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HS-SPME-GC/ MS ANALYSIS OF 3 LAMIACEAE PLANTS: *AJUGA IVA* (L.) SCHREB., *SALVIA VERBENACA* L. AND *THYMUS ALGERIENSIS* BOISS. & REUT.

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ABSTRACT

The chemical analysis of three Algerian plants was conducted by using Headspace Solid-Phase Micro-Extraction coupled with Gas Chromatography-Mass Spectrometry (HS-SPME-GC/ MS). This analysis was performed directly on the dried aerial parts of the plants. The results revealed that the major compounds are 1,8-cineol (27.3%) in *Ajuga iva*, *cis*-muurola-3,5 diene (14.6%) in *Salvia verbenaca* and 2,3-dehydro-1,4-cineol (36%) in *Thymus algeriensis*. The oxygenated monoterpenes are the major class representing 62.1% and 78.4% in *Ajuga iva* and *Thymus algeriensis*, respectively, whereas the sesquiterpene hydrocarbons are the major class in *Salvia verbenaca* representing 50.1%. Identification of naturally rare monoterpene ether, 2,3-dehydro-1,4-cineol was the first time in the *Ajuga iva*, *Salvia verbenaca* and *Thymus algeriensis*.

Keywords: *Ajuga iva*; *Salvia verbenaca*; *Thymus algeriensis*; Lamiaceae.

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1. INTRODUCTION

Algeria has a privileged place in production and export of medicinal and aromatic plants due to its different bioclimatic zones (humid, sub humid, semiarid, arid or desert) and its geographical situation [1]. Medicinal plants are of major importance for all African region, both for their use in traditional medicine and for trade [2]. More than 200 000 of the 300 000 plant species found today in our entire planet live in tropical countries in Africa and elsewhere. Among the potential users of these plants, traditional medicine and pharmacopoeia come at the head of those who practice them, namely 70% of Third World populations [3]. The Lamiaceae is a family yielding a high number of medicinal taxa, especially due to their high content of essential oil [4]**.** Headspace (HS) analysis involves the direct analysis of the volatiles in the gas phase above a sample. It is an inherently simple technique which offers numerous advantages over more traditional sample preparation techniques such as extraction, adsorption, precipitation and distillation [5]. Solid Phase Micro-Extraction (SPME) was introduced as a solvent-free sample preparation technique. The basic principle of this approach is to use a small amount of the extracting phase, usually less than 1 μL [6]**.** Headspace and Solid Phase Micro-Extraction (HS-SPME) are two methods of sample preparation widely used in industrial fields and biology. Their combination helps to increase the sensitivity of analytes and consequently to obtain lower limits of quantification and detection [7]**.** *Ajuga iva* (L.). Schreb. *Salvia verbenaca* L. and *Thymus algeriensis* Boiss. & Reut are the medicinal plants in which the essential oils have been used as antirheumatic, antiseptic, antispasmodic, antimicrobial, carminative, diuretic and expectorant antioxidant etc [8-10]. The objective of this study is to analyse the volatile constituents of the Algerian Lamiaceae plants: *Ajuga iva* (L.). schreb, (*A. iva*), *Salvia verbenaca* L. (*S. verbenaca*) and *Thymus algeriensis* Boiss. & Reut (*T. algeriensis*) using the HS-SPME coupled with GC/MS. This paper is the first to study the HS SPME GC/MS analysis of the three Lamiaceae plants.

2. MATERIAL AND METHODS

2.1. PLANT COLLECTING

Aerial parts of *A. iva*, *S. verbenaca* and *T. algeriensis* were collected in March 2019 from plants growing in Djelfa (Algeria). The plant samples were purified and dried at room

temperature for one week, kept separately and transferred to the laboratory then stored in low temperature (-15 °C) until the analysis. Voucher specimens were deposited at the laboratory of exploration and valorization of steppic ecosystems of Djelfa (Algeria).

