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ASN, Vol 6, No 2, Pages 62 - 70, 2019

Acta Scientifica Naturalis
Former Annual of Konstantin Preslavsky University – Chemistry, Physics, Biology, Geography
Journal homepage: asn.shu.bg

Chemical Composition of the Essential Oil of Aerial Parts of *Thymus ciliatus* (Desf.)

Hanane Achoub[1], Lahcene Zaiter[1], Fadila Benayache[1*], Samir Benayache[1], Jean Claude Chalchat[2], Pierre Chalard[3], Gilles Figueredo[4] and Salah Akkal[1]

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

[2]*AVAHEA, La Laye 7, 63500 Saint Babel, France.*

[3]*Université Clermont Auvergne, CNRS, SIGMA Clemen,* ***ICCF,*** *F-63000 Clermont–Ferrand, France.*

[4]*Laboratoire d'Analyses des Extraits Végétaux et des Arômes (LEXVA Analytique), 460 Rue du Montant, 63110 Beaumont, France.*

Abstract: *The essential oil of the aerial parts of Thymus ciliatus (Desf.) belonging to the Lamiaceae family, was obtained by steam distillation and analyzed by GC-FID and GC-MS. 75 components were identified corresponding to 95.57% of the total oil. The major constituents of the oil were: elemol (6.80%), carvacrol (5.86%), γ-muurolene (5.18%), β-sesquiphellandrene (5.09%), bicyclogermacrene (5.04%) , β-pinene (4.49%) and curcumene (4.20%), together with other compounds at relatively low levels: 1,8-cineol (3.66%), β-eudesmol (2.92%), β-bisabolene (2.81%), β-silinene (2.75%), camphor (2.64%), germacrone (2.34%), α-zingiberene (2.12%), δ-cadinene (2.08%), caryophyllene oxide (1.90%), spathulenol (1.88%), □-caryophyllene (1.88%), ar-turmerone (1.79%), α-pinene (1.52%) , limonene (1.52%), selina-4,11-diene (1.46%), curzerenone (1.41%), germacrone B (1.37%), bornyl acetate (1.31%), β-farnesene (1.28%), borneol (1.23%), myrtenal (1.16%), zingiberenol (1.15%) and sabinene (1.13%). These results differ from those of previous studies reported on this species collected from other regions of Algeria and Morocco.*

Keywords: ***Thymus ciliatus*** **(Desf.), Lamiaceae, Essential oil composition.**

Introduction

The Lamiaceae or Labiatae are a family of flowering plants with a cosmopolitan distribution containing about 236 genera and has been stated to contain 6900 to 7200 species [1]. In Algerian flora, the genus *Thymus* from this

Corresponding author: fbenayache@yahoo.fr

DOI: 10.2478/asn-2019-0019

sciendo

ASN, Vol 6, No 2, Pages 62 - 70, 2019

family has 12 species from which 9 are endemic [2]. *Thymus ciliatus* (Desf.) Benth. which is an endemic species for North Africa, includes three sub-species: ssp. eu-*ciliatus* Maire, ssp. *coloratus* (Boiss. et Reut.) Batt. and ssp. *munbyanus* (Boiss. et Reut.) Batt. Many *Thymus* species are used in various regions of the world in the treatment of bronchial, pulmonary, digestive and urinary infections and possess spasmolytic, antitussive and expectorant properties [3]. The essential oils of several species of thyme have already proved their antibacterial and antifungal properties [4-7]. In continuation of our studies on *Thymus* species [8, 9], we report herein, our results concerning the chemical composition of the essential oil of the aerial parts of *Thymus ciliatus* (Desf.) Benth. collected from the area of M'Sila, which were different from those previously reported on this species [10-17].

Materials and Methods

Plant Material

The aerial parts of *T. ciliatus* (Desf.) were collected from M'Sila (GPS coordinates in DMS: 35°42'20.99" N 4°32'30.98" E) on April 2013 and authenticated on the basis of Quezel and Santa (1963) [2] by professor Mohamed Kaabeche, Université Ferhat Abbas, Setif 1, Setif, Algeria. A voucher has been deposited in the Herbarium of the Research Unit VARENBIOMOL, Université Frères Mentouri-Constantine 1, Algeria.

Extraction of the essential oil

The aerial parts of *Thymus ciliatus* (Desf.) were subjected to steam distillation in a Kaiser Lang apparatus for three hours. The obtained essential oil was collected and dried over anhydrous sodium sulphate and kept at 4°C until analysis. The yield of the oil was calculated in relation of the dry weight of the plant.

GC-FID Analysis

The essential oil was analyzed on an Agilent gas chromatograph (GC-FID) Model 6890, equipped with a HP-5MS fused 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 oil was 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 (C_8-C_{20}) were analyzed under the same conditions to calculate retention indices (RI) with Van del Dool and Kratz equation.

