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Abstract: ***Background*:** The present work investigates the chemical composition, the antioxidant and the antimicrobial activities of the Essential Oils (EOs) of three species of the *Lamiaceae* family gowning in Algeria: *Thymus vulgaris* L., *Thymus algeriensis* Boiss. & Reut. and *Mentha pulegium* L.

***Methods*:** Essential Oils (EOs) obtained by hydrodistillation of the aerial parts of the studied plants were analyzed by GC and GC-MS. The antioxidant activity of the EOs was determined using two different assays: free radical scavenging activity of DPPH$^{\bullet}$ (*2,2-diphenyl-1-picrylhydrazyl*) and Phosphomolybdenum reducing power. The EOs were also tested for their antibacterial and antifungal activities against eight pathogenic bacteria (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli, Bacillus cereus, Staphylococcus aureus, Methicillin resistant strain of S. aureus "MRSA" and Enterococcus faecalis*); a yeast (*Candida albicans*)*;* and a fungi (*Fusarium oxysporum*).

***Results*:** For DPPH assay, *T. vulgaris* presented very interesting activity. At the opposite, *T. algeriensis* (Aflou) and *M. pulegium*, were the most active EOs in term of Phosphomolybdenum assay. The antimicrobial activity of *T. vulgaris* was found to be the most active EO and exhibited important resistance against most of studied bacteria. For disc diffusion test, the most active EO plant was *T. algeriensis.* Alternatively, and for antifungal activity, *T. vulgaris* presented the highest value of MFC.

***Conclusion*:** The antioxidant activity test's results showed that the EOs exhibited important reducing powers but weak scavenging activities. On the other hand, it was found that some EO samples have shown very interesting antimicrobial activities. Actually, among the investigated EOs, *T. vulgaris* presented the strongest antibacterial and antifungal activities.

Keywords: *Thymus vulgaris* L., *Thymus algeriensis* Boiss. & Reut., *Mentha pulegium* L., DPPH assay, phosphomolybdenum assay, antimicrobial activity, essential oil.

1. INTRODUCTION

To cure its ills, man had always relied on animals and plants. These have always been the basis of the traditional systems of medicine for thousands of years and continue to date to provide new remedies [1]. Recently, the introduction of traditional medicine, as an alternative form, as well as the development of microbial resistance to antibiotics has led researchers to study the antimicrobial activity of medicinal plants [2]. On the other hand, the different synthetic antioxidants used in food, were suspected to have negative health effects. It is for this reason that the interest for the study of natural products such as antioxidants is increasingly important. Thus, various sources of antioxidants of plant origin have been studied in recent years [3].

The use of essential oils as functional ingredients in foods, drinks, toiletries and cosmetics is gaining momentum [4]. Additionally, the use of essential oils is becoming popular to increase the shelf-life of food products, since consumers are more conscious about the health problems caused by several synthetic preservatives [5, 6].

The purpose of this research was to investigate, firstly the chemical composition and the antioxidant properties of three EOs (*T. vulgaris* L., *T. algeriensis* Boiss. & Reut. and *M. pulegium* L.) collected from two regions in the center of Algeria; and secondly, to examine their antimicrobial activities against eight pathogenic bacteria (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Escherichia*

coli, Bacillus cereus, Staphylococcus aureus, Methicillin resistant strain of *S. aureus* "MRSA" and *Enterococcus faecalis*); a yeast (*Candida albicans*)*;* and a fungi (*Fusarium oxysporum*).

Only very few reports on the determination of the antioxidant activity of the essential oil using Phosphomolybdenum assay and applied for of *T. vulgaris* were found in literature [7-9]. For these reports, the antioxidant values are difficult to use for comparison purpose since different methods of expressions or different antioxidant of reference were engaged. In the same context, and as best of our knowledge, there are no reports dealing with the Phosphomolybdenum assay of the EO for *T. algeriensis* Boiss. & Reut. and *M. pulegium* L. As far as we know, this is the first report that investigates the antioxidant antimicrobial activities of the essential oils of the plants from these regions of collection (Djelfa and Aflou "Laghouat") from Algeria. Furthermore, we have used for the first time a special form of *Fusarium oxysporum* which was isolated from a fragment of date palm from Ghardaia region to investigate the antifungal activity. In addition, the antioxidant activity of the EOs determined by DPPH and Phosphomolybdenum assays were examined for the first time for these regions. Finally, correlations between chemical compositions of the EOs and their antioxidant and antimicrobial activities were investigated using statistical analysis; this was done in order to determine the nature of the chemical component(s) responsible(s) for the different activities of the EOs.

