

## CHEMICAL CONSTITUENTS OF THE ESSENTIAL OIL FROM *SALVIA VERBENACA* SSP. *CLANDESTINA* FROM ALGERIAN PRE-SAHARA

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**Abstract:** The essential oil obtained by hydrodistillation from the aerial parts of *Salvia verbenaca* (L.) Briq. ssp. *clandestina* (L.) Pugsl. (*Lamiaceae*) growing wild in Bou Saâda, pre-Saharan region of Algeria, was analyzed by GC-MS. Sixty-four compounds were detected, representing 95.6% of the whole oil, among them forty five compounds are identified in this sample for the first time. The essential oil of *S. verbenaca* ssp. *clandestina* showed the predominance of sesquiterpenes (56.4%) followed by monoterpene derivatives (35.5%). The main constituents were  $\beta$ -pinene (10.2%), spathulenol (8.7%), caryophyllene oxide (6.1%),  $\alpha$ -pinene (5.2%), germacrene D (5%) and  $\alpha$ -gurjunene (4.9%). Chemical composition of the essential oil from our sample may be categorized as sesquiterpene and monoterpene chemotype among the four chemotypes identified for *Salvia* species.

**Keywords:** *Salvia verbenaca* ssp. *clandestina*, essential oil, GC-MS,  $\beta$ -pinene, spathulenol.

### 1. Introduction

*Salvia*, commonly known as sage, is one of the largest and most important aromatic genera of the *Lamiaceae* family, comprising about 800 species (Hao et al., 2015). It grows in Central and South America, Asia and Mediterranean regions (Tenore et al., 2010). Several traditional uses of *Salvia* species have been reported, for instance, reducing perspiration and fever, relieving digestion and spasmodic pain and treating liver disorders. Phytochemical composition of plants belonging to this genus has been previously described, it confirms the richness of these species in monoterpenes,

diterpenoids, sterols, flavonoids and essential oils (Lu and Foo, 2002). Essential oils of plants from this genus showed antibacterial (Akin et al., 2010), antifungal (Fraternali et al., 2005), anti-inflammatory (Kamatou et al., 2005; Chan et al., 2011), anticholinesterase (Orhan et al., 2007; Kivrak et al., 2009) and antioxidant activities (Bozin et al., 2007; Miguel et al., 2011). Furthermore, volatile oils obtained from *Salvia* species were also used in cosmetic industry, in perfumery and also as condiments (Delamare et al., 2007). Among the most known constituents identified in *Salvia* oils,

1,8-cineole, spathulenol, borneol,  $\beta$ -caryophyllene, germacrene D, bicyclgermacrene and caryophyllene oxide (Flamini et al., 2007; Kelen et al., 2008; Oztürk et al., 2009).

In Algeria, *Salvia* includes 23 species, five of which are endemic (Quezel and Santa, 1963). Moreover, this genus is represented in Algerian Sahara only by three species: *S. chudaei*, *S. aegyptiaca* and *S. verbenaca* (ssp. *clandestina* and ssp. *Pseudo-jaminiana*) (Ozenda, 1991). Aiming to continue our investigations on Algerian Saharan species (Smaili et al., 2011; Flamini et al., 2013, Belkassam et al., 2019, Smaili et al., 2021), the chemical characterization of the essential oil from *Salvia verbenaca* ssp. *clandestina* growing wild in Bou Saâda, pre-Saharan region in Algeria, is described.

## 2. Materials and methods

### *Plant material*

Aerial parts of *Salvia verbenaca* ssp. *clandestina* (Fig. 1) were collected during the ripening stage from pre-Saharan area of Bou

Saâda, South-east of Algeria. Identification of collected samples was confirmed by Pr. Tahar Smaili and a voucher sample was placed at the Department of Natural and Life Sciences, Faculty of Sciences, University of M'sila, Algeria (*S. verbenaca* ssp. *clandestina* voucher number ST/RK N°10).

### *Isolation of the essential oil*

The air dried plant material (200 g) was coarsely cut and subjected to hydrodistillation for 2 h using a Clevenger type apparatus. Obtained essential oil was then preserved in a sealed vial at 4 °C until further analysis (Shafaie et al., 2019). **GC-MS analysis**

The chromatographic analysis (GC-MS) was carried out using a Varian CP-3800 GC containing a DB-5 capillary column (30m  $\times$  0.25 mm; coating thickness 0.25  $\mu$ m) and equipped with a mass spectrometry detector (Varian Saturn 2000).