GC-MS Analysis

Corresponding author: fbenayache@yahoo.fr

DOI: 10.2478/asn-2019-0019

sciendo
ASN, Vol 6, No 2, Pages 62 - 70, 2019

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 oil constituents were identified by matching their mass spectra and retention indices (RI) with those of reference compounds from libraries such as Adams [18] and Mc Lafferty & Stauffer [19]. The proportions of the identified compounds were calculated by internal normalization.

Results and Discussion

The yield of steam distillation was 0.46% (w/w) in relation to the dry weight of the plant. A total of 75 constituents were determined which account for about 95.57% of the essential oil of *Thymus ciliatus* (Desf.). The identified components (Table 1) are listed in order of their experimental retention times and retention indices. The major constituents of the oil were: elemol (6.80%), carvacrol (5.86%), γ-muurolene (5.18%), β-sesquiphellandrene (5.09%), bicyclogermacrene (5.04%), β-pinene (4.49%) and curcumene (4.20%) together with other compounds at relatively low levels: 1,8 -cineol (3.66%), β-eudesmol (2.92%), β-bisabolene (2.81%), β-silinene (2.75%), camphor (2.64%), germacrone (2.34%), α-zingiberene (2.12%), δ-cadinene (2.08%), caryophyllene oxide (1.90%), spathulenol (1.88%), □-caryophyllene (1.88%), ar-turmerone (1.79%), *α*-pinene (1.52%) , limonene (1.52%), selina-4,11-diene (1.46%), curzerenone (1.41%), germacrone B (1.37%), bornyl acetate (1.32%), β-farnesene (1.28%), borneol (1.23%), myrtenal (1.16%), zingiberenol (1.15%) and sabinene (1.13%).

Table 1. Composition of the essential oil of *Thymus ciliatus* (Desf.)

PK	[b]RT	[b]RI	[a] Components	%
1	5.937	923	*α*-Thujene	0.15
2	6.108	931	*α*-Pinene	1.52
3	6.308	947	Camphene	0.39
4	6.579	951	Thuja-2,4(10)-diene	0.10
5	7.028	971	Sabinene	1.13
6	7.143	976	β-Pinene	4.49
7	7.410	987	Myrcene	0.98
8	8.019	1014	*α*-Terpinene	0.15
9	8.201	1023	*ortho*-Cymene	0.40
10	8.299	1027	Limonene	1.52

Corresponding author: fbenayache@yahoo.fr

DOI: 10.2478/asn-2019-0019

11	8.370	1031	1,8 -Cineol	3.66
12	8.660	1044	β-Ocimene	0.38
13	8.922	1056	γ-Terpinene	0.21
14	9.198	1069	*cis*-Sabinene hydrate	0.20
15	9.487	1082	Terpinolene	0.07
16	9.811	1097	Linalool	0.40
17	9.941	1104	NI	0.13
18	10.362	1125	*α*-Campholenal	0.28
19	10.606	1138	Nopinone	0.10
20	10.661	1141	*trans*-Pinocarveol	0.64
21	10.760	1146	*trans*-Verbenol	0.94
22	10.791	1147	Camphor	2.64
23	1.982	1157	Sabina ketone	0.14
24	11.073	1162	Pinocarvone	0.56
25	11.281	1172	Borneol	1.23
26	11.432	1180	Terpinen-4-ol	0.42
27	11.567	1187	*p*-Cymen-8-ol	0.18
28	11.719	1195	Myrtenal	1.16
29	11.942	1207	Verbenone	0.43
30	12.158	1219	*trans*-Carveol	0.23
31	12.317	12228	NI	0.11
32	12.610	1244	Carvone	0.22
33	13.320	1283	Bornyl acetate	1.32
34	13.628	1300	Carvacrol	5.86
35	14.146	1331	Bicycloelemene	0.28
36	14.389	1345	δ-Elemene	0.18
37	14.683	1362	Carvacrol acetate	0.06
38	14.901	1375	α-Copaene	0.43
39	14.994	1381	α-Elemene	0.03
40	15.042	1384	β-Bourbonene	0.38
41	15.122	1388	β-Elemene	0.79