2.2. HS-SPME-GC/ ANALYSIS

The three Lamiaceae plants were subjected to the HS-SPME-GC/MS analysis according to Asakawa et *al.* [11] with slight modification. The dried aerial parts of *A.iva., S. verbenaca,* and *T. algeriensis* were cut into small pieces (about 0,1g), and they were kept in a vial (20 mL) which was completely closed. The HS gas was absorbed on a PDMS type fiber (100 µm film thickness, Supelco) at 25 °C for 1 h. Then, the fiber was introduced into an injector of 6890 GC system (Agilent Technologies) equipped with a DB-1MS column (60 m × 0.25 mm id., 0.25 µm film thickness, Agilent Technologies). The oven temperature program was set at 60 °C with 3 min initial hold and then raised to 100 °C at a rate of 10 °C/min and then raised to 200 °C at a rate of 5 °C/min and hold with 3 min and raised to 260 °C at a rate of 15 °C/min and then raised to 300 °C at a rate of 20 °C/min. The post run oven temperature program was set at 310 °C with 3 min hold. The carrier gas was helium with a constant flow of 1 mL/min with no split. A 5973 Mass selective detector (Agilent Technologies) was operated under electron impact condition at an ionization energy of 70 eV in scan mode at *m/z* 40-500 with 3 scans. The injector, the transfer line and the ion source temperatures were set at 250 °C, 280 °C and 230 °C, respectively. The retention indices were calculated relative to C8-C15 n-alkanes. Compounds were identified using a computer supported library [12], mass spectra of references compounds [13, 14] and mass spectra from the japanese library databases.

3. RESULTS AND DISCUSSION

The identified volatile compounds in *A. iva*, *S. verbenaca* and *T. algeriensis* are listed in tables 1, 2, 3 and 4, respectively in which the retention times, percentages and retention indices of the compounds are given. The GC profiles of *A. iva*, *S. verbenaca* and *T. algeriensis* are presented in figures 1, 2 and 3, respectively. The total identification of compounds in A. *iva* is 81.0% (8 compounds), *S.verbenaca* is 69.3% (18 compounds) and *T. algeriensis* is 93.4% (20 compounds). Five compounds appear in the three plants, and these are α-pinene, camphene, 1-octen-3-yl acetate, bornyl acetate and 2,3-dehydro-1,4-cineol.

Three compounds appear in *A.iva* and *T.algeriensis*, these are *p*-cymene, 1,8-cineol and camphor. Six compounds appear in *T.algeriensis* and *S. verbenaca*, these are β-pinene, borneol, α-gurjunene, β-caryophyllene, α-cubebene and *cis*-muurola-3,5-diene.

Table 1. Volatile Compounds in *Ajuga iva* (L.) Schreb.

Number of peak	Compounds	Retention times	Retention indices	%
1	α-pinene	9.95	935	9.7
2	camphene	10.26	950	6.4
3	*p*-cymene	10.84	977	2.8
4	**1,8-cineol**	**11.94**	**1025**	**27.3**
5	1-octen-3-yl acetate	13.53	1092	1.5
6	camphor	14.42	1128	9.2
7	unknown	14.84	1145	1.7
8	Bornyl acetate	18.10	1274	8.0
9	2,3-dehydro-1,4-cineol	19.65	1335	16.1

Table 2. Volatile Compounds in *Salvia verbenaca* L.

Number of peak	Compounds	Retention times	Retention indices	%
1	α-pinene	9.95	935	2.8
2	camphene	10.69	970	0.7
3	β-sabinene	10.83	976	3
4	β-pinene	10.96	982	4.2
5	limonene	11.93	1125	2.8
6	borneol	14.41	1128	0.5
7	1-octen-3-yl acetate	15.92	1188	0.7
8	Bornyl acetate	18.09	1274	0.8
9	2,3-dehydro-1,4-cineol	19.65	1335	3.7

10	Bicycloelemene	19.77	1340	4.3
11	γ-amorphene	20.85	1383	10.5
12	γ-cadinene	21.09	1392	4.8
13	α-gurjunene	21.72	1417	2.3
14	β-ylangene	21.87	1423	1.2
15	β-caryophyllene	21.93	1426	2.6
16	unknown	22.08	1432	2.6
17	α-cubebene	22.36	1443	3.0
18	unknown	22.91	1466	10.5
19	***cis*-muurola-3,5-diene**	**23.33**	**1483**	**14.6**
20	bicyclogermacrene	23.69	1497	6.8

Table 3. Volatile Compounds in *Thymus algeriensis* Boiss. & Reut.

