

Acta Scientifica Naturalis

Former Annual of Konstantin Preslavsky University of Shumen: Chemistry, Physics, Biology, Geography
Journal homepage: asn.shu.bg

Chemical Composition of Algerian *Thymus algeriensis* Boiss. & Reut. and *Marrubium vulgare* L. (Lamiaceae) Essential Oils from the Aures Region

Sara Kebbi¹, Hamza Fadel^{1*}, Jean-claude Chalchat², Gilles Figueredo³, Pierre Chalard⁴, Hichem Hazmoune¹, Fadila Benayache¹, Samir Benayache¹ and Ramdane Seghiri¹

¹ *Unité de Recherche Valorisation des Ressources Naturelles, Molécules Bioactives et Analyses Physicochimiques et Biologiques. Université des Frères Mentouri, Constantine, Route d'Ain El Bey, 25000, Constantine, Algérie*

² *Association de Valorisation des Huiles Essentielles et des Arômes (AVAHEA, La Haye 7, 63500 Saint Babel, France*

³ *Laboratoire d'Analyses des Extraits Végétaux et des Arômes (LEXVA Analytique), 7 Rue Henri Mondor, Biopôle, Clermont-Limagne, 63360, Saint Beuzir, France*

⁴ *Université Clermont Auvergne, CNRS SIGMA Clermont ICC, F-63000 Clermont Ferrand France*

Abstract: This study was aimed to determine the chemical composition of essential oils obtained from aerial parts of *Thymus algeriensis* Boiss. & Reut. and *Marrubium vulgare* L. collected in the Aures region of Algeria. Essential oils were extracted by steam distillation method and analyzed using GC-MS and GC-FID techniques. Thirty-five (35) and twenty-nine (29) components were identified respectively in *T. algeriensis* and *M. vulgare* essential oils to represent respectively 94.8% and 75.7% of the total oil composition. The main compounds of *T. algeriensis* oil were germacrene D (29.6%), β -caryophyllene (11.0%), E- β -farnesene (7.8%), bicyclogermacrene (4.4%) and δ -cadinene (4.0%), while, β -bisaboene (36.3%), β -caryophyllene (7.8%), phytol (6.2%), nonacosane (4.0%) and heptacosane (3.3%) were the main components of *M. vulgare* oil. The richness of these two oils in germacrene D and β -bisaboene respectively could suggest a new bioactivity.

Keywords: Essential oil, *Thymus algeriensis*, *Marrubium vulgare*, germacrene D and β -bisaboene

Introduction

The essential oils obtained from various parts of the plants have considerable interests in the pharmaceutical industry due to their biological potential [1, 2]. The species of Lamiaceae family have been used in traditional medicine to treat several diseases, thus, it should be also highlighted for its essential oils potential [3]. Among the genera of this family of great biological interest and which is growing in Mediterranean area, we distinct the genus *Thymus* and *Marrubium* [4, 5]. *Thymus algeriensis* Boiss. & Reut. is an herbaceous annual plant which called in Algeria “Djertil” [6]. It is largely used in traditional medicine to treat respiratory disorders, illnesses of the digestive tube and as anti-abortion [7]. *Marrubium vulgare* L. is commonly known as “white horehound”, and in Algeria as “Marriout” [8]. It is used in folk medicine to treat the digestive tract, diarrhea, diabetes, rheumatism, coughs and cold [9, 10].

In fact, many authors have investigated the chemical composition of *T. algeriensis* [6, 11-22] and *M. vulgare* essential oils [10, 23-36]. In addition, several studies proved that some factors such geographic origin [37] genetic and extraction methods [38] could influence the variability of essential oils composition. Therefore, the present study is an attempt to identify and enrich the knowledge on the variation of the chemical composition of essential oils from two species of Lamiaceae family of the Aures area, named *Thymus algeriensis* and *Marrubium vulgare* using GC-MS and GC-FID analyzes.

Material and Methods

Plant materials

Plant materials were collected from north eastern of Algeria (Batna, Aures region). *M. vulgare* was collected in January 2015 from El-Kantina of Yabous (61 km south-west from Khenchla, 35°24'29" Nord, 6°38'31" East, Altitude: 1167 m), and *T. algeriensis* was collected in June 2015 from Foug-Toub region (57 km south east from Batna, 35°24'18" Nord, 6°32'59" East, Altitude: 1164 m). The authenticated voucher specimens (TA/107/VAR/06-15 and MV/102/VAR/01-15) were deposited in the Herbarium of Research Unit (VARENBIOMOL), University of Mentouri Brothers, Constantine1, Algeria.

Extraction of the essential oils

Dried aerial parts of *T. algeriensis* (186 g) and of *M. vulgare* (130 g) were subjected to steam distillation in a Kaiser Lang apparatus for three hours. The obtained essential oils (94 and 50 mg, respectively) were collected and dried over anhydrous sodium sulphate and kept at 4°C until analysis. The oils yields of essential oils were calculated according to the dry plant materials of the species.

