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Chemical composition and biological activities of Algerian *Thymus* oils

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ABSTRACT

The compositions of essential oils isolated from nine samples of three *Thymus* species (*Thymus algeriensis*, *Thymus pallescens* and *Thymus dréatensis*) were analysed by GC and GC–MS, and a total of 114 components were identified. *T. pallescens* collected from various regions showed a great similarity in their compositions and were characterised by carvacrol (44.4–57.7%), *p*-cymene (10.3–17.3%) and γ-terpinene (10.8–14.2%) as the major components for four samples; only one sample was thymol-rich (49.3%) with a small amount of carvacrol (9.0%). On the other hand, *T. algeriensis* showed a chemical polymorphism, even for samples from the same location, and two new chemotypes for this species were proposed. Oxygen-containing monoterpenes were the predominant class (76.3%) in *T. dreatensis* oil, with linalool (30.4%), thymol (20.2%) and geraniol (19.6%) as the principal constituents. The oils were screened for their possible antioxidant activities by four complementary assays, namely DPPH free radical scavenging, hydroxyl radical scavenging, inhibition of lipid peroxidation and reducing power. The two new chemotypes of *T. algeriensis* exhibited strong hydroxyl radical scavenging (IC_{50} = 2.2–3.3 μg/ml), but were not or only slightly active against the other radicals and exhibited a weak reducing power. Despite their chemical similarity, *T. pallescens* oils sometimes produced significant differences in their antioxidant activities. The essential oils were also screened for their antimicrobial activity against five bacteria (three Gram-positive and two Gram-negative) and one yeast (*Candida albicans*). The tested essential oils showed antimicrobial activity against the microorganisms used, in particular against two important pathogens, *C. albicans* and *Helicobacter pylori*.

1. Introduction

Thymus (Lamiaceae) is a large genus divided in eight sections, comprising about 215 species particularly prevalent in the Mediterranean area. This genus is represented by eleven species in the flora of Algeria; among them are *Thymus algeriensis* Boiss. et Reut., *Thymus pallescens* de Noé and *Thymus dreatensis* Batt. (Morales, 2002).

T. algeriensis is the most widespread North African species; *T. pallescens* is common and endemic to northern Algeria, while *T. dreatensis* is rare and endemic to the Aures mountains (Batna region) and Djurdjura mountains (Kabylie region) of eastern Algeria (Quezel & Santa, 1963).

T. algeriensis is largely used, fresh or dried, only as a culinary herb, whereas the two other species, mainly *T. pallescens*, are widely used in Algerian folk medicine for their antitussive, antiseptic, expectorant, anti-helmintic and antispasmodic properties.

The chemical compositions have been previously established for *T. algeriensis* (Aboutabl & El-dahmy, 1995; Benjilali, Hammoumi, M'hamedi, & Richard, 1987; Benjilali, Hammoumi, & Richard, 1987; Houmani, Azzoudj, Naxakis, & Skoula, 2002), and for *T. pallescens* (Hazzit, Baaliouamer, Faleiro, & Miguel, 2006), while *T. dreatensis* is assessed for the first time. Except for *T. pallescens*, the antimicrobial and/or the antioxidant activities of these species from Algeria have not been reported before.

Due to the application of *Thymus* species growing wild in Algeria as a culinary herb and in folk medicine, the purpose of the present work was to evaluate the antioxidant and antimicrobial activities of their essential oils and relate them with their chemical composition, for further application in food and pharmaceutical industries as natural valuable products.

2. Materials and methods

2.1. Plant material

Fresh aerial parts of *Thymus* samples were collected in July 2005 at full flowering stage. The five samples of *T. pallescens* were from

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Sidi Aissa (Kef Ennaga mountain) and Boussaada (Theniet Eddem) at 180 and 300 km south of Algiers, respectively; Kadiria and El-Asnam (Bouira region) at 80 and 210 km east of Algiers, respectively, and Oued Rhiou at 250 km west of Algiers. The samples of *T. algeriensis* were collected from Chrea National Park at 800 and 1500 m altitude (Blida region) and El-Asnam, while *T. dreatensis* was collected from Takoucht in the Djurdjura mountains (Kabylie region), 180 km east of Algiers. Voucher specimens of the different samples were stored in the herbarium of the Department of Botany, National Institute of Agronomy (INA), Algiers. The taxonomic identity of the plants was confirmed by comparing voucher specimens with those of known identity already deposited in the herbarium previously. Afterwards, M. Abdelkader Beloued, Department of Botany, authenticated the plants.

2.2. Isolation of the essential oils

Leaves and inflorescences of plant material (100 g) were submitted for 3 h to steam distillation, using a Clevenger-type apparatus. The oil yields from fresh material of *T. pallescens* from Sidi-Aissa, Boussaada, Oued Rhiou, Kadiria and El Asnam were 3.2, 2.8, 6.2, 3.3, and 2.7%, respectively, whereas those of *T. algeriensis* from Chrea National Park collected at 800 and 1500 m altitude and from El Asnam were 0.4, 0.7 and 0.5%, respectively, while that of *T. dreatensis* was 2.3% (all v/w).

2.3. Gas-chromatography (GC) analysis

GC analyses were performed using a Chrompack CP-9002 gas chromatograph equipped with flame ionisation detector (FID) and a Stabilwax (PEG) column (30 m × 0.32 mm i.d., film thickness = 1 μm). Oven temperature was programmed at 50–220 °C at 2 °C min^{-1} and finally held isothermally for 15 min; injector and detector temperatures were 250 and 280 °C, respectively; carrier gas was N_2 at a flow rate of 1 ml min^{-1}. Volumes of 0.2 μl were injected. The percentage composition of the oils was computed by the normalisation method from the GC peak areas, calculated as mean values of two injections from each oil, without using correction factors.

2.4. Gas-chromatography–mass spectrometry (GC–MS) analysis

GC–MS analyses were performed using an Agilent 6890 series GC systems (Agilent Technologies, Santa Clara, CA) coupled to a quadrupole mass spectrometer (model HP 5973) equipped with an HP5-MS (cross-linked 5% phenyl methyl siloxane) capillary column (30 m × 0.25 mm, 0.25 μm film thickness). For GC–MS detection electron ionisation with ionisation energy of 70 eV was used over a scan range of 30–550 atomic mass units. Helium was the carrier gas, at a flow rate of 0.5 ml min^{-1}. Injector and detector temperatures were set at 250 and 280 °C, respectively; the temperature of the ion source was 175 °C. Column temperature was initially kept at 60 °C for 8 min, then gradually increased to 280 °C at 2 °C min^{-1} and finally held isothermally for 30 min. Samples of 0.2 μl of hexane-oil solution were injected in splitless mode. The oils components were identified by matching their recorded mass spectra with the data bank mass spectra (Wiley 7N and NIST 2002 libraries) and by comparing their retention indices relative to a series of *n*-hydrocarbons (C_7–C_{23}) with literature values (Adams, 1995; Figueredo, Cabassu, & Chalchat, 2006; Figueredo, Chalchat, & Pasquier, 2006; Jennings & Shibamoto, 1980; Loziene & Venskutonis, 2006; Tuberoso et al., 2005; İscan et al., 2006). Some structures were further confirmed by available authentic standards analysed under the conditions described above.

