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Chemical composition, antimicrobial and antioxidant activities of *Thymus fontanesii* Boiss. et Reut. and *Origanum glandulosum* Desf. essential oils

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Abstract

The chemical composition, and antioxidant and antimicrobial activities of the essential oil (EO) of two lamiaceae (*Origanum glandulosum* Desf. and *Thyms fontanesii* Boiss. et Reut.) have been investigated. The essential oils were isolated with hydrodistillation method using Clevenger-type apparatus and characterized by GC and GC/MS method. The major compounds of *T. fontanesii* and *O. glandulosum* oils were carvacrol (67.5-12.9%) and ɣ-terpinene (13.0-29.4%), respectively. The antimicrobial activity of these EOs has been studied against 6 strains using disc diffusion method and microdilution plate method. The results revealed that all the oils exhibited a strong antimicrobial activity against the germs, with inhibition zone diameters varied from 29.61 to 48.56 mm and minimal inhibition concentration (MIC) of 0.11 µL/mL for all the strains tested. Antioxidant properties were tested by means of DPPH assay and β-carotene bleaching tests. EO of *T. fontanesii* had the highest scavenging effect against DPPH free radical, and the strongest anti-bleaching activity with IC_{50} values of 51.56 ± 0.25 mg/mL and 1.48 ± 0.02 mg/mL, respectively. The essential oils studied shows strong antimicrobial activity and can be considered as potential source of antioxidant.

Introduction

The presence and growth of micro-organisms in food may cause spoilage and result in a reduction in nutritional and microbiological quality also decrease of storage stability of food. Therefore an exploration of naturally occurring antimicrobials for food preservation receives increasing attention due to consumer awareness of natural food products and a growing concern of microbial resistance toward conventional preservatives (Gould, 1995). Antimicrobial properties of herbs and spices have been recognized and used since ancient times for food preservation and in medicine. Use of essential oils as antimicrobial agents in food systems may be considered as an additional intrinsic determinant to increase the safety and shelf life of foods (Nejad Ebrahimi *et al*., 2008). Some of these compounds are classified as Generally Recognized as Safe (GRAS) substances and therefore could be used to prevent post-harvest growth of native and contaminant bacteria (Burt, 2004). Numerous studies have documented the antifungal (Bouchra *et al*., 2003; Ouraïni *et al*., 2005) and antibacterial (Vagi *et al*., 2005; Yesil Celiktas *et al*., 2007) effects of plant essential oils, their antimicrobial activity is attributed mainly to its major components, although the synergistic or antagonistic effect of one compound in minor percentage of mixture has to be considered (Burt, 2004). Also an important characteristic of these compounds is their hydrophobicity which enables them to partition the lipids of the bacterial cell membrane thus disturbing its structure and rendering the cells more permeable.

Thymus species are known to be used in traditional medicine for the treatment of various illnesses and have been found to possess significant pharmacologic activities (Ghannadi *et al*., 2004). Indeed twelve species are distributed in Algerian flora among them

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Table 1. Location and the ecological traits of harvesting plants

Locality	Species	Latitude	Longitude	Altitude (m)	Bioclimatic zone
Aguemoune (Bejaïa)	*O.glandulosum*	N 36° 38 12.79	E5° 12 19.60	22	Mediterranean
Mechraa Safa (Tiaret)	*T. fontanesii*	N 35° 23 2	E1° 3 12	596	Continental

T. fontanesii Boiss. et Reut. or *T. pallescens* de Noé (Lamiaceae) is common and endemic to northern Algeria (Quezel and Santa, 1963). This plant is widely used in Algerian folk medicine for their antitussive, antiseptic, expectorant, anti-helmintic and antispasmodic properties (Hazzi *et al*., 2009).