GC-FID Analysis

The essential oils were analyzed on an Agilent gas chromatograph (GC-FID) Model 6890, equipped with a HP-5MSf used silica capillary column (5%-diphenyl-95%-dimethylpolysiloxane, 25 m x 0.25 mm, film

thickness 0.25 μm), programmed from 50°C (5 min) to 250°C at 3°/min and held for 10 min. Injector and flame ionization detector temperatures were 280 and 300°C, respectively. The essential oils were diluted in acetone (3.5%, v/v) and injected in split mode (1/60) helium was used as a carrier gas (1.0 ml/min). Solutions of standard alkanes (C8-C20) were analyzed under the same conditions to calculate retention indices (RI) with Van del Dool and Kratz equation [38, 39].

GC-MS Analysis

Mass spectrometry was performed on an Agilent gas chromatograph-mass spectrometer (GC-MS) Model 7890/5975, equipped with HP-5MS capillary column (25 m x 0.25 mm, film thickness 0.25 μm) programmed with the same conditions as for GC-FID. The mass spectrometer (MS) ionization was set in positive electron impact mode at 70 eV and electron multiplier was set at 2200 V. Ion source and MS quadrupole temperatures were 230°C and 180°C, respectively. Mass spectral data were acquired in the scan mode in the m/z range 33-450. The essential oils constituents were identified by matching their mass spectra and retention indices (RI) with those of reference compounds from libraries [40, 41]. The proportions of the identified compounds were calculated by internal normalization.

Results and Discussion

The steam distillation of *T. algeriensis* aerial part produced sharp odorous yellow oil with a yield of 0.05% (w/w). The GC-MS (Table 1) and GC-FID (Figure 1) results showed the presence of 35 identified components (listed in order of their experimental retention times and retention indices), representing 94.8% of the total oil composition. Sesquiterpenes were found highly dominated to represent 81.0% of the total composition, where hydrocarbons sesquiterpenes were the predominant class (67.0%), followed by oxygenated sesquiterpenes (14.0%), oxygenated monoterpenes (7.7%), hydrocarbon monoterpenes (3.2%) and other compounds (2.6%). The major components were respectively germacrene D (29.6%), β -caryophyllene (11.0%), E- β -farnesene (7.8%), β -eudesmol (5.3%), bicyclogermacrene (4.4%), δ -cadinene (4.0%), α -humulene (3.5%), α -bulnesene (2.4%), E-nerolidol (2.4%), α -guaiane (2.3%), phytol (2.3%), spathulenol (2.2%) and verbenol (2.0%).

According to the previously reported data, the results of some Algerian *T. algeriensis* oils from different regions showed variability in chemical profiles. Indeed, our sample was characterized by high content of germacrene D (29.6%) and β -caryophyllene (11.0%), while, Houmani et al. [12] found that *T. algeriensis* oil samples purchased from different herbalists of Blida, were rich in linalool (78.8%) and thymol (62.7%). Moreover, Dob et al. [6] and Giordiani et al. [14] revealed that linalool (47.3%) and α -pinene (25.5% to 27.1%) were respectively the main constituents of *T. algerienis* oil from Media and Souk-aharas regions. However, Hazzit et al. [13] found that geranyl acetate (16.4%) was the major component of *T. algerienis* oil

from Blida and Bouira regions, while in 2009, the same author reported thymol (29.5%) and terpinyl acetate (18.0%) as the predominant components for samples from the same location. Touhami et al. [42] reported that camphor was the chemotype of the oil before flowering stage with (33.3%) and *p*-cymen-7-ol in flowering period (27.0%). Recently, it has been reported by Bendjabeur et al. [22] that carvacrol (43.2%) and *p*-cymene (18.7%) were the major components in *T. algeriensis* oil from Relizane and Bouira regions, and similar results were obtained by Kouache et al. [19] in the same sample from Ain-Defla region, whereas, Benabed et al. [21] found in their studies on two samples collected from Djelfa and Laghouat, that the main compounds were camphor (17.7%) and α -terpinenyle acetate 27.3%. Furthermore, Benabdallah et al. [20] found that elemol (18.4%) and camphor (14.2%) were the main component in a sample collected from the national park of Bellazma (Batna). However, thymol (71.4 and 38.5%) was the predominant component in Algerian *T. algeriensis* oils from Hamam-Alwan of Blida [43] and in a Libyan sample [18]. Thus, Benjilali et al. [11] found thymol (14.4 to 65.1%) and carvacrol (22.8 to 70.3%) as the predominant compounds, while, Ait-Ouazzou et al. [44] found borneol (23.5%) as the major compounds of Moroccan samples. But, according to the results of Amarti et al. [16] and Zouari et al. [17] camphor (27.7%) and 1,8-cineole (7.55 to 22.07%) were the main components in *T. algeriensis* oil from Morocco and Tunisia, respectively.

In fact, the chemical composition of our sample was rather different, where we observed the presence of germacrene D, β -caryophyllene, E- β -farnesene, bicyclogermacrene and α -humulene with high contents. It is also interesting to note again that α -bulnesene, α -guaiene, phytol, β -copaene and nonacosane were identified for the first time in our *T. algeriensis* essential oil.

