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ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF THE ESSENTIAL OILS AND VARIOUS EXTRACTS OF *Salvia sahendica* IN DIFFERENT PHENOLOGICAL STAGES

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Salvia, the largest genus of the Lamiaceae family, is represented in the flora of Iran by 58 species, of which 17 species are endemic [1]. Sage (*S. officinalis*) has been credited with a long list of medicinal uses: e.g., spasmolytic, antiseptic, and astringent [2]. Some of the phenolic compounds of plants belonging to this genus have also shown excellent antimicrobial and radical scavenging activity [3], as well as inhibition of lipid peroxidation [4, 5]. We studied the essential oil composition isolated from *S. sahendica*.

The yield of essential oils (w/w %) obtained from aerial parts of *S. sahendica* in different phenological stages based on dry weight of plant were in the order full flowering (1.1%)> fruiting set (0.6%)> floral budding (0.5%)> and vegetative (0.3%). A total number of 35, 35, 32, and 32 compounds, representing 99.8%, 99.9%, 98.4%, and 98.5% of the total oils, were identified, respectively. The results are listed in Table 1 along with the retention indices of the identified compounds, where all constituents are arranged in order of their elution on the DB-1 column. A comparison among the compositions of the essential oils revealed both quantitative and qualitative differences.

The results showed that α-pinene (18.9–28.5%), β-pinene (18.5–26.1%), 1,8-cineole (4.9–13.9%), germacrene-D (0.4–9.6%), bicyclogermacrene (3.7–8.2%), linalyl acetate (0.2–8.4%), and linalool (1.4–5.3%) were the principal constituents in these essential oils. α-Pinene was the major compound in all samples. In the oil of the full flowering stage the content of some main compounds such as 1,8-cineole (13.9%), linalool (5.3%), linalyl acetate (8.4%), and bicyclogermacrene (8.2%) reached the maximum. Germacrene-D was found to be in high amounts in the floral budding (10.4%) and vegetative (9.6%) stages and then subsequently decreased in the reproductive stage. Monoterpene hydrocarbons were the main group of constituents in the essential oil in all growing stages. Oxygenated sesquiterpenes comprised less than 2.0% of the total oil.

The oils showed a wide antimicrobial spectrum of action (Table 2). An interesting observation was the strong activity against Gram-positive bacteria of the essential oils in all stages. The absolute oil (15 μL) exhibited moderate to high activity against the tested microorganisms, of which *S. epidermidis*, *S. aures*, and *B. subtilis* were more sensitive than the others.

The oil inhibited the growth of two Gram-negative bacteria, *E. coli* and *K. pneumoniae*, only at high concentrations, while *P. aeruginosa* was resistant at all of the oil concentrations tested. Furthermore, the results obtained from two main constituents of the oil, α-pinene and β-pinene, at 10 μL concentration showed moderate antibacterial activity. All extracts of *S. sahendica* were found to possess moderate to high activity against *B. subtilis* and moderate activity against *S. aureus* and *E. coli* (Table 2). When compared to extracts, the essential oils exhibited stronger and broader activity. No significant differences were evident between activities of essential oils in different phenological stages in terms of the antimicrobial spectrum.

The antioxidant activity of *S. sahendica* extracts and its essential oils was evaluated in a series of *in vitro* test. In the DPPH test, the ability of extract and essential oil to act as a donor of hydrogen atoms or electrons in the transformation of DPPH° into its reduced form DPPH-H was measured spectrophotometrically. The concentration of extracts providing 50% inhibition is included in Table 3. The free radical scavenging activity of methanol extract (ME) was superior to all other extracts (IC_{50} = 17.0 μg/mL). Polar extracts exhibited stronger activity than nonpolar ones. When compared to BHT, the extracts from methanol (ME), acetone (AE), chloroform (CE), and ethanol (EE) were more effective radical scavengers. The nonpolar *n*-hexane extract (HE) showed a low RSC (IC_{50} = 230.0 μg/mL).

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TABLE 1. Chemical Composition of the Essential Oil of *S. sahendica* in Different Phenological Stages

