Phytochemical Composition and Antibacterial Properties of Surface Extracts of Six *Salvia* Species

Somayeh Zare, Maryam Parish, Mehdi Zare and Amir Reza Jassbi*
Medicinal and Natural Products Chemistry Research Center,
Shiraz University of Medical Sciences, Shiraz, Iran.
jassbiar@sums.ac.ir*

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Summary: The chemistry and biological activity of cuticular waxes and the secondary metabolites produced in the indumentum of different plant organs has been attracting the interest of botanists. This work was aimed to investigate the chemistry and antibacterial activity of the surface extracts (SE) of different organs of six *Salvia* species. The cuticles and indumenta of the leaves, calyxes, and stems of *Salvia atropatana* Bunge., *S. lachnocalyx* Hedge., *S. ceratophylla* L., *S. palaestina* Benth., *S. persepolitana* Boiss., and *S. hydrangea* DC. ex Benth. were extracted with dichloromethane. The SEs were analyzed using gas chromatography-mass spectrometry (GC/MS). The antibacterial activity of each extract was assessed against *Escherichia coli*, *Staphylococcus epidermidis*, and *Bacillus subtilis* strains using the agar disc diffusion bioassay. The yields of extracts were in the range from 1.1- 2.3% w/w, while the detected compounds constituted 59.3-95.4% of the total extract composition. All species except *S. persepolitana* contained the sesquiterpenes, including β-caryophyllene (0.5-19.5%) and germacrene D (0.4-12.9%), while sclareol (2.1-75.6%) was the major labdane diterpenoid in the examined *Salvia* species excepting *S. hydrangea*. In addition to terpenoids, the analyzed SEs contained long-chain *n*-alkanes, fatty acids, alcohols, and aldehydes in the range from 4.8-78.5%. *E. coli* was the most susceptible microorganism to the tested extracts of *S. persepolitana* calyxes and stems (20 and 18 mm) and the *S. lachnocalyx* stems (17 mm), respectively. The antibacterial properties of the SEs of the plants suggested their protective role against pathogenic microorganisms, which can be attributed to their major phytochemicals such as sclareol and abienol.

Key-words: Cuticular phytoconstituents, Surface extract, *Salvia atropatana*, *Salvia lachnocalyx*, *Salvia hydrangea*, *Salvia ceratophylla*, *Salvia palaestina*, *Salvia persepolitana.*

Introduction

The cuticular waxes are mixtures of soluble and insoluble long-chain fatty acids, aldehydes, alcohols, and hydrocarbons that are absorbed into the cuticles as the hydrophobic layer on the plant's surface. The cuticles and the trichome indumenta vary morphologically not only between different plant species but also among distinct organs of the same species [1-4]. The trichome characters of 46 Iranian *Salvia* species were examined and showed capitate glandular and long simple non-glandular for those of *S. atropatana*, S. *palaestina*, *S. hydrangea*, and *S. ceratophylla* [5]. In addition to cuticular waxes, other types of phytochemicals such as monoterpenoids [6], sesquiterpenoids [7], diterpenoids [8], and triterpenoids [9] are exuded by glandular trichomes. These compounds may be absorbed on the cuticular wax layers [10] or accumulate as epicuticular crystals [11]. On the other hand, surface-originated flavonoids accumulate on the epicuticular layer of the plants [2, 4, 12].

Most investigations on the chemical composition of trichomes and surface exudates have been conducted on the Solanaceae and Lamiaceae families, whose species show considerable diversity of trichome types and chemistry [5, 13]. The chemistry, biological activity as well as ecological roles of phytochemicals exudated from the trichomes of *Nicotiana* species have been studied thoroughly [13]. Cembranoid and labdane-type diterpenoids, sucrose, and glucose esters of fatty acids are exerted from the glandular trichomes of the leaves of different *Nicotiana* species, which protect the plants against microbial infections and herbivores [13]. Also, some of the cembranoid diterpenoids showed anticancer activity *in vitro* and *in vivo* [14]. Moreover, antimicrobial and phytotoxic clerodane [15, 16] and icetaxane [17] diterpenoids are provided from surface extracts (SEs) of different *Salvia* species. In addition, pentacyclic triterpenoids were detected at high concentrations in the cuticular wax mixtures of *S. corrugata and S. argentea* [17, 18]. Salvinorin A is a neoclerodane diterpenoid with hallucinogenic activity that is synthesized in peltate glandular trichomes of *S. divinorum* [8]. The chemical analysis of leaf surface extract of *S. blepharophylla* has led to the isolation of flavonoids, nuchensin and pedalitin, the neoclerodane diterpenoid, salvianduline D, and the triterpenoids, ursolic acid and α-amyrin [19]. The leaf glandular trichomes of *S. pomifera* L. accumulate monoterpenes and labdane diterpenes as major metabolites [20].

