Chemical composition and antioxidant activity of *Thymus fontanesii* essential oil from Algeria

Lamia Sidali[a*], *Moussa Brada*[a], *Marie-Laure Fauconnier*[b], *Georges Lognay*[c]

[a] *Valuation of Natural Substances Laboratory, Djilali Bounaama University of Khemis-Miliana, Road of Theniet El-Had, 44225, Algeria*

[b]*Agro-Bio Chem Department, Laboratory of General and Organic Chemistry, University of Liège, Gembloux Agro-Bio Tech, 2, Passage of Deportees, B-5030 Gembloux, Belgium*

[c]*Agro-Bio Chem Department, Laboratory of Analytical Chemistry, University of Liège, Gembloux Agro-Bio Tech, 2, Passage of Deportees, B-5030 Gembloux, Belgium*

Abstract: *Background*: *Thymus fontanesii* is one of the importance Algerian plant, used traditionally to treat the cough and cold. In addition, it may help to protect the people against lipid peroxidation and oxidative stress and can be used as an antioxidant agent for the preservation of processed food.

***Objective*:** The aim of this study was to determine the chemical composition of Algerian *Thymus fontanesii* essential oil and to test its antioxidant activity.

***Method*:** The oil was extracted by electromagnetic induction (EMI) heating assisted extraction and by hydrodistillation, and was analysed by gas chromatography with flame ionization detector (GC/FID) and gas chromatography/mass spectrometry (GC/MS). The antioxidant activity was evaluated by three assays mainly: DPPH assay, reducing power and β-carotene/linoleic acid.

***Results*:** The yield of the essential oil was varied from 2.1 ± 0.3 to 3.1 ± 0.1% (w/w), and from 1.8 ± 0.01 to 2.6 ± 0.02% (w/w), for the electromagnetic induction heating assisted extraction and hydrodistillation, respectively. Twenty seven components were identified representing 95.6 - 99.9% of the oil. Carvacrol (54.7 ± 1.2 - 63.9 ± 1.9%) was the major compound followed by *p*-cymene (9.2 ± 1.2 - 17.5 ± 1.2%) and γ-terpinene (8.8 ± 0.9 - 14.9 ± 0.8%). The *Thymus fontanesii* essential oil was found as a significant antioxidant with IC_{50} values were ranging from 57.3 ± 1.4 to 236.7 ± 1.4 µg/mL, which were higher than that of butylated hydroxyl toluene (BHT) choosing as reference (9.1 ± 1.2 to 67.8 ± 0.1 µg/mL).

***Conclusion*:** The obtained results encourage the use of *Thymus* species with bioactive compounds for further food applications.

Keywords

Thymus fontanesii, electromagnetic induction heating, hydrodistillation, essential oil, chemical composition, antioxidant activity.

1. Introduction

Lamiaceae among which *Thymus* species are very common in the Mediterranean regions especially in dry and arid environments. The diversity of their essential oils biological properties may be a consequence of their rich chemical diversity, and in part explains the large number of studies investigating their chemical composition [1-2]. *Thymus* genus is divided in eight

sections, comprising 215 species and eleven of them are in the flora of Algeria [3]. *Thymus fontanesii* Boiss. & Reut, a spontaneous aromatic plant, endemic to Algeria and Tunisia [4] is used in Algerian traditional medicine, as antispasmodic, carminative, stomachic, expectorant, antitussive, antiseptic and anthelminitic remedy in some gastrointestinal diseases [5], and widely used to treat respiratory infections, irritation of throat and respiratory organs [6].

Some reports on the chemical composition of *Thymus fontanesii* essential oil (TFEO) revealed that carvacrol and thymol represent the most important compounds in the species flowed by *p*-cymene and γ-terpinene [5-9]. Concerning the biological properties of *T. fontanesii* essential oil, there are some studies on the antimicrobial activity [7-9], antidermatophytic activity [10] and anti-inflammatory activity [6, 8], but there are not studies on their antioxidant activity.

The present study has therefore been designed to characterize the essential oil of *Thymus fontanesii* from North Algeria obtained by two different extraction techniques and evaluate its antioxidant activity.