Number of peak	**Compounds**	**Retention times**	**Retention indices**	**%**
1	α-pinene	9.96	935	5.3
2	camphene	10.26	950	3.2
3	β-pinene	10.83	977	0.8
4	Dehydro-1,8-cineol	10.98	983	0.4
5	*p*-cymene	11.72	1016	0.4
6	1,8-cineol	11.94	1025	13.3
7	t*rans*-sabinene hydrate	12.7	1057	0.4
8	unknown	13.35	1274	0.4
9	1-octen-3-yl acetate	13.52	1092	1.0
10	unknown	13.64	1340	0.9
11	α-campholenal	13.96	1110	0.4
12	camphor	14.42	1128	11.9
13	unknown	14.53	1133	1.8

14	unknown	14.84	1145	0.7
15	borneol	15.1	1156	1.6
16	α-terpineol	15.64	1177	1.1
17	verbenone	15.9	1188	1.0
18	unknown	17.22	1240	0.4
19	bornyl acetate	18.1	1274	11.7
20	**2,3-dehydro-1,4-cineol**	**19.68**	**1336**	**36.0**
21	Neryl isobutyrate	19.81	1341	1.7
22	α-cubebene	20.85	1382	1.2
23	unknown	21.09	1392	0.5
24	α-gurjunene	21.72	1417	0.5

Table 4. Volatile Compounds in *Thymus algeriensis* Boiss. & Reut.(continued)

Number of peak	**Compounds**	**Retention times**	**Retention indices**	**%**
25	β-caryophyllene	21.93	1426	0.6
26	*cis*-muurola-3,5-diene	23.32	1482	0.9

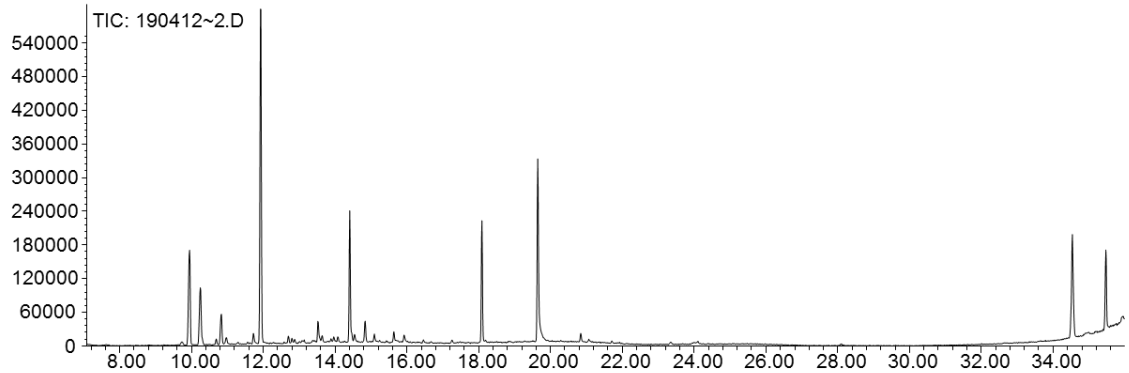

Fig.1. GC profile of *Ajuga iva* (L.) Schreb.