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[*] **To whom all correspondence should be addressed.**

Table-1: Collection coordinates and voucher specimens of the examined Iranian *Salvia* species.

Plant names	Locality (Province, Region)	Longitude, Latitude	Altitude (m)	Date of collection	Herbarium numbers
S. atropatana	Fars, Sepidan, Poladkaf ski resort	30 22.37.9, 51 54 59.0	2400	June 2020	PC-99-3-8-12.5
S. lachnocalyx	Fars, Eghlid, Kuh-e Bul	30 51.130, 52 42.025	2290	June 2020	PC-99-3-8-3.4
S. ceratophylla	Fars, before Safashahr	30 34.080, 53 11.395	2292	June 2020	PC-99-3-8-9.5
S. palaestina	Road of Shiraz-Qaemyeh	29 43.660, 51 47.156	1231	May 2020	PC-99-3-8-14.4
S. persepolitana	Fars, Kazerun, Tang-e Abolhayat	29 42.221, 51 47.096	1252	May 2020	PC-99-3-8-4.3
S. hydrangea	Fars, Eghlid, Kuh-e Bul	30 51.130, 52 42.025	2290	June 2020	PC-93-3-8-1-1

Despite to the few phytochemicals from the surface extracts of *Salvia* species, there are many reports on natural compounds from Iranian-long time-extracted *Salvias*, including terpenoids [21, 22] and phenolics [23, 24]. However, to the best of our knowledge, this is the first time we have reported phytoconstituents from surface short-time extracts from different organs of six Iranian *Salvia* species, including *S. atropatana*, *S. lachnocalyx*, *S. ceratophylla*, *S. palaestina*, *S. persepolitana*, and *S. hydrangea*. Since *Salvia* species are rich in the production of antibacterial essential oils [25], in addition to phytochemical analyses of the surface extracts, their antibacterial activities were measured using agar disc diffusion (ADD) bioassays.

Experimental

Plant material and surface extraction

Fresh aerial parts of the plants were collected from different localities of Fars Province, Iran in the spring of 2020 (Table-1). One of us, MZ, identified the plant material and a voucher specimen was kept for each species at the herbarium of our institution (Table-1). The aerial parts including leaves, calyces, and stems of each species were separated and extracted individually. For the extraction of the cuticular waxes, we have used washing of the plant's surface with the solvent [15]. For doing so, the above organs (5 g) were soaked in 50 mL of dichloromethane for 30 seconds at room temperature [4, 26]. After filtration, the surface extract of each sample was evaporated to remove traces of solvents under vacuum, and at 40 ºC (EYELA Rotary Evaporator, N-1001S-W, Japan), the dried extracts were kept in the freezer until the time of performing bioassay and GC/MS analyses. The percentage yield of each extract was calculated and shown in Table-2. The dried extracts were dissolved in dichloromethane (CH_2Cl_2) and for removing the water, the obtained extracts were dried over anhydrous sodium sulfate (anhydrous Na_2SO_4). Therefore, each extract was prepared at the concentration of 10 mg/mL for GC/MS analyses and the antibacterial bioassay.

GC/MS analysis of the SEs

Qualitative and quantitative analyses of the constituents of the SEs were carried out by GC/MS. The GC/MS analyses were performed using an Agilent 7890A GC coupled to a 5975C inert MSD operating in EI mode at 70 eV. The GC was equipped with a J&W DB-5 ms ScientificIC column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The oven temperature was initially set and maintained at 100 °C for 5 min, then increased to 250 °C with a gradient of 5 °C/min, and then retained for 15 min at the final temperature. Helium (He) was used as carrier gas with a flow rate of 1 mL/ min, and the temperature of the injector was 260 °C in the split mode (1:20) for the surface extract analyses. The injection volume was one µL. The SE constituents were identified by comparing their mass spectra and the calculated relative retention indices (RRI) with those of the presented credible samples in the literature [27]. The RRI of compounds was calculated using the retention times relative to the series of C_8–C_{20} *n*-alkanes (Fluka Analytical) according to the Van Den Dool formula [28].

