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Composition of the Essential Oils of *Thymus* and *Origanum* Species from Algeria and Their Antioxidant and Antimicrobial Activities

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The composition of the essential oils of *Origanum* and *Thymus* species restricted to Algeria and the North Africa region was determined. Antioxidant and antibacterial activities of the isolated essential oils were also determined. The oils of oregano plants were strongly characterized by *p*-cymene (16.8−24.9%), γ-terpinene (16.8−24.9%), thymol (8.4−36.0%), and carvacrol (1.1−29.7%), a thymol chemotype for *Origanum floribundum* and a α-terpineol chemotype for *Thymus numidicus* being described for the first time. The strains of *Listeria monocytogenes* tested were relatively resistant to the action of essential oils of either *Origanum* or *Thymus* species. All essential oils possessed antioxidant activity, but this was dependent on the specific chemical composition and the method employed to determine such activity.

KEYWORDS: ***O. glandulosum*; *O. floribundum*; *T. guyonii*; *T. pallecens*; *T. numidicus*; *T. munbyanus*; antioxidant activity; antimicrobial activity; essential oil**

INTRODUCTION

Origanum and *Thymus* belonging to the Lamiaceae family are widespread in the Mediterranean area. Although >75% of oregano plants are located in this region, they are used as a very popular spice all over the world. Despite oregano's economic importance, its genetic resources, variability, and potential utilization have not been fully explored (*1*). This is due mainly to its complex taxonomy and the fact that the concept of "typical oregano" from a quality point of view is still under debate (*2*). The *Origanum* genus in Algeria includes two species: *O. floribundum* Munby, which is rare and endemic to the north-central part of Algeria, and *O. glandulosum* Desf., which is common over northern Algeria and endemic to the Algerian and Tunisian areas (*3*). According to the taxonomic revision of the genus carried out by Ietswaart (*4*), *O. floribundum* was classified into the section Elongatispica, whereas *O. glandulosum* has to be considered synonymous to *O. vulgare* L. subsp. *glandulosum*.

The genus *Thymus* is noteworthy for the numerous species and varieties of wild-growing plants. Many of these species are characteristic of the Mediterranean area. Eleven *Thymus* species have been recorded for Algeria included in four sections of this genus (*5*); among them are *T. guyonii* De Noe, considered to be rare and endemic to the northern Sahara; *T. pallescens* De Noe, which is common and endemic to northern Algeria; *T. numidicus* Poiret, which is endemic to the Algerian and Tunisian areas, growing in two regions, Constantine (northeastern Algeria) and Kabylie (north-central Algeria) (*3*); and *T. munbyanus*, which is endemic to the Algerian and Moroccan areas.

Many studies reviewed by Stal-Biskup (*6*) investigated the chemical composition of the essential oils from different countries and different species of the genus *Thymus*. Furthermore, the biological properties of *Thymus* essential oils have been studied by several authors (*7*−*12*). As far as our literature survey could ascertain, the chemical composition and antimicrobial and/or antioxidant activities of *T. pallescens*, *T. guyonii*, and *T. munbyanus* have not previously been published. There is one study on the chemical composition of *T. numidicus* from the Constantine region (*13*) and another on the chemical composition and antifungal activity against *Candida albicans* (*14*) for a sample from the same region.

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Concerning Algerian *Origanum* species essential oils, there are no studies on their antibacterial activity, but there is a report on the antioxidant activity of *O. glandulosum* from the Setif region (northeastern Algeria) (*15*). Studies on the chemical composition of the essential oils of Algerian *Origanum*, mainly those of *O. floribundum*, are also rather scarce.

The use of antioxidants from natural sources has become more popular as a means of increasing the shelf life of food products rich in lipids, improving their stability. Thus, volatile oils may be a rich source of phytochemicals that possess beneficial antiperoxidative and free radical scavenging characteristics. The search for natural and more health-friendly compounds against microorganisms to overcome the resistance induced by antibiotics or to prevent some infectious diseases mainly owing to contaminated foods is another priority. *Listeria monocytogenes* is a ubiquitous bacterium and a recognized foodborne pathogen. It is characterized by unusual ability to overcome different food preparation processes due to its extraordinary capacity to grow at low temperature, over a wide range of pH values (4.4−9.6), and in the presence of high salt content (*16*, *17*). The high mortality rate distinguishes *L. monocytogenes* from other foodborne pathogens such as *Escherichia coli* O157:H7 and *Salmonella* spp. The consumption of minimally processed and ready-to-eat foods contaminated with *L. monocytogenes* may constitute a risk, especially to the elderly, the very young, and pregnant women. For the reasons indicated above the search for natural compounds such as essential oils from aromatic plants never or poorly studied either chemically or biologically, particularly from the Mediterranean area, is one of our priorities. The large chemical variability among the essential oils with the consequent differences in the biological activities requires a proper investigation. In our study, five essential oils of *Origanum* and four of *Thymus* were chemically studied and their biological activities explored. The most promising oils in terms of biological activities were selected for application in food systems.