2.5. Antioxidant activity

2.5.1. Hydroxyl radical scavenging

The ability of *Thymus* oils to scavenge hydroxyl radical was assessed using the deoxyribose degradation assay, as described by Halliwell, Gutteridge, and Aruoma (1987). When the complex EDTA-iron (III) is incubated with reducing agent and H_2O_2 in the assay, hydroxyl radicals are generated in free solution, which attack the deoxyribose substrate fragmenting it. An aliquot (1.0 ml) of the reaction mixture contained 50 mM 2-deoxy-D-ribose (dissolved in 50 mM KH_2PO_4/K_2HPO_4 buffer at pH 7.4), a 25 μl solution of various concentrations of *Thymus* oils, 10 mM EDTA-10 mM $FeCl_3$ (premixed immediately before its addition to the reaction mixture) and 10 mM H_2O_2. The reaction was commenced by adding 1 mM ascorbic acid. After an incubation period of 1 h at 37 °C, the extent of deoxyribose degradation was measured by the thiobarbituric acid (TBA) reaction. A total of 1.0 ml of TBA (1% in 50 mM NaOH) and 1.0 ml of trichloroacetic acid, TCA (2.8%), was added to the reaction mixture, and the tubes were heated at 100 °C for 20 min. After cooling, the absorbance was read at 532 nm against a blank containing only buffer and deoxyribose. Reactions were carried out in triplicate. Mannitol, the well known hydroxyl radical scavenger was used as positive control. Inhibition (I) of deoxyribose degradation in per cent was calculated in the following way:

$$I = (1 - A_1/A_0) \times 100,$$

where A_0 is the absorbance of the control reaction (containing all reagents except the test compound) and A_1 is the absorbance of the test compound. Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against oil concentration.

2.5.2. DPPH assay

The antioxidant activity of thyme oils and tested substances was measured in terms of hydrogen-donating or radical-scavenging ability, using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent (Burits & Bucar, 2000; Şahin et al., 2004). Fifty microlitres of various concentrations of the sample in methanol (both essential oil and control substance) were added to 2 ml of a 60 μM methanolic solution of DPPH. Absorbance measurements were read at 517 nm, after 20 min of incubation time at room temperature. Absorption of a blank sample containing the same amount of methanol and DPPH solution acted as the negative control. All determinations were performed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula:

$$\%\ \text{Inhibition} = [(A_B - A_A)/A_B] \times 100,$$

where A_B is the absorption of the blank sample (t = 0 min) and A_A is the absorption of the tested oil or substance solution (t = 20 min).

2.5.3. Reducing power determination

The reductive potential of the oils and the standards (BHT and ascorbic acid) was determined according to the method of Oyaizu (1986). The different methanolic samples were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide ($K_3Fe(CN)_6$; 2.5 ml, 1%). The mixture was then incubated at 50 °C for 20 min. Afterwards, 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm. Finally, the upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1% w/v), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. The assay was carried out in triplicate.

2.5.4. Inhibition of lipid peroxidation

Inhibition of lipid peroxidation was carried out as previously described elsewhere by using a modified thiobarbituric acid reactive species (TBARS) assay (Hazzit et al., 2006). Briefly, the reaction mixture contained 0.5 ml of 10% (w/v) egg yolk homogenate in 1.15% (v/w) KCl, 0.1 ml of various concentrations of methanolic oils or positive controls and 0.05 ml of 2,2′-azobis(2-amidinopropane) dihydrochloride (ABAP, 0.07 M) as radical inducer and made up to 1 ml with distilled water. Then, 1.5 ml of 20% acetic acid (pH 3.5), and 1.5 ml of 0.8% (w/v) TBA in 1.1% (w/v) sodium dodecyl sulfate (SDS) were added. The resulting mixture was vortexed and heated at 95 °C for 60 min. After cooling, at room temperature, 5 ml of 1-butanol were added to each tube, stirred and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm. The percentage inhibition of lipid peroxidation was determined by comparing the results of the test compounds with those of controls not treated with oils or positive control. Calculations were done as mentioned for the hydroxyl radical scavenging. The antioxidant capacity measurements were carried out in triplicate.

2.6. Antimicrobial activity

In this study five different bacteria and one yeast (*Candida albicans*) were used. The bacterial group included three Gram-positive, namely *Staphylococcus aureus* CFSA2, an environmental isolate, *Listeria monocytogenes* EGD (a clinical isolate from Trudeau Institute) and *Bacillus cereus* C1060 (a food isolate from INETI-DTIA, Lisbon, Portugal), and two Gram-negative, *Salmonella* sp. (a gift from Regional Health Administration Service, Faro, Portugal) and *Helicobacter pylori* strains J99 and 26695 (a gift from the National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal). The antimicrobial activity was determined by the agar diffusion method as previously described (Faleiro et al., 2003; Faleiro et al. 2005; Hazzit et al., 2006). Briefly, bacterial strains were kept at −80 °C and the strains of *Salmonella* sp. *L. monocytogenes* and *B. cereus* maintained in tryptic soy agar (TSA), whereas *S. aureus* was maintained in brain heart infusion agar (BHIa) at 4 °C during the study. The *H. pylori* strains were kept at −80 °C and recovered from freezing on Columbia agar (Oxoid, Basingstoke, UK) supplemented with 10% (v/v) blood and incubated in a microaerophilic environment for 48 h at 37 °C prior to each assay. *C. albicans* was kept at −80 °C and recovered on yeast malt broth. Prior to the assay, recovered microbial cultures were firstly grown in the appropriate media plate for 24 h at 37 °C (30 °C for *L. monocytogenes)* and from this plate a loopful was used to inoculate 50 ml of each appropriate broth and incubated for a further 2 h at 37 °C (or 30 °C), in order for the culture growth to reach the exponential phase. The selected media plates were inoculated with 0.1 ml of the previously prepared microbial suspensions. Sterile filter paper discs (Oxoid) containing 3 μl of essential oil, sterile water (used as negative control) and 30 μg of the antibiotic chloramphenicol per disc or cyclohexamide (100 μg) (used as positive control) were distributed across the inoculated plates. Inhibition zones were determined in triplicate after an incubation period of 24–48 h at 37 °C (or 30 °C).