Antibacterial activity of the SE using disc diffusion method

To examine the antibacterial effects of the extracts, one Gram-negative bacteria (*Escherichia coli*: PTCC1330) and two Gram-positive bacteria (*Staphylococcus epidermidis*: PTCC1114, *Bacillus subtilis*: PTCC1023) were chosen, and tested in agar disc diffusion ADD bioassays. Briefly, the suspensions of bacteria were grown in nutrient broth media (Merck) overnight at 37 ºC. Following that period, the bacterial optical density was measured at OD 600 nm using a spectrophotometer and adjusted to 0.1 absorption unit (au). The extracts were diluted in CH_2Cl_2 to obtain two concentrations; 5 and 2.5 mg/10µL. The sterile paper discs, which were 6 mm in diameter (dia.), were loaded with 10 µL of different concentrations of each extract's solutions, and CH_2Cl_2 was applied as the negative control. The bacterial suspensions with OD=0.1 au were inoculated over the surface of solidified agar media in 9 cm dia. Petri dishes. The antibiotic paper discs of gentamicin and ampicillin (10 µg/disc) were used as the positive control. When the impregnated papers got dry, they were put down on the seeded media on the Petri dishes. Finally, all of the Petri dishes were put in a refrigerator (4 °C) for 3 h to spread the metabolites in the agar media and afterward were incubated for 18 h at 37 °C. The dia. of inhibition zones (IZ) was calculated in millimeters (mm) and the experiments were accomplished in triplicate [24, 25].

Table-2: Chemical composition of the surface extracts of the examined *Salvia* species analyzed by GC/MS.