MATERIALS AND METHODS

Materials. Samples of *Origanum* and *Thymus* were harvested in July 2004 during flowering phase. Two populations of *O. glandulosum* were collected from the Blida region at Ouled Slama (200 m altitude) and Souhane (300 m) and another from the Kabylie region at Ighzer Amokrane (800−100 m). *O. floribundum* samples were collected from the Blida region at Chrea National Park (1500 m) and Hammam Melouane (200 m). *T. munbyanus* and *T. numidicus* were collected from the Kabylie region at Ighzer Amokrane and Azzazga (Yakouren forest), respectively, whereas *T. guyonii* and *T. pallescens* were from the Djelfa (Haoues mountain) and Medea (Tablat) regions. For each species, the collective sample typically constituted 35−50 plants. A voucher specimen of each plant was deposited in the Herbarium of the Department of Botany, National Institute of Agronomy (INA), Algiers. Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), 2,2′-azobis(2-amidinopropane) dihydrochloride, 1,1-diphenyl-2-picrylhydrazyl, and all of the standard compounds were purchased from Sigma-Aldrich (Steinheim, Germany). α-Tocopherol, 1,8-cineole, thymol, linalool, and potassium chloride were purchased from Merck (Darmstadt, Germany). Thiobarbituric acid (TBA) was purchased from Acros (Geel, Belgium). The culture medium tryptic soy agar (TSA) and sterile blank disks were purchased from Oxoid (Basingstoke, Hampshire, U.K.).

Extraction of the Essential Oils. Leaves and inflorescences of plant material (100 g) were submitted to hydrodistillation for 3 h using a Clevenger-type apparatus. The oil yields from fresh material of *T. munbyanus*, *T. numidicus*, *T. guyonii*, and *T. pallescens* were 1.8, 2.4, 1, and 3.7% (v/w), respectively, whereas those from *O. floribundum* (Chrea National Park) and Hammam Melouane) and *O. glandulosum* from Souhane, Ouled Slama, and Ighzer Amokrane were 4.5, 2.9, 3.2, 2.5, and 1.8% (v/w), respectively.

Gas Chromatography Analysis (GC). GC analyses of essential oils obtained from fresh material were performed using a Chrompack 9002 gas chromatograph equipped with a flame ionization detector (FID) and a Stabilwax (PEG) column (30 m × 0.32 mm i.d., film thickness = 1 μm). The operating conditions were as follows: injector and detector temperatures, 250 and 280 °C, respectively; carrier gas, N_2 at a flow rate of 1 mL/min; oven temperature program, 3 min isothermal at 50 °C, raised at 2 °C/min to 220 °C, and finally held isothermal for 15 min. The identities of the separated components on the polar column were determined by comparing their retention indices relative to aliphatic hydrocarbons injected under the above temperature program with literature values measured on columns with identical polarities (*18*).

Gas Chromatography−Mass Spectrometry Analysis (GC-MS). The GC-MS analysis was performed using a Hewlett-Packard 6890 series GC systems (Agilent Technologies) coupled to a quadrupole mass spectrometer (model HP 5973) equipped with a HP5 MS capillary column (5% phenyl methylsiloxane, 30 m × 0.25 mm, 0.25 μm film thickness). For GC-MS detection an electron ionization system with an ionization 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. Injector and detector MS transfer line 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, and finally held isothermal for 30 min. The volume of injections was 0.2 μL of a hexane−oil solution, injected in the splitless mode. The identity of the components was assigned by matching their spectral data with those detailed in the Wiley 7N, NIST 02, and NIST 98 libraries. The results were also confirmed by the comparison of their retention indices, relative to C_7-C_{29} *n*-alkanes assayed under GC-MS in the same conditions as the oils (*18*, *19*). Some structures were further confirmed by available authentic standards analyzed under the same conditions described above. The percentage composition of the oils was computed by the normalization method from the GC peak areas, calculated as the mean value of two injections from each oil, without using correction factors.

Antioxidant Activity Measurement. *TBARS Assay*. The ability of the oils to inhibit malondialdehyde formation, and therefore lipid peroxidation, was determined by using a modified thiobarbituric acid reactive species (TBARS) assay. Two sets of experiments were used, in the absence and in the presence of a radical inducer (ABAP). In both cases egg yolk homogenates were used as a lipid-rich medium obtained as described elsewhere (*8*). Briefly, 0.5 mL of 10% (w/v) homogenate and 0.1 mL of sample containing essential oil or the control substances (BHA, BHT, and α-tocopherol acted as positive controls), soluble in methanol, were added to a test tube and made up to 1 mL with distilled water; 0.05 mL of 2,2′-azobis(2-amidinopropane) dihydrochloride (0.07 M) was added to the set of samples with ABAP to induce lipid peroxidation. 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 butan-1-ol was added to each tube; the contents of the tubes were stirred and centrifuged at 8172*g* for 10 min. The absorbance of the organic upper layer was measured at 532 nm using a Shimadzu 160-UV spectrophotometer. All of the values were based on the percentage antioxidant index (AI%), whereby the control was completely peroxidized and each oil demonstrated a degree of change; the AI% was calculated using the formula $(1 - T/C) \times 100$, where C is the absorbance value of the fully oxidized control and T is the absorbance of the test sample. The samples were used at concentrations of 50, 100, 250, 500, 750, and 1000 mg/L. The antioxidant capacity was determined from four replicates.

DPPH Radical Scavenging Assay. The antioxidant activities of the oregano and thyme oils or control substances were measured in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH as a reagent (*20*, *21*). A methanolic stock solution (50 μL) of the antioxidant (each essential oil and control substance was

assayed at list concentration values 50−1000 mg/L) was placed in a cuvette, and 2 mL of 60 μM methanolic solution of DPPH was added. Absorbance measurements were read at 517 nm, after 5 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 following formula: % inhibition = $[(A_B - A_A)/A_B] \times 100$, where A_B is the absorption of blank sample, which contains essential oil ($t = 0$ min), and A_A is the absorption of the tested oil control solution ($t = 5$ min). Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against oil concentration.

