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## Biological activities and composition of the essential oil of *Thymus ciliatus* subsp. *coloratus* from Bir Chouhada (Algeria).

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### ABSTRACT

The chemical composition of the essential oil of *Thymus ciliatus* subsp. *coloratus*, collected from Bir Chouhada (Northern Oum El Bouaghi, Eastern Algerian), was analyzed by GC and GC/MS. The main components of the essential oil were viridiflorol (16.02%),  $\alpha$ -pinene (8.75%), camphor (7.5%) and nerolidol (6.95%). The antibacterial activity of the essential oil was tested against 10 gram-positive and gram-negative bacteria by the use of the disc diffusion method. The antioxidant activity was also investigated by the use of  $\beta$ -carotene bleaching method.

**Keywords:** *Thymus ciliatus* subsp. *coloratus*, Antioxidant activity, Linoleic acid,  $\beta$ -carotene, Antibacterial activity.

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### INTRODUCTION

*Thymus* genus (Lamiaceae) is widely distributed; it comprises about 400 species. In North African folk medicine, *Thymus* plants are used as remedies in various diseases e.g. bronchitis, pulmonary infection, flu, cough and some gastrointestinal disorders [1]. In Algerian flora, there are 12 *Thymus* species from which 9 are endemic [2]. In continuation of our works on Lamiaceae [3-27], we report here the GC and GC/MS analyses, the antibacterial and the antioxidant activities of the essential oil of the endemic specie *Thymus ciliatus* subsp. *coloratus* collected from Bir Chouhada (Northern Oum El Bouaghi, Eastern Algerian).

### MATERIALS AND METHODS

#### Plant Material

Fresh aerial parts of *Thymus ciliatus* subsp. *coloratus*, growing in Bir Chouhada (Northern Oum El Bouaghi, Eastern Algerian) was collected in May 2012. A voucher specimen were deposited at the herbarium of the University of Constantine 1, Algeria (LOST Tc Bir/05/12).

#### Extraction of the essential oil

The hydrodistillation of fresh aerial parts (100 g) of *Thymus ciliatus* subsp. *coloratus*, collected from Bir Chouhada, for 3 h in a Clevenger-type apparatus, yielded 2 % of a yellow good smell essential oil.

### Gas chromatography-mass spectrometry (GC/MS) analysis

The oil was analyzed by GC/MS using a Hewlett Packard 6890 mass selective detector coupled with a Hewlett Packard 6890 gas chromatograph, equipped with a cross-linked 5% PH ME siloxane HP-5MS capillary column (30 m \ 0.25 mm, film thickness 0.25  $\mu$ m). Operating conditions were as follows: carrier gas, helium with a flow rate of 2 ml/ min; column temperature, 60D275 °C at a rate of 4°C/min; injector temperature, 280°C; injected volume, 0.1  $\mu$ l of the oil; split ratio, 1:50.

The MS operating parameters were as follows: ionization potential, 70 eV; ion source temperature, 200 °C; resolution, 1000.

### Identification of components

Essential oil components were identified based on their retention indices (determined with reference to a homologous series of normal alkanes), and by comparison of their mass spectral fragmentation patterns with those reported in the literature [31, 32] and with authentic compounds for major components.

### Antibacterial activity

The antibacterial activity of the essential oil was tested against a range of microorganisms, namely *Escherichia coli* ATCC 25922, *Escherichia coli* (HS), *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* (HS), *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa* (HS), *Klebsiella pneumonia* (HS), *Enterobacter aerogenes* (HS), *Proteus mirabilis* (HS), *Streptococcus enterococcus* (HS). The reference strains were obtained from the Pasteur Institute (Algiers). The hospital strains (HS) were obtained from the laboratory of bacteriology, Benbadis- Hospital, Constantine, using conventional methods (clinical isolation) [33].

### Antioxidant activity: $\beta$ -carotene bleaching test

The antioxidant activity of the essential oils was evaluated by  $\beta$ -carotene–linoleic acid model system [34].

## RESULTS AND DISCUSSION

### Chemical composition of the essential oil

The hydrodistillation of aerial parts of *Thymus ciliatus* subsp. *coloratus* collected from Bir Chouhada yielded 2% (w/w) of a good smell yellowish oil. 52 compounds were identified by GC and GC/MS, representing 86.06% of the total essential oil mainly characterized by the presence of viridiflorol (16.12%),  $\alpha$ -pinene (8.75%), camphor (7.5%) and nerolidol (6.95%) (Table 1). Comparatively, 28 compounds were identified in the essential oil of *T. ciliatus* collected from Ain Mlila [24] which was mainly characterized by thymol (54.98 %), *p*-cymene (6.66%),  $\gamma$ -terpinene (11.33%) and carvacrol (4.96%).  $\alpha$ -Pinene has been reported as a main component of many *Thymus* oils in particular in the essential oils of *T. cilicicus* Boiss. [35], *T. algeriensis* [36], *T. marschallianus* Willd. [37]. Camphor has been found to be a major component of essential oils of *T. sipyleus* (15%), *T. serpyllum* L. (16.7%), *T. algeriensis* Boiss. (27.7%) and *T. moroderi* (22.8%), growing in Central Turkey [38], Vilnius (Lithuania) [39], Morocco [40] and Spain [41], respectively. In addition, the present oil was characterized by the main presence of nerolidol (6.95%) which was also mainly found in the essential oil of *T. algeriensis* (12.6-), *T. pulegioides* L. (8.2-13.8%), *T. zygoides* Griseb. var. *zygoides* (12.5%) and *T. albanus* subsp. *albanus* H. Braun (20.3-48.4%) growing in Algeria [42], Lithuania [43], Turkey [44] and Macedonia [45], respectively.