2. Materials and Methods

2.1. Plant material and isolation procedure

The aerial parts of *T. fontanesii* were collected during the flowering period from three different regions Miliana (M, latitude: N36°17'; longitude: W2°13'; altitude: 723 m), Tarik Ibn Ziad (TIZ, latitude: N36°00'; longitude: W2°09'; altitude: 630 m) and Oued El Chorfa (OEC, latitude: 36°12'00''; longitude: W2°31'00''; altitude: 460 m) located in Northern Algeria. Voucher specimens were deposited in the herbarium of the Agronomic Department of Khemis Miliana University (references: T_M, T_{TIZ} and T_{OEC}, respectively).

The essential oil was extracted from each of the samples by two extraction methods, in order to introduce the electromagnetic induction (EMI) heating assisted extraction as a new technique of extraction. Therefore, the yield and chemical composition of TFEO extracted by EMI heating were studied and compared with those obtained by hydrodistillation.

- Hydrodistillation (HD): Fifty five grams of aerial parts of *T. fontanesii* (cut into small pieces of 0.5 to 1 cm and dried at room temperature ~ 20 °C for 10 days) were submitted to hydrodistillation with a Clevenger apparatus, and extracted for 2 h The essential oil was collected by decantation and stored in closed dark vials at 4°C until analysis.

- Electromagnetic induction heating assisted extraction (EMI): The aerial parts of collected plants were subjected an EMI heating assisted extraction. The system was equipped with a pressure cooker (5 L capacity), placed on an induction plate (1800 W), whereas the extraction was carried out in magnetizable conditions. The essential oil was collected by decantation and dried over anhydrous sodium sulfate, the yield measured, and stored in a freezer at 4°C in dark glass bottles until use.

The following formula was used to determine the essential oils yield:

Essential oil yield (%) = (W_1/W_2) x 100

Where W1 is the net weight of TFEO (g) and W2 is the total weight of dried aerial parts of *T. fontanesii* (g)

2.2. *Essential oil analyses*

5 mg of oil was dissolved in 2.5 mL pure diethyl ether and further analyzed by gas chromatography/flame ionization detector (GC-FID) and gas chromatography/mass spectrometry (GC-MS).

GC-FID: The analysis of the extracted oil was carried out by means of a HP 6890A gas chromatograph fitted with FID. Using a capillary column coated with 5% phenyl-methylsiloxane (30 m x 0.25 mm x 0.25 μm film thickness, Agilent Technologies, Hewlett-Packard, CA, USA); column temperature program was the following: from 40 °C (1 min) to 200 °C at 6 °C/min, 200 - 280 °C at 30 °C/min, 280 °C (final hold of 2 min). The injections have been performed in splitess mode and injector temperature was set at 280 °C;

detector temperature 300 °C; volume injected was 1 µL of diluted oil in diethyl ether. The carrier gas was helium at flow rate of 1 mL/min.

GC-MS: GC-MS was carried out using an Agilent 5973 GC-MS coupled to an Agilent 6890 gas chromatograph fitted with a split-splitless injector at 250 °C (splitless mode). The analytical conditions have been fixed as follows: Agilent HP-5MS capillary column (30 m x 0.25 mm, df = 0.25 µm), temperature program: from 40 – 250 °C at 6 °C/min. The carrier gas was helium at flow rate of 1 mL/min. The mass spectra have been recorded in EI mode at 70 eV (scanned mass range: 35 to 500 amu). The source and quadrupole temperatures were fixed at 230 °C and 150 °C, respectively. The identification of the components was performed on the basis of chromatographic retention indices (RI) and by comparison of the recorded spectra with a computed data library (Wiley 275.L). For sesquiterpene hydrocarbons, further confirmations were obtained by comparing the mass spectra with data from the literature [11,12]. RI values were measured on an HP-5MS column. RI calculations were performed in temperature programmation mode according to [13], a mixture of homologues *n*-alkanes (C_7–C_{30}) was used, under the same chromatographic conditions. Main components have been confirmed by comparison of their retention data with co-injected pure (commercially available) references.

2.3. Antioxidant activity

2.3.1. *DPPH assay*

The stable 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\cdot}$) was used for determination of free radical scavenging activity of the essential oils [14]. Fifty microliters of various concentrations of the samples (25, 50, 75 and 100 µg/mL) in methanol (essential oil and BHT) were added to 2 mL of 60 µM methanol solution of $DPPH^{\cdot}$. After 20 min at room temperature, the absorbance was recorded at 517 nm, absorption of the blank sample containing the same amount of methanol and $DPPH^{\cdot}$ solution acted as the negative control. Butylated hydroxyl toluene (BHT) at (25, 50, 75 and 100 µg/mL) was used as positive control The percentage inhibition of the $DPPH^{\cdot}$ radical by the samples was calculated according to the formula:

$$\% \text{ Inhibition} = [(A_b - A_a)/A_b] \times 100 \quad (1)$$

where A_b is the absorbance of the blank sample and A_a is the absorbance of the test sample.