Antibacterial Assay. In total, 13 strains of *L. monocytogenes* were used, namely, the type strain NCTC 7973, 3 clinical strains (Scott A, SLU 2157, and SLU 1922), and 9 food isolates (C681, C759, C779, C782, C830, C882, C895, C897, and G14). Antilisterial activity was determined according to the agar diffusion method as described previously (*7*, *22*). Briefly, bacterial strains were kept at −70 °C and maintained in TSA) at 4 °C during the study. The cultures were recovered from freezing by growth on TS broth (TSB). Recovered cultures, prior to the antibacterial assays, were first grown on TSA plates for 24 h at 30 °C. A loopful of the overnight culture was transferred to 10 mL of fresh TSB and incubated for a further 2 h at 30 °C in order for growth to reach exponential phase. TSA plates were then inoculated with ≈0.1 mL of this bacterial suspension. Sterile filter paper disks containing 3 μL of essential oil, sterile water (negative control), or 30 μg of chloramphenicol (positive control) were then added to the inoculated plates. Inhibition zones were determined in triplicate after an incubation period of 24 h at 30 °C.

The collected data were subjected to two-way analysis of variance and Tuckey's test analysis.

RESULTS

Chemical Composition of the Essential Oils. The yields obtained from the five samples (**Table 1**) of oregano varied over the range of 2.5−4.5% (v/w). Concerning thyme plants, the oils analyzed were obtained in yields of 1.0−3.7% (v/w) from fresh material at full-flowering phase (**Table 1**). The lowest yield was found for *T. guyonii* and the highest one for *T. pallescens*.

Table 1 lists the chemical composition of the five samples of both species oregano essential oils as well as those isolated from the four species of *Thymus*. The oils are characterized by a very high content of monoterpene hydrocarbons (44−60%). Except for the sample of *O. floribundum* from Chrea constituted by carvacrol (29.6%) and thymol (8.4%), all other samples are rich in thymol (23.9−36.0%) and poor in carvacrol (1.1−8.0%). The major compounds of the five oils were thymol, carvacrol, *p*-cymene, and γ-terpinene.

The analysis showed that the oils are rich in monoterpenes (82−92%) with comparable amounts between oxygen-containing and monoterpene hydrocarbons in *T. munbyanus*, *T. guyonii*, and *T. pallescens*, whereas in *T. numidicus* the oxygen-containing monoterpenes (61%) dominated. The analytical data showed that *T. numidicus* belongs to the α-terpineol chemotype, with a high level of α-terpineol (34.4%) compared with the other components, especially those that characterize the other chemotypes of the genus *Thymus*.

T. guyonii was characterized by *p*-cymene (18.6%) and γ-terpinene (13.0%) as the main constituents. In addition, in comparison with the three other species here reported, the high amounts of thymol methyl ether (10.7%) and borneol (6.1%) must be pointed out. Carvacrol (41.5%) and γ-terpinene (16.6%) were the major components found in *T. pallescens*, whereas thymol (37.7%) and *p*-cymene (14.2%) dominated in *T. munbyanus*.

Antioxidant Activity. Tables 2 and **3** represent the antioxidant indices (percentage) of the essential oils of oregano and thyme oils as well as those of BHT, BHA, and α-tocopherol, in different concentrations, using the TBARS assay in the absence and in the presence of the radical inducer ABAP, respectively. In the absence of the oxidative catalyst ABAP, the majority of the tested oils presented some capacity for preventing lipid peroxidation, mainly when they were present in high concentrations. Generally, the effectiveness of the samples was characterized by an increase over the concentration range tested. The sample oil from *O. glandulosum* Ouled Slama at concentrations ranging from 50 to 750 mg/L and the oil of *T. numidicus* at 50 mg/L presented the poorest capacities to prevent lipid peroxidation, in contrast to the essential oils of *T. mumbyanus* and *T. pallescens*, which had the higher antioxidant activity, mainly from 250 mg/L, even superior to that of the synthetic antioxidants. When ABAP was added to reaction mixture, in the set (two) of experiments, generally the oils and substances tested demonstrated a decrease in their antioxidant capacity (**Table 3**) with the exception of BHA, which increased its activity over the concentration range, and α-tocopherol, especially at the concentrations of 100−250 mg/L. Regarding the essential oils, the addition of ABAP reduced greatly the antioxidant capacity of the essential oils from *O. glandulosum* from Souhane and *T. guyonii*, being almost always inferior to the remaining samples.

As shown in **Table 4**, the DPPH radical scavenging activities of oregano and thyme essential oils in methanol exist, because they are able to reduce the stable radical DPPH to yellow diphenylpicrylhydrazine with values of IC_{50} ranging from 378.2 to 826.5.1 mg/L, nevertheless significantly inferior to the capacity of BHA (126.4 mg/L). The lowest capacity to reduce DPPH was observed in *T. numidicus* and *T. guyonii* oils (597.7 and 826.5 mg/L, respectively), that is, in the poorest carvacrol plus thymol samples. Nevertheless, the highest level of these components (46.1%) in the essential oils of *T. mumbyanus* was not enough to obtain a low IC_{50}. This value was quite similar to that observed for *T. numidicus* in which the sum of the percentages of thymol and carvacrol in the essential oil was 21.8%.

Antibacterial Activity. Data of the antibacterial activity of *Origanum* and *Thymus* essential oils against *L. monocytogenes* strains are summarized in **Table 5**. The antilisterial activity of either *Origanum* or *Thymus* essential oils was in the range of less than 10 mm and not higher than 14 mm. Besides, to be possible to distinguish the susceptibilities of the *L. monocytogenes* strains to the different essential oils, the registered values of inhibition indicate a generalized resistance of *L. monocytogenes* to the essential oils tested. The antibiotic used (chloramphenicol) demonstrated a significantly higher activity ($P < 0.05$) than the essential oils of *Origanum* and *Thymus* against all *L. monocytogenes* strains.