Our essential oil obtained from *M. vulgare* oil was produced as a pale yellow color with a yield of 0.04% (w/w). The results of analyzes by GC-MS (Table 2) and GC-FID (Figure 2) showed the presence of 29 identified compounds to represent 81.3% of the total oil components. The oil was characterized by domination of sesquiterpene (53.8%) where hydrocarbons sesquiterpenes were predominant class (50.0%), followed by oxygenated hydrocarbons (13.9%), hydrocarbons (12.5%), oxygenated monoterpenes (1.8%) and oxygenated sesquiterpens (2.6%). The main constituents were respectively β -bisabolene (36.3%), β -caryophyllene (7.8%), phytol (6.2%), nonacosane (4.0%), heptacosane (3.3%), nonanal (2.8%), 6,10,14-trimethyl-2-pentadecanone (2.6%) and α -humulene (2.0%).

Several studies have described the chemical composition of *M. vulgare* essential oils from different regions of Algeria, for example, Elbali et al. [37] reported β -bisabolene (43.4%) and δ -cadinene (10.2%) as the major constituents of *M. vulgare* collected from Tazoult region of Batna. However, in Setif region, Belhattab et al. [29] reported that eugenol (50.1%) and β -bisabolene (28.8%) were found as major components in *M. vulgare* oil, whereas, Abadi et al. [10] reported that 4,8,12,16-tetramethyl heptadecan-4-ol (17.0%) and germacrene D-4-ol (9.6%) were the major constituents of the same sample from Tebessa region.

Moreover, eugenol (21.5%) and β -caryophyllene were the main constituents of the sample from Medea region [45]. Furthermore, Kadri et al. [30] reported that γ -eudesmol (11.9%) and β -citronellol (9.9%) were found as the main components of Tunisian *M. vulgare* oil. In a Libyan *M. vulgare* oil, [33] thymol (20.1%) and E- β -farnesene (15.6%), respectively, were found as the major compounds, whereas, in Egypt, *M. vulgare* oil was found rich in thymol (34.5%) and γ -cadinene (17.7%) by Salama et al. [31], while, Said-Al-Ahl et al. [35] found that carvacrol (36.3%) and β -phellandrene (15.5%) were the major compounds of *M. vulgare* oil from Netherlands. Moreover, in different Iranian regions, β -bisabolene (25.4%) and β -caryophyllene (11.6%) were found as the major components of *M. vulgare* oil. [27] Similar results was found in another Tunisian *M. vulgare* oil by Hamdaoui et al. [34] where they found β -bisabolene (28.3%) and β -caryophyllene (7.8%) as the main compounds. Thus, Morteza-Semnani et al. [26] found that β -bisabolene (20.4%) and δ -cadinene (19.1%) were the major components, whereas, another Iranian group research found caryophyllene oxide (18.7%) and *trans*-caryophyllene (12.8%) as the main components [28]. However, Bokaeian et al. [46] reported γ -eudesmol (11.0%), germacrene D (10.0%) and citronellyl formate (10.0%) as the major compounds, while, Golparvar et al. [36] found that β -caryophyllene (32.2%) and (E)- β -farnesene (11.4%) were the major constituents. In addition, tricyclene and β -pinene were found as the main compounds of the same species oil from another Egyptian region [23]. Similar results were obtained by Nagy and Svajdlenska [24] where they found β -caryophyllene (45.8%) and germacrene D (14.4%) as the major constituents of *M. vulgare* oil from Slovakia. Moreover, E-caryophyllene (24.8 to 44.5%) and germacrene D (23.8 to 43.4%) was found as the major compound in Polish *M. vulgare* oil [32]. Weel et al. [25] reported that (Z)- β -farnesene (9.6%), β -caryophyllene (8.5%) and (E)-hex-2-enal (4.7%) were the main components of *M. vulgare* oil from a Lithuanian region. However, Bayir et al. [47] reported that α -pinene (28.8%), β -pinene (18.3%), β -phellandrene (17.4%) and 2-hexenal (14.8%) were the major compounds of *M. vulgare* oil from a Turkish area.

According to the previous published data of *M. vulgare* oil composition, the results revealed a significant variability for our sample, where we noticed that β -bisabolene was identified as a major component in many samples from different regions [26, 27, 29, 34] but we noticed that pulegone, undecanal, E- β -ionone, 3,5,7-trimethyl-2E,4E,6E,8E-undecatetraene, megastigma-5,7(E),9-trien-4-one, 1-heptadecene, pentadecanal, 6,10,14-trimethyl-2-pentadecanone, octacosane, nonacosane and unctane were identified for the first time in our *M. vulgare* oil. This variability found in essential oils composition could be linked to several factors such as genetic, environmental or extraction methods [4, 38].