Compound	RRI[a]	*S. atropatana*			*S. lachnocalyx*			*S. ceratophylla*			*S. palaestina*			*S. persepolitana*			*S. hydrangea*	
		L[b] %	C[c]	S[d]	L	C	S	L	C	S	L	C	S	L	C	S	L	S
α- pinene	934	-	-	-	2.4	-	-	-	-	-	-	-	-	-	-	-	0.7	0.8
sabinene	974	-	-	-	3.8	-	-	-	-	-	-	-	-	-	-	-	-	0.4
β- pinene	977	-	-	-	2.8	-	-	-	-	-	-	-	-	-	-	-	-	2.1
n- nonanal	1100	-	-	-	-	-	-	-	-	-	0.2	-	0.2	-	-	-	-	-
1,8- cineol	1030	-	-	-	1.8	-	-	-	-	-	-	-	-	-	-	-	2.1	10.4
β-ocimene	1054	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6
linalool	1096	-	-	-	-	-	-	-	-	-	-	6.8	-	-	-	-	-	-
1, 3, 8- *p*- menthatriene	1097	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.8	2.7
β-terpineol	1134	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.9
camphor	1146	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6	0.5
borneol	1169	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.5	-
α- terpineol	1199	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6.6	-
bornyl acetate	1288	-	0.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5
δ- elemene	1338	-	-	-	7.1	0.6	-	-	-	-	-	-	-	-	-	-	-	-
α- cubebene	1348	-	-	-	-	-	-	-	-	-	5.0	-	2.3	-	-	-	-	-
terpinyl acetate	1349	-	-	-	8.5	0.3	0.6	-	-	-	-	-	-	-	-	-	-	-
β- bourbonene	1390	-	-	-	-	-	-	-	-	-	0.1	-	0.2	-	-	-	0.6	0.6
β- cubebene	1393	-	-	-	-	-	-	-	-	-	0.9	-	0.3	-	-	-	-	-
β- caryophyllene	1419	9.6	1.1	1.8	7.8	1.2	0.5	-	1.8	-	10.2	3.6	4.1	-	-	-	19.5	8.9
γ- elemene	1436	-	-	-	-	-	-	-	4.3	-	-	-	-	-	-	-	-	-
α- humulene	1454	-	-	-	0.5	-	-	-	-	-	-	-	-	-	-	-	0.8	-
trans β-farnesene	1456	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5.5	0.4
germacrene D	1485	8.1	1.1	2.5	9.8	1.0	0.4	-	5.1	-	12.9	3.1	4.4	-	-	-	-	0.4
trans β-guaiene	1502	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	-
germacrene B	1561	-	-	-	-	-	-	-	6.6	-	-	-	-	-	-	-	-	-
bicyclogermacrene	1493	-	-	-	25.7	2.9	1.1	-	-	-	-	1.1	-	-	-	-	-	-
trans- *α*- farnesene	1505	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
δ- cadinene	1525	-	-	-	-	-	-	-	-	-	1.3	-	-	-	-	-	1.3	-
spathulenol	1578	-	-	-	2.7	0.4	0.7	-	-	-	-	-	-	-	-	-	-	3.2
caryophyllene oxide	1583	1.7	-	-	-	-	-	-	-	-	5.4	1.6	4.9	-	-	-	1.5	5.6
viridiflorol	1592	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.6	-
n- hexadecane	1600	-	-	-	-	-	0.7	-	-	-	-	-	-	-	-	-	-	-
n- tetradecanal	1612	3.7	19.1	16.3	-	-	-	14.5	12.2	8.9	1.8	2.2	2.3	0.3-	1.2	-	-	-
aromadendrene epoxide	1641	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.9
valeranone	1675	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20.9	-
n- heptadecane	1700	-	-	-	-	-	0.4	-	-	-	-	-	-	-	-	-	-	-
benzyl benzoate	1760	-	-	-	-	-	-	-	-	-	-	-	-	3.8	6.3	5.0	-	10.6
1-pentadecanol	1773	-	1.3	1.2	-	-	-	-	2.8	2.2	-	-	-	-	-	-	-	-
n- octadecane	1800	-	-	-	-	-	0.5	-	-	-	-	-	-	-	-	-	-	-
n- hexadecanal	1826	-	0.9	1.3	-	-	-	3.2	2.7	2.3	-	-	-	-	-	-	-	-
1- pentadecene	1896	-	3.2	1.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
n- nonadecane	1900	-	-	-	-	-	0.6	-	-	-	-	-	-	-	-	-	-	-
sclareoloxide	1903	-	-	-	-	-	-	-	-	-	1.1	1.1		0.3	0.5	0.5	-	-
methyl palmitate	1921	-	-	-	-	-	0.4	-	-	-	-	-	-	-	-	-	-	0.7
1 - heptadecene	1977	-	-	-	-	-	-	-	3.4	2.6	-	-	-	-	-	-	-	-
1- eicosene	1988	-	-	-	-	-	-	3.9	-	-	-	-	-	-	-	-	-	-
n- eicosane	2000	-	-	-	-	-	0.6	-	-	-	-	-	-	-	-	-	2.5	0.5
n- octadecanal	2030	-	8.7	11.9	-	-	-	11.0	6.7	5.1	-	-	-	-	-	-	-	-
manool	2076	-	-	-	-	-	-	-	-	-	-	-	-	0.6	1.2	0.6	-	-
1-docosanol	2091	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5	-	-	-
1-eicosanol	2089	-	0.6	0.7	-	-	-	7.9	9.0	7.3	-	-	-	-	-	-	-	0.4
n- heneicosane	2100	-	-	-	-	-	1.5	-	-	-	1.9	0.6	-	-	-	-	-	-
methyl octadecanoate	2124	-	-	-	-	-	0.5	-	-	0.5	-	-	-	-	-	-	-	-
abienol	2149	-	-	-	-	-	-	-	-	-	-	2.5	-	2.2	1.6	3.1	-	-
n- docosane	2200	-	-	-	-	-	0.8	-	-	-	-	-	-	-	-	-	-	-
sclareol	2223	11.6	2.1	-	3.8	72.9	4.8	-	5.1	3.0	2.8	15.3	4.2	56.3	75.6	62.8	-	-
n- tricosanr	2300	-	0.6	0.8	3.1	1.2	10.1	5.4	-	4.6	1.1	-	0.9	-	-	-	-	-
n- tetracosane	2400	-	-	-	-	0.2	0.7		-	-	1.6	-	1.7	-	-	-	-	-
n- pentacosane	2500	4.9	1.1	0.5	0.1	-	0.2	2.4	-	-	-	11.5	-	3.7	3.6	3.0	-	0.5
n- hexacosane	2600	-	-	-	-	-	0.9	9.5	-	-	-	-	-	0.3	-	-	-	8.8
n- heptacosane	2700	19.1	8.9	14.3	1.4	2.6	27.3	3.9	3.1	7.4	16.5	19.6	48.8	7.4	1.4	7.9	-	-
n- octacosane	2800	26.9	0.9	-	-	0.2	30.1	-	11.7	14.6	-	-	0.9	-	-	-	-	-
n- nonacosane	2900	5.7	9.4	11.8	3.5	0.6	3.2	12.9	1.9	4.4	6.0	2.7	14.9	6.9	3.5	4.4	-	-
Total monoterpenoids		-	0.9	-	19.3	0.3	0.6	-	-	-	-	6.8	-	-	-	-	14.3	18.9
Total sesquiterpenoids		19.8	2.2	4.3	53.6	6.0	2.7	-	17.8	-	35.8	9.4	16.2	-	-	-	52.9	20.0
Total diterpenoids		11.6	2.1	-	3.8	72.9	4.8	-	5.1	3.0	3.9	18.9	4.2	59.4	78.9	67.0	-	-
total terpenoids		31.4	5.2	4.3	76.7	79.2	8.1	-	22.9	3.0	39.7	35.1	20.4	59.4	78.9	67	67.2	38.9
Total C_{16}-C_{29} hydrocarbons		56.6	20.9	27.4	8.1	4.8	77.6	34.1	16.7	31	27.1	34.4	67.2	18.3	8.5	15.3	2.5	9.8
Total long-chain hydrocarbons and derivatives [e]		60.3	54.0	60.3	8.1	4.8	78.5	74.6	53.7	59.4	29.2	36.6	69.7	18.6	10.2	15.4	2.5	10.9
Unidentified		8.3	40.2	35.4	15.2	16.0	12.2	25.4	23.0	37.6	26.1	28.4	9.9	17.7	3.4	12.3	29.4	39.6
Total identified		91.7	59.3	64.6	84.8	84.0	86.6	74.6	76.5	62.4	68.8	71.6	90.1	81.8	95.4	87.4	69.7	60.4
extract yields (w/w%)		1.9	2.1	2.0	1.7	1.9	1.5	2.0	2.3	1.8	1.2	1.7	1.1	1.4	1.5	1.1	1.8	1.3