DISCUSSION

The highest oil yield of *O. floribundum* from Chrea is very different from that reported (*23*) from the leaves for *O. floribundum* collected from the same region in June 1998 (0.7%). The different harvesting period and the aerial parts treated could be partially responsible for this difference. Blidean oil yields increased with altitude for each species; the same observation was already noted by some authors (*24*) for Calabrian (Italy) *O. vulgare* ssp. *hirtum*. The oil yield of *T. numidicus* from the Kabylie region (2.4%) was higher than that reported by Hadef et al. (*14*) for a sample from the Constantine

Table 1. Chemical Composition (Percent) of the Essential Oils from Fresh Aerial Parts of *O. glandulosum* and *O. floribundum*, *T. munbyanus*, *T. guyonii*, *T. pallescens*, and *T. numidicus* Collected during Full-Flowering Phase

compound[b]	KI[a] 1	KI[a] 2	OF1[c]	OF2[c]	OG1[c]	OG2[c]	OG3[c]	TMu[c]	TGu[c]	TPa[c]	TNu[c]	identification[d]
methyl isovalerate	765		0.1	tr[e]	tr	tr	tr	tr				MS, RI
(*E*)-2-hexenal	850		tr	tr	0.1		0.1	tr			tr	MS, RI
3-heptanone	887		tr	tr	0.1	tr	0.1	tr	tr		tr	MS, RI
α-thujene	925	1029	3.7	3.1	4	8	4.1	1.5	2.1	2.7	0.4	MS, RI
α-pinene	939	1026	2.2	1.8	2.4	0.3	1.2	4.6	1.7	2.6	2.5	MS, RI, co-GC
camphene	951	1072	0.1	tr	0.1		0.2	tr	2.5	0.3	tr	MS, RI, co-GC
sabinene	973	1132	0.2	0.6	0.6		0.6	tr	0.2	0.6	1.2	MS, RI, co-GC
β-pinene	978	1119	0.6	0.1	0.6	0.3	0.4	1	1.7	0.4	tr	MS, RI, co-GC
1-octen-3-ol	986	1462						0.2			tr	MS, RI
β-myrcene	992	1173	2.9	4.8	4	2.6	4.1	7.1	0.1	3.2	3.7	MS, RI, co-GC
α-phellandrene	1007	1177					1.2	0.5		0.8	0.2	MS, RI, co-GC
α-terpinene	1017	1192	2.5	2.1	2.9	1.6	2.5	0.5	2.2	1	0.8	MS, RI, co-GC
p-cymene	1026	1290	18.5	24.9	17.2	23	16.8	14.2	18.6	16.2	6.7	MS, RI, co-GC
limonene	1044	1212	tr				tr	0.3	0.2	0.3	tr	MS, RI, co-GC
β-phellandrene	1045	1222		tr			tr	0.2	0.2	0.3		MS, RI, co-GC
1,8-cineole	1046	1223	tr	tr			tr	0.1	0.5	0.2		MS, RI, co-GC
γ-terpinene	1060	1261	13.7	22.3	23.9	11.5	16.5	10.1	13	16.6	8.4	MS, RI, co-GC
cis-sabinene hydrate	1075	1567	tr	tr	0.7	0.1	0.2	0.5	0.4	0.3	0.6	MS, RI
1-nonen-3-ol	1088		tr	tr	0.2	tr	tr		0.1			MS, RI
terpinolene	1093	1300	0.2	0.2	0.3	0.4	0.2	0.2	0.3	0.3	0.1	MS, RI, co-GC
α-*p*-dimethylstyrene	1095	1452	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.1		MS, RI
trans-sabinene hydrate	1101	1482	0.1	tr	tr	0.1	tr	tr	tr	tr	tr	MS, RI, co-GC
linalool	1104	1558	3.8	2.4	5	2.6	2.5	2.2	6.1	4.5	1.6	MS, RI, co-GC
borneol	1166	1732		tr	0.2		tr	tr	6.1	tr	0.1	MS, RI, co-GC
terpinen-4-ol	1177	1613	0.3	0.1	0.4		0.1	0.1		0.1	tr	MS, RI, co-GC
α-terpineol	1190	1723		0.4	0.6		0.4	tr	tr		34.4	MS, RI, co-GC
trans-dihydrocarvone	1200		tr	0.1								MS, RI
thymol methyl ether	1235	1611	0.4	0.6	1.1	0.3	0.8	1.4	10.7	tr	2.6	MS, RI
carvacrol methyl ether	1245	1976	6.9	3.6	2	1.8	1.1	0.2	0.3	0.1	0.1	MS, RI
thymoquinone	1260	2176									0.2	MS, RI
thymol	1297	2212	8.4	27.9	23.9	31.6	36	37.7	10.9	0.1	15	MS, RI, co-GC
carvacrol	1317	2238	29.6	1.6	1.1	2.9	8	8.4	4.2	41.5	6.8	MS, RI, co-GC
thymyl acetate	1362		tr		0.1	0.1	tr				tr	MS, RI
terpenyl acetate	1367								0.1	0.2		MS, RI
eugenol	1373	2192	0.1		0.1	tr	tr	tr	tr	0.1		MS
α-copaene	1376	1509						0.1	0.1	0.1	tr	MS, RI
β-bourbonene	1384	1536						0.1	tr	0.1	0.2	MS, RI
carvacryl acetate	1391		0.1	tr		tr	tr					MS
geranyl acetate	1392	1778	tr		tr		tr	0.2				MS
β-elemene	1394	1600						tr	0.1		tr	MS, RI
tetradecane	1400	1400									0.3	MS, RI, co-GC
α-gurjunene	1408	1549								0.8	0.1	MS, RI
methyl eugenol	1410								0.1			MS, RI
β-caryophyllene	1417	1620	0.7	1.4	1.3	2.2	1.4	2.9	3.7	1.4	2.6	MS, RI, co-GC
trans-α-bergamotene	1438			tr	tr	0.1	tr	tr	0.1		0.1	MS, RI
aromadendrene	1439	1625							0.1	tr		MS, RI
α-humulene	1452	1687	tr	0.4	tr	tr		0.2	0.3	0.2	tr	MS, RI
1,6,6-trimethylbicyclo[3.3.0]octan-3-one[f]	1454		1.1	0.3	0.8	2.3	0.8	0.2	tr	0.1	1.1	MS
allo-aromadendrene	1457	1663							0.4	0.3		MS, RI
γ-muurolene	1474	1704						tr	0.1	tr		MS, RI
germacrene D	1480	1742	tr	0.1			tr	1	0.2	0.7	1.7	MS, RI
α-curcumene	1483	1790				0.1						MS, RI
epi-bicyclosesquiphellandrene	1488							tr			0.3	MS,RI
bicyclogermacrene	1495	1766		0.1					0.5	0.4		MS, RI
α-muurolene	1497						tr	0.1	0.2	0.1		MS, RI
pentadecane	1500										0.3	MS, RI, co-GC
β-bisabolene	1506	1748	0.1	0.2	0.6	0.9	0.1	0.1	2.5	0.5	3.4	MS, RI
γ-cadinene	1511	1776	tr	0.1	tr	tr	tr	0.1	0.3	0.1	0.2	MS, RI
δ-cadinene	1519	1772	tr	0.2	tr		0.1	0.2	0.5	0.2	0.4	MS, RI
β-sesquiphellandrene	1525		tr		0.2	0.7	tr				tr	MS, RI
trans-cadina-1,4-diene	1527	1799						tr	0.1			MS, RI
α-bisabolene	1540		tr	tr	tr	tr	tr		0.1	0.1	0.4	MS, RI
(*E*)-nerolidol	1551	2055								0.1		MS, RI, co-GC
palustrol	1567									0.1		MS, RI
cis-3-hexenylbenzoate	1568				0.1	tr	tr					MS, RI
germacrene D-4-ol	1569			0.1			tr		0.2	0.1	0.1	MS, RI
spathulenol	1571	2154		0.1			tr		1.4	0.2		MS, RI
caryophyllene oxide	1578	2014	0.2	0.4	0.5	0.7	0.2	0.6	0.5	0.2	0.3	MS, RI, co-GC
viridiflorol	1587	2104							0.1	0.1		MS, RI
caryophylladienol[f]	1629							tr	0.1			MS
T-cadinol	1639	2198	tr	0.1		tr		tr	0.4	0.1	0.1	MS, RI
T-muurolol	1640		tr	0.1				tr	0.2		tr	MS, RI
β-eudesmol	1645	2260						0.1	0.2	tr	0.1	MS, RI

