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Chemical composition, angiotensin I-converting enzyme inhibitory, antioxidant and antimicrobial activities of essential oil of Tunisian *Thymus algeriensis* Boiss. et Reut. (Lamiaceae)

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ABSTRACT

The present study describes chemical composition, angiotensin I-converting enzyme (ACE) inhibitory, antioxidant and antimicrobial activities of the essential oil of wild growing *Thymus algeriensis* Boiss. et Reut. (Lamiaceae), a traditional medicinal plant which is mainly endemic in Tunisia and Algeria. The essential oil from the fresh leaves and flowers of *T. algeriensis* were extracted by hydrodistillation and analysed by GC and GC/MS. Fifty-seven compounds were identified accounting for 97.71% of the total oil, where oxygenated monoterpenes constituted the main chemical class (44.85%). The oil was dominated by camphor (7.82%), 4-terpineol (7.36%), α-pinene (6.75%), 1,8-cineole (5.54%) and *cis*-sabinene hydrate (5.29%). The *T. algeriensis* essential oil was found to possess an interesting inhibitory activity towards ACE with an IC50 value of 150 μg/ml. The obtained results also showed that this oil can act as radical scavengers (IC_{50} = 0.8 mg/ml) and displayed a lipid peroxidation inhibitory activity (IC_{50} = 0.5 mg/ml) as evaluated by 2,2-diphenyl-1-picrylhydrazyl and β-carotene bleaching methods, respectively. Furthermore, the oil was tested for antimicrobial activity against six bacterial strains and two fungal strains. The inhibition zones and minimal inhibitory concentration values of microbial strains were in the range of 13.5–64 mm and 1–6 μl/ml, respectively. The oil exhibited remarkable inhibitory activity against fungal and Gram-positive bacteria strains.

Keywords: *Thymus algeriensis*; Essential oil; Chemical composition; Angiotensin I-converting enzyme; Antioxidant activity; Antimicrobial activity

1. Introduction

Lipid peroxidation of food lipid components produced during the manufacturing process and storage of food, leads to changes in taste, smell and color and therefore the loss of food quality (Mau et al., 2004). Synthetic antioxidants were used in food industry to retard the oxidation of lipids. In the last years, several questions are raised concerning the safety of chemicals used for food preservation or in medicine. In fact, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) commonly used in food industry are suspected to have negative effects on consumer's health (Namki, 1990). Natural antioxidants from fruits and medicinal plants have become highly demanded in recent decades because of their interest for human health. The regular intake of natural antioxidants can decrease the risks of cancer, cardiovascular

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disease, diabetes, and other ageing-related diseases by reducing oxidative stress (Kris-Etherton et al., 2002). Several fruits and herbs were found to exhibit antioxidant properties, which are mainly attributed to a variety of active natural antioxidants including flavonoids, polyphenols, alkaloids, anthocyanins, terpenoids, carotenoids and vitamins (Lee et al., 2004; Espin et al., 2007). Moreover, spice and herb extracts have been reported to contain compounds with antioxidant activity, and were used in many food systems (Shahidi and Champaign, 1998). Therefore, interest in finding natural antioxidants such as essential oils able to protect humans from oxidative stress damage or to protect foods from oxidation is growing.

The angiotensin I-converting enzyme (ACE, EC 3.4.15.1) is a zinc metallopeptidase that can increase blood pressure by converting the inactive decapeptide angiotensin I to the potent vasoconstrictor angiotensin II (octapetide) and by the degradation of a hypotensive peptide, bradykinin (Skeggs et al., 1956). This enzyme plays a key physiological role in blood pressure control. Consequently, inhibition of ACE activity is considered to be a useful therapeutic approach to tackle hypertension troubles. Several chemically synthesized ACE inhibitors are currently used in the treatment of hypertensive patients, but these drugs may provoke various undesirable side effects (Kapel et al., 2006). A number of compounds from plants have been identified to possess an *in vitro* ACE inhibitory activity, including hydrolysable tannins, phenylpropanes, proanthocyanidins, flavonoids, xanthones, fatty acids, terpenoids, alkaloids, oligosaccharides and peptides/amino acids, among others (Nyman et al., 1998). Moreover, various plants such as *Calophyllum brasiliense*, *Combretum fruticosum*, *Leea rubra*, *Phoenix roebelinii* and *Terminalia catappa* showed rates of ACE inhibition to about 50% at a concentration of 0.10 mg/ml of ethanol extract (Braga et al., 2007). Therefore, research to find natural ACE inhibitors could be considered as an interesting alternative for the treatment of high blood pressure.

Thymus algeriensis Boiss. et Reut. (Lamiaceae), which is endemic to Tunisia and Algeria, is an herbaceous fragrant plant largely used, fresh or dried, as a culinary herb (Pottier-Alapetite, 1981; Le Floc'h and Boulos, 2008). Furthermore, this plant is also widely used in folk medicine against illnesses of the digestive tube and antiabortion (Le Floc'h, 1983). In Tunisia, *T. algeriensis* populations are distributed from the sub-humid to the lower arid bioclimates at altitudes ranging from 120 to 1100 m. The species grows on poor fertile calcareous soils and occurs in scattered, small populations, showing different levels of destruction, mainly due to overharvesting and overgrazing (Ben El Hadj Ali et al., 2010).

T. algeriensis is a short lived, diploid ($2n = 2x = 30$) and gynodioecious shrub (Morales, 1986). It reproduces by seeds and can reach 20–50 cm in height. The leaves are opposite and linear/lanceolate (6–12 mm). The flowers, with ovate bracts and pink purplish or whitish purple corolla, are small (5–7 mm). Flowering takes place between April and June (Ben El Hadj Ali et al., 2010).