a) RRI: relative retention index, b) L= leaves, c) C= calyxes, d) S= stems, e) long chain hydrocarbons and derivatives containing *n*-alkanals, *n*-alkenes, primary alcohols, and methyl esters.

Results and Discussion:

The surface of the leaves, calyxes, and stems of the six examined *Salvia* species were extracted shortly in dichloromethane to yield volatile and non-volatile phytochemicals ranging from 1.1 to 2.3% (w/w). The GC/MS analyses of the above-mentioned extracts resulted in the identification of 63 volatile and semi-volatile constituents of the surface extracts (SEs, Table-2). Various types of metabolites including different classes of terpenoids, long-chain hydrocarbons, *n*-aldehydes, *n*-alcohols, and esters were detected in the SEs.

The SEs of the analyzed *Salvia* species resulted in the detection of monoterpenoids, for instance, *α*- pinene (2.4, 0.7, 0.8%), *β*- pinene (2.8, -, 2.1%), 1,8-cineol (1.8, 2.1, 10.4%) in the leaves of *S. lachnocalyx* and leaves and stems of *S. hydrangea*, respectively. *β*-Caryophyllene (0.5-19.5%) and germacrene D (0.4-12.9%) are the two major sesquiterpene hydrocarbons of all species except *S. persepolitana*, often found in the leaves of the plants. On the other hand, sclareol (2.1- 75.6%), a labdane-type diterpenoid, was among the most abundant constituents of all species except *S. hydrangea*. It is detected in the leaves and stems of *S. atropatana*, and leaves of *S. ceratophylla*. Sclareol (56.3 to 75.6%) and abienol (1.6 to 3.1%) were detected in all parts of *S. persepolitana*. Glandular trichomes are widely distributed on the surface of aerial reproductive and vegetative organs of *Salvia* species [5]. The biosynthesis of terpenoids in the glandular trichomes of Lamiaceae plants, including *Salvia* species, was reviewed [6, 7, 29]. The biosynthesis of abietane diterpenoids carnosic acid, carnosol, pisiferic acid, and salviol were reported in the glandular trichome of *S. pomifera* [30] and *S. fruticose* [31]. Also, the biosynthesis of labdane diterpenoid, sclareol was reported in the trichomes of clary sage, *S. sclarea* [32]. In fact, Sclareol is accumulated in a crystalline epicuticular form, mostly on calyces of *S. sclarea* [11]. The identified mono-, sesqui-, and di-terpenoids in the present investigated *Salvia* species are compatible with the previous data in the literature.