The sample concentration providing inhibition (IC_{50}) was calculated by plotting the inhibition percentages against the concentration of sample.

2.3.2. Reducing power determination

The reducing power of the essential oil and standards was determined according to the protocol of Hseu *et al.* [15]. In brief, 1 mL of different concentrations of the samples (25, 50, 75 and 100 µg/mL) was mixed with phosphate buffer (1 mL, 0.2 M, pH = 6.6) and 1% potassium ferricyanide [$K_3Fe(CN)_6$] solution (1 mL). The mixture was incubated at 50 °C for 20 min. Trichloroacetic acid (TCA) (1 mL, 10%) was added to the solution, which was then centrifuged for 10 min at 3000 g. The supernatant was recovered and mixed with distilled water (1.5 mL) and 0.1% $FeCl_3$ (150 µL) and the absorbance was measured at 700 nm. Absorbance increase of the reaction mixture (according to the blank) indicated increased reducing power.

2.3.3. β-carotene/linoleic acid bleaching assay

The β-carotene/linoleic acid test evaluated the inhibitory effect of a compound or a mixture on the oxidation of β-carotene in the presence of molecular oxygen (O_2). Assay of β-carotene gives an estimation of the antioxidant potential of the sample. The method described by Miraliakbari and Shahidi [16] was used; a mixture of β-carotene and linoleic acid was prepared by adding together 0.5 mg of β-carotene in 1 mL of chloroform, 25 µL pure linoleic acid and 200 mg Tween 40. The chloroform was then completely evaporated under vacuum and 100 mL of oxygenated water was subsequently added to the residue and mixed

to form a clear yellowish emulsion. Three hundred and fifty microliters of various concentrations of sample (25, 50, 75 and 100 µg/mL) in methanol (essential oil and BHT) were added to 2.5 mL of the above emulsion in test tubes and mixed. The test tubes were incubated in water bath at 50°C for 2 h together with a negative control (blank) containing pure methanol instead of sample.

The absorbance values were measured at 470 nm. Antioxidant activity (percentage inhibition, % I) of the samples was calculated as follows:

$$\% I = [A (\beta\text{-carotene after 2 h assay})/A (\text{initial } \beta\text{-carotene})] \times 100 \quad (2)$$

where A (β-carotene after 2 h assay) is the absorbance value of β-carotene after 2 h assay remaining in the samples whereas A (initial β-carotene) is the absorbance value of β-carotene from the standard freshly prepared solution. The activity was calculated as 50% inhibition concentration (IC_{50}).

All experiments were repeated three times on independent samples and the data were expressed as mean ± standard deviation (SD).

3. Results and Discussion

3.1. *Essential oil yield and chemical composition*

T. fontanesii samples gave yellow liquid with sharp odor and yield ranging from 2.1 ± 0.3 % to 3.1 ± 0.1%, (w/w) for EMI heating and 1.8 ± 0.01% to 2.6 ± 0.02%, (w/w) for HD. Figure 1 showed the variation of the extraction yield of TFEO from Tarik Ibn Ziad according to the extraction time. Three phases were observed in the process of the EMI heating. The first phase (0 – 10 min) represented the preheating time from room temperature to 140 °C. The second phase (10 – 55 min) was presented by increasing yields, representing 2.9% of oil relative to the initial amount of TFEO. The third phase corresponds to a plateau, which marked the end of extraction process. Therefore, the max yiled was 2.9% after 55 min of extraction.

HD lead to a max of 2.1% essential oil yield and the end of extraction process was reached after 85 min. The higher yield was found for the samples from Miliana (from 2.6 ± 0.02% to 3.1 ± 0.1%), while the lower yield was found for the samples from Oued El Chorfa (from 1.8 ± 0.01% to 2.1 ± 0.3%), and for *T. fontanesii* from Tarik Ibn Ziad (from 2.1 ± 0.01% to 2.9 ± 0.1%). These results were in line with those obtained by Haddouchi, Lazouni and Meziane [17] (2%) and Ghannadi, Sajjadi and Kabouche [5] (1.9%), but they were higher than those obtained by Dob *et al.* [9] (0.9%). The EMI heating assisted extraction showed better results, which can be considered quite an innovate isolation and efficiency technique comparative by the Clevenger apparatus, this efficiency was probably based on the interaction between the speed of the EMI heating and evaporation of the essential oil from vegetable material.