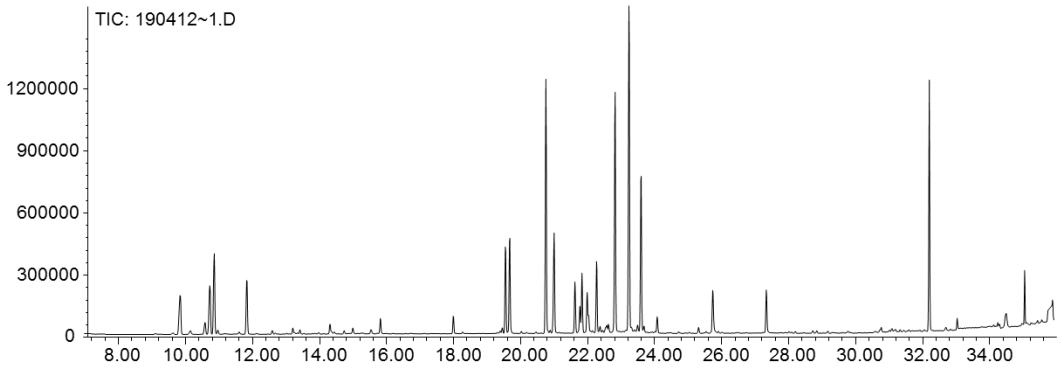

Fig.2. GC profile of *Salvia verbenaca* L.

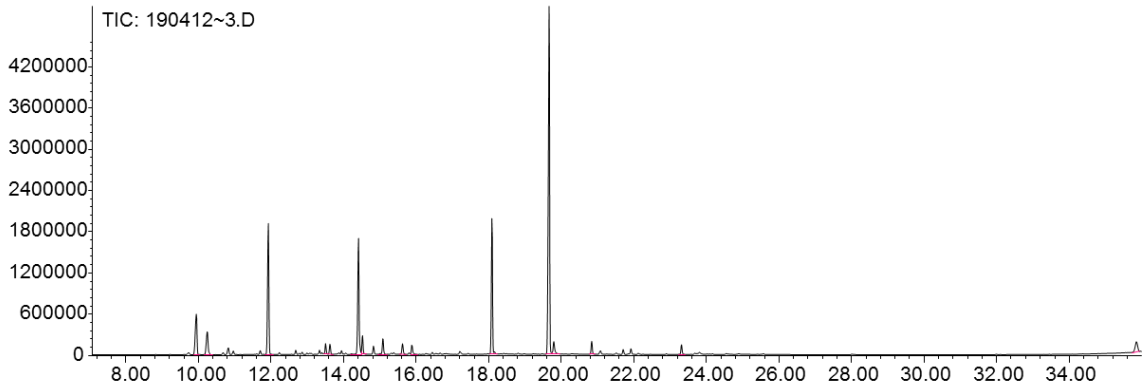

Fig.3. GC profile of *Thymus algeriensis* Boiss. & Reut.

The results showed that the major compounds in *A. iva*, *S.verbenaca* and *T.algeriensis* are 1,8-cineol (27.3%), *cis*-muurola-3,5 diene (14.6%) and 2,3-dehydro-1,4-cineol (36.0%), respectively. The different classes of compounds are presented in the table 5. The results revealed that oxygenated monoterpenes are the major class of compounds in both *A. iva* and *T. algeriensis* representing 62.1% and 78.4%, respectively followed by the monoterpene hydrocarbons representing 18.9% in *A. iva* and 10.1% in *T. algeriensis.* Sesquiterpene hydrocarbons are the major class of compounds in *S. verbenaca* representing 50.1%, followed by the monoterpene hydrocarbons representing 13.5% and oxygenated monoterpenes 5.7%.