Origanum glandulosum Desf. (Lamiaceae) is an endemic plant that grows naturally in North African region (Algeria and Tunisia) (Quezel and Santa, 1963; Leclerc, 1999) it's used as a medicinal plant in the following cases: lack of appetite, distendings, chronic bronchitis, pulmonary tuberculosis, acute or chronic rheumatisms (Valnet, 1984). To our knowledge there are few studies on the chemical composition, antioxidant and antimicrobial activities of *O. glandulosum* EO (Sari *et al*., 2006; Bendahou *et al.*, 2008) and *T. fontanesii* EO (Haddouchi *et al*., 2009; Hazzi *et al*., 2009). Therefore we suggested studying these two plants which belongs to the Lamiaceae family in order to better know the chemical composition and the biological activities of Algerian medicinal plants. So the aim of this work was (I) to evaluate the antimicrobial activity of *O. glandulosum* and *T. fontanesii* EOs, (II) to determine the antioxidant activity of these oils by two methods DPPH and β-carotene bleaching tests, (III) and finally the characterization of their chemical composition by the means of GC/MS method, for further application in food and pharmaceutical industries as valuable natural products.

Materials and Methods

Plant materials

Aerial parts of *O. glandulosum* and *T. fontanesii* were collected in North Algeria from two different regions: Aguemoune (Bejaïa) and Mechraa safa (Tiaret) in Jun and July 2013, respectively. All the plants were air dried in shady place at room temperature until the stabilization of their weight. The location and the ecological traits of harvesting plants are listed in Table 1.

Extraction of essential oils

The extraction of EOs was made by hydrodistillation method, using a Clevenger-type apparatus for 03 h. The EOs was collected, dried over anhydrous sodium sulfate and stored at - 20°C until used. The yields of extracted EOs were 5.0% and 2.9% (v/w)(%) for *T. fontanesii* and *O. glandulosum*, respectively.

GC and GC/MS analysis

The essential oils of O. glandulosum (1:5000) (v/v) and *T. fontanesii* (1:10000) (v/v) were diluted in ethyl acetate before analysis with GC and GC/MS method. The identification of the EOs was performed using Shimadzu QP2010 gas chromatography coupled to mass spectrometry (GC/MS) equipped with a ZB5MS column (30 m x 0.25 µm x 0.25µm). The carrier gas was helium at flow rate of 1.03 mL/min. 1µL of each sample diluted was injected in the split-less mode using following conditions: injection temperature at 230°C, The oven temperature program was initially at 60°C and increased at rate of 3°C/min to 240°C, then held at 240°C for 5 min. GC/MS condition were the same as described above with the use of electronic impact mode at 70 eV, ion source temperature at 200°C and the interface temperature at 245°C.

Components identification

The constituents were identified by comparison of their GC Kovats retention indices (RI), determined with reference to an homologous series of C5-C28 n-alkanes and with those of authentic standards. The identification was confirmed when possible by comparison of their mass spectral fragmentation patterns with those stored in the MS database (National Institute of Standards and Technology and Wiley libraries).

Strains and growth conditions

The antibacterial activity tests of different EOs included five food-borne pathogenic bacteria and one species of yeast supplied by Pasteur institute (Algiers, Algeria), identified with the ATCC number (American Type Culture Collection). The Gram-negative bacteria: *Escherichia coli* ATCC 25922 and the Gram-positive bacteria: *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, Methicillin-Resistant *S. aureus* ATCC 43300 (MRSA), *Listeria innocua* CLIP 74915 and *Candida albicans* ATCC 10231 were studied. All strains were subcultured in Brain Heart Infusion (BHI) agar and incubated at 37°C for 18-24 h until the stationary growth phase was reached.

Disc diffusion method

Antibacterial activity of oils was analyzed by the disc diffusion method described by Belaiche (1979), which is normally used as a preliminary check and to select among efficient essential oils, against six pathogenic microbial strains. All strains were grown on the nutrient agar plate at 37 ± 1°C for 18-24 h for bacteria and at 30 ± 1°C for 24-48 h for *Candida albicans* in order to obtain freshly cultured microbial suspensions for tests. Sterile filter paper discs (6 mm in diameter) were impregnated with 20 µL of the oil and then placed on to Mueller Hinton plates (25 mL, pH 7) previously inoculated with a microbial suspension (10^7 CFU/mL) using a sterile cotton swap. After incubation for 2 h at 4°C, the treated Petri dishes were incubated at 37 ± 1°C for 18-24 h for bacteria and at 30 ± 1°C for 24-48 h for yeast. The antimicrobial activity was evaluated by measuring the diameter of the growth inhibition zone around the discs (including the diameter (6 mm) of the paper disc) with an electronic calliper. Each experiment was carried out in triplicate, and the mean diameter of the inhibition zone was recorded.