### Antibacterial activity

The essential oil exhibited the best antibacterial activity against *Pseudomonas aeruginosa* ATCC 27853 and *Pseudomonas aeruginosa* (HS) with 15 mm and 14 mm, inhibition zone diameters respectively (Table 2). Comparatively, the essential oil of *T. ciliatus* collected at Ain Mlila [24] exhibited the best antibacterial activity against *Enterobacter aerogenes* (HS), *Escherichia coli* ATCC, *Pseudomonas aeruginosa* ATCC, *Staphylococcus aureus* ATCC, *E. coli* (HS) and *S. aureus* (HS) with 25, 23, 20, 20, 20, 20 mm inhibition zone diameters, respectively. The difference of the antibacterial activities of these two essential oils of the same plant collected from two regions of the same province may be due to the difference of the compositions which depend on the nature of the soil and climate.

**Table 1: Chemical composition, Retention indices and Percentage composition of the essential oils *Thymus ciliatus* subsp. *coloratus* from Bir Chouhada.**

Pic	Compounds <sup>a</sup>	IR <sup>b</sup>	(%) <sup>c</sup>	(%)
1	$\alpha$ -thujene	930	2.43	0.5
2	$\alpha$ -pinene	937	2.32	<b>8.75</b>
3	camphene	952	0.12	2.12
4	verbenene	967	/	0.15
5	sabinene	974	0.33	0.78
6	$\beta$ -pinene	978	/	3.37
7	3-octanone	984	/	0.05
8	$\beta$ -myrcene	989	2.67	2.56
9	3-octanol	991	0.05	/
10	$\alpha$ -phellandrene	1002	0.48	0.30
11	$\delta$ -3-carene	1010	0.12	/
12	$\alpha$ -terpinene	1016	2.80	0.08
13	<i>p</i> -cymene	1024	<b>6.66</b>	0.88
14	limonene	1028	/	1.87
15	$\beta$ -phellandrene	1029	1.02	/
16	1,8-cineole	1030	0.05	<b>4.50</b>
17	<i>cis</i> - $\beta$ -ocimene	1037	/	0.11
18	<i>trans</i> - $\beta$ -ocimene	1047	0.15	1.10
19	$\gamma$ -terpinene	1060	<b>11.33</b>	0.20
20	<i>cis</i> -sabinene hydrate	1068	0.55	0.67
21	<i>cis</i> -linalool oxide	1075	/	0.13
22	linalool	1095	3.81	2.77
23	$\alpha$ -terpinolene	1091	0.10	/
24	camphor	1146	/	<b>7.50</b>
25	$\alpha$ -phellandrene-8-ol	1150	/	0.08
26	isomenthone	1167	/	0.57
27	borneol	1169	0.12	<b>3.10</b>
28	4-terpineol	1179	0.44	0.72
29	<i>p</i> -cymen-8-ol	1185	/	0.35
30	$\alpha$ -terpineol	1191	/	0.90
31	myrtenol	1197	/	0.88
32	verbenone	1207	/	0.54
33	<i>trans</i> -carveol	1220	/	0.41
34	<i>cis</i> -carveol	1233	/	0.05
35	$\beta$ -fenchyl acetate	1226	0.17	/
36	thymyl methyl ether	2237	0.78	0.12
37	pulegone	1240	/	0.31
38	neral	1244	0.13	/
39	<i>trans</i> -geraniol	1260	/	0.59
40	borneol acetate	1289	/	0.16
41	thymol	1294	<b>54.98</b>	<b>3.38</b>
42	carvacrol	1302	<b>4.96</b>	0.22
43	$\alpha$ -terpinenyl acetate	1358	/	2.14
44	$\alpha$ -copaene	1385	/	0.08
45	geranyl acetate	1389	/	2.62
46	$\beta$ -elemene	1399	/	0.12
47	$\beta$ -bourbonene	1390	0.05	/
48	germacrene -D	1491	0.47	0.44
49	$\alpha$ -gurjunene	1416	/	0.15
50	$\beta$ -caryophyllene	1425	/	0.89
51	$\alpha$ -humulene	1461	/	0.11
52	bicyclogermacrene	1502	/	1.12
53	$\delta$ -cadinene	1530	/	1.94
54	nerolidol	1569	/	<b>6.95</b>
55	spathulenol	1584	/	2.01
56	$\beta$ -caryophyllene	1589	/	0.11
57	caryophyllene oxide	1599	1.06	0.35
58	$\alpha$ -muurolene	1625	0.34	0.17
59	viridiflorol	1608	/	<b>16.12</b>
	<b>Identified compounds</b>	<b>Total</b>	<b>98.49</b>	<b>86.06</b>