Few studies investigated the chemical composition and biological properties of *T. algeriensis* Boiss. et Reut. essential oil from some regions of North Africa (Giordiani et al., 2008; Hazzit et al., 2009; Ben Bnina et al., 2009; Ben El Hadj Ali et al., 2010). Recently, investigations on the chemical composition of *T. algeriensis* essential oil wildly growing in Tunisia, allowed the identification of six chemotypes according to the main compounds. In fact, a high variation among populations for the majority of the compounds was shown (Ben El Hadj Ali et al., 2010). Therefore, the aim of the present work is to provide more information on the chemical composition of the essential oil obtained from aerial part of *T. algeriensis* originated from South-Western region of Tunisia. For the first time, the *in vitro* angiotensin I-converting enzyme inhibitory activity of essential oil from *Thymus* essential oil was measured. Furthermore, the antioxidant, antifungal and antibacterial activities of the *T. algeriensis* essential oil were also investigated.

2. Materials and methods

2.1. Chemicals

Angiotensin I-converting enzyme (ACE) from rabbit lung, the ACE synthetic substrate hippuryl-L-histidyl-L-leucine (HHL), 2,2-diphenyl-1-picrylhydrazyl (DPPH), butyl-hydroxyanisole (BHA), β-carotene, linoleic acid and Twenn 40 were from Sigma Chemical (St. Louis, USA). All culture media and standard antibiotics were from Bio-Rad (France). All other chemicals were of analytical grade.

2.2. Plant material

The aerial parts of three individuals of *T. algeriensis* Boiss. et Reut. spaced at a distance of about 20 m were collected from the South-Western of Tunisia (Gafsa: Ayaycha mountain at 192 m altitude, latitude 34°21′05″N, longitude 09°23′32″E, with an arid climate characterized by a mean rainfall of 150 mm/year) at the flowering stage (April 2009). The harvested samples size does not exceed 20 cm. After harvest, the fresh vegetable matter was first weighted and then dried on the shadow, until constancy of the weight (20 days). Finally, leaves and flowers, were separated from stems and subjected for essential oil extraction.

2.3. Essential oil extraction

The dry matter was submitted to hydrodistillation for 4 h, using a Clevenger-type apparatus. The average yield of oil was found to be 2.25% (v/w). The essential oil was dried over anhydrous sodium sulphate and stored in sealed vials protected from the light at −20 °C until analysis.

2.4. Essential oil analysis

2.4.1. Gas chromatography (GC)

A Hewlett-Packard 5890 series II gas chromatograph equipped with HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm; Hewlett-Packard) and connected to a flame ionization detector (FID) was used. The column temperature was programmed at 50 °C for 1 min, then 7 °C/min to 250 °C, and then left at 250 °C for 5 min. The injection port temperature was 240 °C and that of the detector 250 °C (split ratio: 1/60). The carrier gas was helium (99.995% purity) with a flow rate of 1.2 ml/min and the analysed sample volume was 2 μl. Percentages of the constituents were calculated by electronic integration of FID peak areas, without the use of response factor correction. Mean percentage of compounds in *T. algeriensis* essential oil represents the average calculated on three individuals. Retention indices (RI) were calculated for separate compounds relative to C_8–C_{17} n-alkanes mixture (Aldrich Library of Chemicals Standards) (Kovàts, 1958).

2.4.2. Gas chromatography/mass spectrometry (GC/MS)

The isolated volatile compounds were analysed by GC/MS, using a Hewlett-Packard 5890 series II gas chromatograph. The fused HP-5MS capillary column (the same as that used in the GC analysis) was coupled to a HP 5972A masse-selective detector (Hewlett-Packard, Palo Alto, CA, USA). The oven temperature was programmed at 50 °C for 1 min, then 7 °C/min to 250 °C, and then left at 250 °C for 5 min. The injection port temperature was 250 °C and that of the detector 280 °C (split ratio: 1/100). The carrier gas was helium (99.995% purity) with a flow rate of 1.2 ml/min and the analysed sample volume was 2 μl. The mass spectrometer conditions were as follow: ionization voltage, 70 eV; ion source temperature, 150 °C; electron ionization mass spectra were acquired over the mass range 50–550 *m*/z.

2.4.3. Volatile compounds identification

The essential oil compounds of *T. algeriensis* were identified by comparing the mass spectra data with spectra available from the Wiley 275 mass spectra libraries (software, D.03.00). Further identification confirmations were made referring to retention indices (RI) data generated from a series of known standards of n-alkanes mixture (C_8–C_{17}) (Kovàts, 1958) and to those previously reported in the literature (Adams, 2001; Baranauskiene et al., 2003; Asuming et al., 2005; Hazzit et al., 2006; Jalali-Heravi et al., 2006; Kim et al., 2006; Ferhat et al., 2007; Liu et al., 2007; Vagionas et al., 2007).

2.5. Determination of the angiotensin I-converting enzyme (ACE) inhibitory activity

The ACE inhibitory activity was assayed as reported by Nakamura et al. (1995). A volume of 80 μl containing different concentrations (50, 100, 150 or 200 μg/ml) of *T. algeriensis* essential oil was added to 200 μl of 5 mM hippuryl-L-histidyl-L-leucine (HHL) and preincubated for 3 min at 37 °C. *T. algeriensis* essential oil and HHL were prepared in 100 mM borate buffer, 300 mM NaCl, pH 8.3. Then, the reaction was initiated by adding 20 μl of 0.1 U/ml ACE prepared in the same buffer and incubated for 30 min at 37 °C. The enzyme reaction was stopped by adding 250 μl of 0.1 M HCl. After that, the released hippuric acid (HA) was extracted by the addition of 1.7 ml ethyl acetate and mixing using vortex for 15 s. One ml of the upper layer was transferred into a glass tube and evaporated at 90 °C for 15 min. Finally, the released HA was redissolved in 1 ml of distilled water and the absorbance was measured at 228 nm using spectrophotometer (T70, UV/VIS spectrometer, PG Instruments Ltd., China). The average value from three determinations at each concentration was used to calculate the ACE inhibition percentage as follows:

$$\text{ACE inhibition (\%)} = \left[\frac{B - A}{B - C}\right] \times 100$$

where *A* is the absorbance of HA generated in the presence of ACE inhibitor component; *B* is the absorbance of HA generated without ACE inhibitor and *C* is the absorbance of HA generated without ACE (corresponding to HHL autolysis in the course of enzymatic assay). Oil concentration (μg/ml) providing 50% inhibition of the ACE activity (IC_{50}) was calculated from the graph plotting residual activity against oil concentration.