Minimum inhibitory concentration (MIC)

The MIC was determined by the microdilution plate method as recommended by NCCLS (NCCLS, 1999). The minimal inhibition concentration (MIC) values were determined for the microbial strains which were sensitive to the essential oil in disc diffusion assay. All tests were performed in Mueller Hinton Broth (MHB) supplemented with Tween 80 detergent (final concentration of 0.5% (v/v)), except for yeast (Sabouraud dextrose broth: SDB + Tween 80). Bacterial strains were cultured overnight at 37°C in MHA and in SDB at 30°C for 48 h for *Candida albicans* in SDB. Test strains were suspended in MHB to give a final density of $5x10^5$ CFU/mL and these were confirmed by viable counts. Different dilutions ranging from 0.05 µL/mL to 100 µL/mL of the EOs were prepared in a 96-well plate. The latter was prepared by dispensing into each well 95 µL of Mueller Hinton Broth (MHB) and 5 µL of the inoculums then 100 µL from the diluted solutions of different EOs was added into the corresponding well. The last well containing 195 µL of nutrient broth without essential oil and 5 µL of the inoculums on each strip was used as negative control. The final volume in each well was 200 µL. The plate was covered with a sterile plate sealer and was incubated under normal atmospheric conditions at 37°C for 24 h for bacteria and at 30°C for 48 h for *Candida albicans* isolates. The bacterial growth was indicated by the presence of a white ''pellet'' on the well bottom. The EOs tested in this study were screened three times against each organism. The MIC was defined as the lowest concentration of the oil to inhibit the growth of microorganisms.

DPPH scavenging activity

The hydrogen atom or electron donation abilities of the pure oils tested were measured from the bleaching of the purple-coloured methanol solution of 2,2-diphenylpicrylhydrazyl (DPPH), according to method described by Gachkar *et al*. (2007). Fifty microlitres of the essential oils appropriate diluted in methanol were added to 5 mL of a 0.004% methanol solution of DPPH. BHA and BHT, stable antioxidants, were used as a synthetic reference. The dilutions used for the EOs were 20, 40, 80, 120 and 160 mg/mL and for the synthetic antioxidants (BHA and BHT), were 0.2, 0.4, 0.6, 0.8 and 1 mg/mL. After 30 minutes incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical by DPPH in percent [I (%)] was calculated using the equation: $I\ \% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$;

where, A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Tests were carried out in triplicate.

The concentration of extract (standard) which scavenge fifty percent of the DPPH free radical (IC_{50}, mg/mL) was determined with the use of the percentage inhibition curve of the DPPH free radical obtained with different concentrations.

β- carotene bleaching test

Antioxidant activity of essential oils was determined using the β-carotene bleaching test. A stock solution of β-carotene/linoleic acid mixture was prepared as follows: 0.5 mg β-carotene was dissolved in 1 mL of chloroform, and 25 µL linoleic acid and 200 mg Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator at 40°C. Then, 100 mL distilled water, saturated with oxygen, were added with vigorous shaking (Tepe *et al*., 2006). The determination of β-Carotene bleaching test was carried out as described by Koleva *et al.* (2002). 3 mL of this reaction mixture were dispersed into test tubes and 200 µL portions of the EOs prepared in methanol at different concentrations (1, 2, 4, 6 and 8 mg/mL), were added and the emulsion system was incubated for 1 h at 50°C. The same procedure was repeated with the synthetic antioxidants, BHA, BHT as positive control, and a blank. The concentrations of BHA and BHT used for the determination of the IC_{50} (mg/mL) were 0.1, 0.2, 0.4, 0.6, 0.8 and 1 mg/

mL. After this incubation period, the absorbance was measured at 470 nm. Readings of all samples were performed immediately (t = 0 min) and after 60 min of incubation. All determinations were performed in triplicate. The antioxidant activity was expressed as inhibition percentage with reference to control after 60 min of incubation, using the following formula given by Kulisic *et al*. (2004).