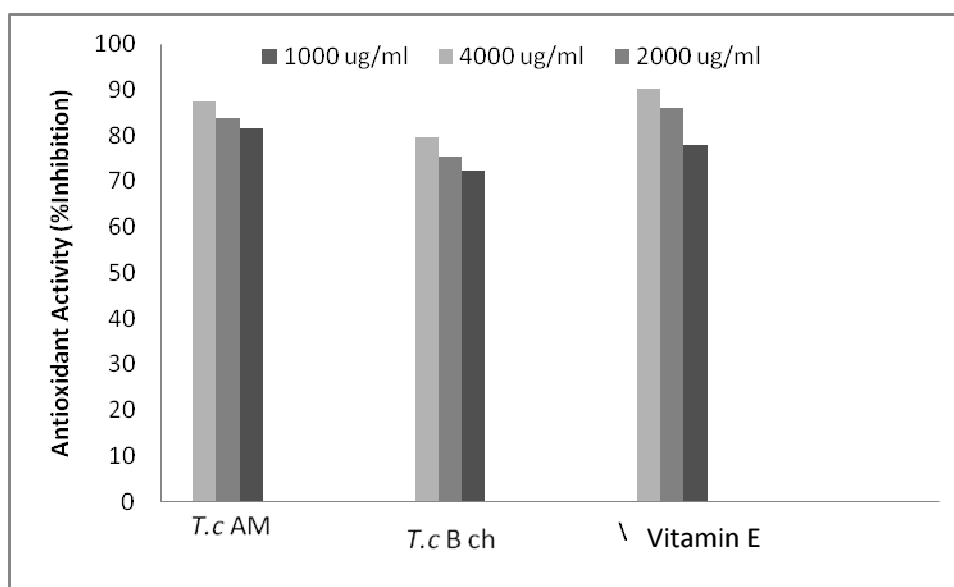
<sup>a</sup>Compounds listed in order of their RI<sup>b</sup>RI (retention index) measured relative to *n*-alkanes (C<sub>6</sub>-C<sub>24</sub>) using HP-5MS column<sup>c</sup>composition of the essential oil of *T. ciliatus* subsp. *coloratus* collected from Ain Miila (Eastern Algerian) [24]

**Table 2: Antibacterial activity (inhibition zones and MIC) of the essential oil of *Thymus ciliatus* subsp. *coloratus* collected from Bir Chouhada.**

Microrganism	Inhibition zone <sup>a</sup> (mm)	MIC (µg/ml)
<i>Escherichia coli</i> ATCC 25922	11	80
<i>Escherichia coli</i> (HS) <sup>b</sup>	13	80
<i>Pseudomonas aeruginosa</i> ATCC27853	15	40
<i>Pseudomonas aeruginosa</i> (HS)	14	40
<i>Klebsiella pneumoniae</i> (HS)	11	80
<i>Staphylococcus aureus</i> ATCC 2913	13	80
<i>Staphylococcus aureus</i> (HS)	12	80
<i>Streptococcus enterococcus</i> (SH)	11	80

<sup>a</sup>280 µg/ml<sup>b</sup> Hospital Strain**Antioxidant activity**

Total antioxidant activity was carried on by the use of by  $\beta$ -carotene bleaching method [31] assays. The activity was increased as dose dependent. The essential oil exhibited a high antioxidant activity (79.5% inhibition percentage) but lower than of the plant collected at Ain Mlila (*T.c* AM) (87,39% inhibition percentage) [24] (Figure (1)).

**Figure (1): Inhibition (%) of lipid peroxidation of the essential oils of *Thymus ciliatus* subsp. *coloratus* from Bir Chouhada (*T.c* B ch), from Ain Mlila (*T.c* AM) [24] and Vitamin E by the  $\beta$ -carotene bleaching method.****CONCLUSION**

The essential oil of *Thymus ciliatus* subsp. *coloratus*, collected from Bir Chouhada (Northern Oum El Bouaghi, Eastern Algerian), is characterized by the main presence of viridiflorol (16.12%),  $\alpha$ -pinene (8.75%), camphor (7.5%) and nerolidol (6.95%). Viridiflorol is reported here for the first time as a major component of *Thymus* oil; this may be due to the differences in soils and climates nature. The essential exhibited the best antibacterial activity against *Pseudomonas aeruginosa* ATCC 27853 and *Pseudomonas aeruginosa* (HS), by the use of the disc diffusion method, and a high antioxidant activity by the use of  $\beta$ -carotene bleaching method.

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