Fig 1. Variation of *T. fontanesii* essential oils yields from Tarik Ibn Ziad according to extraction time.

The results of TFEO characterization are gathered in Table 1. Twenty six and twenty four compounds were identified in TFEO from TIZ extracted by the EMI and HD, represented 99.8% and 95.6% of EO (Table 1). Higher amounts of oxygenated monoterpenes were presented in the TFEO isolated by EMI heating (67.6%) compared to HD (59.6%). Whereas, the monoterpene hydrocarbons were presented in high proportions in HD (35.71%) compared to EMI heating (31%) essential oil, which there were less valuable than oxygenated compounds in terms of their contribution to fragrance of the essential oil. Conversely, the oxygenated compounds are highly odoriferous. Carvacrol is the main component in the TFEO but the relative amounts differed for the two isolation methods: 63.9.8 ± 1.9% for EMI heating and 54.7 ± 1.2% for HD, respectively. *p*-Cymene and γ-terpinene were the two other main components in TFEO. The highest proportion of *p*-cymene was found in HD essential oil (17.5 ± 1.2%) compared with EMI heating essential oil (10.5 ± 0.2%) and γ-terpinene is more abundant in EMI heating (14.9 ± 0.8%) against

8.8 ± 0.9% in HD. Therefore, the EMI heating, highly accelerated the extraction process, without major difference in proportions of compounds.

Twenty seven compounds representing 99.9, 99.8 and 96.9% of M, TIZ and OEC oils, respectively. Oxygenated monoterpenes was the predominant chemical group (62.1 - 67.6%), followed by the monoterpenes (31 - 35.1%), whereas the sesquiterpenes (0.9 - 1.2%) and oxygenated sesquiterpenes (0.1 - 0.2%) proportions were very low. The major components were carvacrol (55.1 ± 0.8 - 63.9 ± 1.9%), a phenolic molecule with significant antioxidant activity [18-20], *p*-cymene (9.2 ± 1.2 - 14.3 ± 1.3%) and γ-terpinene (11.6 ± 1.5 - 14.9 ± 0.8%).

These results are in agreement with those of Bekhechi *et al.* [7] and indicate that *Thymus fontanesii* of the present study belongs to a "carvacrol chemotype" [6]. In other essential oils of *T. fontanesii* from Algeria [5,9], the major components were thymol (67.8%), *p*-cymene (13%) and γ-terpinene (15.9%) with a low content of carvacrol (1.7%).

This large variability in the chemical composition of different species of Algerian TFEO samples could be due that several factors including abiotic (local climate and environment like temperature, sun, location and nutriments) [21], biotic factors as intraspecific chemotypes [22] and harvesting (phenological stage) season. In fact, the comparison of the oil composition obtained from the three *T. fontanesii* samples showed some quantitative differences. Other previous study of some *Thymus* species confirmed that the environmental conditions play an important role in the chemical variations [22,23].

Table 1. Chemical composition of the essential oil of *T. fontanesii* from Algeria (mean of triplicates)