Table 1 (Continued)

compound[b]	KI[a] 1	KI[a] 2	OF1[c]	OF2[c]	OG1[c]	OG2[c]	OG3[c]	TMu[c]	TGu[c]	TPa[c]	TNu[c]	identification[d]
α-cadinol	1650	2259	tr			tr	tr	0.1	0.4	0.1	0.1	MS, RI
α-bisabolol	1680										0.4	MS, RI
n-hexadecanol	1883								tr		0.1	MS, RI
trans-phytol	2122								tr		tr	MS, RI
identified components (%)			96.6	99.7	95.2	94.3	99.8	97.1	95.3	98.6	97.7	
monoterpene hydrocarbons			44.7	60	56.2	47.8	47.9	40.3	43	45.4	24	
oxygen-containing monoterpenes			49.7	36.1	34.1	39.5	49.1	50.8	39.4	47.1	61.4	
sesquiterpene hydrocarbons			0.8	2.5	2.1	4	1.6	4.8	9.3	5	9.4	
oxygen-containing sesquiterpenes			0.2	0.8	0.5	0.7	0.2	0.8	3.5	1	1.1	
others			1.2	0.3	2.3	2.3	1	0.4	0.1	0.1	1.8	

[a] Kovats indices relative to *n*-alkanes C_7-C_{29} on nonpolar column HP 5MS (1) and polar column Stabilwax (PEG) (2). [b] Components listed in order of elution from HP 5MS capillary column. [c] OF1, OF2, *O. floribundum* from Chrea Nationl Park and Hammam Melouane respectively; OG1, OG2, OG3, *O. glandulosum* from Souhane, Ouled Slama, and Ighzer mokrane, respctively; TMu, TGu, TPa, TNu., *T. munbyanus*, *T. guyonii*, *T. pallescens*, and *T. numidicus*, respectively. [d] Identification: co-GC, co-injection with an authentic sample; MS, comparison of mass spectra with MS libraries; RI, comparison of retention index with bibliography. [e] tr = concentration <0.05%. [f] Correct isomer not identified.