2.6. Antioxidant activity

2.6.1. Determination of DPPH radical-scavenging activity

The antioxidant activity of *T. algeriensis* essential oil was measured in terms of hydrogen-donating or radical-scavenging ability, using the stable DPPH as reagent according to the method described by Kirby and Schmidt (1997). A volume of 500 μl of diluted essential oil in methanol at different concentrations (0.1–1.0 mg/ml) was added to 375 μl of methanol and 125 μl of DPPH solution (0.2 mM in methanol) as free radical source. Absorbance measurements were read at 517 nm after 60 min of incubation time at room temperature and in the dark. Absorbance of a blank sample containing the same amount of methanol and DPPH solution without the sample acted as the negative control. BHA was used as positive control. Scavenging activity was measured by monitoring the decrease in absorbance at 517 nm. In its radical form, DPPH has an absorption band at 517 nm which disappears upon reduction by an antiradical compound. Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity. The percentage inhibition of the DPPH radical (scavenging activity) was calculated according to the formula:

$$\text{scavenging activity (\%)} = \left[\frac{Ac - As}{Ac}\right] \times 100$$

where *Ac* is the absorbance of the control reaction and *As* is the absorbance of *T. algeriensis* essential oil. Oil concentration (mg/ml) providing 50% inhibition (IC_{50}) was calculated from the graph plotting scavenging activity against oil concentration. All tests were carried out for three sample replications and the results were averaged.

2.6.2. Determination of antioxidant activity using β-carotene/linoleic acid assay

In this assay, oxidation of linoleic acid produces many oxidation products (lipid hydroperoxides, conjugated dienes, and volatile by-products) which attack the chromophore of β-carotene, resulting in bleaching of its characteristic color. However, the presence of antioxidant inhibits the β-carotene bleaching by the linoleic oxidation products formed. The capacity of *T. algeriensis* essential oil to prevent the bleaching of β-carotene was investigated out as described by Koleva et al. (2002). An emulsion of β-carotene/linoleic acid was prepared as follows: 0.5 mg of β-carotene in 1 ml of chloroform was mixed with 25 μl of linoleic acid and 200 μl of Twenn 40. The chloroform was completely evaporated under vacuum using a rotary evaporator at 40 °C. Then, 100 ml of distilled water was added and the resulting mixture was vigorously stirred. Aliquots (2.5 ml) of the β-carotene/linoleic acid emulsion (freshly prepared before each experiment) were transferred to test tubes containing different essential oil concentrations (0.1–1.0 mg/ml) diluted in methanol and the systems were incubated for 2 h at 50 °C. The same procedure was repeated with BHA used as positive standard. Finally, absorbance's of mixtures was measured at 470 nm and the relative antioxidant activity was calculated according to the formula:

$$\text{antioxidant activity (\%)} = \left[1 - \frac{A_0 - A_t}{A_{00} - A_{0t}}\right] \times 100$$

where A_0 is the absorbance at the beginning of the incubation with the essential oil; A_t is the absorbance after incubation with the essential oil; A_{00} is the absorbance at the beginning of the incubation without the essential oil and A_{0t} is the absorbance after incubation without the essential oil.

Samples were read against a blank containing the emulsion β-carotene/linoleic acid. Oil concentration (mg/ml) providing 50% inhibition (IC_{50}) was calculated from the graph plotting antioxidant activity against oil concentration. All tests were carried out for three sample replications and the results were averaged.

2.7. Antimicrobial activity

2.7.1. Microbial strains

Antibacterial activities of *T. algeriensis* essential oil were tested against 6 strains of bacteria: four Gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883, *Salmonella typhimurium* NRRLB 4420) and two Gram-positive (*Bacillus cereus* ATCC 11778, *Enterococcus faecalis* ATCC 29212). Antifungal activities were tested using *Fusarium solani* and *Aspergillus niger*. Microorganisms were obtained from the culture collection of the ("Centre de Biotechnologie de Sfax", Tunisia).

2.7.2. Disc diffusion method

The paper disk agar diffusion method was made according to Vanden Berghe and Vlietinck (1991). Culture suspension (200 μl) of the tested microorganisms (10^6 colony forming units (cfu)/ml of bacteria cells and 10^8 spores/ml of fungal strains) were spread on the Mueller–Hinton broth (MHB) and potato dextrose agar (PDA) medium, respectively. Then, absorbent disks (Whatman disk No. 3 of 6 mm diameter) impregnated with 10 μl of essential oil were applied on the surface of plates (90 mm) inoculated with different microbial strains. Negative controls were prepared using a disk impregnated with sterile water. Gentamycin and cycloheximide were used as positive references for bacteria and fungi, respectively. Before incubation, all plates were stored in the dark at 4 °C for 2 h, to allow the diffusion of the oil from disc to medium without microbial growth. At the end of incubation time (24 h at 37 °C for bacteria strains) or (72 h at 30 °C for fungal strains), positive antibacterial and antifungal activities were established by the presence of measurable zones of inhibition. The antimicrobial activity was recorded as the width (in mm, diameter of the disc included) of the inhibition zones after incubation. All tests were carried out for three sample replications and the results were averaged.

2.7.3. Determination of the minimum inhibitory concentration (MIC)

MIC values, which represent the lowest essential oil concentration that preventing visible growth of microorganisms, were determined as described previously (Ben Bnina et al., 2009). All tests were performed in MHB or PDA supplemented with 5% dimethylsulfoxide (DMSO). Bacterial strains were cultured overnight in MHB at 37 °C. Tubes of MHB and PDA containing various concentrations of oils were inoculated with 10 μl bacterial inoculums adjusted to 10^6 cfu/ml of bacteria cells and 10^8 spores/ml of fungal strains, respectively. Then, they were incubated under shaking conditions (120 rpm) (24 h at 37 °C for bacteria strains) or (72 h at 30 °C for fungal strains). Control tubes without tested samples were essayed simultaneously. All tests were carried out for three sample replications and the results were averaged.

2.8. Statistical analysis

Values were expressed as means ± standard deviation. Analysis of variance was conducted and differences between variables were tested for significance by one-way ANOVA with a SPSS 11 (Statistical Package for the Social Sciences) programme. Differences at $P < 0.05$ were considered statistically significant.