Components	RI	Vegeta-tive	Floral budding	Full flowering	Fruiting	Components	RI	Vegeta-tive	Floral budding	Full flowering	Fruiting
α-Thujene	926	0.6	4.1	2.3	2.6	Pulegone*	1222	-	-	0.1	-
α-Pinene*	938	28.5	27.3	18.9	27.4	Linalyl acetate	1244	0.2	0.3	8.4	1.7
Camphene	950	2.1	1.8	1.8	2.7	Bornyl acetate	1276	1.2	0.9	1.6	1.4
Sabinene	971	10.3	8.1	7.6	9.1	Terpinenyl acetate	1335	0.4	-	-	-
β-Pinene*	979	26.1	23.4	18.5	23.8	δ-Elemene	1338	0.1	3.2	2.2	0.8
Myrcene	983	-	0.5	-	-	Geranyl acetate	1362	0.1	0.1	0.7	0.3
α-Phellandrene	1004	0.1	0.1	0.1	0.1	α-Copaene	1383	0.2	0.2	-	-
α-Terpinene	1015	0.4	0.3	0.2	0.5	β-Elemene	1393	0.5	0.5	0.2	0.1
p-Cymene	1018	-	-	0.1	0.2	α-Gurjunene	1418	-	0.2	0.2	-
1,8-Cineole*	1027	4.9	5.0	13.9	8.9	β-Caryophyllene	1427	0.2	0.1	-	-
Z-β-Ocimene	1038	-	-	0.3	0.1	*allo*-Aromadendrene	1457	-	0.1	-	-
γ-Terpinene*	1052	0.7	0.8	0.3	0.9	α-Humelene	1468	-	0.1	-	0.1
trans-Sabinene hydrate	1057	0.1	0.1	0.1	0.2	Germacrene D	1487	9.6	10.4	1.6	0.4
Terpinolene	1084	-	-	1.2	-	Bicyclogermacrene	1502	3.8	5.8	8.2	3.7
Linalool*	1089	1.9	1.4	5.3	5.2	δ-Cadinene	1522	1.1	0.5	0.2	-
β-Thujone	1093	-	-	0.2	-	Spathulenol	1577	-	0.2	0.6	1.8
cis-Menth-2-en-1-ol*	1113	0.1	-	-	0.1	Ledol	1605	-	-	-	0.1
Camphor	1131	0.1	-	0.1	0.3	γ-Eudesmol	1622	0.4	-	-	0.2
trans-Verbenol	1134	0.3	0.2	0.1	0.2	α-Eudesmol	1650	0.6	-	0.1	0.2
δ-Terpineol	1146	0.1	0.1	0.1	0.1	(*E*,*E*)-Farnesol	1690	1.0	0.3	0.2	-
Borneol*	1158	1.6	1.2	1.6	2.3	Monoterpene hydrocarbons		68.8	67.4	50.1	67.4
4-Terpineol	1169	1.4	1.0	0.8	1.9	Oxygenated monoterpenes		12.5	9.5	34.1	25.1
α-Terpineol	1180	0.3	0.2	1.9	1.4	Sesquiterpene hydrocarbons		16.2	21.1	15.3	5.5
Myrtenol	1186	-	-	-	0.2	Oxygenated sesquiterpene		1.0	0.4	0.3	1.9
Nerol	1214	-	-	0.2	0.1	Total		98.5	98.4	99.8	99.9

Method of identification: MS, RI; *MS, RI, CoI. RI, retention indices relative to C_6-C_{24} *n*-alkanes on the DB-1 column; MS, mass spectrum; CoI, coinjection with an authentic sample.

TABLE 2. Antibacterial Activity of the Essential Oil of *S. sahendica* in Different Phenological Stages and Various Extracts of *S. sahendica*

Microorganisms	Essential oil[a] (15 µL/disc)				Main compounds (10 µL/disc)		Antibiotics (10 µg/disc)		Extracts (2.5 mg/disc)[a]					
	vegetative	floral budding	full flowering	fruiting	α-pinene	β-pinene	pen	amp	ME	EE	AE	E	CE	HE
B. subtilis	16	20	16	18	10	15	22	14	14	13	16	12	16	13
S. aureus	17	15	20	16	8	9	32	13	9	8	-	10	12	9
S. epidermidis	16	14	16	15	9	12	13	12	-	-	-	10	12	11
E. faecalis	12	10	10	-	-	7	17	11	-	-	-	-	8	-
K. pneumoniae	-	-	9	9	-	-	-	12	-	8	-	8	14	-
E. coli	-	10	13	10	11	10	8	12	-	9	10	10	12	11
P. aeruginosa	-	-	-	-	-	-	-	10	-	-	-	-	8	-

[a]Diameter of inhibition zone including diameter of disc 6 mm; (-), not active; (8-14), moderately active; (>14), highly active. Penicillin, pen; ampicillin, amp. Methanol extract (ME); acetone extract (AE); chloroform extract (CE); ethanol extract (EE); ethyl acetate extract (E); *n*-hexane extract (HE).

TABLE 3. Total Phenolic Compounds and Radical Scavenging Capacity of Various Extracts of *S. sahendica* against DPPH (IC_{50})

Extracts	Gallic acid equivalents (mg/L)	IC_{50} (μg/mL)
Methanol extract (ME)	250.0±0.8	17.0±1.1
Acetone extract (AE)	283.3±1.3	18.5±0.8
Chloroform extract (CE)	231.4±1.1	21.0±1.5
Ethanol extract (EE)	216.8±3.5	25.0±0.6
Ethyl acetate extract (E)	83.3±2.1	140.0±2.0
n-Hexane extract (HE)	45.3±1.3	230±3.4
Control (BHT)	-	26.5±1.0

Results are given as mean ± standard deviation of three different experiments.

Based on the absorbance values of the various extract solutions reacting with Folin-Ciocalteu reagent and compared with the standard solution of gallic acid equivalents, the amounts of total phenolics were in the order ME (25.0%) > AE (23.8%) > CE (23.1%) > EE (21.6%) > E (8.3%) > HE (4.5%). The scavenging activity of polar extracts could be attributed to their higher phenolic compounds than nonpolar extracts. Total phenolics was highest in ME (25.0%), AE (23.8%), CE (23.1%), EE (21.6%), and ethyl acetate extract (8.3%). The lowest amount of total phenolics was recorded in nonpolar extracts HE (4.5%). The capability of different phenolic substances to scavenge various types of oxidation-initiating radicals has been reported in the polar phase [6, 7]. Plants belonging to the Lamiaceae family are very rich in polyphenolic compounds. Polyphenolic compounds have been shown to have antioxidant activity and it is likely that the activity of the examined plants is due to these compounds [8].

It can be concluded that, since the biological activities of *S. sahendica* have not been previously investigated, testing of the antibacterial and antioxidant properties of its essential oil and extracts is important, primarily in order to find new promising sources for natural antioxidants, functional foods, and pharmaceuticals.

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