Table 2. Antioxidant Index of the Essential Oils, α-Tocopherol, BHT, and BHA, in Different Concentrations Using TBARS Assay in the Absence of ABAP

oil/substance	antioxidant index[a] (%, mean ± SD) at concn of 50 mg/L	100 mg/L	250 mg/L	500 mg/L	750 mg/L	1000 mg/L
BHA	52.3 ± 3.7bc	61.5 ± 3.3bc	69.4 ± 3.1b	75.9 ± 2.6ab	81.2 ± 2.1abc	83.4 ± 2.0abc
BHT	61.0 ± 3.7cd	63.9 ± 3.3cd	76.7 ± 3.1bcd	77.8 ± 2.6ab	78.6 ± 2.1ab	80.4 ± 2.0a
tocopherool	70.8 ± 3.7d	77.5 ± 3.6d	82.3 ± 2.8cd	89.2 ± 2.6cd	89.4 ± 2.0def	90.2 ± 2.0cde
O. glandulosum Ighzer Amokrane	45.1 ± 3.4ab	60.1 ± 3.6bc	75.1 ± 2.8bcd	80.8 ± 2.6abc	83.1 ± 2.1abcd	85.5 ± 2.0abc
O. glandulosum Ouled Slama	37.4 ± 3.4a	46.2 ± 3.3a	56.9 ± 2.8a	73.8 ± 2.6a	76.3 ± 2.1a	82.6 ± 1.9ab
O. glandulosum Souhane	44.1 ± 3.4ab	60.6 ± 3.3bc	72.1 ± 2.8bc	76.8 ± 2.8ab	83.1 ± 2.0abcd	85.0 ± 1.9abc
O. floribundum Chrea	53.9 ± 3.7bc	71.8 ± 4.1cde	76.6 ± 3.5bcd	84.7 ± 2.8bcd	89.9 ± 2.0def	90.1 ± 1.9cde
O. floribundum Hammam Melouane	47.1 ± 3.4ab	62.7 ± 3.3bcd	81.2 ± 3.1cd	76.8 ± 2.6ab	86.3 ± 2.0cde	88.7 ± 1.9bcd
T. munbyanus	62.4 ± 3.4cd	75.2 ± 3.3de	85.8 ± 3.1d	94.2 ± 2.8d	96.1 ± 2.1f	96.6 ± 1.9e
T. pallescens	47.4 ± 3.7ab	66.3 ± 3.6cde	84.5 ± 3.1d	92.6 ± 2.8d	93.4 ± 2.1ef	94.1 ± 2.0de
T. numidicus	39.5 ± 3.4a	50.7 ± 3.3ab	66.2 ± 2.8ab	80.6 ± 2.8abc	80.4 ± 2.0abc	82.4 ± 1.9ab
T. guyonii	68.2 ± 3.7d	67.0 ± 3.3cde	80.8 ± 3.1cd	84.4 ± 2.6bcd	84.5 ± 2.1bcd	85.9 ± 2.0abc

[a] In each column, means with different letters are significantly different ($P < 0.05$).

Table 3. Antioxidant Index of the Essential Oils, α-Tocopherol, BHT, and BHA, in Different Concentrations, Using TBARS Assay in the Presence of ABAP

oil/substance	antioxidant index[a] (%, mean ± SD) at concn of 50 mg/L	100 mg/L	250 mg/L	500 mg/L	750 mg/L	1000 mg/L
BHA	62.6 ± 2.7e	70.9 ± 3.7fg	80.9 ± 3.1d	86.5 ± 2.6f	87.0 ± 3.6d	89.6 ± 2.4e
BHT	42.0 ± 2.7d	50.5 ± 3.7de	63.0 ± 3.1bc	66.0 ± 2.6bcde	73.4 ± 3.6abc	72.9 ± 2.4ab
α-tocopherool	65.7 ± 2.7e	83.7 ± 3.4g	88.0 ± 2.9d	88.5 ± 2.6f	90.0 ± 3.6d	91.3 ± 2.6e
O. glandulosum Ighzer Amokrane	21.8 ± 2.7ab	40.8 ± 3.7bcd	51.5 ± 2.9a	63.1 ± 2.6abc	68.6 ± 3.3ab	77.0 ± 2.6bcd
O. glandulosum Ouled Slama	44.6 ± 2.5d	59.2 ± 3.4ef	55.4 ± 2.9ab	63.1 ± 2.4abc	71.1 ± 3.3abc	76.4 ± 2.4bcd
O. glandulosum Souhane	17.7 ± 2.5a	33.4 ± 3.4ab	49.6 ± 2.9a	56.7 ± 2.4a	71.5 ± 3.3abc	74.0 ± 2.4bc
O. floribundum Chrea	43.7 ± 2.7d	57.8 ± 3.7e	68.3 ± 2.9c	73.5 ± 2.4e	80.9 ± 3.3cd	84.2 ± 1.4de
O. floribundum Hammam Melouane	36.0 ± 2.7cd	48.0 ± 3.7cde	55.1 ± 2.9ab	72.2 ± 2.4de	80.9 ± 3.3cd	82.4 ± 2.4cde
T. munbyanus	30.1 ± 2.5bc	43.3 ± 3.4bcd	56.7 ± 2.9ab	70.3 ± 2.4cde	69.6 ± 3.3abc	82.9 ± 2.4cde
T. pallescens	18.2 ± 2.7a	33.4 ± 3.4ab	55.2 ± 2.9ab	64.3 ± 2.4abcd	78.2 ± 3.6bcd	79.9 ± 2.6bcd
T. numidicus	23.1 ± 2.7ab	36.8 ± 3.7abc	54.2 ± 2.9ab	65.4 ± 2.4bcde	64.7 ± 3.6a	75.2 ± 2.4bc
T. guyonii	17.7 ± 2.5a	26.0 ± 3.7a	51.1 ± 2.9a	60.8 ± 2.5ab	65.1 ± 3.6a	64.7 ± 2.6a

[a] In each column, means with different letters are significantly different ($P < 0.05$).

region (1.1%) collected during the vegetative phase. As reported above for oregano plants, the different maturation stages and the different environmental conditions in which each sample of *Thymus* was grown could be partly responsible for this difference.