3. Results and discussion

3.1. Chemical composition of the T. algeriensis essential oil

The chemical composition of *T. algeriensis* essential oil was investigated using both GC and GC/MS techniques. The percentages and the retention indices of the identified oil components were listed in Table 1 in the order of their elution on the HP-5MS column. Fifty-seven compounds were identified, accounting for 97.71% of the total oil content. These compounds were divided into five classes that are monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and phenyl propanoid (Table 1). This oil was characterized by very high percentage of monoterpenes (74.34%) and especially the oxygenated ones (44.85%) which constituting the predominant class as was found for the majority of *T. algeriensis* (Hazzit et al., 2009; Ben El Hadj Ali et al., 2010). Camphor (7.82%), 4-terpineol (7.36%), 1,8-cineole (5.54%) and *cis*-sabinene hydrate (5.29%) were found to be the major components among the oxygenated monoterpenes. *cis*-Sabinene hydrate was present at relatively high rate in the essential oil in comparison to Algerian chemotypes (0.15–2.30%) (Giordiani et al., 2008; Hazzit et al., 2009). Moreover, this compound was not detected in Tunisian chemotypes of *T. algeriensis* (Ben Bnina et al., 2009; Ben El Hadj Ali et al., 2010). Furthermore, 4-terpineol was also found in the studied essential oil at relatively high rate as compared to Tunisian chemotypes (<1.9%) (Ben Bnina et al., 2009; Ben El Hadj Ali et al., 2010). The monoterpene hydrocarbons fraction accounting for 29.49% of the total oil, were represented by 16 compounds: the most important were α-pinene (6.75%) and γ-terpinene (3.50%). In contrast, the sesquiterpene fraction (22.67%) was distributed equally between hydrocarbons and oxygenated ones. Among sesquiterpenes hydrocarbons, epibicyclophellandrene (2.22%), γ-cadinene (1.81%) and germacrene D (1.72%) were the main ones. Among oxygenated sesquiterpenes, viridiflorol was the major compound (3.94%) as was found in several Tunisian *T. algeriensis* populations (tr-5.5%) (Ben El Hadj Ali et al., 2010). Nevertheless, this compound was not found in the essential oil of Algerian *T. algeriensis* chemotypes (Hazzit et al., 2009). Thymol and carvacrol were absent in the studied essential oil. These results were similar to those of Tunisian chemotypes (Ben El Hadj Ali et al., 2010). Nevertheless, the percentage of thymol in Algerian *T. algeriensis* is ranged from 0.2 to 29.5% (Hazzit et al., 2009). Jaafari et al. (2007) also reported that carvacrol (49.33–80.40%) was the major compound for Moroccan *T. algeriensis* populations.

One can notice that the studied *T. algeriensis* chemotype can be classified into the 1,8-cineole/α-pinene/camphor chemotype as described by Ben El Hadj Ali et al. (2010). Besides, this chemotype was also characterized by a relatively high rate of 4-terpineol and *cis*-sabinene hydrate (Table 1) in comparison

Table 1 – Mean percentage of compounds of *T. algeriensis* (*n* = 3) essential oil (Gafsa city, South-Western of Tunisia).

Compounds[a]		(%)[b]	RI[c]
1	Tricyclene	0.27	924
2	α-Thujene	0.56	929
3	**α-Pinene**[d]	**6.75**	939
4	Camphene	2.88	952
5	Verbenene	0.28	956
6	Sabinene	2.49	977
7	β-Pinene	2.24	981
8	β-Myrcene	0.73	993
9	α-Phellandrene	0.23	1007
10	α-Terpinene	2.46	1020
11	p-Cymene	2.57	1030
12	**1,8-Cineole**[d]	**5.54**	1037
13	*cis*-Ocimene	0.5	1040
14	*trans*-β-Ocimene	2.4	1051
15	γ-Terpinene	3.5	1063
16	***cis*-Sabinene hydrate**[d]	**5.29**	1076
17	Camphenilone	0.14	1088
18	Terpinolene	1.41	1091
19	Linalool	3.65	1106
20	p-Menth-2-en-1-ol	1.34	1129
21	**Camphor**[d]	**7.82**	1153
22	p-Menth-4(8)-ene	0.22	1164
23	Pinocarvone	0.48	1168
24	Borneol	3.49	1177
25	**4-Terpineol**[d]	**7.36**	1189
26	p-Cymen-8-ol	0.49	1194
27	α-Terpineol	1.74	1200
28	Myrtenal	1.38	1202
29	Verbenone	0.95	1217
30	*cis*-Carveol	0.27	1226
31	Thymyl methyl ether	1.19	1237
32	Linalyl acetate	0.83	1256
33	Chrysantenyl acetate	0.16	1263
34	Bornyl acetate	1.91	1289
35	Sabinyl acetate	0.18	1293
36	Thymol	0.64	1304
38	α-Copaene	0.41	1380
39	β-Bourbonene	0.12	1390
40	β-Elemene	0.24	1395
41	Unidentified	1.44	1404
42	α-Gurjunene	0.95	1415
43	*trans*-Caryophyllene	1.35	1426
44	Alloaromadendrene	0.35	1468
45	Germacrene D	1.72	1489
46	Bicyclogermacrene	0.65	1503
47	γ-Cadinene	1.81	1521
48	δ-Cadinene	0.48	1528
49	*cis*-α-Bisabolene	0.38	1544
50	Elemol	2.21	1559
51	Caryophyllene oxide	2.01	1594
52	Viridiflorol	3.94	1607
53	Epiglobulol	0.66	1616
54	2-Isopropyl-5-methyl-9-methylene bicyclo[4.4.0]dec-1-ene	0.33	1625
55	Diallapiole	0.7	1631
56	γ-Eudesmol	0.85	1643
57	Epibicyclophellandrene	2.22	1653
58	α-Eudesmol	1.99	1667
59	Unidentified	0.74	1673
Total identified		99.89	
Grouped components (%)			
Monoterpene hydrocarbons		29.49	
Oxygenated monoterpenes		44.85	
Sesquiterpene hydrocarbons		11.01	
Oxygenated sesquiterpenes		11.66	
Phenyl propanoid (diallapiole)		0.7	
Unidentified		2.18	

[a] Compounds are listed in order of their elution from a HP-5MS column.
[b] Percentages obtained by FID peak-area normalization.
[c] RI, retention indices calculated against C_8–C_{17} n-alkanes mixture on the HP 5MS column.
[d] Main compound in bold font.