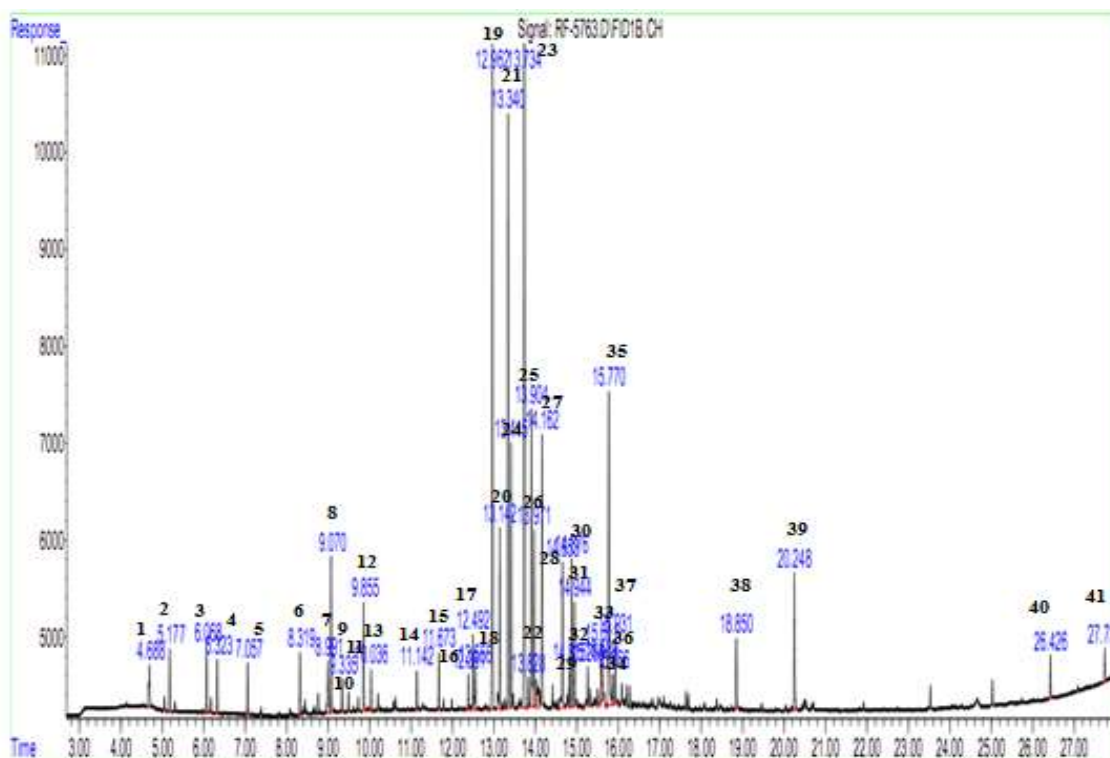


Figure 1. GC-FID Chromatogram of *T. algeriensis* essential oil

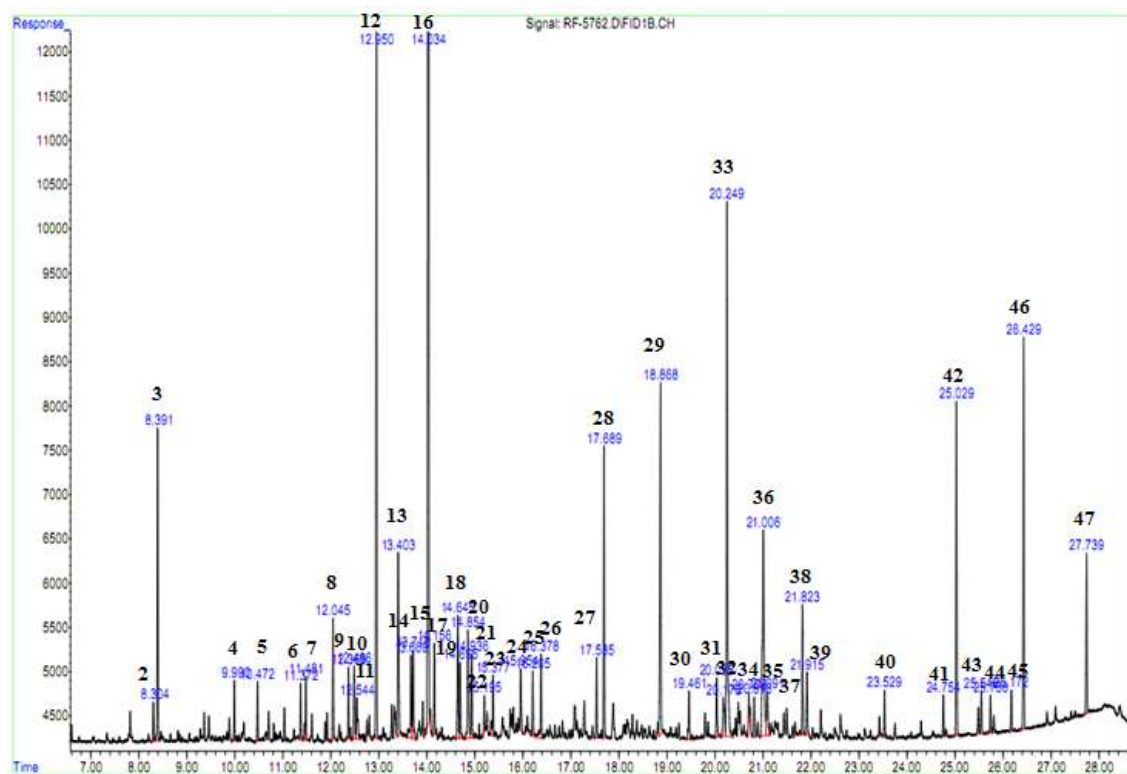


Figure 2. GC-FID Chromatogram of *M. vulgare* essential oil

Table 1. Chemical composition (%) of *Thymus algeriensis* essential oil

Peak N°	RT	^b RI	^a Components	%
1	4.688	910	Unknown	0.9
2	5.177	934	α -Pinene	0.9
3	6.068	978	β -Pinene	0.9
4	6.323	991	Myrcene	0.7
5	7.057	1031	Limonene	0.7
6	8.319	1102	Linalol	0.8
7	8.991	1144	Trans -pinocarveol	1.0
8	9.070	1149	trans-Verbenol	2.0
9	9.335	1166	Pinocarpone	0.4
10	9.492	1176	Borneol	tr
11	9.727	1191	α -Terpineol	tr
12	9.855	1199	Myrtenol	1.7
13	10.036	1211	Verbenone	0.6
14	11.142	1288	Bornyl acetate	0.5
15	11.673	1326	Myrtenyl acetate	0.7
16	12.381	1379	α -Copaene	0.4
17	12.492	1388	β -Copaene	1.0
18	12.556	1393	β -Elemene	0.6
19	12.962	1424	β -Caryophyllene	11.0
20	13.142	1439	α -Guaiene	2.3
21	13.340	1455	(E)- β -Farnesene	7.8
22	13.415	1461	α -Humulene	3.5
23	13.734	1486	Germacrene D	29.6
24	13.828	1494	Unknown	0.4
25	13.904	1500	Bicyclogermacrene	4.4
26	13.971	1505	α -Bulnesene	2.4
27	14.162	1522	δ -Cadinene	4.0
28	14.653	1563	E-Nerolidol	2.4
29	14.815	1577	Germacrene D-4-ol	0.5
30	14.876	1582	Spathulenol	2.2
31	14.944	1588	Caryophyllene oxide	1.6
32	15.265	1616	Humuleneepoxide II	0.6
33	15.589	1645	Unknown	0.4
34	15.611	1647	epi- α -Cadinol	0.9
35	15.770	1661	β -Eudesmol	5.3
36	15.866	1670	α -Eudesmol	0.5
37	15.931	1676	Unknown	0.9

Table 1. Chemical composition (%) of *Thymus algeriensis* essential oil (continued)

38	18.850	1959	Unknown	1.0
39	20.248	2098	Phytol	2.3
40	26.426	2900	Nonacosane	0.6
41	27.739	3101	Unknown	0.5
Total components				98.9
Total identified				94.8
Total not identified				4.1
Hydrocarbon monoterpenes				3.2
Oxygenated monoterpenes				7.7
Hydrocarbon sesquiterpenes				67.0
Oxygenated sesquiterpenes				14.0
Others				2.9

^aCompounds are listed in order of their RI