The higher levels of thymol than carvacrol in almost all oregano oils were partially similar to those of two essential oils of *O. glandulosum* of the Setif region previously cited (*15*), which are rich in thymol (36.7−37.8%) and poor in carvacrol (18.3−22.6%). However, they are very different from that reported by other authors (*25*) for two essential oils of *O. glandulosum*, which are particularly rich in carvacrol (79−83%), and in other oils in which the levels of this component ranged from 45 to 57% in the same species collected in Setif (Algeria) during July 2002 (*26*). Up to now, all of the studies reported for the chemical composition of essential oils of *O. floribundum* have shown that this species is rich in carvacrol and poor in thymol. Thus, 35% carvacrol and 9.9% thymol were cited by some authors (*27*), whereas others (*23*) reported 40% for carvacrol and 1.1% for thymol. These results are partially similar

Table 4. Effects of Oregano and Thyme Essential Oils, BHT, and BHA on in Vitro Free Radical (DPPH) Scavenging

oil/substance	IC_{50}[a] (mg/L)
BHA	126.4 ± 18.0a
BHT	369.1 ± 18.0b
O. glandulosum Ighzer Amokrane	480.2 ± 18.0de
O. glandulosum Ouled Slama	378.2 ± 18.0bc
O. glandulosum Souhane	434.1 ± 18.0cd
O. floribundum Chrea	477.3 ± 18.0de
O. floribundum Hammam Melouane	521.1 ± 18.0ef
T. munbyanus	542.6 ± 18.0fg
T. pallescens	410.2 ± 18.0bc
T. numidicus	597.7 ± 18.0g
T. guyonii	826.5 ± 18.0h

[a] Concentration for a 50% inhibition. Means with different letters are significantly different ($P < 0.05$).

to those of our sample from Chrea. In contrast, the Hammam Melouane sample was characterized by thymol (27.3%) as the main component and carvacrol in smaller amounts (3.6%); this result appears to be new for *O. floribundum* species.

The *T. numidicus* oil from the Kabylie region differed from those from the Constantine region that were particularly rich in thymol. Thus, Hadef et al. (*14*) reported thymol (60.8%) and *p*-cymene (10.3%) as the main components, whereas other authors (*13*) indicated thymol (68.2%) and carvacrol (16.9%) as the main components.

The relative efficiency of the oils of thyme and oregano in the absence or in the presence of ABAP may be partially ascribed to the presence of the same main components, principally the two phenols, thymol and carvacrol (*28*). However, the significant differences detected for some essential oils cannot be explained by only the presence of these two phenol compounds. For instance, the essential oils of *T. guyonii* and *T. numidicus* in which the levels of carvacrol and thymol were not so important as for the remaining oil samples generally presented some antioxidant activity in the absence of ABAP in contrast to *O. glandulosum* Ouled Slama oils, which presented the most inferior antioxidant indices. In the presence of ABAP, *T. guyonii* oils that were poor in thymol or carvacrol were revealed to be the poorest antioxidants, as expected. Nevertheless, *T. numidicus*, which also presented low amounts of these two components, had a different behavior. At least two factors can be highlighted that may be related to such results: (a) it is not only the major compounds of the essential oils that are responsible for the antioxidant activity, but some minor components can interact either synergistically or antagonistically; (b) several compounds can react with 2-thiobarbituric acid under identical conditions. In fact, some authors have also reported that some components could be responsible for the lowest antioxidant activity of *O. vulgare* oil as concentrations increased (*22*). The presence of ABAP within the media induced an increase of antioxidant activities of BHA and α-tocopherol samples in comparison with BHT, which diminished its ability. According to some authors (*29*, *30*), the explanation of this behavior may be structural.

Different values of antioxidant activities were detected for essential oils according to the antioxidant method. According to this, the *O. glandulosum* essential oil from Ouled Slama had a significantly higher ability to reduce DPPH radicals than the remaining oil samples; such a capability was even comparable to that of BHT. Such behavior was not noticeable when the TBARS method was used in the absence of ABAP. Differences in the antioxidant capability of the samples according to the method used were also already referred to (*31*) for *O. vulgare*