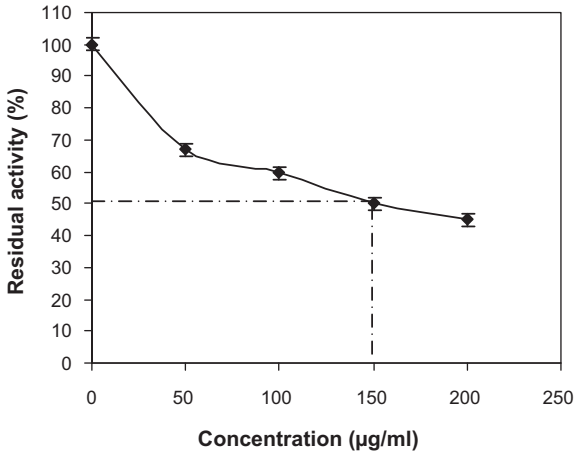

Fig. 1 – Effect of *T. algeriensis* essential oil on angiotensin I-converting enzyme activity.

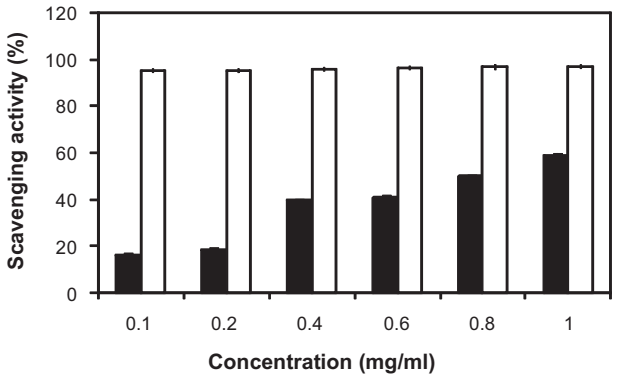

Fig. 2 – Free radical-scavenging activities (%) of *T. algeriensis* essential oil (■) and BHA (□) as positive control measured in DPPH assay.

to Tunisian chemotypes (Ben Bnina et al., 2009; Ben El Hadj Ali et al., 2010).

3.2. *ACE inhibitory activity*

It has been well demonstrated that the inhibition of ACE is considered to be a useful therapeutic approach in the treatment of high blood pressure (Skeggs et al., 1956). Due to the application of *T. algeriensis* growing wild in South Tunisia in folk medicine to treat hypertension, the *in vitro* ACE inhibitory activity of its essential oil was investigated. *T. algeriensis* essential oil exhibited dose-dependant ACE inhibitory activity (Fig. 1). The IC_{50} value, defined as the concentration of inhibitor required to abolish 50% of the ACE activity, was found to be 150 μg/ml. These results suggest that *T. algeriensis* essential oil could be used as ACE inhibitor for hypertension prevention and remedy. Few studies described the potential of plant essential oils to inhibit ACE activity. Nevertheless, several chemical classes of ACE inhibitors compounds derived from plant solvent extracts have been described such as terpenoids, tannins, fatty acids, oligosaccharides, peptides/amino acids, alkaloids and flavonoids (Nyman et al., 1998; Lacaille-Dubois et al., 2001; Braga et al., 2007). For comparative purposes, *C. brasiliense*, *C. fruticosum*, *L. rubra*, *P. roebelinii* and *T. catappa* showed rates of ACE inhibition to about 50% at a concentration of 0.10 mg/ml of ethanol extract (Braga et al., 2007). Furthermore, The flavonoids vitexin and isovitexin at 0.33 mg/ml isolated from various plants inhibited the ACE activity by 20% and 45%, respectively (Lacaille-Dubois et al., 2001). These compounds were suggested to inhibit the ACE activity by competing with the substrate for the enzyme active site. Some authors suggested that the inhibitory effect of flavonoids is due to the formation of chelate complexes with the zinc atom within the active site of zinc-dependant metallopeptidase (Chen et al., 1992).

3.3. *Antioxidant activity*

3.3.1. *DPPH radical scavenging activity*

The effect of antioxidants on DPPH radical scavenging activity was thought to be due to their hydrogen-donating ability (Shimada et al., 1992). Free radical scavenging activities of *T. algeriensis* essential oil, measured by DPPH assay were shown in Fig. 2. *T. algeriensis* oil was able to reduce the stable free radical DPPH with an IC_{50} value of 0.8 mg/ml. This value is comparable with those exhibited by various Algerian chemotypes of *Thymus* species oils measured by the same test (Hazzit et al., 2009). Nevertheless, these activities are significantly lower than that of BHA used as positive control. The DPPH radical scavenging activity of Algerian *Thymus* species oils was claimed to be attributed to the presence of phenolic constituents, especially thymol or carvacrol as major components (29.5–57.7%) (Hazzit et al., 2009). This is in contradiction with our findings that Tunisian *T. Algeriensis* chemotype displayed relatively high DPPH radical scavenging activity despite the absence of thymol and carvacrol. Theses results imply that other non-phenolic compounds present in oil are responsible for this activity.

Table 1 shows that essential oil of *T. algeriensis* is markedly rich in oxygenated monoterpenes (44.85%) which may act as radical scavenging agents. It seems to be a general trend that the essential oils which contain oxygenated monoterpenes have greater antioxidative properties (Tepe et al., 2004). In line with our findings, few studies reported that some essential oils containing low amounts of phenolic compounds also have interesting antioxidant potentials (El-Massry et al., 2002). For example, the high scavenging activity observed for two *Rosmarinus officinalis* L. varieties could be explained partially by the high amounts of camphor, linalyl acetate and α-thujene recorded for these oils (Zaouali et al., 2010). However, its difficult to attribute the antioxidant effect of a total essential oil to one or few active compounds. Both minor and major compounds should make a significant contribution to the oil's activity (Wang et al., 2008).