The collected data were subjected to two-way analysis of variance and the Tukeýs test analysis.

3. Results and discussion

3.1. Essential oil yield and chemical composition

The yields of the different samples of the three *Thymus* species ranged from 0.4 to 6.2% (v/w), with a particularly high yield for *T. pallescens* from Oued Rhiou (6.2%) and low yields for *T. algeriensis* samples. The percentage composition and modes of identification of the oils components are listed in Table 1. The chromatographic analyses resulted in the identification of 114 components, representing 91.2–98.9% of the oils, among which 96 were quantified, the others being only in trace amounts (<0.1%). Except for some slight differences, four *T. pallescens* oils (**TP1, TP2, TP4** and **TP5**) have highly similar chemical compositions characterized by carvacrol (44.4–57.7%), *p*-cymene (10.3–17.3%) and γ-terpinene (10.8–14.2%) as the major components. These results were in agreement with our previous report for this species from another region (Hazzit et al., 2006); meanwhile the sample collected from Oued Rhiou (**TP3**) was not only different because of its remarkably high oil yield but also because thymol (49.3%) was the major component instead of carvacrol (9.0%).

T. algeriensis oils showed a large variability and displayed different chemical profiles, even for the two samples collected from the same location at two different levels (**ALG1** and **ALG2**). Thus, sample collected at 800 m altitude from Chrea National Park was characterised by thymol (29.5%) as predominant component. The combined predominance of terpinyl acetate (18.0%), nerolidol (12.6%), α-pinene (11.1%), borneol (9.0%) and bornyl acetate (7.7%) characterised the sample collected from the same location at 1500 m. These observations are partly in accordance with those reported by Houmani et al. (2002) for two *T. algeriensis* samples also collected from the same location, of which one was thymol-rich (62.7%) and the other linalool-rich (78.8%). Recently, Dob, Dahmane, Benabdelkader, and Chelghoum (2006) found in a sample from Medea (north-central Algeria) that linalool (47.3%) was the most important component, while Hazzit and Baaliouamer (2007), and Giordiani, Hadef, and Kaloustian (2008) reported geranyl acetate (16.4%) and α-pinene (25.5–27.1%) as the main constituents, respectively. Regarding the sample collected from El Asnam, 4-terpineol (10.6%), camphor (10.1%), *p*-cymene (9.9%), α-pinene (6.5%) and 1,8-cineole (6.5%) were the predominant constituents. Compositions of samples from other North African countries showed that thymol and carvacrol were individually or jointly the predominant components. Thus, Aboutabl and El-dahmy (1995) reported thymol (36.8%) and myrcene (20.2%) as the major components for *T. algeriensis* from Libya, while other authors (Benjilali, Hammoumi, M'hamedi, & Richard, 1987; Benjilali et al., 1987) cited thymol (14.4–65.1%) and carvacrol (22.8–70.3%) as the major compounds for Moroccan samples. Our findings suggest the occurrence of two new chemotypes (**ALG2** and **ALG3**), which do not contain thymol, carvacrol, linalool, α-pinene or geranyl acetate as major components, but only as trace or in small amounts. It seems that the identified chemotypes occur do not due to the morphological diversity of the plant, as the samples studied have identical morphology.

T. dreatensis oil was dominated by oxygenated monoterpenes which accounted for 76.3% of the oil, among which linalool (30.4%), thymol (20.2%) and geraniol (19.6%) were preponderant. The presence of geraniol at a high percentage is noteworthy, since that its presence has not yet been revealed in other Algerian *Thymus* species.

3.2. Antioxidant activity

Numerous techniques are available to evaluate the antioxidant activities of compounds or complex mixtures, such as essential oils or plant extracts. Despite the various methods, just one procedure cannot identify all possible mechanisms characterising an antioxidant. Therefore, oils were screened for their possible antioxidant activities by using four complementary *in vitro* assays: reductive potential, inhibition of DPPH free radical, oxygen radicals, such as lipid peroxides, and hydroxyl radicals.

Table 1
Percentage composition of the essential oils of *T. pallescens*, *T. algeriensis* and *T. dreatensis* at flowering stage.