% Inhibition = $[A_{A(60)} - A_{C(60)} / A_{C(0)} - A_{C(60)}] \times 100$;
Where, $A_{A(60)}$ is the absorbance of the antioxidant at t = 60 min, $A_{C(60)}$ is the absorbance of the control at t = 60 min, and $A_{C(0)}$ is the absorbance of the control at t = 0 min.

The concentration of extract (standard) which inhibit fifty percent of an oxidation of oleic acid (IC_{50}, mg/mL) was determined with the use of the percentage inhibition curve of an oxidation of oleic acid obtained with different concentrations.

Statistical analysis

The experiments were performed in triplicate. The data were recorded as mean ± standard deviation and analysed by Statistica (version 5.5). Statistical differences were examined using the analysis of variance (ANOVA/MANOVA) and the least significant difference (LSD) test was performed to compare the means ($P < 0.05$).

Results and Discussion

Chemical composition of the essential oils

The chemical composition of the essential oils of *T. fontanesii* and *O. glandulosum* identified by GC/MS method are presented in Table 2. Twenty-seven and twenty-four compounds were identified in the EOs of *T. fontanesii* and *O. glandulosum*, respectively. The major compounds found in *T. fontanessii* EO were carvacrol (67.5%), ɣ-terpinene (13.0%), and ρ-cymene (7.4%); while in *O. glandulosum* EO the main compounds were ɣ-terpinene (29.4%), thymol (24.1%), ρ -cymene (19.2%) and carvacrol (12.9%). The oils studied were characterized by a high content of hydrocarbons and oxygenated monoterpenes. The amounts of monoterpene hydrocarbons obtained were 27.2%, 55.7% for *T. fontanessii* and *O. glandulosum* EOs, respectively. On the other hand, the oxygenated monoterpenes content of *T. fontanessii* EO was 71.8% (Table 2). Belhattab *et al.* (2005) found the following major compounds in *O. glandulosum* EO of Setif region (Algeria) extracted by hydrodistillation and distillation - extraction: carvacrol (47.0-57.1%), ɣ-terpinene (13.4-13.2%), ρ-cymene (11.2-10.3%) and thymol (6.6-6.7%), respectively. While Ruberto

Table 2. Chemical composition (%) of *T. fontanesii* and *O. glandulosum* essential oils extracted by hydrodistillation method.

N°	Compounds	LRI[a]	CRI[b]	*T. fontanesii*	*O. glandulosum*
1	α-Thujene	931	926	0.6	0.9
2	α-Pinene	939	933	0.8	1.0
3	Camphene	954	948	0.1	-
4	β-Pinene	978	977	0.3	-
5	β-Myrcene	991	989	1.8	2.1
6	I-Phellandrene	-	1005	0.2	0.2
7	α- Terpinene	1018	1017	2.0	2.9
8	**ρ-Cymene**	1027	1024	**7.4**	**19.2**
9	I-Limonene	1032	1028	1.0	-
10	1,8-Cineole	1037	1031	0.3	-
11	(Z) -β-Ocimene	-	1035	-	t
12	(E) β-Ocimene	1059	1046	0.1	t
13	**γ-Terpinene**	1061	1058	**13.0**	**29.4**
14	α -Terpinolene	1104	1088	0.1	-
15	Linalool	1100	1099	2.2	0.7
16	Borneol	1177	1168	0.2	0.2
17	Terpinene-4-ol	1184	1179	0.5	0.5
18	α -Terpineol	1194	1192	0.1	0.4
19	**Thymol methyl ether**	1230	1235	-	1.2
20	Pulegone	-	1241	0.1	-
21	Carvacrol methyl ether	1246	1244	-	0.6
22	**Thymol**	1291	1294	0.9	**24.1**
23	Thymoquinone	-	1251	-	0.1
24	**Carvacrol**	1297	1304	**67.6**	**12.9**
25	α -Gurjunene	1394	1413	0.3	-
26	trans-Caryophyllene	-	1423	0.2	0.9
27	(+)-Aromadendrene	1452	1443	0.2	-
28	α-Humulene	1437	1458	-	0.1
29	Alloaromadendrene	1444	1465	t	-
30	β-Bisabolene	1481	1510	-	0.7
31	γ-Cadinene	1503	1518	t	t
32	Δ-Cadinene	1519	1526	0.1	-
33	Spathulenol	1577	1582	0.1	-
34	Caryophyllene oxide	1586	1588	-	0.1
	Total			**99.9**	**98.9**
	Monoterpene hydrocarbons			27.2	55.7
	Oxygenated monoterpenes			71.8	40.5
	Sesquiterpenes			0.9	1.8
	Others			0.0	0.9
	Yield % (v/w)			5.0	2.9