3.3.2. *β-Carotene bleaching by linoleic acid assay*

The potential of *T. algeriensis* oil to inhibit lipid peroxidation was evaluated using the β-carotene/linoleic acid bleaching test, which measures the oil capacity for inhibiting the conjugated diene hydroperoxides formation upon linoleic acid oxidation. *T. algeriensis* essential oil was efficient to inhibit the oxidation of linoleic acid (Fig. 3). The antioxidant activity of *T. algeriensis* oil increased with increasing essential oil concentration. The IC_{50} value was found to be 0.5 mg/ml. The potential of *T. algeriensis* oil to inhibit lipid peroxidation was found to be more important than *Thymus. caramanicus* which essential oil showed 79% inhibition at 2 mg/ml, measured by the same test (Safaei-Ghomi et al., 2009). Furthermore, the IC_{50} value obtained for *T. algeriensis* oil antioxidant activity was also found to be similar to the values, measured by the same test, exhibited by *Cymbopogon schoenanthus* oil (IC_{50} = 0.47 mg/ml) (Khadri et al., 2008) or *Mosla chinensis* oil (IC_{50} = 0.59 mg/ml)

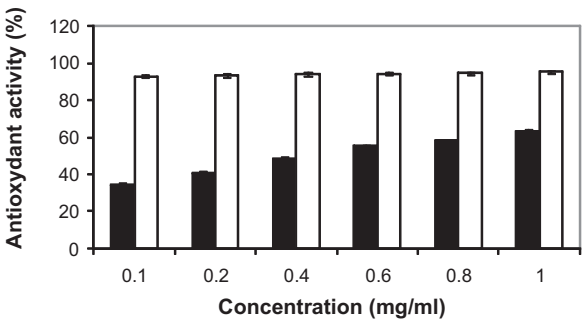

Fig. 3 – Antioxidant activities (%) of *T. algeriensis* essential oil (■) and BHA (□) as positive control, measured by β-carotene bleaching assay.

(Cao et al., 2009), but less effective than *Bidens pilosa* oil (IC_{50} = 0.0497 mg/ml) (Deba et al., 2008).

3.4. *Antimicrobial activity*

The antimicrobial activity of *T. algeriensis* essential oil against eight species of microorganisms was assessed by evaluating the inhibition zones and the determination of MIC values (μl of oil/ml of medium). As can be seen in Table 2, *T. algeriensis* essential oil showed varying degrees of antimicrobial activity against all strains tested. The inhibition zones and MIC values of microbial strains were in the range of 13.5–64 mm and 1–6 μl/ml, respectively. Gram-positive bacteria were shown to be more sensitive to the *T. algeriensis* essential oil than Gram-negative bacteria. In fact, the most susceptible bacterium for this oil was *B. cereus* (30 mm, MIC = 1 μl/ml). It has frequently been reported that Gram-negative bacteria were resistant to the inhibitory effects of essential oil and their components. This resistance has been attributed to the presence of cell wall lipopolysaccharides that can screen out the essential oil (Bezić et al., 2003). Furthermore, *T. algeriensis* essential oil exhibited remarkable inhibitory activity against fungal strains tested.

The antimicrobial activity of *T. algeriensis* essential oil might be related to its oxygenated monoterpenes and monoterpene hydrocarbons components which constitute respectively about 44.85% and 29.49% of this oil as was previously suggested (Cox et al., 2000). In fact, it was shown, that monoterpenes in essential oils are able to affect cellular integrity resulting in inhibition of respiration and alteration in permeability. Previous works focusing on the antimicrobial activities of different *Thymus* essential oils tried to correlate these activities to one or many major components. In fact, antifungal activities of some *Thymus* oils was previously explained by the high phenols (thymol and carvacrol) content. Giordiani et al. (2008) related the strong antifungal activity of *T. vulgaris* essential oil to its high amount of thymol (25.57%). Effective antifungal activity of a *T. pallescens* from Oued Rhiou or El-Asnam regions in Algeria was also explained by their high content in thymol (49.3%) and carvacrol (57.7%), respectively (Hazzit et al., 2009). However, it is difficult to attribute the antimicrobial activity of *T. algeriensis* essential oil, characterized by a complex mixture, to a single or particular constituent. In fact, some studies have concluded that whole essential oils have a greater antibacterial activity than the major components mixed (Gill et al., 2002). Synergistic and antagonistic effects between several components in the oil are possible and should also be taken into consideration. In fact, Faleiro et al. (2003) assessed the antimicrobial activities of the pure components (linalool, 1,8-cineole and linalool/1,8-cineole (1:1)) which represent the major oil components of the *Thymus* species growing wild in Portugal. These results demonstrated a higher antimicrobial activity of linalool compared with 1,8-cineole. They also demonstrated that possible antagonistic and synergistic effects may occur, namely in the case of the strain of *E. coli* that is not susceptible to the mixture andida of linalool/1,8-cineole but is susceptible to linalool and for *C albicans*, the mixture of linalool/1,8-cineole slightly increased the antimicrobial activity whereas 1,8-cineole alone had a diminished activity.

Table 2 – Antibacterial and antifungal activities of *T. algeriensis* essential oil.

Tested microorganisms	Inhibition zone diameter[a] (mm)		MIC (μl/ml)
	Essential oil	Antibiotics[b]	
Bacteria (Gram-negative)			
S. typhimurium	15 ± 0.5	24 ± 2	6
E. coli	14 ± 1	20 ± 1	6
K. pneumoniae	13.5 ± 0.5	22 ± 1	6
P. aeruginosa	14.5 ± 0.5	26 ± 2	5
Bacteria (Gram-positive)			
E. faecalis	18.5 ± 0.5	21 ± 2	3
B. cereus	30 ± 2	21 ± 1	1
Fungi			
F. solani	31 ± 1.5	39 ± 2	1
A. niger	64 ± 3	31 ± 1	2

[a] Values represent averages ± standard deviations for triplicate experiments.
[b] Gentamycin (10 μg/disc) and cycloheximide (10 μg/disc) were used as positive controls for bacteria and fungi, respectively.