**Fig. 1.** *Salvia verbenaca* ssp. *clandestina* plants (Original).

The analysis was performed using analytical grade helium as carrier gas at a flow rate of 1 mL/min; injector and transfer line temperature were fixed at 220°C and 240°C, respectively; the oven temperature was programmed from 60°C to 240°C at 3°C/min, using helium at 1 mL/min as carrier gas; injection of 0.2 µL of a 10% hexane solution; split ratio 1:30 (Da Silva *et al.*, 2013).

### Identification of volatile components

The chemical compounds of essential oil were identified based on the retention time on capillary column in comparison with injected standards; retention indices were compared to those given in the literature; computer matching identified compounds used as references (NIST 2000 and ADAMS 2007) and

was also performed against homemade library of mass spectra built up from pure substances and MS literature data (Adams, 2007; Davies, 1990).

### 3. Results and discussions

The yield of isolated essential oil from aerial parts of *S. verbenaca* ssp. *clandestena* was 0.95% (w/w). In general, 64 compounds were identified; representing 95.6% of the whole oil, 45 compounds of them are identified in *S. clandestena* volatile oil for the first time in this study. The detailed chemical composition of the studied essential oil is summarized in **Table 1**.

**Table 1.** Percentage composition of the essential oil from *S. verbenaca* ssp. *clandestena*.

R.t (min)	Compound name	Content %	I.r.i
3.24	(E)-2-hexenal*	0.5	854
3.45	1-hexanol*	0.2	867
4.59	α-thujene	0.3	931
4.75	<b>α-pinene</b>	<b>5.2</b>	939
5.11	camphene*	0.2	953
5.24	thuja-2,4(10)-diene	0.3	957
5.39	benzaldehyde*	0.1	961
5.72	sabinene	0.9	976
5.81	<b>β-pinene</b> *	<b>10.2</b>	980
6.07	6-methyl-5-hepten-2-one*	0.3	985
6.18	myrcene	0.9	991
6.37	(E,Z)-2,4-heptadienal*	0.1	1000
6.80	(E,E)-2,4-heptadienal*	0.2	1016
6.95	α-terpinene*	0.2	1020
7.20	p-cymene*	0.3	1028
7.33	limonene	1.2	1033
7.62	(Z)-β-ocimene*	0.7	1042
7.83	benzene acetaldehyde*	0.6	1045
7.96	(E)-β-ocimene*	0.5	1052
8.33	γ-terpinene*	0.5	1063
9.39	terpinolene*	0.4	1090
9.83	linalool*	0.5	1100
10.00	nonanal*	1.1	1104
10.84	α-campholenal*	0.8	1127
11.32	trans-pinocarveol*	2.7	1143
11.58	cis-verbenol*	1.8	1145
12.29	pinocarvone*	1.8	1163

12.48	p-mentha-1,5-dien-8-ol*	0.6	1166
12.90	4-terpineol*	0.9	1179
13.47	$\alpha$ -terpineol*	0.3	1191
13.70	myrtenal*	3.0	1195
14.29	verbenone*	0.2	1206
14.66	trans-carveol*	0.2	1219
15.72	carvone*	0.3	1245
16.53	(E)-2-decenal*	0.4	1263
16.94	geranial*	0.2	1272
18.90	(E,E)-2,4-decadienal*	0.2	1316
20.94	(E)-2-undecenal*	0.3	1368
21.39	$\alpha$ -copaene	2.9	1377
21.77	$\beta$ -bourbonene	1.2	1384
22.02	$\beta$ -cubebene	1.0	1391
22.82	<b><math>\alpha</math>-gurjunene*</b>	<b>4.9</b>	1411
23.22	$\beta$ -caryophyllene	4.7	1420
23.64	$\beta$ -copaene*	0.1	1433
24.38	cis-muurolo-3,5-diene*	0.2	1448
24.65	$\alpha$ -humulene	0.8	1454
24.73	(E)-geranyl acetone*	0.4	1455
24.95	alloaromadendrene*	1.8	1461
25.07	cis-muurolo-4(14),5-diene	0.8	1462
25.82	<b>germacrene D</b>	<b>5.0</b>	1482
26.11	$\beta$ -selinene*	0.9	1486
26.46	bicyclogermacrene	2.2	1495
26.79	$\alpha$ -bulnesene*	0.4	1505
27.20	trans- $\gamma$ -cadinene	4.1	1514
27.58	$\delta$ -cadinene	1.5	1525
29.33	ledol*	0.5	1565
29.73	<b>spathulenol</b>	<b>8.7</b>	1577
29.95	<b>caryophyllene oxide</b>	<b>6.1</b>	1582
30.74	Guaiol*	1.2	1595
30.98	humulene oxide II*	0.8	1608
31.23	1,10-di epi-cubenol	1.2	1615
32.23	tau-cadinol*	4.4	1642
32.77	$\alpha$ -cadinol*	0.7	1655
33.99	epi- $\alpha$ -bisabolol*	0.3	1686
	<b>Total identified</b>	<b>95.6%</b>	

\*Compound identified for the first time in the studied essential oil. **I.r.i** = Linear retention indices (HP-5 column). **R.t** = retention time.