[a]LRI: literature retentions index, [b]CRI: calculated retentions index relative to C_5–C_{28} n-alkanes on the ZB5MS column, t: trace (<0.05%).

et al. (2002) reported that the major compounds of *O. glandulosum* EO were thymol (7.7-36.7%), carvacrol (18.3-63.7%), ρ-cymene (3.6-25.8%) and ɣ-terpinene (4.8-13.2 %). Moreover Thymol (41.6%), ɣ-terpinene (27%), ρ-cymene (17.1%) and carvacrol (2.2%) were the main components determined in *O. glandulosum* EO extracted by hydrodistillation method found by Bendahou *et al.* (2008).

According to the major compounds found in the *Origanum* species essential oil composition, the essential oils of this species are classified in three groups: the first group is constituted by Linalool, terpinen-4-ol, and sabinene hydrate, the second group contains the phenolic compounds, namely carvacrol and/or thymol, and the third group constituted by sesquiterpenes (Kokkini, 1996). Thus

Table 3. Antimicrobial activity of *O. glandulosum* and *T. fontanesii* oils expressed by the diameter inhibition zones and MIC methods

Microorganisms	DD (mm)		MIC (µL/mL)	
	Essential oils		Essential oils	
	O.G	T.F	O.G	T.F
Bacillus subtilis	31.35 ± 0.11^a	30.66 ± 0.13^b	0.11^a	0.11^a
Staphylococcus aureus	35.86 ± 0.15^a	36.26 ± 0.19^a	0.11^a	0.11^a
Methicillin-resistant *Staphylococcus aureus*	40.43 ± 0.11^b	41.34 ± 0.16^a	0.11^a	0.11^a
Listeria innocua	29.61 ± 0.07^b	40.06 ± 0.08^a	0.11^a	0.11^a
Escherichia coli	35.25 ± 0.16^a	35.53 ± 0.11^a	0.11^a	0.11^a
Candida albicans	48.56 ± 0.07^a	44.99 ± 0.01^b	0.11^a	0.11^a

DD: agar disc diffusion method. Diameter of inhibition zone (mm) including diameter paper disc of 6 mm.
MIC: minimum inhibitory concentration. Values given as µL/mL.
O.G: *Origanum glandulosum* essential oil, T.F: *Thymus fontaneseii* essential oil.
Means followed by the same letter are not significantly different at $P \leq 0.05$.

O. glandulosum EO, from Bejaia region, should belong to the phenolic compound group. Bendahou *et al.* (2008) found, for the first time, thymoquinone as trace in the *O. glandulosum* EO extracted by microwave assisted extraction (MAE),and it was totally absent in hydrodistillation and solvent free microwave extraction (SFME) EO, while, in this study, this compound was found with a percentage of 0.1%.

Hazzit *et al.* (2009) reported that the major components of *T. pallescens* EO from Oued Rhiou (west region of Algeria) were thymol (49.3%), ρ-cymene (11.2%), ɣ-terpinene (10.9%) and carvacrol (9%). From this data, we note a difference of EO composition with *T. fontanesii* EO of this study from Tiaret region, which was rich of carvacrol (67.6%), ɣ-terpinene (13.0%) and ρ-cymene (7.4%), while thymol (0.9%) was found as minor compound. The EOs composition could be influenced by environmental factors, harvesting period (Williams and Lusunzi, 1994; Gardeli *et al.*, 2008) and part of plants studied (Flamini *et al.*, 1999), as well as, by the extraction method used (Bendahou *et al.*, 2008).