No.	Compounds[a]	KI[b]		**TP1**[c]	**TP2**[c]	**TP3**[c]	**TP4**[c]	**TP5**[c]	**ALG1**[c]	**ALG2**[c]	**ALG3**[c]	TDR[c]	Identification[d]
		1	2										
1	Methyl 2-methylbutyrate	773		–	–	–	–	t	0.2	–	–	–	RI, MS
2	Ethyl *n*-valerate	847		–	t	t	t	–	–	–	–	–	RI, MS
3	(*E*)-2-Hexenal	850	1090	–	–	–	–	–	t	–	–	–	RI, MS
4	3-Hexen-1-ol	857		–	t	t	–	–	t	–	–	–	RI, MS
5	1-Hexanol	867		–	t	t	t	–	–	–	–	–	RI, MS
6	3-Heptanone	886		–	t	t	t	–	–	–	–	–	RI, MS
7	Tricyclene	923	1017	t	t	t	t	t	t	0.3	0.1	–	RI, MS
8	α-Thujene	925	1029	2.5	2.7	2.2	2.2	1.4	1.5	0.5	0.2	0.2	RI, MS, co-GC
9	α-Pinene	935	1026	2.3	2.0	1.6	5.1	1.1	5.8	**11.1**	**6.5**	1.2	RI, MS, co-GC
11	2,4(10)-Thujadiene	945		–	t	t	t	–	0.1	–	–	–	RI, MS
12	Camphene	948	1072	0.1	0.1	0.1	0.2	0.1	0.7	**5.9**	2.3	t	RI, MS, co-GC
13	Verbenene	953	1121	–	t	t	t	–	0.1	0.1	0.6	–	RI, MS
14	Sabinene	972	1132	t	0.2	t	0.2	0.1	0.1	0.8	0.4	0.1	RI, MS, co-GC
15	β-Pinene	976	1119	0.3	0.4	0.3	0.5	0.1	3.7	3.2	2.0	0.1	RI, MS, co-GC
16	1-Octen-3-ol	982	1411	t	0.3	0.2	0.3	0.1	0.5	t	–	–	RI, MS
17	3-Octanone	987	1225	t	t	t	t	0.1	0.1	t	t	t	RI, MS
18	β-Myrcene	992	1173	2.0	2.8	2.4	3.2	1.5	1.9	3.0	0.3	2.5	RI, MS, co-GC
19	3-Octanol	1001	1393	t	t	t	0.1	0.1	0.1	0.1	0.1	0.1	RI, MS
20	α-Phellandrene	1005	1177	0.3	0.5	0.3	0.4	0.2	0.2	0.2	0.2	0.1	RI, MS, co-GC
21	δ-3-Carene	1009	1163	0.1	0.1	0.1	0.1	0.1	0.1	–	–	t	RI, MS, co-GC
22	α-Terpinene	1017	1192	2.7	3.7	2.6	2.3	1.8	1.2	0.2	2.8	0.4	RI, MS, co-GC
23	*p*-Cymene	1026	1290	**17.3**	**10.0**	**11.2**	**12.0**	**10.3**	**13.0**	0.4	**9.9**	**4.0**	RI, MS, co-GC
24	Limonene	1029	1212	0.1	0.1	0.8	0.1	–	0.7	2.8	0.1	0.3	RI, MS, co-GC
25	β-Phellandrene	1030	1222	0.2	0.1	t	0.2	0.8	–	–	–	t	RI, MS
26	1,8-Cineole	1033	1223	0.1	0.1	t	0.1	–	1.5	1.0	**6.5**	–	RI, MS, co-GC
27	*cis*-β-Ocimene	1039	1246	t	t	t	0.1	–	0.1	0.1	–	–	RI, MS
28	*trans*-β-Ocimene	1048	1272	0.1	0.1	0.1	0.4	0.1	0.9	1.5	t	t	RI, MS
29	γ-Terpinene	1061	1261	**12.3**	**14.2**	**10.9**	**10.8**	**13.4**	**6.9**	0.8	**5.5**	4.4	RI, MS, co-GC
30	*cis*-Sabinene hydrate	1069	1567	0.3	0.4	0.4	0.5	0.2	1.8	0.3	2.3	0.1	RI, MS
31	*cis*-Linalol oxide	1072	1480	–	–	–	0.1	–	0.1	–	t	–	RI, MS
32	1-Nonen-3-ol	1085	1525	t	0.1	0.1	0.1	t	–	–	0.2	t	RI, MS
33	Terpinolene	1087	1300	0.1	0.2	0.2	0.1	t	0.2	0.3	1.6	t	RI, MS, co-GC
34	*p*-Cymenene	1089	1452	t	t	0.1	0.1	–	0.1	–	0.6	–	RI, MS
35	*trans*-Sabinene hydrate	1099	1482	t	t	t	0.1	t	0.2	0.1	0.5	–	RI, MS
36	Linalool	1104	1558	2.2	2.8	1.7	3.2	3.9	3.6	2.0	1.2	**30.4**	RI, MS, co-GC
37	Nonanal	1106	1401	0.1	0.2	0.3	–	–	–	–	–	–	RI, MS, co-GC
38	Chrysanthenone	1110	1493	–	–	–	–	–	–	0.1	–	–	RI, MS
39	1-Octen-3-yl acetate	1116	1388	–	–	–	–	–	–	0.3	–	–	RI, MS
40	α-Campholenal	1125	1502	t	t	t	0.1	t	0.1	0.7	1.0	–	RI, MS, co-GC
41	*trans*-Pinocarveol	1138	1664	t	–	–	0.1	t	0.4	0.4	–	–	RI, MS
42	Camphor	1144	1556	–	–	–	–	–	0.2	2.3	**10.1**	–	RI, MS, co-GC
43	*trans*-Verbenol	1146	1683	–	–	–	t	–	0.4	1.2	3.6	t	RI, MS
44	*trans*-Chrysanthemal	1149		–	–	–	–	–	–	–	–	0.1	RI, MS
45	Pinocarvone	1162	1586	–	–	–	–	–	–	0.3	0.6	–	RI, MS
46	Borneol	1167	1732	0.2	0.1	0.1	0.2	0.3	–	**9.0**	1.6	t	RI, MS, co-GC
47	*p*-Mentha-1,5-dien-8-ol	1169	1674	–	–	–	–	–	–	–	1.3	–	RI, MS
48	Terpinen-4-ol	1177	1613	0.4	0.3	0.2	0.4	0.1	1.0	0.4	**10.6**	0.1	RI, MS, co-GC
49	*p*-Cymen-8-ol	1187	1864	–	–	–	–	–	–	t	1.1	–	RI, MS
50	α-Terpineol	1194	1723	–	–	–	–	–	0.3	1.0	0.9	2.5	RI, MS, co-GC
51	Myrtenal	1196	1649	–	–	–	–	–	–	0.4	1.0	–	RI, MS
52	*cis*-Dihydrocarvone	1197	1626	–	–	–	–	–	–	–	–	0.1	RI, MS
53	Myrtenol	1198	1804	–	–	–	–	–	0.2	0.2	0.9	–	RI, MS
54	*trans*-Dihydrocarvone	1203	1647	–	–	–	–	–	0.1	–	t	t	RI, MS
55	Verbenone	1210	1734	–	–	–	–	–	–	0.1	0.9	–	RI, MS
56	*trans*-Carveol	1221	1845	–	–	–	–	–	–	0.1	–	–	RI, MS
57	α-Fenchyl acetate	1227	1490	t	t	0.2	t	–	–	0.2	–	–	RI, MS
58	Nerol	1232	1808	–	–	–	–	–	–	t	–	0.2	RI, MS, co-GC
59	Thymol methyl ether	1235	1611	–	0.1	0.1	–	–	1.2	0.1	–	0.7	RI, MS
60	Cuminaldehyde	1241	1815	–	–	–	–	–	0.1	t	0.2	0.3	RI, MS, co-GC
61	Neral	1242	1694	–	–	–	–	–	0.1	–	0.6	0.6	RI, MS, co-GC
62	Carvacrol methyl ether	1242	1976	0.3	0.5	–	0.1	0.1	0.3	–	–	–	RI, MS
63	Carvone	1246	1755	–	–	0.3	–	t	t	0.1	–	–	RI, MS
64	Lanalyl acetate	1258	1663	–	–	–	–	–	–	1.9	0.3	–	RI, MS, co-GC
65	Geraniol	1259	1820	–	–	–	–	–	t	–	–	**19.6**	RI, MS, co-GC
66	Bornyl acetate	1285	1608	–	–	–	–	–	–	**7.7**	0.7	–	RI, MS, co-GC
67	Thymol	1302	2212	1.7	1.7	**49.3**	0.6	t	**29.5**	t	0.2	**20.2**	RI, MS, co-GC
68	Carvacrol	1318	2238	**50.9**	**46.9**	9.0	**44.4**	**57.7**	3.3	–	1.0	1.1	RI, MS, co-GC
69	Thymyl acetate	1352	1949	–	0.4	–	0.5	–	0.1	–	–	–	RI, MS
70	Terpinyl acetate	1356	1714	–	–	–	–	–	–	**18.0**	0.7	–	RI, MS, co-GC
71	Neryl acetate	1366	1733	–	–	–	–	–	–	0.2	–	–	RI, MS
72	α-Copaene	1372	1509	–	0.2	–	–	0.1	t	0.2	0.1	t	RI, MS
73	β-Bourbonene	1380	1536	–	–	–	0.1	0.1	0.2	0.1	0.3	0.1	RI, MS
74	Geranyl acetate	1385	1770	–	–	–	–	–	–	0.2	–	0.3	RI, MS, co-GC
75	β-Cubebene	1386	1549	–	–	–	–	–	–	0.1	–	–	RI, MS