4. Conclusion

This study deals with the development of medicinal and aromatic plants of the Tunisian flora, in order to find new bioactive natural products. The main components of the essential oil of *T. algeriensis* collected from the South-Western of Tunisia (Gafsa) were camphor (7.82%), α-pinene (6.75%), 1,8-cineole (5.54%), 4-terpineol (7.36%) and *cis*-sabinene hydrate (5.29%) which confirm the important intraspecific chemical variability in *T. algeriensis*. Interestingly, our results demonstrate the capacity of the *T. algeriensis* essential oil to inhibit ACE activity, suggesting the possibility to use this plant as an interesting alternative for the treatment of high blood pressure. The *T. algeriensis* oil also exerted an interesting inhibitory activity against fungal and Gram-positive bacteria strains.

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References

Adams, R.P., 2001. Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectrometry. Allured Publishing Corporation, Carol Stream, USA.

Asuming, W.A., Beauchamp, P.S., Descalzo, J.T., Dev, B.C., Dev, V., Frost, S., Ma, C.W., 2005. Essential oil composition of four *Lomatium* Raf. species and their chemotaxonomy. Biochemical Systematics and Ecology 33, 17–26.

Baranauskiene, R., Venskutonis, P.R., Viskelis, P., Dambrauskiene, E., 2003. Influence of nitrogen fertilizers on the yield and composition of thyme (*Thymus vulgaris*). Journal of Agriculture and Food Chemistry 51, 7751–7758.

Ben El Hadj Ali, I., Zaouali, Y., Bejaoui, A., Boussaid, M., 2010. Variation of the chemical composition of essential oils in Tunisian populations of *Thymus algeriensis* Boiss. et Reut (Lamiaceae), and implication for conservation. Chemistry and Biodiversity 7 (5), 1276–1289.

Ben Bnina, E., Hammami, S., Daami-Remadi, M., Cheraif, I., Hajjlaoui, H., Ben Jannet, H., Ben Said, M., Mighri, Z., 2009. Composition and antimicrobial activities of essential oils from the aerial parts and flowers of *Thymus hirtus* W. growing in Tunisia. Journal of Essential Oil Research 21 (6), 567–572.

Bezić, N., Skocibusić, M., Dunkić, V., Radonić, A., 2003. Composition and antimicrobial activity of *Achillea clavennae* L. essential oil. Phytotherapy Research 17, 1037–1040.

Braga, F.C., Serra, C.P., Viana Júnior, N.S., Oliveira, A.B., Côrtes, S.F., Lombardi, J.A., 2007. Angiotensin-converting enzyme inhibition by Brazilian plants. Fitoterapia 78, 353–358.

Cao, L., Si, J.Y., Liu, Y., Sun, H., Jin, W., Li, Z., Zhao, X.H., Pan, R.L., 2009. Essential oil composition, antimicrobial and antioxidant properties of *Mosla chinensis* Maxim. Food Chemistry 115, 801–805.

Chen, C.H., Lin, J.Y., Lin, C.N., Hsu, S.Y., 1992. Inhibition of angiotensin I-converting enzyme by tetrahydroxyxanthones isolated from *Tripterosperum lanceolatum*. Journal of Natural Products 55, 691–695.

Cox, S.D., Mann, C.M., Markham, J.L., Bell, H.C., Gustafson, J.E., Warmington, J.R., Wyllie, S.G., 2000. The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). Journal of Applied Microbiology 88, 170–175.

Deba, F., Xuan, T.D., Yasudaa, M., Tawata, S., 2008. Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from *Bidens pilosa* Linn. var. Radiata. Food Control 19, 346–352.

El-Massry, K.F., El-Ghorab, A.H., Farouk, A., 2002. Antioxidant activity and volatile components of Egyptian *Artemisia judaica* L. Food Chemistry 79, 331–336.

Espin, J.C., Garcia-Conesa, M.T., Tomas-Barberan, F.A., 2007. Nutraceuricals: facts and fiction. Phytochemistry 68, 2986–3008.

Faleiro, M.L., Miguel, M.G., Ladeiro, F., Venâncio, F., Tavares, R., Brito, J.C., 2003. Antimicrobial activity of essential oils isolated from Portuguese endemic species of *Thymus*. Letters in Applied Microbiology 36, 35–40.

Ferhat, M.A., Meklati, B.Y., Chemat, F., 2007. Comparison of different isolation methods of essential oil from *Citrus* fruits: cold pressing, hydrodistillation and microwave "dry" distillation. Flavour and Fragrance Journal 22, 494–504.

Gill, A.O., Delaquis, P., Russo, P., Holley, R.A., 2002. Evaluation of antilisterial action of cilantro oil vacuum packed ham. International Journal of Food Microbiology 73, 83–92.

Giordiani, R., Hadef, Y., Kaloustian, J., 2008. Compositions and antifungal activities of essential oils of some Algerian aromatic plants. Fitoterapia 79, 199–203.

Hazzit, M., Baaliouamer, A., Faleiro, M.L., Miguel, M.G., 2006. Composition of the essential oils of *Thymus* and *Origanum* species from Algeria and their antioxidant and antimicrobial activities. Journal of Agriculture and Food Chemistry 54, 6314–6321.

Hazzit, M., Baaliouamer, A., Verissimo, A.R., Faleiro, M.L., Miguel, M.G., 2009. Chemical composition and biological activities of Algerian *Thymus* oils. Food Chemistry 116, 714–721.

Jaafari, A., Ait Mouse, H., Rakib, E.M., Ait M'barek, L., Tilaoui, M., Benbakhta, C., Boulli, A., Abbad, A., Zyad, A., 2007. Chemical composition and antitumor activity of different wild varieties of Moroccan thyme. Revista Brasileira de Farmacognosia 17, 477–491.

Jalali-Heravi, M., Zekavat, B., Sereshti, H., 2006. Characterization of essential oil components of Iranian geranium oil using gas chromatography–mass spectrometry combined with chemometric resolution techniques. Journal of Chromatography A 1114, 154–163.