On the other hand, plant's cuticular waxes are typically mixtures of unbranched, fully saturated aliphatic compounds, including a homologous series of primary *n*-alcohols, *n*-aldehydes, and fatty acids as well as *n*-alkanes with chain lengths ranging from 20 to almost 40 carbons [4]. The SEs of the analyzed *Salvia* species were composed of major portions of long-chain hydrocarbons, *n*-alkanes, fatty alcohols, and fatty aldehydes. The stems of *S. lachnocalyx*, constituted 78.5% lipids, while its calyxes' SE was composed of a minor portion of long-chain hydrocarbon (4.8%) derivatives. *n*-Tetradecanal is the fatty aldehyde of all species except *S. hydrangea* and *S. lachnocalyx* and was in higher levels in the leaves, calyxes, and stems SE of *S. atropatana* (3.7, 19.1, and 16.3%) and *S. ceratophylla* (14.5, 12.2, and 8.9%), respectively. Not only the chemical composition of SEs of the examined *Salvia* species were qualitatively and quantitatively different, but also, they were divergent regarding the various organs of one specie. The sesquiterpenes, *β*-caryophyllene and germacrene D are at higher levels in the leaves, while sclareol is dominant in the calyxes of the plants. Although leaves and calyxes of *S. lachnocalyx* are rich in production of terpenoids (76.7 and 79.2% respectively), the stems are prevailing in long-chain hydrocarbons and derivatives (78.5%).

Table-3: Antimicrobial potential of different parts of plant surface extracts by agar disc diffusion bioassay.

Plants	**Part used**	***E. coli* IZ**^{a}		***B. subtilis***		***S. epidermidis***	
Concentrations ^{b}		**5**	**2.5**	**5**	**2.5**	**5**	**2.5**
S. atropatana	**leaves**	**11**	**5**	**15**	**10**	**10**	**10**
	calyxes	**12**	**5**	**18**	**12**	**10**	**8**
	stems	**11**	**7**	**17**	**12**	**10**	**7**
S. lachnocalyx	**leaves**	**13**	**15**	**12**	**12**	**NA**	**NA**
	calyxes	**6**	**5**	**10**	**10**	**NA**	**NA**
	stems	**17**	**14**	**10**	**8**	**NA**	**NA**
S. ceratophylla	**leaves**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
	calyxes	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
	stems	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
S. palaestina	**leaves**	**NA**	**NA**	**10**	**10**	**6**	**5**
	calyxes	**NA**	**NA**	**13**	**9**	**8**	**4**
	stems	**NA**	**NA**	**6**	**4**	**8**	**5**
S. persepolitana	**leaves**	**15**	**12**	**9**	**8**	**10**	**7**
	calyxes	**20**	**15**	**10**	**10**	**9**	**8**
	stems	**18**	**12**	**6**	**3**	**11**	**8**
S. hydrangea	**leaves**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
	stems	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
Antibiotic (10 µg/disc)	**Ampicillin**	**-**		**20**		**19**	
	Gentamycin	**21**		**-**		**-**	

^{a}Inhibition Zone, ^{b}values in mm (each extract tested at 5 and 2.5 mg/10 µl per disc), All of the tests have been performed in triplicate.

Antibacterial activity of the SEs obtained from leaves, calyxes, and stems of *Salvia* species was assessed against one Gram-negative and two Gram-positive bacteria; *E. coli*, *Staph. epidermidis*, and *B. subtilis* using ADD bioassays, respectively. The tested bacterial strains showed a different pattern of inhibition in the presence of each SEs of the investigated *Salvia* species (Table-3).

All of the SEs were antibacterial against the tested microorganism except those of *S. hydrangea* and *S. ceratophylla*, which did not exhibit considerable effect at the tested concentrations (2.5 and 5.0 mg dose amounts). The SEs of *S. atropatana* (5-18 mm IZ) and *S. persepolitana* (3-20 mm IZ) were active against all tested bacterial strains, while those of *S. palaestina* inhibited only the Gram-positive bacteria in the range of 4-13 mm IZ (Table-3). Finally, *S. lachnocalyx* was antibacterial against both *E. coli* (5-17 mm IZ) and *B. subtilis* (8-12 mm IZ).