Table 1 (*continued*)

No.	Compounds[a]	KI[b]		**TP1**[c]	**TP2**[c]	**TP3**[c]	**TP4**[c]	**TP5**[c]	**ALG1**[c]	**ALG2**[c]	**ALG3**[c]	TDR[c]	Identification[d]
		1	2										
76	β-Elemene	1389	1600	–	–	–	–	–	t	0.1	–	–	RI, MS
77	α-Gurjunene	1403	1549	0.2	0.6	0.3	0.5	0.5	t	0.1	t	0.1	RI, MS
78	β-Caryophyllene	1415	1612	0.1	1.1	0.4	1.5	1.6	5.0	0.4	t	3.0	RI, MS, co-GC
79	β-Gurjunene	1426	1532	–	–	–	–	–	0.1	–	–	0.1	RI, MS
80	α-Bergamotene	1435	1563	–	–	–	–	–	t	t	–	t	RI, MS
81	Aromadendrene	1437	1625	0.2	0.1	0.1	0.1	t	–	–	–	t	RI, MS
82	α-Humulene	1450	1687	–	0.1	t	0.1	0.1	0.3	t	–	0.1	RI, MS
83	*trans*-β-Farnesene	1456	1641	–	–	–	–	–	0.2	–	–	–	RI, MS
84	allo-Aromadendrene	1457	1663	0.1	0.2	0.1	0.2	–	–	0.1	–	0.1	RI, MS
85	γ-Muurolene	1472	1690	–	t	0.1	0.1	–	–	–	–	–	RI, MS
86	Germacrene D	1477	1726	–	0.7	0.2	0.5	1.0	0.7	1.1	1.3	1.8	RI, MS
87	Ledene	1486	1713	0.2	0.1	0.1	0.1	t	–	–	–	–	RI, MS
88	Bicyclogermacrene	1492	1766	–	0.6	0.4	0.4	0.6	–	0.1	0.3	t	RI, MS
89	α-Muurolene	1495	1740	–	t	t	t	t	t	t	0.2	t	RI, MS
90	β-Bisabolene	1505	1748	–	0.2	t	0.4	0.1	0.1	0.1	t	0.7	RI, MS
91	γ-Cadinene	1511	1776	0.1	0.1	0.1	0.1	0.1	–	0.1	–	t	RI, MS
92	Geranyl isobutyrate	1513		–	–	–	–	–	t	–	–	–	RI, MS
93	δ-Cadinene	1518	1772	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.2	0.1	RI, MS
94	β-Sesquiphellandrene	1525		–	–	–	–	–	–	–	–	t	RI, MS
95	*trans*-Cadina-1,4-diene	1526	1757	–	t	–	t	–	–	–	–	t	RI, MS
96	α-Bisabolene	1537	1776	0.1	0.1	0.1	0.1	0.1	–	0.2	–	0.1	RI, MS
97	Palustrol	1558	1953	t	t	t	t	t	–	–	–	0.3	RI, MS
98	(*trans*)-Nerolidol	1565	2050	–	–	–	–	–	–	**12.6**	0.6	–	RI, MS, co-GC
99	Germacrene D-4-ol	1568		–	t	–	t	0.2	–	0.6	t	0.2	RI, MS
100	Spathulenol	1570	2154	0.5	0.2	0.4	0.2	0.2	–	–	1.8	–	RI, MS
101	Caryophyllene oxide	1573	2014	0.5	0.2	0.4	0.2	0.3	5.0	1.0	3.9	0.2	RI, MS, co-GC
102	Globulol	1574	2098	–	t	t	t	t	–	–	0.2	–	RI, MS
103	Ledol	1580	2057	t	–	t	t	t	–	–	0.4	–	RI, MS
104	Caryophylladienol[e]	1628		–	–	–	–	–	0.4	–	–	–	MS
105	Isospathulenol	1629	2251	0.1	t	t	0.1	t	–	–	–	–	RI, MS
106	T-Cadinol	1635	2198	t	0.1	0.1	0.1	t	–	0.1	–	t	RI, MS
107	T-Muurolol	1636		–	t	–	t	–	–	–	–	–	RI, MS
108	β-Eudesmol	1644	2255	t	0.1	–	t	0.1	–	0.1	–	t	RI, MS
109	α-Cadinol	1650	2259	t	0.1	t	0.1	t	–	0.2	0.7	t	RI, MS
110	*cis, trans*-Farnesol	1701	–	–	–	–	–	–	0.1	–	–	–	MS
111	Hexadecanol	1887	–	–	–	–	–	–	0.1	–	–	–	RI, MS
112	Manoyl oxide	1992	–	–	–	–	–	–	–	0.1	–	–	RI, MS
113	*trans*-Phytol	2121	–	–	–	–	–	–	t	–	–	–	RI, MS
114	Linoleic acid	2148	–	t	0.1	0.1	t	–	–	–	–		RI, MS
	Identified components (%)			98.8	96.1	97.8	93.9	98.9	95.0	97.1	91.2	96.6	
	Monoterpene hydrocarbons			40.4	37.2	32.9	38.0	31.0	37.3	31.2	33.1	13.3	
	Oxygen-containing monoterpenes			56.1	53.3	61.3	50.4	62.3	44.5	48.0	47.8	76.3	
	Sesquiterpene hydrocarbons			1.1	4.2	2.0	4.3	4.5	6.7	2.8	2.4	6.2	
	Oxygen-containing sesquiterpenes			1.1	0.7	0.9	0.7	0.8	5.5	14.7	7.6	0.7	
	Others			0.1	0.7	0.7	0.5	0.3	1.0	0.4	0.3	0.1	

[a] Components quantified on the HP 5MS capillary column and listed in order of elution from the same column.
[b] Kovats indices relative to *n*-alkanes C_7–C_{23} on non-polar column HP 5MS (1) and polar column Stabilwax (PEG) (2).
[c] **TP1**-**TP5**, *T. pallescens* from Sidi Aissa, Boussaada, Oued Rhiou, Kadiria and El-Asnam respectively; ALG1, ALG2 and ALG3, *T. algeriensis* from Chrea National Park at 800 m and 1500 m altitude, El-Asnam respectively; TDR, *T. dreatensis*.
[d] Identification: co-GC, comparison with authentic compounds; MS, comparison of mass spectra with MS libraries; RI, comparison of retention index with bibliography.
[e] Correct isomer not identified. *t* = concentration < 0.1%.