Table 5. Classes of compounds in the three plants

	A. iva %	*S. verbenaca* %	*T. algeriensis* %
Total identification	81.0	69.3	93.4
Monoterpene hydrocarbons	18.9	13.5	10.1
Oxygenated monoterpenes	**62.1**	5.7	**78.4**
Sesquiterpene hydrocarbons	-----	**50.1**	4.9

Only a few studies have been conducted, using the HS-SPME GC/MS method, on plants belonging to the genus *Salvia* and *Thymus*. According to D'Auria and Racioppi [15], the main compounds in *Salvia officinalis* L. collected from Italy are 1,8-cineole (22.3 %), β-thujone, camphor (11.2%), β-pinene (8.9%), and *trans*-caryophyllene (5.0%). Another study conducted on the same species from Iran, the main compounds are n-hexyl benzoate (40.2%), benzyl benzoate (24.1%), linalool (7.4%) and butyl benzoate (5.4%) [16]. The main compounds of the volatile fraction of *Salvia* species from Poland are identified as α-pinene, camphene, β-pinene, thujol, camphor, β-chamigrene, and cadina-3,9-diene [17]. In addition, the analysis revealed that 1-octen-3-ol (46.5 %), 2-hexenal (18.0 %), benzaldehyde (8.8 %) and aromadendrene (7.2 %) are the main compounds of the aerial parts of *Salvia farinacea* Benth collected from Egypt [18]. The main compounds of *Thymus serpyllum* (Italy) are *p*-cymene (30.3 %) and γ-terpinene (11.8%) [15]. According to Almeida et *al.* [19], linalool is the most important compound in *Thymus-mastichina* from Sesimbra (Portugal) (26-30%) and 1,8-cineol or eucalyptol from Algarve (Portugal) (23-33%).

Several studies have been published on the composition of the volatile constituents of the three plants using the hydrodistillation and GC/MS techniques. Many compounds have been identified in these studies. According to Chouitah et *al.* [20] dienestrol is the main compound in *A. iva* from Mascara (Algeria) representing 54.0%, whereas carvacrol is the main compound (35.1%) in the essential oil of *A.iva* collected in Libya [21]. Sabinene is the main monoterpene detected in *S.verbenaca* from Saudi Arabia representing 16.0% [22]. Hexadecanoic acid is the most abundant compound (23.1%) in the essential oil of *S.*

verbenaca collected in Italy [10]. In regard to the composition of the essential oil of *T.algeriensis,* the previous studies revealed that Borneol is the major compound representing 28.0% (Morocco) [23] and 18.3% [24] (Morocco). Benabed *et al.*[25] reported α-terpinyl acetate (27.3%) as main compound in *T.algeriensis* from Djelfa (Algeria). Thymol is the major compound in *T. algeriensis* collected in Algeria (29.5%) [26], in Libya (38.5%) [27]. α-pinene is the major compound representing 27.1% and 25.5% (Algeria) [28].

The comparison of the current results with those of previous studies on the three Lamiaceae plants revealed that oxygenated monoterpenes are the major class in *T.algeriensis* 48.0% [24], 79.5% [29], 54.7% [27], 88.5% [30]. Monoterpenes hydrocarbons are the major class in *S.verbenaca* [22]. In the study of Al-Jaber [31], sesquiterpenes hydrocarbons represent 62.7% in dried *S.verbenaca*, whereas in the fresh plant, oxygenated monoterpenes represent 61.3%. In *A. iva*, the oxygenated monoterpenes represent 52.3% [21]. The previous studies carried out on these plants showed a high diversity in the chemical composition. The chemovariation might be influenced by the source of the plant material, a genetic and environemental factors [32-34]. 2,3-Dehydro-1,4-cineol, a very rare natural product, was identified in the present *Ajuga iva, Salvia verbenaca* and *Thymus algeriensis* the first time.

4. CONCLUSION

The HS SPME GC/MS analysis of the three Lamiaceae plants from Djelfa (Algeria) allowed to identify 46 compounds. The main compounds are 1,8-cineol, *cis*-muurola-3,5-diene and 2,3- dehydro-1,4-cineol. The results of this investigation revealed that *Ajuga iva* (L.) Schreb., *Salvia verbenaca* L. and *Thymus algeriensis* Boiss. & Reut. are rich sources of bioactive constituents. These compounds are natural products make them more attractive for further studies about the biological activities of the isolated compounds.

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