According to obtained results, the essential oil is mainly composed by sesquiterpene derivatives (56.4%), with hydrocarbon derivatives (32.5%) as the major sesquiterpenes fraction, largely represented by germacrene D (5%),  $\alpha$ -gurjunene (4.9%) and  $\beta$ -caryophyllene (4.7%), while oxygenated sesquiterpenes (23.9%) are mainly represented by spathulenol (8.7%) and caryophyllene oxide (6.1%). The

second major fraction in the studied sample is represented by monoterpenes derivatives (35.5%): Monoterpene hydrocarbons (21.8%) are principally presented by  $\beta$ -pinene (10.2%),  $\alpha$ -pinene (5.2%) and limonene (1.2%) and oxygenated monoterpenes (13.7%) are mainly represented by myrtenal (3%) and *trans*-pinocarvol (2.7%).

**Table 2.** Chemical groups of the essential oil from *Salvia clandestena*

Chemical groups	%
Monoterpene hydrocarbons	21.8
Oxygenated monoterpenes	13.7
<b>Total monoterpenes</b>	<b>35.5</b>
Sesquiterpene hydrocarbons	32.5
Oxygenated sesquiterpenes	23.9
<b>Total sesquiterpenes</b>	<b>56.4</b>
Aldehydes	03.5
Alcohols	00.2
<b>Total non-terpene fraction</b>	<b>03.7</b>

Non-terpene compounds form a minor part of the sample (3.7%) and are constituted by aldehydes (3.5%) and alcohols (0.2%). The principle chemical groups found in *S. clandestena* volatile oil are summarized in **table 2**. Therefore, chemical composition of *S. clandestena* in this study may be categorized as sesquiterpene and monoterpene chemotype among the four chemotypes identified for *Salvia* species (Jassbi *et al.*, 2012).

Only few data exist in literature about the chemical composition of *S. verbenaca* ssp. *clandestena* essential oil. In a study realized by Belloum *et al.* (2014), the volatile oil of the studied plant collected from a different region (Bechar) in Algerian Sahara, was found to contain germacrene D as a major constituent (20.5%), followed by  $\alpha$ -copaene (10.4%),  $\beta$ -phellandrene (3.8%) and  $\beta$ -caryophyllene (3.8%). However,  $\beta$ -pinene which is the major constituent in the volatile oil of *S. verbenaca* ssp. *clandestena* in the present analysis is minor constituent of the same species growing in Bechar, permitting the hypothesis that this constituent is a chemical marker of our sample.

Similar differences were observed in the chemical composition of essential oils from *Salvia* species. In a study reported by Mohammadi *et al.* (2014) about *S. aegyptiaca* aerial parts essential oil, where the plant was collected from Bechar region in the South-west of Algeria,  $\beta$ -caryophyllene (10.2%), selina-4,11-diene (9.7%), bornyl acetate (8.5%) and

$\beta$ -gurjunene (7.6%) were identified as main constituents. Whereas the volatile oil of the same plant collected from other regions like Saudi Arabia in the study of Basaif in 2004, which showed a rich composition in 1,10-aristolene (19.3%), and also collected from Egypt in the study of El-Sawi in 2003, which confirmed the presence of trans, *trans*-farnesol, phytol, spathulenol and *cis*, *trans*-farnesol as major constituents of the oil.

## Conclusions

In the present research, the chemical composition of the essential oil from aerial parts of *Salvia verbenaca* ssp. *clandestena* is reported. Obtained results were partially different from those previously described, the relatively high level of  $\beta$ -pinene seems characteristics of our sample and these differences may be due to the different growth habitat and environmental factors. Furthermore, the chemical composition of *Salvia verbenaca* ssp. *clandestena* volatile oil from Bou Saâda (Algeria) may be categorized as sesquiterpene and monoterpene chemotype among the four chemotypes identified for *Salvia* species. Further studies are required to evaluate biological activities of this volatile oil to know its possible applications.

## Conflict of interest

None declared.

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