Antimicrobial activity

The results of the antimicrobial activity of *O. glandulosum* and *T. fontanesii* EOs extracted by hydrodistillation method are gathered in Table 3. The results obtained from the disc diffusion method, indicated that the oils studied exhibited a stronger antimicrobial activity against the germs tested with inhibition zone diameters ranged from 29.61 to 48.56 mm. Methicillin-resistant *Staphylococcus aureus* which is bacterium responsible for several infections in humans, shown strong sensibility with inhibition zone diameters of 41.34, 40.43 mm with *T. fontanesii* and *O. glandulosum* oils, respectively. On the other hand, *O. glandulosum* EO exhibited the powerful antimicrobial effect on *C. albicans* strain with 48.56 mm of inhibition zone diameter followed by *T. fontanesii* EO with 44.99 mm of inhibition zone diameter, respectively (Table 3). In contrast, the lowest antimicrobial effect was obtained in the case of *O. glandulosum* EO against *L. innocua* with inhibition zone diameter of 29.61 mm.

The results obtained for MICs with microdilution method are the same for all the oils tested which is 0.11µL/mL. The strong antimicrobial activity of *O. glandulosum* and *T. fontanesii* EOs investigated in this study, was apparently related to their major phenolic compounds, such as thymol, carvacrol, and their precursors ɣ-terpinene and ρ-cymene. Indeed phenolic components (thymol and carvacrol) and their precursors (*p*-cymene and γ-terpinene) present in essential oils are responsible for the strong antifungal activity (Giordani *et al.*, 2008). Also they sensitize the phospholipid bilayer of the cell membrane, causing an increase of permeability and leakage of vital intracellular constitutes or impairment of bacterial enzyme systems (Singh *et al.*, 2002). In addition, Oyedemi *et al.* (2009) have shown that EO components such as γ -terpinene exert bactericidal effects against both Gram positive and Gram negative bacteria by disrupting their outer membranes.

Sari *et al.* (2006) found that the inhibition zones varied from 8 mm to 18 mm against four bacteria *(S. aureus, E. hirae, E. coli, P. aerugenosa)* and two yeasts (*C. albicans* and *C. tropicalis*) tested by disc diffusion method for *O. glandulosum* oil. While Bendahou *et al.* (2008) noticed in their study that *O. glandulosum* oil extracted by hydrodistillation method exhibited antimicrobial activity against seventeen strains with inhibition zones which varied from 8 mm to 34 mm. The MICs of *O. glandulosum* oils extracted by hydrodistillation method are 31.25-

Table 4. Antioxidant activities of *O. glandulosum* and *T. fontanesii* EOs extracted by hydrodistillation method.

	DPPH scavenging activity (IC50 mg/mL)	BCBT (IC50 mg/mL)
T. fontanesii	51.56 ± 0.25^{b}	1.48 ± 0.02^{c}
O. glandulosum	92.39 ± 0.89^{c}	3.72 ± 0.29^{d}
BHA	0.40 ± 0.01^{a}	0.41 ± 0.04^{a}
BHT	0.72 ± 0.12^{a}	0.98 ± 0.01^{b}

Means followed by the same letter are not significantly different at $P \leq 0.05$

125 µg/mL and 36-120.50 µg/mL obtained by Sari *et al.* (2006) and Bendahou *et al*. (2008), respectively. From this data it's noticeable that *O. glandulosum* oil from Aguemoune (Bejaïa) has high inhibition zones and less MICs from those reported by Sari *et al.* (2006) and Bendahou *et al*. (2008), and this strong activity can be attributed to the presence of carvacrol and thymol in balanced quantity in this oil. The antimicrobial activity of *T. fontanesii* oil studied, corroborate with the inhibition zone diameters found by Haddouchi *et al.* (2009) for the same plant species.