Corresponding author: fbenayache@yahoo.fr

DOI: 10.2478/asn-2019-0019

42	15.307	1399	Sesquithujene	0.63
43	15.423	1409	NI	0.14
44	15.645	1420	□-Caryophyllene	1.88
45	15.765	1428	γ-Elemene	0.22
46	15.804	143	β-Copaene	0.12
47	15.863	1434	α-Guaiene	0.15
48	16.114	1450	β-Farnesene	1.29
49	16.221	1457	α-Humulene	0.52
50	16.288	1461	□-Caryophyllene	0.56
51	16.450	1471	Aristolochene	0.17
52	16.486	1473	Selina-4,11-diene	1.46
53	16.607	1481	Curcumene	4.20
54	16.644	1483	γ-Muurolene	5.18
55	16.691	1486	NI	0.81
56	13.952	1491	β-Silinene	2.75
57	16.804	1493	α-Zingiberene	2.12
58	16.864	1497	Bicyclogermacrene	5.04
59	17.000	1506	β-Bisabolene	2.81
60	17.111	1513	γ-Cadinene	0.36
61	17.175	1518	δ-Cadinene	2.08
62	17.264	1524	β-Sesquiphellandrene	5.09
63	17.561	1543	α-Calacorene	0.32
64	17.674	1551	Elemol	6.80
65	17.824	1561	Germacrone B	1.37
66	18.101	1579	Spathulenol	1.88
67	18.188	1585	Caryophyllene oxide	1.90
68	18.38	1598	*trans*-β-Elemenone	0.15
69	18.419	1601	Curzerenone	1.41
70	18.515	1607	NI	0.23
71	18.605	1614	Zingiberenol	1.15
72	18.851	1631	NI	0.36

Corresponding author: fbenayache@yahoo.fr

DOI: 10.2478/asn-2019-0019

73	18.894	1634	γ-Eudesmol	0.75
74	19.03	1644	α-Muurolol	0.22
75	19.058	1645	*epi*-α-Muurolol	0.19
76	19.245	1659	β-Eudesmol	2.92
77	19.309	1663	ar-Turmerone	1.79
78	19.663	1688	NI	0.90
79	19.768	1695	Germacrone	2.34
80	19.84	1701	NI	0.78
81	20.407	1742	(6S,7R)-Bisabolene	0.84
82	20.467	1747	NI	0.91
83	20.651	1761	Aristolone	0.20
84	21.651	1836	2-Pentadecanone-6,10,14-trimethyl	0.46
Total identified				**95.57**
Not identified				**4.37**

[a]Compounds are listed in order of their RI

[b]RI (retention index) measured relative to *n*-alkanes (C_8-C_{20}) using HP-5MS

NI: not identified

The comparison of our results with literature data shows important qualitative and quantitative differences in compositions. Indeed, *T. ciliatus*, growing in Batna (Eastern Algeria) was characterized mainly by thymol (79.1%) and *p*-cymene (5.6%) [10]. Thymol (44.2%), β-E-ocimene (25.8%) and α-terpinene (12.3%) were mainly found in the essential oil of *T. ciliatus* growing in Azrou (89 km south of Fes), Morocco [11]. Other studies reported that essential oil of *T. ciliatus*, collected from Guelma (northeastern Algeria) was dominated by thymol (62.41%), *p*-cymene (15.51%) and carvacrol (6.12%) [20]. On the other hand, essential oil of *T. ciliatus* collected from Ain Mlila in the wilaya of Oum El Bouaghi (Eastern Algerian) was reported to contain thymol (54.98%), γ-terpinene (11.33%), *p*-cymene (6.66%) and carvacrol (4.96%) as dominant compounds [21]. Moreover, *Thymus ciliatus* growing in Imilchil (High Atlas of Morocco) contained as most representative components: carvacrol (26.2%), *p*-cymene (19.6%), thymol (17.3%) and γ -terpinene (14.6%) [22]. We also note that the chemical composition of the essential oil of *T. ciliatus* growing in Remchi, wilaya of Tlemcen in the north west of Algeria, gave a similar profile: carvacrol (80.3%) and p-cymene (6.1%) [16]. Thus, the results obtained in this study revealed significant differences in the composition of the essential oil compared with previous studies which were high-thymol chemotypes. Indeed, the present essential oil is characterized by the presence of elemol (6.80%), γ-muurolene

Corresponding author: fbenayache@yahoo.fr

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(5.18%), β-sesquiphellandrene (5.09%) and bicyclogermacrene (5.04%) which have not been found in any reported essential oil of *T. ciliatus*. The variations in the chemical composition of the essential oils from the qualitative and quantitative point of view, may be due to certain ecological factors, to the part of the plant used, to the age of the plant and to the period of the vegetative cycle or even genetic factors [23-26].

Conclusion

The essential oil of *Thymus ciliatus* (Desf.), collected from M'Sila Algeria is characterized by the main presence of Elemol (6.80%), carvacrol (5.86%), γ-muurolene (5.18%), β-sesquiphellandrene (5.09%), bicyclogermacrene (5.04%), β-Pinene (4.49%) and curcumene (4.20%) as major components. It is interesting to note that the chemical composition of the present essential oil differs from those reported in the literature. Our chemotype is exclusively characterized by high level of elemol, γ-muurolene β-sesquiphellandrene and bicyclogermacrene which have not been found in any reported essential oil of *T. ciliatus*. These variations may be explained by the different climates, seasons, geographic and soil conditions as well as altitude differences and harvest periods of the plant.

Acknowledgements

We are grateful to professor Mohamed Kaabeche (Setif 1 University, Algeria) for the identification of the plant material and MESRS (DGRSDT) for financial support.

Disclosure statement

No potential conflict of interest was reported by the authors.

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DOI: 10.2478/asn-2019-0019