Kapel, R., Rahhou, E., Lecouturier, D., Guillochon, D., Dhulster, P., 2006. Characterization of an antihypertensive peptide from an Alfalfa white protein hydrolysate produced by a continuous enzymatic membrane reactor. Process Biochemistry 41, 1961–1966.

Khadri, A., Serralheiro, M.L.M., Nogueira, J.M.F., Neffati, M., Smiti, S., Araujo, M.E.M., 2008. Antioxidant and antiacetylcholinesterase activities of essential oils from *Cymbopogon schoenanthus* L. Spreng. Determination of chemical composition by GC–mass spectrometry and ^{13}C NMR. Food Chemistry 109, 630–637.

Kim, M.R., Abd El-Aty, A.M., Kim, I.S., Shim, J.H., 2006. Determination of volatile flavor components in danggui cultivars by solvent free injection and hydrodistillation followed by gas chromatographic–mass spectrometric analysis. Journal of Chromatography A 1116, 259–264.

Kirby, A.J., Schmidt, R.J., 1997. The antioxidant activity of Chinese herbs for eczema and placebo herbs. Journal of Ethnopharmacology 56, 103–108.

Koleva, I.I., van Beek, T.A., Linssen, J.P.H., de Groot, A., Evstatieva, L.N., 2002. Screening of plant extracts for antioxidant: a comparative study on three testing methods. Phytochemical Analysis 13, 7–8.

Kovàts, E., 1958. Characterization of organic compounds by gas chromatography. Part 1. Retention indices of aliphatic halides, alcohols, aldehydes and ketones. Helvitica Chimica Acta 41, 1915–1932.

Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, S.M., Binkoski, A.E., Hilpert, K.F., 2002. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. American Journal of Medicine 30, 71–88.

Lacaille-Dubois, M.A., Frank, U., Wagner, H., 2001. Search for potential angiotensin converting enzyme (ACE)-inhibitors from plants. Phytomedicine: International Journal of Phytotherapy and Phytopharmacology 8, 47–52.

Lee, J., Koo, N., Min, D.B., 2004. Reactive oxygen species, aging, and antioxidative nutraceuticals. Comprehensive Reviews in Food Science and Food Safety 3, 1–3.

Le Floc'h, E., 1983. Contribution à une étude ethnobotanique de la flore tunisienne Ministère de l'Enseignement Supérieur et de la Recherche Scientifique (Ed.). Imprimerie Officielle de la République Tunisienne, 402 pp.

Le Floc'h, E., Boulos, L., 2008. Flore de la Tunisie, Catalogue Synonymique Commenté, Montepellier. p. 461.

Liu, L., Song, G., Hu, Y., 2007. GC/MS analysis of the essential oils of *Piper nigrum* L. and *Piper longum* L. Chromatographia 66, 785–790.

Mau, J.L., Huang, P.N., Huang, S.J., Chen, C.C., 2004. Antioxidant properties of methanolic extracts from two kinds of *Antrodia camphorata* mycelia. Food Chemistry 86 (1), 25–31.

Morales, R., 1986. Taxonomia de los géneros *Thymus* (excluida la sección *Serpyllum*) y Thymbra en la Península Ibérica. Ruizia 3, 1–324.

Nakamura, Y., Yamamoto, N., Sakai, K., Okubo, A., Yamazaki, S., Takano, T., 1995. Purification and characterization of angiotensin I-converting-enzyme inhibitors from sour milk. Journal of Dairy Science 78, 777–783.

Namki, M., 1990. Antioxidants/antimutagens in food. Critical Reviews in Food Science and Nutrition 29, 273–300.

Nyman, U., Joshi, P., Madsen, L.B., Pedersen, T.B., Pinstrup, M., Rajasekharan, S., George, V., Pushpangadana, P., 1998. Ethnomedical information and *in vitro* screening for angiotensin-converting enzyme inhibition of plants utilized as traditional medicines in Gujarat, Rajasthan and Kerala (India). Journal of Ethnopharmacology 60, 247.

Pottier-Alapetite, G., 1981. 'Flore de la Tunisie', I. O. R. Tunisienne, Tunis, vol. 2, p. 809.

Safaei-Ghomi, J., Ebrahimabadi, A.H., Djafari-Bidgoli, Z., Batooli, H., 2009. GC/MS analysis and *in vitro* antioxidant activity of essential oil and methanol extracts of *Thymus caramanicus* Jalas and its main constituent carvacrol. Food Chemistry 115, 1524–1528.

Shahidi, F., Champaign, I.L., 1998. Natural Antioxidants Chemistry Health Effects and Application. AOCS Press, USA, pp. 1826–3489.

Shimada, K., Fujikawa, K., Yahara, K., Nakamura, T., 1992. Antioxidative properties of xanthum on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry 40, 945–948.

Skeggs, L.T., Kahn, J.E., Shumway, N.P., 1956. The preparation and function of the angiotensin I-converting enzyme. Journal of Experimental Medicine 103, 295–299.

Tepe, B., Daferera, D., Sokmen, M., Polissiou, M., Sokmen, A., 2004. The *in vitro* antioxidant and antimicrobial activities of the essential oil and various extracts of *Origanum syriacum* L. var. bevanii. Journal of the Science of Food and Agriculture 84, 1389–1396.

Vagionas, K., Graikou, K., Ngassapa, O., Runyoro, D., Chinou, I., 2007. Composition and antimicrobial activity of the essential oils of three *Satureja* species growing in Tanzania. Food Chemistry 103, 319–324.

Vanden Berghe, D.A., Vlietinck, A.J., 1991. Screening methods for antibacterial and antiviral agents from higher plants. In: Hostettmann, K. (Ed.), Methods in Plant Biochemistry. Academic Press, London, UK, pp. 47–69.

Wang, W., Wu, N., Zu, Y.G., Fu, Y.J., 2008. Antioxidative activity of *Rosmarinus officinalis* L. essential oil compared to its main components. Food Chemistry 108, 1019–1022.

Zaouali, Y., Bouzaine, T., Boussaid, M., 2010. Essential oils composition in two Rosmarinus officinalis L. varieties and incidence for antimicrobial and antioxidant activities. Food and Chemical Toxicology 48, 3144–3152.