^bRI (retention index) measured relative to *n*-alkanes (C8-C20) using HP-5MS column

RT (retention time)

Table 2. Chemical composition (%) of *M. vulgare* essential oil

Peak N°	RT	^b RI	^a Components	%
1	4.675	910	Unknown	0.5
2	8.304	1101	Linalol	0.3
3	8.391	1106	Nonanal	2.8
4	9.990	1208	Decanal	0.5
5	10.472	1241	Pulegone	0.5
6	11.372	1304	Undecanal	0.4
7	11.481	1312	Unknown	0.6
8	12.045	1354	Eugenol	1.0
9	12.369	1379	Elphacopaene	0.6
10	12.486	1387	β-Bourbonene	0.9
11	12.544	1392	β-Elementene	0.6
12	12.950	1424	β-Caryophyllene	7.8
13	13.403	1460	α-Humulene	2.0
14	13.669	1481	Germacrene D	0.9
15	13.716	1485	E-β-Ionone	1.0
16	14.034	1511	β-Bisabolene	36.3
17	14.156	1521	δ-Cadinene	0.8
18	14.645	1563	E-Nerolidol	1.3

Table 2. Chemical composition (%) of *M. vulgare* essential oil (continued)

19	14.695	1567	Unknown	0.7
20	14.854	1580	3,5,7-Trimethyl-2E,4E,6E,8E-undecatetraene	1.6
21	14.936	1587	Caryophyllene oxide	0.9
22	15.195	1610	Unknown	0.3
23	15.377	1626	Megastigma-5,7(E),9-trien-4-one	0.5
24	15.954	1678	1-Heptadecene	0.6
25	16.205	1700	Heptadecane	0.6
26	16.378	1716	Pentadecanal	0.9
27	17.535	1827	Unknown	0.8
28	17.689	1842	6,10,14-Trimethyl-2-pentadecanone	2.6
29	18.868	1961	Unknown	4.0
30	19.461	2021	Unknown	0.5
31	20.035	2077	Unknown	0.6
32	20.176	2091	Unknown	0.7
33	20.249	2098	Phytol	6.2
34	20.708	2155	Unknown	0.1
35	20.818	2169	Unknown	0.4
36	21.006	2192	Unknown	3.9
37	21.091	2203	Unknown	0.7
38	21.823	2288	Unknown	1.4
39	21.915	2299	Unknown	0.8
40	23.529	2499	Pentacosane	0.4
41	24.754	2662	Unknown	0.4
42	25.029	2700	Heptacosane	3.3
43	25.546	2772	Unknown	0.4
44	25.738	2799	Octacosane	0.4
45	26.172	2863	Unknown	0.4
46	26.429	2901	Nonacosane	4.0
47	27.739	3101	Uncontane	1.6
Total components				98.5
Total identified				81.3
Total not identified				17.2
Oxygenated monoterpene				1.8
Sesquiterpene hydrocarbons				50.0
Oxygenated sesquiterpenes				2.6
Hydrocarbons				12.5
Oxygenated hydrocarbons				13.9

