00 (C-2), 102.71 (C-3), 181.42 (C-4), 161.34 (C-5), 98.68 (C-6), 164.20 (C-7), 93.81 (C-8), 157.08 (C-9), 103.49 (C-10), 121.14 (C-1'), 116.96 (C-2'), 145.95 (C-3'), 152.11 (C-4'), 116.46 (C-5'), 122.04 (C-6'), 103.75 (C-1''), 73.03 (C-2''), 75.77 (C-3''), 71.82 (C-4''), 74.15 (C-5''), 172.41 (C-6'') [24].

In vitro screening of *Salvia sarawschanica* samples for antibacterial and antifungal activity was performed using the agar disk diffusion method [20]. The following bacterial strains were used as test cultures: *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (RKMUz - 5), *Pseudomonas aeruginosa* (ATCC 27879), *Escherichia coli* (RKMUz - 221) and the fungal strain *Candida albicans* (RKMUz - 247). The drugs ampicillin/sulbactam, gentamicin and fluconazole (Himedia) were used as positive controls, and the extraction solvents as negative controls.

Table 2. Antibacterial and antifungal activity of samples from the aboveground part of *S. sarawschanica*

Specimens from the aboveground part S. sarawschanica	Inhibition zone diameter (mm, ± SD, P≤0.05)				
	Bacillus subtilis	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Candida albicans*
Essential oil	10.04±0.10	7.04±0.10	na*	6.08±0.12	na
Benzene extract	6.04±0.10	7.04±0.10	na	na	na
Ethyl acetate extract	na	na	na	7.08 ± 0.12	na
Ampicillin/Sulbactam (10 μg+10 μg disk)	31.04±0.10	29.04± 0.10	nt	nt	nt
Gentamicin (10 μg/disk)	nt	nt	22.04±0.10	27.08 ± 0.12	nt
Fluconazole (25 μg/disk)	nt	nt	nt	nt	30.04±0.10

nt – not tested, na - not active

The test results showed that among the studied samples, only the essential oil from the above-ground parts of the *S. sarawschanica* plant exhibits a noticeable antibacterial effect against the gram-positive bacterial strain *B. subtilis* (10.04±0.10 mm) (Table 2).

The antibacterial and antifungal properties of essential oil and hexane extract were studied by Doctor of Biological Sciences S.A. Sasmakov in the laboratory of molecular genetics.

Conclusion. The component composition of the EO from the air-dried above-ground part of *S. sarawschanica* was studied using the GC-MS method. Oxidized

monoterpenes and monoterpenes predominate in the EO. The main components of the essential oil are 1,8-cineole, camphene, camphor, aromadendrene and limonene. The above-ground part of *S. sarawschanica* can serve as a rich source of 1,8-cineole. Four flavonoids and two sterols were isolated from the above-ground part of *S. sarawschanica*, which, based on the study of spectral data, were identified with acacetin, luteolin, cynaroside, luteolin-3'-O-β-D-glucuronide.

The antibacterial and antifungal effects of root extracts and isolated substances were studied in vitro using the disk diffusion method in agar. It has been established that the essential oil exhibits an antibacterial effect against the gram-positive strain of bacteria B. subtilis.

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