Table 5. Inhibitory Activity of *Origanum* and *Thymus* Essential Oils

	inhibition zone[a] (mm)									
	O. floribundum		*O. glandulosum*							
L. monocytogenes	Chréa	Hamman Mélouane	Ighzer Amokrane	Souhane	Ouled Slama	*T. munbyanus*	*T. pallescens*	*T. numidicus*	*T. guyonii*	antibiotic
NCTC 7973	10.00 ± 0.0a	9.68 ± 0.58a	9.67 ± 0.58a	10.67 ± 2.08a	11.67 ± 2.08a	10.00 ± 0.00a	9.67 ± 2.08a	9.67 ± 0.58a	9.33 ± 0.58a	24.67 ± 0.58b
Scott A	11.00 ± 2.0bc	9.70 ± 1.53bc	10.00 ± 1.0bc	11.33 ± 0.58c	10.00 ± 0.0b	8.67 ± 1.15ab	10.67 ± 1.15bc	8.67 ± 0.58ab	7.00 ± 1.00a	28.00 ± 1.73d
C681	11.67 ± 1.53b	10.33 ± 0.58b	12.00 ± 0.0b	10.33 ± 0.0b	7.33 ± 1.15a	7.67 ± 1.53a	8.00 ± 2.00a	6.00 ± 0.00a	7.33 ± 1.15a	26.67 ± 0.58c
C759	11.67 ± 1.53bc	10.33 ± 0.58abc	11.33 ± 1.15abc	12.67 ± 1.53c	8.67 ± 0.58abc	8.33 ± 1.53ab	8.33 ± 1.15ab	10.00 ± 2.65abc	7.67 ± 1.15a	21.67 ± 5.77d
C779	11.00 ± 1.73a	10.00 ± 3.0a	10.67 ± 1.15a	11.33 ± 3.06a	12.00 ± 2.65a	9.33 ± 3.21a	12.33 ± 2.52a	9.33 ± 0.58a	12.33 ± 2.52a	27.67 ± 0.58b
C782	10.00 ± 0.00a	9.67 ± 0.58a	12.67 ± 2.52a	11.33 ± 1.53a	8.00 ± 1.00a	9.33 ± 0.58a	11.67 ± 2.89a	8.67 ± 1.53a	9.33 ± 1.15a	20.00 ± 5.0b
C830	11.00 ± 1.00abc	9.67 ± 0.58abc	10.00 ± 1.00c	11.67 ± 0.58bc	10.33 ± 1.53ab	9.33 ± 0.58abc	9.67 ± 0.58abc	8.00 ± 1.00ab	7.33 ± 0.58a	26.33 ± 2.31d
C882	10.00 ± 1.00ab	9.33 ± 0.58a	10.00 ± 0.00ab	11.00 ± 1.00b	10.33 ± 0.58ab	9.67 ± 0.58ab	9.67 ± 0.58ab	9.67 ± 0.58ab	9.00 ± 1.00a	25.67 ± 1.15c
C895	10.33 ± 0.58bc	9.33 ± 0.58ab	10.00 ± 0.00bc	11.00 ± 1.00cd	12.00 ± 1.00d	14.00 ± 1.73e	11.33 ± 0.58cd	10.00 ± 0.00bc	8.33 ± 0.58a	25.33 ± 0.58f
C897	11.67 ± 0.58e	10.00 ± 0.00abc	10.33 ± 0.58cd	11.33 ± 0.58de	10.00 ± 1.00bc	9.00 ± 1.0b	11.00 ± 0.00cde	8.00 ± 0.00a	8.33 ± 0.58a	27.00 ± 0.00f
G14	11.00 ± 1.00a	11.67 ± 3.06a	9.33 ± 0.58a	12.67 ± 0.58a	11.67 ± 2.89a	11.00 ± 1.00a	10.33 ± 0.58a	10.33 ± 0.58a	11.00 ± 2.65a	24.67 ± 4.51b
SLU 2157	10.33 ± 0.58b	10.67 ± 0.58b	10.67 ± 0.58b	9.67 ± 2.08b	9.67 ± 1.15b	9.00 ± 1.00ab	8.67 ± 1.15ab	7.33 ± 1.15a	7.33 ± 1.15a	24.67 ± 0.58c
SLU 1922	10.00 ± 0.00ab	10.33 ± 0.58b	9.00 ± 0.00ab	10.00 ± 0.00ab	9.33 ± 0.58ab	9.00 ± 0.00ab	10.00 ± 0.00ab	8.67 ± 0.58a	10.00 ± 0.00ab	27.33 ± 2.08c

[a] Data are the mean of three independent experiments ± standard deviation. Data with different letters are significantly different ($P < 0.05$). Inhibition zone includes the diameter of the disk.

L. ssp. *hirtum* essential oil. As reported for the former method, other oil constituents seem to influence, in some way, the ability of reducing the radical DPPH. Therefore, this method, which is considered to be very rapid, simple, sensitive, and reproducible and does not require special instrumentation (*32*), cannot be used to detect antioxidant activities of some oils or extracts.

According to our previous data on the antilisterial activity of the essential oil of *O. vulgare*, the capacity of this essential oil to inhibit the growth of *L. monocytogenes* strains is comparable to that of the antibiotic, which in this present study using the species *O. floribundun* and *O. glandulosum* was not verified (*22*). This discrepancy on antilisterial activity of the essential oils of *O. floribundun* and *O. glandulosum* in comparison to the essential of *O. vulgare* from Portugal is possibly related to different concentrations of some components other than thymol that influence the antimicrobial activity. For instance, *O. vulgare* possessed the lower levels of *p*-cymene than the species of oregano studied in this work. With regard to the activity of the essential oil of *Thymus* species, the inhibition activity registered in this study is according to previous values obtained with Portuguese endemic species of *Thymus* (*7*) and other species growing in other Mediterranean countries (*9*). The antibacterial activity of *Thymus* essential oils seems to be more homogeneous with regard to the origin than that of *Origanum* species.

The results reported here provide information, not yet available in most cases, on the chemical composition and antimicrobial and antioxidant activities of some Algerian *Thymus* and *Origanum* species. In both species it was possible to detect differences in the oil composition and yields. Generally, the oils of all species demonstrated a relatively high degree of preventing lipid peroxidation in the absence and presence of the radical inducer ABAP as well as in the DPPH radical scavenging assay. However, the differences detected among the samples assume that carvacrol and thymol, phenolic compounds generally considered to be primary antioxidants, were not solely responsible for such dissimilarities. Fractionation of the oils and complementary methods for evaluating the antioxidant activities are needed to establish which components are responsible for the antagonism or synergism detected in the raw material.

The utilization of the more active species is recommended in microbial control. The present study showed that due to the relative chemical variability of oregano and thyme oils from Algeria, it is advisable to consider more carefully the use of these plants as their biological activities differ greatly. Therefore, the results of this study suggest the use of the most efficient natural products such as aromatic and medicinal plants.

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