^aCompounds are listed in order of their RI

^bRI (retention index) measured relative to *n*-alkanes (C8-C20) using HP-5MS column

RT (retention time)

Conclusions

The chemical composition of *Thymus algeriensis* and *Marrubium vulgare* essential oils collected from the Aures area of Algeria was characterized by high contents of sesquiterpenes (67.0% and 50.5%, respectively). The major compounds of *T. algeriensis* oil were germacrene D (29.6%), β -caryophyllene (11.0%), E- β -farnesene (7.8%), bicyclogermacrene (4.4%) and δ -cadinene (4.0%), while, the major components of *M. vulgare* oil were β -bisaboene (36.3%), β -caryophyllene (7.8%), phytol (6.2%), nonacosane (4.0%) and heptacosane (3.3%). Indeed, the present results revealed a relative variability according to the literature data. It was the first time that germacrene D and β -caryophyllene, E- β -farnesene, bicyclogermacrene and α -humulene were described with high contents in our *T. algeriensis* oil, as we observed that pulegone, undecanal, E- β -ionone, 3,5,7-trimethyl-2E,4E,6E,8E-undecatetraene, megastigma-5,7(E),9-trien-4-one, 1-heptadecene, pentadecanal, 6,10,14-trimethyl-2-pentadecanone, octacosane, nonacosane and unctane were identified for the first time in our *M. vulgare* oil. Therefore, these results will certainly contribute to enrich the knowledge on the variation of essential oils composition of Lamiaceae species, which suggest a new activity and may act as an alternative to discover new biological drugs.

Acknowledgements

The authors would like to thank MESRS (DGRSDT) for the financial support and Pr. Mohamed Kaabeche (Setif 1 University, Algeria) for the identification of the plant materials.

References

- [1]. Franco Mancarz, G.F.; Laba, L.C.; Morais Silva, T.A.; de Santi Pazzim, M.; de Souza, D.; Maria Machado Prado, M.; de Souza, L.M.; Nakashima, T.; Guetter Mello, R., Chemical composition and biological activity of *Liquidambar styraciflua* L. leaf essential oil, *Ind Crop Prod*, **2019**, 138, 111-146.
- [2]. Kulaksiz, B.; Sevda, E.R.; Üstündağ-Okur, N.; Saltan-İşcan, G., Investigation of antimicrobial activities of some herbs containing essential oils and their mouthwash formulations, *Turk J Pharm Sci*, **2018**, 15(3), 370-375.
- [3]. Ahmed, J.H.; Ezer, N., Anatomical features of genus *Prunella* l. growing in turkey, *Turk Pharm Sci*, **2008**, 5(1), 17-27.
- [4]. Mehalaine, S., Seed germination and propagation for regeneration of some medicinal plants growing wild in semiarid region of Algeria, *Handbook of Environmental Materials Management*, **2018**, 1-24.

- [5]. Mesquita, L.S.S.; Luz, T.R.S.A.; Mesquita, J.W.C.; Coutinho, D.F.; Amaral, F.M.M.; Ribeiro, M.N.S.; Malik, S., Exploring the anticancer properties of essential oils from family Lamiaceae, *Food Rev Int*, **2018**, 35(2), 105-131.
- [6]. Dob, T.; Dahmane, D.; Benabdelkader, T.; Chelghoum, C, Studies on the essential oil composition and antimicrobial activity of *Thymus algeriensis* Boiss. Et Reut, *Int J Aromather*, **2006**, 16(2), 95-100.
- [7]. Boutaoui, N.; Zaiter, L.; Benayache, F.; Benayache, S.; Carradori, S.; Cesa, S.; Giusti, A.M.; Campestre, C.; Menghini, L.; Innosa, D.; Locatelli, M., Qualitative and quantitative phytochemical analysis of different extracts from *Thymus algeriensis* aerial parts, *Molecules*, **2018**, 23, 463.
- [8]. Fadel, H.; Benayache, F.; Benayache, S., Antioxidant properties of four Algerian medicinal and aromatic plants *Juniperus oxycedrus* L., *Juniperus phoenicea* L., *Marrubium vulgare* L. and *Cedrus atlantica* (Manetti ex Endl), *Pharm Lett*, **2016**, 8(3), 72-79.
- [9]. Boudjelal, A.; Henchiri, C.; Siracusa, L.; Sari, M.; Ruberto, G., Compositional analysis and *in vivo* anti-diabetic activity of wild Algerian *Marrubium vulgare* L. infusion, *Fitoterapia*, **2012**, 83(2), 286-292.
- [10]. Abadi, A.; Hassani, A., Essential oil composition and antioxidant activity of *Marrubium vulgare* L. growing wild in Eastern Algeria, *ILCPA*, **2013**, 14, 17-24.
- [11]. Benjilali, B.; Hammoumi, M.; Richard, H., Chemical polymorphism of Moroccan thyme essential oils 1. compounds characterization, *Sci Aliments*, **1987**, 7(1), 77-91.
- [12]. Houmani, Z.; Azzoudj, S.; Naxakis, G.; Skoula, M., The essential oil composition of Algerian Zaâtar: *Origanum* ssp. And *Thymus* ssp, *J Herbs Spices Med Plants*, **2002**, 9(4), 275-280.
- [13]. Hazzit, M.; Baaliouamer, A., Composition of essential oils of *Thymus algeriensis* Boiss. Et Reut. and *Thymus numidicus* Poiret from Algeria, *Rivista Italiana, EPPOS*, **2007**, 43, 11-18.
- [14]. Giordiani, R.; Hadeif, Y.; Kaloustian, J., Compositions and antifungal activities of essential oils of some Algerian aromatic plants, *Fitoterapia*, **2008**, 79(3), 199-203.
- [15]. Hazzit, M.; Baaliouamer, A.; Verissimo, A.R.; Faleiro, M.L.; Miguel, M.G., Chemical composition and biological activities of Algerian *Thymus* oils, *Food Chem*, **2009**, 116(3), 714-721.
- [16]. Amarti, F.; Satrani, B.; Ghanmi, M.; Farah, A.; Aafi, A.; Aarab, L.; El-Ajjouri, M.; Chaouch, A., Composition chimique et activité antimicrobienne des huiles essentielles de *Thymus algeriensis* Boiss. & Reut. et *Thymus ciliatus* (Desf.) Benth. du Maroc, *Biotechnol Agron Soc Environ*, **2010**, 14(1), 141-148.
- [17]. Zouari, N.; Fakhfakh, N. ; Zouari, A.; Bougatef, A.; Karray, A.; Neffati, M.; Ayadi, M.A., Chemical composition, angiotensin I-converting enzyme inhibitory, antioxidant and antimicrobial activities of essential oil of Tunisian *Thymus algeriensis* Boiss. Et Reut. (Lamiaceae), *J Food Bioprod Process*, **2011**, 89(4), 257-265.