3.2. Antioxidant activity of T. fontanesii essential oil

The antioxidant activity of TFEO was assessed by three antioxidant assays: the DPPH· assay, evaluating the H-donating or radical scavenging ability of the oil using the stable radical 2,2-diphenyl-1-picrylhydrazyl as a reagent, an assay estimating the ferric-reducing capacity of the oil and the β-carotene/linoleic acid bleaching assay. The concentration that led to 50% inhibition or effectiveness (IC_{50}) values reflected better protective actions. As shown in Table 2, the classification order of antioxidant power was the same for all assays, with in decreasing order, TIZ sample, followed by M and OEC. The TFEO sample of TIZ showed the higher capacity for scavenging free radicals (IC_{50} = 57.3 ± 1.4 µg/mL), while TM and TOC (IC_{50}= 83.8 ± 0.5 µg/mL and IC_{50}= 91.2 ± 1.1 µg/mL, respectively), but all three samples were less effective than the reference antioxidant butylated hydroxyl toluene (BHT) (18.3 ± 0.8 µg/mL). Some reports have carried out evaluations of antioxidant activity based on the DPPH· in different *Thymus* species such as *T. pallescence* from Algeria, *T. capitatus* from Tunisia, *T. saturejoides* from Morocco rich in carvacrol [24-26] and other *Thymus* species from Lybia rich in thymol [27]. In most such studies, phenolic compounds, due to their chemical structures that allow then to donate hydrogen to free radicals, were introduced as the major factor contributing to the antioxidant activity of EO [28]. The antioxidant activity of TFEO was also assessed by the reducing power assay and the results are gathered in Table 2. For the measurements of the EOs reductive abilities, the transformation of Fe^{3+} - Fe^{2+} in the presence of oil were investigated. Where, the greatest effectiveness was shown by TIZ sample (63.8 ± 0.17 µg/mL), slightly lower for M sample (103.9 ± 0.4 µg/mL), and the lowest for OEC sample (112.6 ± 1.8 µg/mL), compared to positive control, BHT (9.7 ± 1.23 µg/mL). These results showed that the reducing power of TFEO was in line with *Thymus marocanus* Ball essential oil from Morocco (61.4 ± 1.58 µg/mL) rich in carvacrol [14]. Previous studies had indicated that the high reducing power of the *Thymus* EO was not directly related to its carvacrol and thymol contents, but the substitution of hydroxyl group in the aromatic ring might have contributed to its antioxidant activity [29]. TFEO and BHT prevented the

bleaching of β-carotene, whereas TIZ oil (IC_{50} = 148.9 ± 0.45 µg/mL) was better than that of M (167.2 ± 0.9 µg/mL) and OEC (236.7 ± 1.4 µg/mL) oils. These results were less potent than the references BHT (67.8 ± 0.1 µg/mL) and almost similar to *T. saturejoides* essential oil (213.61 ± 1.74 µg/mL) from Morocco rich in carvacrol and borneol [25]. β-carotene bleaching inhibition based on the loss of the yellow color of β-carotene due to its reaction with radicals produced during linoleic acid auto-oxidation in an emulsion [30]. TFEO of the three collection regions was found to be relatively active in all assays. While it appears that there was a very positive correlation between carvacrol content and high antioxidant potential, also the literature review showed that the presence of carvacrol in essential oil of *Thymus* species may be the main cause of its high antioxidant activity [31,32]. On the other hand, the data obtained from the evaluation of antioxidant activity of TFEO in this research were comparable to the results reported on some Algerian *Thymus* [26,33] and other *Thymus* species [27,28,34,35], which the antioxidant potential was addressed to the phenolic compounds (thymol and carvacrol). Monoterpene hydrocarbons, particulary α-terpinene and γ-terpinene, could be also taken into account for the antioxidant activity [36].

Table 2: IC_{50} values (µg/mL) of *T. fontanesii* essential oil and BHT

4. Conclusion

In this study, the EMI assisted heating was presented as an extraction method suitable for essential oil extraction. It resulted a reduced extraction time. Moreover, alteration of essential oil constituents is surely limited in comparison with classical hydrodistillation process (Clevenger apparatus). After 55 min of EMI assited extraction at 140°C, it was possible to collect almost all the existing essential oils from the three samples with a yield from 2.1 ± 0.3 to 3.1 ± 0.8% (w/w), but the high temperature of electromagnetic induction heating is probably causing the degradation of compounds. Carvacrol was found as a major compound in all TFEOs samples with a higher amount in the oil from Tarik Ibn Ziad, followed by *p*-cymene and γ-terpinene. The antioxidant activity of TFEO was evaluated and IC_{50} values were raining from 57.3 ± 1.4 to 236.7 ±1.4 µg/mL. These results revealed the importance of *T. fontanesii* as an antioxidant, which, is may help to protect the people against lipid peroxidation and free radical damage. In addition, the essential oil of *T. fontanesii* can be used as an antioxidant agent for the preservation of processed food and as functional food components.

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Table 1. Chemical composition of the essential oil of *T. fontanesii* from Algeria (mean of triplicates)