3.2.1. Hydroxyl radical scavenging

The highly reactive hydroxyl radical can cause oxidative damage to DNA, lipids and proteins (Spencer et al., 1994). The hydroxyl radical-scavenging capacity of the oils (Table 2) was assessed by the classic deoxyribose assay. Hydroxyl radicals formed in the Fenton reaction were detected by their capacity to degrade 2-deoxy-D-ribose into fragments. Protective effects of the essential oils were evaluated by their ability to remove hydroxyl radicals from the test solution and prevent the degradation of deoxyribose. All the oils tested showed a strong inhibition of its degradation, higher than that of mannitol. The highest and similar IC_{50} values were found for the samples **TP1** and **AlG2**, followed by **AlG3** and **TDR**. Except for one sample (**TP1**), *T. pallescens* oils showed IC_{50} values ranging from 4.8 to 8.6 μg ml^{-1}.

3.2.2. Inhibition of lipid peroxidation

In the aqueous phase, ABAP undergoes steady state decomposition into carbon-centred free radicals. These primordial radicals

Table 2
IC_{50} values (μg ml^{-1}) of *Thymus* essential oils and positive controls in lipid peroxidation and hydroxyl radical scavenging.

Sample	Abbreviations	Hydroxyl	Lipid peroxidation
T. pallescens Sidi Aissa	**TP1**	2.3 ± 0.1[a]	416.3 ± 6.5[d]
T. pallescens Boussaada	**TP2**	8.6 ± 0.3[f]	192.5 ± 9.1[b]
T. pallescens Oued Rhiou	**TP3**	4.8 ± 0.3[c]	314.3 ± 6.8[c]
T. pallescens Kadiria	**TP4**	7.3 ± 0.2[e]	467.4 ± 3.8[d]
T. pallescens El-Asnam	**TP5**	6.4 ± 0.1[d]	614.2 ± 3.3[e]
T. algeriensis Chréa (800m)	**ALG1**	8.5 ± 0.1[f]	106.7 ± 8.4[a]
T. algeriensis Chréa (1500 m)	**ALG2**	2.2 ± 0.03[a]	na[*]
T. algeriensis El-Asnam	**ALG3**	3.3 ± 0.08[b]	911.6 ± 7.4[f]
T. dréatensis	TDR	3.3 ± 0.1[b]	277.3 ± 13.6[b]
Mannitol		26.4 ± 0.4[g]	nt[**]
BHT		nt[**]	173.4 ± 4.2[b]

In each column, means of three independent experiments (±SD) with different superscript letters are significantly different ($p < 0.05$).
[*] na: not active.
[**] nt: not tested.

Table 3
Scavenging ability of *Thymus* essential oils, BHA and BHT on DPPH radicals (%).

Sample	Scavenging ability (%, mean ± SD), concentrations (mg ml^{-1})					
	0.1	0.2	0.4	0.6	0.8	1.0
TP1	19.0 ± 0.2^{c}	34.9 ± 1.0^{c}	62.4 ± 1.7^{c}	77.0 ± 0.1^{b}	93.2 ± 0.0^{a}	93.4 ± 0.0^{a}
TP2	13.1 ± 0.3^{d}	24.7 ± 0.4^{d}	39.5 ± 0.6^{e}	49.5 ± 0.4^{d}	54.8 ± 0.4^{e}	59.9 ± 0.4^{f}
TP3	12.3 ± 0.2d	26.5 ± 0.4^{d}	41.3 ± 0.3^{de}	51.6 ± 0.1^{d}	58.0 ± 0.7^{d}	62.0 ± 0.1^{e}
TP4	12.2 ± 0.2^{d}	25.9 ± 0.2^{d}	42.3 ± 0.6^{d}	58.7 ± 0.6^{c}	69.7 ± 0.6^{c}	75.1 ± 0.2^{c}
TP5	10.1 ± 0.4^{e}	22.3 ± 0.1^{e}	34.7 ± 0.2^{f}	46.0 ± 0.0^{e}	52.1 ± 0.1^{f}	63.3 ± 0.1^{d}
ALG1	8.3 ± 0.4^{f}	19.6 ± 0.7^{f}	32.4 ± 0.4^{g}	41.8 ± 0.3^{f}	47.6 ± 0.3^{h}	53.4 ± 0.2^{h}
ALG2	3.1 ± 0.2^{g}	3.4 ± 0.2^{g}	3.8 ± 0.1^{h}	4.2 ± 0.2^{g}	5.3 ± 0.5^{j}	6.3 ± 0.3^{j}
ALG3	1.6 ± 0.0 ^{h}	2.6 ± 0.1^{g}	3.8 ± 0.1^{h}	4.6 ± 0.1^{g}	6.9 ± 0.1^{i}	7.8 ± 0.2^{i}
TDR	10.7 ± 0.1^{e}	18.5 ± 0.1^{f}	32.2 ± 0.2^{g}	42.0 ± 1.2^{f}	50.4 ± 0.7^{g}	58.2 ± 1.0^{g}
BHA	91.7 ± 0.4^{a}	91.4 ± 0.3^{a}	92.1 ± 0.7^{a}	93.1 ± 0.1^{a}	93.3 ± 0.0^{a}	93.3 ± 0.0^{a}
BHT	88.5 ± 0.6^{b}	87.5 ± 0.2^{b}	88.2 ± 0.7 ^{b}	90.5 ± 2.9^{a}	91.8 ± 0.1^{b}	91.3 ± 0.1^{b}

In each column, means of three independent experiments (±SD) with different superscript letters are significantly different ($p < 0.05$).