- [18]. Giweli, A.A.; Dzamic, A.M.; Sokovic, M.D.; Ristic, M.S.; Marin, P.D., Chemical composition, antioxidant and antimicrobial activities of essential oil of *Thymus algeriensis* wild-growing in Libya, *Cent Eur J Biol*, **2013**, 8(5), 504-511.
- [19]. Kouache, B.; Brada, M.; Saadi, A.; Fauconnier, M.L.; Lognay, G.; Heuskin, S., Chemical composition and acaricidal activity of *Thymus algeriensis* essential oil against *Varroa destructor*, *Nat Prod Commun*, **2017**, 12(1), 135-138.
- [20]. Benabdallah, F.Z.; Zellagui, A.; Demirtas, I., Chemical composition of essential oils and antioxidant activities of extracts of two endemic plants from Algeria, *IJPSR*, **2017**, 8(1), 244-250.
- [21]. Benabed, K.H.; Gourine, N.; Ouinten, M.; Bombarda, I.; Yousfi, M., Chemical composition, antioxidant and antimicrobial activities of the essential oils of three Algerian Lamiaceae species, *J Food Sci*, **2017**, 13(2), 97-109.
- [22]. Bendjabeur, S.; Benchabane, O.; Bensouici, C.; Hazzit, M.; Baaliouamer, A.; Bitam, A., Antioxidant and anticholinesterase activity of essential oils and ethanol extracts of *Thymus algeriensis* and *Teucrium polium* from Algeria, *J Food Meas Charact*, **2018**, 12(4), 2278-2288.
- [23]. Saleh, M.M.; Glombitza, K.W., Volatile oil of *Marrubium vulgare* and its anti schistosomal activity, *Planta Med*, **1989**, 55, 105-108.
- [24]. Nagy, M.; Svajdenka, E., Comparison of essential oils from *Marrubium vulgare* L. and *M. peregrinum* L., *J Essent Oil Res*, **1998**, 10, 585-587.
- [25]. Weel, K.G.C.; Venskutonis, P.R.; Pukalskas, A.; Gruzdiene, D.; Linssen, J.P.H., Antioxidant activity of horehound (*Marrubium vulgare*) grown in Lithuania, *Fett Lipid*, **1999**, 101(10), 395-400.
- [26]. Morteza-Semnani, K.; Saeedi, M.; Babanezhad, E., The Essential oil composition of *Marrubium vulgare* L. from Iran, *J Essent Oil Res*, **2008**, 20(6), 488-490.
- [27]. Khanavi, M.; Ghasemian, L.; Hosseiny Motlagh, E.; Hadjiakhoondi, A.; Shafiee, A., Chemical composition of the essential oils of *Marrubium parviflorum* Fisch. & C.A. Mey and *Marrubium vulgare* L. from Iran, *Flav Fragr J*, **2005**, 20(3), 324-326.
- [28]. Asadipour, A.; Mehrabani, M.; Nazeri, V.; Tabarraii, M., Composition of the essential oil of *Marrubium vulgare* L, *Ulm-i-Daroei*, **2005**, 2, 77-82.
- [29]. Belhattab, R.; Larous, L.; Figueiri, A.; Pedro, A., Essential oil composition and glandular trichoma of *Marrubium vulgare* L. growing wild in Algeria, *J Essent Oil Res*, **2006**, 18, 369-373.
- [30]. Kadri, A.; Zarai, Z.; Bekir, A.; Gharsallah, N.; Damak, M.; Gdoura, R., Chemical composition and antioxidant activity of *Marrubium vulgare* L. essential oil from Tunisia, *Afr J Biotechnol*, **2011**, 10(19), 3908-3914.