Antioxidant activities

The results of antioxidant activities of the EOs and positive controls (BHA and BHT) are gathered in Table 4. The highest scavenger effect against DPPH free radical is noted for the *T. fontanesii* EO followed by *O. glandulosum* EO, with IC_{50} values of 51.56 and 92.39 mg/mL, respectively. Sari *et al.* (2006) reported that *O. glandulosum* EO, extracted by hydrodistillation, from three different regions of Bejaia (Kherrata, El-kseur and Teskriout), possess scavenging activity against DPPH free radical, with IC_{50} values, which ranged from 23.5 to 25.6 mg/mL, respectively. This latter is 3.5 to 4 times higher than EO obtained in our study, for the same species. This difference could be explained by the different composition of these essential oils.

The antioxidant activity of EOs from *Origanum* and *Thymus* genera could be due to the presence of phenols (thymol and carvacrol), and by the synergistic effect of the major and minor constituents of these EOs. Indeed, the content of thymol and carvacrol from *O. glandulosum* EO harvested at Bejaia region (Kherrata, El-kseur and Teskriout), were (42% and 41.9%), (44.6% and 13.8%) and (41.9% and 14.6%), respectively (Sari *et al*., 2006). these contents are higher than those found in our study, for the same species, which explains its high scavenger effect on DPPH free radical. Furthermore Ruberto and Baratta, (2000) reported the antioxidant activity of 100 components of essential oils, and showed that ɣ- terpinene, carvacrol and thymol, have antioxidant activity against the oxidation of oleic acid, with inhibition percentages of 61.6%, 59.1% and 25.5%, respectively at a concentration of 100 ppm.

T. caramanicus EO of India region, which contain 85.9% of carvacrol, had an antioxidant activity against DPPH free radical, with an IC_{50} value of 263.09 ± 0.62 µg/mL (Koleva *et al*., 2002). Also *T. pallescens* EO harvested in different region in Algeria had an antioxidant activity against DPPH free radical, with inhibition percentages ranging from 10.1 to 19% at a concentration of 0.1 mg/mL (Hazzit *et al.*, 2009). Therefore Zouari *et al.* (2011), reported that antioxidant activity of *T. algeriensis* EO harvested in Tunisia, against DPPH free radical (IC_{50} = 0.8 mg/mL), was higher than that obtained, with *T. fontanesii* EO studied, Despite the EO of this latter contain high level of carvacrol (67.5%) compared to *T. algeriensis* EO, which possess 0.64% on thymol and the absence of carvacrol in this one. This result confirms the synergistic effect of minor compounds of EO.

T. fontanesii EO have the strong anti-bleaching activity with an IC_{50} value of 1.48 mg/mL, followed by *O. glandulosum* EO. The synthetic antioxidants (BHA and BHT) have strong antioxidant activities than those obtained by the EOs tested, except for *T. fontanesii* EO which showed anti-bleaching activity close to that obtained by BHT (Table 4). *T. fontanesii* EO of Tiaret region showed anti-bleaching activity, 3 times lower than that obtained by Zouari *et al*. (2011), with *T. algeriensis* EO, although this latter contain very low amount in thymol.

The antioxidant ability of studied EOs can be attributed to thymol and carvacrol, which are present as major compounds in these EOs, and the synergistic effect between their minor and major compounds. Indeed Safaei-Ghomi *et al.* (2009), reported that carvacrol at a concentration of 2 mg/mL, have an anti-bleaching activity with value of 50%. Moreover Ruberto and Baratta, (2000) reported that a concentrations of 10^{-3} M of ɣ- terpinene, thymol and carvacrol, showed an anti-bleaching activity with values of 78.5%, 60.9 % and 61.1%, respectively. Therefore the antioxidant efficiency of these oils seems to be related to the activity of some compounds enclosed in, such as oxygenated monoterpenes which have values of 71.8% and 40.5% for *T. fontanesii* and *O. glandulosum* EOs, respectively.

Conclusion

T. fontanesii and *O. glandulosum* EOs, extracted with hydrodistillation method and characterized by means of GC/MS method, showed strong antimicrobial activity, and can be considered as

potential source of antioxidant. According to our data, we suggest the use of these EOs, as valuable natural products to avoid foodborne diseases and food spoilage, and increase the shelf-life of food products. Further experiments are required to prove their safety before introducing them in pharmaceuticals products and food industries.

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