react with dissolved oxygen to form oxidising peroxyl radicals capable of abstracting hydrogen from polyunsaturated fatty acid acyl side chains at the aqueous-lipid interface. Once such reactions have occurred, damage permeates throughout the lipid phase (Dorman & Deans, 2004). A widely-used test for analysing the extent of lipid peroxidation is the measurement of the pink pigment, absorbing at 532 nm, produced through reaction of 2-thiobarbituric acid and oxidation products, not only with malondialdehyde but also with other aldehydes (Kosugi, Kato, & Kikugava, 1987). As can be seen in Table 2, *T. algeriensis* oils followed different patterns, to inhibit 50% of lipid damage; **ALG1** exhibited a strong antioxidant effect superior to that of the positive control (BHT), **ALG2** was not active and **ALG3** showed the weakest activity. Despite the similar compositions of four *T. pallescens* oils, they showed two equivalent (**TP1** and **TP4**) and two very different (**TP2** and **TP5**) activities. Furthermore, the activity of one sample (**TP2**) was equivalent to that of BHT. The disparity in inhibition of lipid peroxidation between the *T. pallescens* oils belonging to the carvacrol chemotype might be due to the reaction between one or some compounds of certain oils with 2-thiobarbituric acid rather than the components demonstrating pro-oxidant characteristics, according to Dorman and Deans (2004).

3.2.3. Free radical scavenging

The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen-donating ability. DPPH radical is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares, Dins, Cunha, & Almeida, 1997). The scavenging ability of essential oils and positive controls (BHA and BHT) are presented in Table 3. Except for *T. pallescens* collected from Sidi Aissa (**TP1**), which exhibited a potent activity at 0.8 and 1.0 mg ml^{-1}, similar to those of the two synthetic antioxidants, all the other samples containing thymol or carvacrol among their main components followed the same concentration-dependent pattern and showed close percentages of inhibition of DPPH radicals and moderate activities. The two new chemotypes of *T. algeriensis* (**ALG1** and **ALG2**) were slightly active, and the weak DPPH radical-scavenging activity of these *Thymus* oils can be attributed to the absence of some components like thymol or carvacrol, which may play an important role according

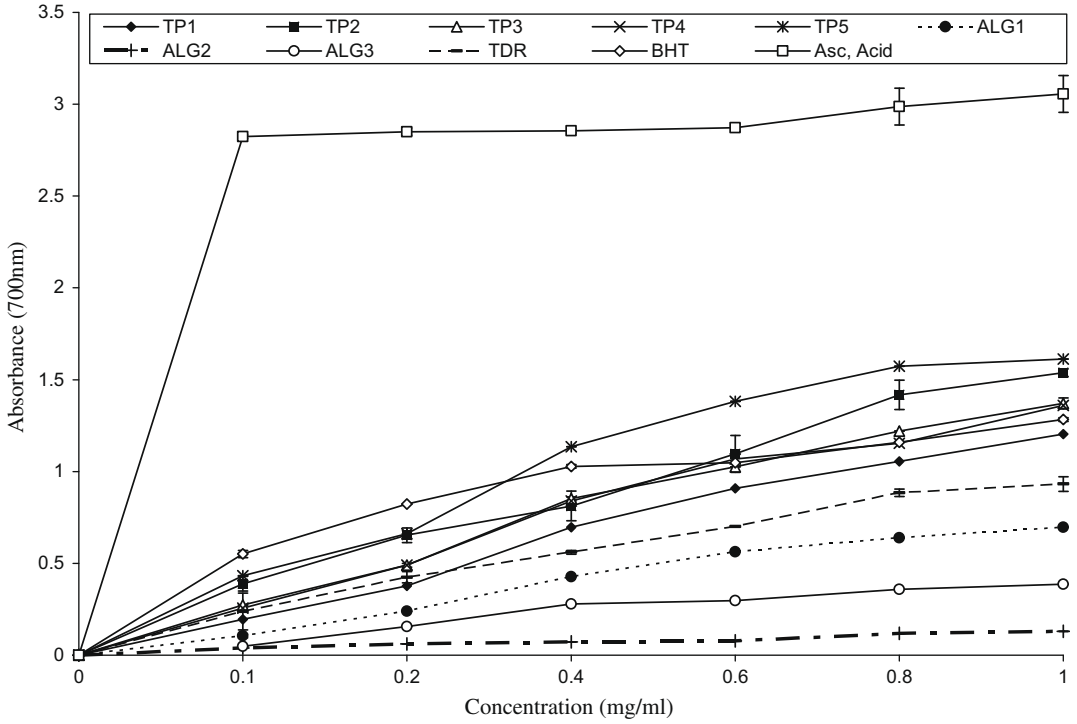

Fig. 1. Reductive potential of essential oils, BHT and ascorbic acid.

to some authors (Kulisic, Radonic, Katalinic, & Milos, 2004; Sokmen et al., 2004; Tepe, Sihoglu-Tepe, Daferera, Polissiou, & Sokomen, 2007; Tepe et al., 2005).

3.2.4. Reductive potential

Reductive ability has been investigated by the Fe^{+3} to Fe^{+2} transformation in the presence of the oils, using the method of Oyaizu (1986). The reducing power of *T. pallescens* oils and reference compounds BHT and ascorbic acid increased with increasing concentration, with a rapid increase for ascorbic acid and similar profiles for *T. pallescens* essential oils and BHT (Fig. 1). The reducing powers of *T. algeriensis* oils poor in thymol or carvacrol were markedly lower than those of the essential oils rich in these two phenols. This result suggests that there may be a close relationship between these two phenolic compounds and reducing power, due to hydroxyl substitutions in the aromatic ring, which possess potent hydrogen donating abilities as described by Shimada, Fugikawa, Yahara and Nakamara (1992). Although **TP3** and **TP4** belong to two different chemotypes, they possessed identical reductive potential over the range of concentrations tested.

Essential oils are complex mixtures and determination of the component(s) responsible for activity is difficult. Antioxidant activity of essential oils has often been attributed to the presence of phenolic constituents, especially thymol and/or carvacrol. This association has been confirmed for the most part of this study, but other compounds also seem to play an important role. For instance, the two samples of *T. algeriensis* poor in these two phenols were the most efficient scavengers of the hydroxyl radical. Furthermore, *T. pallecens* samples, which have similar compositions, with carvacrol, *p*-cymene and γ-terpinene as main components have widely-varying capabilities towards all the radicals. Contrary to *T. algeriensis*, *T. pallescens* oils showed, in most cases, a great stability in their chemical composition and the best antioxidant capability; this may explain partially the wide use of this second species in traditional medicine. The results presented here may contribute to knowledge of the antioxidant potential of *Thymus* species reported elsewhere and provide the first information on an endemic and one of the rarest *Thymus* species.