- [31]. Salama, M.M.; Taher, E.E.; El-Bahy, M.M., Molluscicidal and mosquitocidal activities of the essential oils of *Thymus capitatus* HOFF. ET LINK. and *Marrubium vulgare* L., *Rev Inst Med Trop Sao Paulo*, **2012**, 54(5), 281-286.
- [32]. Zawislak, G., The chemical composition of the essential oil of *Marrubium vulgare* L. from Poland, *Farmacia*, **2012**, 60(2), 287-292.
- [33]. EL-Hawary, S.; EL-Shabrawy, A.; Ezzat, S.; EL-Shibany, F., Gas chromatography-mass spectrometry analysis, hepatoprotective and antioxidant activities of the essential oils of four Libyan herbs, *J Med Plant Res*, **2013**, 7(24), 1746-1753.
- [34]. Hamdaoui, B.; Wannes, W.A.; Marrakchi, M.; Brahim, N.; Marzouk, B., Essential oil composition of two Tunisian horehound species: *Marrubium vulgare* L. and *Marrubium aschersonii* Magnus, *J Essent Oil Bear Pl*, **2013**, 16(5), 608-612.
- [35]. Said-Al-Ahl, H.A.H.; Gendy, A.S.H.; Mahmoud, A.A.; Mohamed, H.F.Y., Essential oil composition of *Marrubium vulgare* L. cultivated in Egypt, *Int J Plant Res*, **2015**, 1(4), 138-141.
- [36]. Golparvar, A.R.; Hadipanah, A.; Mehrabi, A.M.; Armin, A., Chemical composition of the essential oil from the leaves of *Marrubium vulgare* L. from Iran, *JHD*, **2015**, 6(1), 1-5.
- [37]. Elbali, W.; Djouahri, A.; Djerrad, Z.; Saka, B.; Aberrane, S.; Sabaou, N.; Baaliouamer, A.; Boudarene, L., Chemical variability and biological activities of *Marrubium vulgare* L. essential oil, depending on geographic variation and environmental factors, *J Essent Oil Res*, **2018**, 30(6), 470-487.
- [38]. Fadel, H.; Benayache, F.; Chalchat, J.C.; Figueredo, G.; Chalard, P.; Hazmoune, H.; Benayache, S., Essential oil constituents of *Juniperus oxycedrus* L. and *Cupressus sempervirens* L. (Cupressaceae) growing in Aures region of Algeria, *Nat Prod Res*, **2019**, <https://doi.org/10.1080/14786419.2019.1687473>.
- [39]. Ourzeddine, W.; Fadel, H.; Mechehoud, Y.; Chalchat, J.C.; Figueredo, G.; Chalard, P.; Benayache, F.; Benayache, S., Chemical composition and antioxidant activity of the fruit essential oil of *Zizyphus lotus* (L.) Desf. (Rhamnaceae), *IJPPR*, **2017**, 9(2), 228-232.
- [40]. Adams, R.P., Identification of essential oil components by gas chromatography/mass spectroscopy, Allured Publishing Co, Carol Stream; Illinois, **1995**.
- [41]. Mc Lafferty, F.W.; Stauffer, D.B., The Wiley/NBS registry of mass spectral data 5th Edition, J Wiley and Sons, New York, **1991**.
- [42]. Touhami, A.; Chefrour, A.; Boukhari, A.; Ismail, F., Comparative study of chemical compositions and antimicrobial effect of different genius of *Thymus* harvested during two period of development, *J Appl Pharm Sci*, **2016**, 6(8), 51-56.
- [43]. Chemat, S.; Cherfouh, R.; Brahim, Y.; Meklati, B.Y.; Belanteur, K., Composition and microbial activity of thyme (*Thymus algeriensis* genuinus) essential oil, *J Essent Oil Res*, **2012**, 24(1): 5-11.

- [44]. Ait-Ouazzou, A.; Lorán, S.; Bakkali, M.; Laglaoui, A.; Rota, C.; Herrera, A.; Pagán, R.; Conchello, P., Chemical composition and antimicrobial activity of essential oils of *Thymus algeriensis*, *Eucalyptus globulus* and *Rosmarinus officinalis* from Morocco, *J Sci Food Agric*, **2011**, 91(14): 2643-2651.
- [45]. Rezazi, S.; Hanini, S.; Si-Moussa, C.; Abdelmalek, S., Kinetic modeling and parameters identification based on metaheuristic optimization techniques for extraction process of *Marrubium vulgare* L. essential oil, *J Agr Sci Tech*, **2017**, 19, 307-322.
- [46]. Bokaeian, M.; Saboori, E.; Saeidi, S.; Niazi, A.A.; Amini-Borojeni, N.; Khaje, H.; Bazi, S., Phytochemical analysis, antibacterial activity of *Marrubium vulgare* L against *Staphylococcus aureus* in vitro, *Zahedan J Res Med Sci*, **2014**, 16(10), 60-64.
- [47]. Bayir, B.; Gunduz, H.; Usta, T.; Şahin, E.; Ozdemir, Z.; Kayır, O.; Şen, O.; Akşit, H.; Elmastaş, M.; Erenler, R., Chemical composition of essential oil from *Marrubium vulgare* L. leaves, *J New Res Sci*, **2014**, 6, 44-50.