N°	Compounds	RI[a]	RI[b]	Area %			
				EMI			HD
				T_M	T_{OEC}	T_{TIZ}	T_{TIZ}
1	α-Thujene	930	924	0.9 ± 0.07	2.2 ± 0.05	0.8 ± 0.1	1.2 ± 0.08
2	α-Pinene	939	930	4.3 ± 0.8	2.9 ± 0.04	1.2 ± 0.8	3.2 ± 1.01
3	Camphene	954	944	tr	tr	tr	0.2 ± 0.01
4	β-Pinene	979	973	0.3 ± 0.02	0.3 ± 0.03	0.2 ± 0.1	0.1 ± 0.01
5	Myrcene	991	989	1.7 ± 0.08	2.0 ± 0.7	1.6 ± 0.4	1.5 ± 0.03
6	α-Phellandrene	1003	1002	0.2 ± 0.01	0.3 ± 0.01	tr	0.2 ± 0.02
7	δ-3 Carene	1011	1008	tr	tr	tr	tr
8	α-Terpinene	1017	1014	1.8 ± 0.03	2.7 ± 0.02	1.8 ± 0.7	1.7 ± 0.08
9	***p*-Cymene**	**1025**	**1023**	**14.3 ± 1.3**	**9.2 ± 1.2**	**10.5 ± 0.2**	**17.5 ± 1.2**
10	Limonene	1029	1027	tr	1.1 ± 0.03	tr	1.01 ± 0.02
11	**γ-Terpinene**	**1060**	**1059**	**11.6 ± 1.5**	**12.6 ± 0.1**	**14.9 ± 0.8**	**8.8 ± 0.9**
12	*Trans*-Sabinene hydrate	1070	1067	-	0.2 ± 0.02	-	0.3 ± 0.01
13	Linalool	1097	1100	2.3 ± 0.9	3.8 ± 0.8	1.9 ± 0.1	3.7 ± 0.01
14	Borneol	1166	1167	tr	-	tr	0.3 ± 0.05
15	Terpinen-4-ol	1177	1178	0.3 ± 0.01	0.3 ± 0.07	0.2 ± 0.1	0.2 ± 0.01
16	Carvacrol methyl ether	1245	1244	0.5 ± 0.02	1.4 ± 0.09	0.3 ± 0.1	0.4 ± 0.02
17	Thymol	1290	1295	0.6 ± 0.1	1.5 ± 0.3	1.3 ± 0.3	0.3 ± 0.08
18	**Carvacrol**	**1299**	**1311**	**59.8 ± 1.3**	**55.1 ± 0.8**	**63.9 ± 1.9**	**54.7 ± 1.2**
19	α-Gurjunene	1411	1412	0.3 ± 0.05	0.3 ± 0.01	0.3 ± 0.1	0.2 ± 0.01
20	β-Caryophyllene	1419	1422	0.4 ± 0.08	0.6 ± 0.02	0.4 ± 0.1	0.4 ± 0.01
21	Aromadendrene	1447	1442	0.1 ± 0.01	-	0.1 ± 0.06	0.2 ± 0.02
22	allo-Aromadendrene	1460	1464	0.2 ± 0.03	tr	tr	tr
23	Bicyclogermacene	1472	1499	-	-	0.2 ± 0.1	-
24	γ-Cadinene	1514	1515	tr	tr	0.1 ± 0.09	-
25	δ- Cadinene	1523	1526	0.1 ± 0.01	tr	0.1 ± 0.02	-
26	Spathulenol	1587	1583	0.2 ± 0.01	0.1 ± 0.01	tr	0.3 ± 0.06
27	Caryophylleneoxide	1583	1589	tr	tr	tr	1.1 0.03
Monoterpenes (%) :				35.1	33.5	31	35.71
Oxygenated monoterpenes (%) :				63.5	62.1	67.6	59.6
Sesquiterpenes (%) :				1.1	0.9	1.2	0.8
Oxygenated sesquiterpenes (%) :				0.2	0.1	tr	0.4
Identified compounds (%) :				99.9	96.9	99.8	95.6

tr : traces (< 0.1%)

RI[a]:Retention indices (Adams)

RI[b] : Retention indices relative to C_7–C_{30} on the HP-5MS capillary column

Table 2. IC_{50} values (µg/mL) of *T. fontanesii* essential oil and BHT

Assays	T_M	T_{TIZ}	T_{OEC}	BHT
DPPH	83.8 ± 0.5	57.3 ± 1.35	91.2 ± 1.1	18.3 ± 0.8
Reducing power	103.9 ± 0.4	63.8 ± 0.17	112.6 ± 1.8	9.7 ± 1.23
β-carotene	167.2 ± 0.9	148.9 ± 0.45	236.7 ± 1.4	67.8 ± 0.1

T_M: *T. fontanesii* from Miliana; **T_{TIZ}**: *T. fontanesii* from Tarik Ibn Ziad; **T_{OEC}**: *T. fontanesii* from Oued El Chorfa