3.3. Antimicrobial activity

Data on the antimicrobial activity of the different species of *Thymus* essential oils against several bacteria and the yeast *C. albicans* are summarised in Table 4. In general the tested *Thymus* species essential oils have different activities ($p < 0.05$) against the group of bacteria tested. The less active *Thymus* essential oils were *T. algeriensis* collected from El-Asnam (**ALG3**) and from Chrea National Park at 1500 m (**ALG2**). The essential oils isolated from *T. pallescens* collected from the Sidi Aissa region (**TP1**), *T. pallescens* from the Boussaada region (**TP2**) and the *T. pallescens* from Oued Rhiou (**TP3**) had the highest antimicrobial activity. The essential oils of *T. pallescens* from Sidi Aissa region and El-Asnam have particular activity against *H. pylori* strains. The essential oil of *T. algeriensis* from El-Asnam (**ALG3**) showed an intriguing activity, only active against the two strains of *H. pylori* (Table 4). This selective activity against *H. pylori* was previously detected in the essential oil of *Dittrichia viscosa* subsp. *viscosa* (Silva et al., 2005). This anti-*H. pylori* activity may be related with two components, α-cadinol and δ-cadinene, as these two components are major components of *D. viscosa* subsp. *viscosa* and in the subsps. *revoluta* essential oils (Miguel, Faleiro, Cavaleiro, Salgueiro, & Casanova, 2008; Silva et al., 2005), and in the **ALG3** essential oil α-cadinol is present at the highest quantity. Regarding δ-cadinene, **ALG3** and the *T. pallescens* from the same region (**TP5**) showed the highest amount (Table 1).

Table 4
Antimicrobial activity of the essential oils of *T. pallescens*, *T. algeriensis* and *T. dreatensis*.

Microorganism	Inhibition zone[a] (mm)									
	TP1[b]	**TP2**[b]	**TP3**[b]	**TP4**[b]	**TP5**[b]	**ALG1**[b]	**ALG2**[b]	**ALG3**[b]	TDR[b]	Chloramphenicol/cyclohexamide
B. cereus C1060	20.66 ± 2.30^{bc}	22.0 ± 2.00^{b}	20.33 ± 0.57^{c}	16.33 ± 1.52^{c}	9.00 ± 0.00^{a}	17.00 ± 1.00^{d}	9.00 ± 1.00^{a}	NI	14.00 ± 1.00^{cd}	17.66 ± 0.57^{b}
L. monocytogenes EGD	11.33 ± 0.57^{a}	12.66 ± 1.52^{a}	13.00 ± 1.00^{b}	11.33 ± 0.57^{ab}	8.33 ± 0.57^{a}	11.66 ± 1.15^{bc}	NI	NI	10.00 ± 1.00^{abc}	26.33 ± 0.57^{c}
Salmonella sp.	10.66 ± 0.57^{a}	10.33 ± 0.57^{a}	7.00 ± 0.00^{a}	8.00 ± 1.00^{a}	NI	7.00 ± 0.00^{a}	8.33 ± 0.57^{a}	NI	7.00 ± 0.00^{a}	17.33 ± 0.57^{b}
S. aureus CFSA2	11.0 ± 2.00^{a}	14.00 ± 2.00^{a}	11.66 ± 0.57^{b}	11.66 ± 0.57^{ab}	NI	9.33 ± 0.57^{ab}	NI	NI	8.00 ± 1.00 ^{ab}	29.33 ± 0.57^{d}
H. pylori J99	34.67 ± 4.04^{d}	32.33 ± 0.58^{c}	26.00 ± 1.0^{d}	30.00 ± 2.0^{d}	37.66 ± 0.57^{c}	14.33 ± 1.15^{cd}	13.00 ± 1.00^{b}	24.33 ± 0.57^{a}	16.67 ± 4.04^{d}	39.33 ± 0.57^{e}
H. pylori 26695	25.33 ± 0.58^{c}	25.00 ± 2.00^{b}	24.67 ± 2.52^{d}	25.33 ± 1.53^{cd}	40.66 ± 0.57^{d}	17.00 ± 3.00^{d}	15.00 ± 2.00^{b}	30.00 ± 0.00^{b}	12.67 ± 2.08^{bcd}	40.00 ± 0.00^{e}
C. albicans	16.0 ± 1.00^{ab}	12.00 ± 2.00^{a}	18.66 ± 0.57^{c}	12.00 ± 1.73^{bc}	17.00 ± 1.00^{b}	9.33 ± 0.57^{ab}	9.00 ± 1.00^{a}	9.66 ± 0.57	9.00 ± 1.00^{abc}	13.00 ± 1.00^{a}

ND – Not determined.
NI – No inhibition zone.
[a] Data are the mean of three independent experiments ±standard deviation. Data in the same column with different letters are significantly different ($p < 0.05$). Inhibition zone includes the diameter of the disc.
[b] **TP1**–**TP5**, *T. pallescens* from Sidi Aissa, Boussaada, Oued Rhiou, Kadiria and El-Asnam, respectively; ALG1, ALG2 and ALG3, *T. algeriensis* from Chrea National Park at 800 m and 1500 m altitude, El-Asnam, respectively; TDR, *T. dreatensis*.

Another particular activity found concerns the essential oils of *T. pallescens* from Oued Rhiou (**TP3**) and El-Asnam (**TP5**) regions, which were very effective ($p < 0.05$) against *C. albicans*, in comparison to the antifungal agent (Table 4). This antifungal activity may be explained by their composition: **TP3** essential oil is thymol-rich whereas **TP5** contained the highest amount of carvacrol. An effective antifungal activity of a thymol chemotype of *Thymus vulgaris* was previously described by Giordani et al. (2004). Moreover these investigators verified that the activity of amphotericin B against *C. albicans* was strengthened by the *T. vulgaris* thymol chemotype essential oil.

Regarding the susceptibility of different bacteria to the *Thymus* essential oils tested, it was verified that *Salmonella* sp. was the most resistant followed by *S. aureus*, *L. monocytogenes* and *C. albicans*. *B. cereus* was the most susceptible to the tested essential oils ($p < 0.05$).

Our data support the possible use of essential oils of the tested species of *Thymus*, in particular *T. pallescens* from the Sidi-Aissa, Oued Rhiou and El- Asnam regions (**TP1, TP3, TP5**) and *T. algeriensis* from El-Asnam (**ALG3**), against two important pathogenic microorganisms, *H. pylori* and *C. albicans*.

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