The surface-exudate compounds play a vital role in the interactions of plants with their environment, particularly in plant defense against herbivores and pathogens, because of their toxic, antifeedant, anti-fungal, and antibacterial activities [13]. The plant's surface extracts were shown to have antimicrobial activities [33, 34] due to the secretion of glandular trichome's exudates with defensive functions onto the cuticular wax layer [10, 35]. Antibacterial terpenoids, especially diterpenoids with different skeletal types, were identified in the surface extract of *Salvia* species, including clerodane diterpenoids from *S. adenophora* [36] and *S. chamaedryoides* [15], royleanone derivatives from *S. corrugata* [37], sesterterpenoids and labdane diterpenoids such as sclareol and manool from *S. tingitana* [38].

The chemical composition and antibacterial activities of the essential oils (EOs) of Iranian *Salvia* species are reported by different authors [25, 39]. But this study is the first report on the chemical composition profile of quick SEs of the leaves, calyxes, and stems of six *Salvia* species comprising *S. atropatana*, *S. lachnocalyx*, *S. ceratophylla*, *S. palaestina*, *S. persepolitana*, and *S. hydrangea* (excluding calyxes). Among the tested oils, the EO of *S. lachnocalyx* exhibited potent antibacterial activity rich in monoterpenes (34.5%) such as *α*- and *β*- pinene and terpinyl acetate and sesquiterpenes (48.3%) including caryophyllene oxide, *β*- caryophyllene and germacrene D [25]. Like the EO, its SEs also showed high antibacterial activity which is suggested by the presence of higher amounts of sclareol and the above-mentioned mono- and sesquiterpenes in addition to bicyclogermacrene and spathulenol. In another experiment, analyses of the EO of *S. atropatana* exhibited monoterpenes (14.8%), such as *α*- pinene and bornyl acetate and sesquiterpenes (61.5%) including caryophyllene oxide, *β*- caryophyllene and germacrene D [39].

The constituents of SE (Table-2) of *S. atropatana* in the present study is similar to those of its EO in the previous studies, which is compatible with its antibacterial activities against all tested bacterial strains (Table-3). The EOs of *S. hydrangea* showed potent antibacterial activity and were rich in *α*- and *β*-pinene, 1,8-cineol, *β*- caryophyllene, caryophyllene oxide and spathulenol [25, 40, 41]. Unlike its EO, the SEs of *S. hydrangea* were inactive against the tested bacterial strains, which might be due to lower levels of bioactive monoterpenes in the SE compared to the EOs.

The antimicrobial activity of the cuticular waxy material were mostly attributed to different classes of volatile and semi-volatile terpenoids [40, 42]. Therefore, antimicrobial activity of the present SEs may be the results of the presence of active substances such as sclareol [43], *β*-caryophyllene [40, 44], and germacrene D [45]. Sclareol and its epoxy and hydroxyl derivatives exhibited antibacterial activity especially against *B. subtilis* [43]. Accordingly, sclareol can be one of the main antibacterial constituents of the *Salvia* species examined in this work especially in *S. persepolitana*, *S. lachnocalyx* and *S. palaestina*.

Moreover, the long chain hydrocarbons, alcohols, aldehydes and acids have been reported as the significant antibacterial constituents of the plants [46, 47]. In the present work, the antibacterial activity of the SEs can be explained by the presence of high amounts of *n*-alkanes and their derivatives such as *n*-octacosane [47] and *n*-tetradecanal [48].

Conclusion

Considering the results of the current study, there are different types and quantities of volatile and semi-volatile composition profiles of the distinct organ of the investigated *Salvia* species shoots accompanied with diverse patterns of inhibition against bacteria. This fact provides information about the most proper organ's SE for further phytochemical investigation of bioactive constituents. This study also provided proof of the antibacterial activity of SEs against the tested Gram-negative and/or Gram-positive bacteria. In addition to the antibacterial application of the investigated *Salvia* species' SEs, they may be considered as suitable sources in the perfume formulation, having both fragrant volatiles and less volatile fixing agents.

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