2. MATERIALS AND METHODS

2.1. Plants Descriptions

***Thymus vulgaris* L.** is a perennial herb indigenous in central and southern Europe, Africa and Asia. It is widely used in folk medicine in the treatments of variety of diseases such as gastroenteric and bronchopulmonary disorders, as well as due to its anthelmintic, carminative, sedative and diaphoretic properties [10]. It has been reported that its EO possesses numerous biological activities including antiworm, antiseptic, antispasmodic, antimicrobial [11] and antioxidant [12]. In addition, it is a well-known species of the genus Thymus, extensively studied for its chemical and biological activities [13-15].

In Algeria, *T. vulgaris* is one of the most useful popular remedies in the treatment of respiratory affections (colds, flu, angina) and gastric disorders (dyspepsia, cramps) [16].

***Thymus algeriensis* Boiss. & Reut.** is the most widespread North African species, endemic to Libya, Tunisia, Algeria and Marocco. Fresh or dried, it is largely used only as a culinary herb. *T. algeriensis* is also used in traditional medicine, as a fresh or dry seasoning, in respiratory and digestive tube disorders and against abortion [17].

In Algeria, *T. algeriensis* is used as a treatment for stomachic, diaphoretic, antispasmodic and stimulating. The summits and the young flowering twigs are used. Infusion is useful against all infectious diseases, such as influenza, pneumonia and respiratory tract diseases. It is used in friction in cases of neuralgia and sciatica and as odontalgic on decayed toothache [18].

***Mentha pulegium* L.** is one of the *Mentha* species commonly known as pennyroyal. It is native species of Europe, North Africa and in Asia Minor and near East. The flowering aerial parts of *M. pulegium* L. has been traditionally used for its antiseptic for treatment of cold, sinusitis, cholera, food poisoning, bronchitis and tuberculosis, and also as antiflatulent, carminative, expectorant, diuretic, antitussive, menstruate. Some pharmacological effect of *M. pulegium* L. essential oil such as abortifacient effect in rat myometrium, cytotoxic activity against different human cell lines and its antioxidant effect were confirmed [19].

In Algeria it is much appreciated, so much so that it is used to prepare a traditional dish: the potato stew with the pennyroyal "Batata Fliou". Its pleasant odor seems to displease some kind of parasites, and its insecticidal power is well established. Formerly, it was burned in fleece infested premises. It was also used as a lotion on the coat of domestic animals to rid them of their parasitic pests [16].

2.2. Plants Materials Collections

The study was carried out using four EO samples obtained from three species belonging to the *Lamiaceae* family: *M. pulegium* L. (Djelfa), *T. vulgaris* L. (Djelfa) and *T. algeriensis* Boiss. & Reut. (from two different stations: Djelfa and Aflou-Laghouat). Since *T. algeriensis,* wide spreads over a larger region, we have picked up two samples from different locations in order to compare theirs EOs composition differences as well as theirs antioxidant and antibacterial activities behaviors. The different plant samples were collected in 2010 during the flowering stage; (end of May for *M. pulegium* and *T. algeriensis,* and the beginning June for *T. vulgaris*). Voucher specimens (TV/06/10, TA-AFL/06/10, TA-DJF/06/10, MP/06/10) were deposited in the herbarium of the Fundamental Sciences Research Laboratory at Laghouat University.

2.3. Essential Oil Extraction

The different samples of EOs were obtained by hydrodistillation using a Clevenger type apparatus; subsequently the obtained EOs were dried over anhydrous sodium sulfate, and stored in a dark at 4 °C.

2.4. Essential Oil Analysis

The GC analysis was performed using a gas chromatograph type Chrompack CP 9002, equipped with a fused silica capillary column DB-5 (30m×0.32mm, 0.25µm film thickness) and Flame Ionization Detector (FID). The carrier gas was nitrogen at a flow rate of 1mL/min. The column temperature was programmed from 50 °C (3 minutes) to 250 °C at 2 °C/min, and then maintained at 250 °C for 10 min. The temperatures of the injector and detector were set at 250 °C. Volumes of 1µL of diluted samples (1:100 v/v, in ethanol) were injected manually using split less mode. Linear retention indices of the components were calculated using a series of *n*-alkane (C_9-C_{25}) analyzed under the same operating conditions as those of the EOs samples.

The GC/MS analysis was performed on an AGILENT 6890 GC/CMSD 5973 equipped with a capillary column UB-Wax (30m×0.25mm, 0.25µm film thickness) and a 70

eV EI Quadruapole detector. Helium was the carrier gas, at a flow rate of 1mL/min. Injector and MS transfer line temperatures were set at 250 °C and 220 °C, respectively. Column temperature was programmed same as gas chromatography. Diluted samples (1:100 v/v, in ethanol) of 1µL were also injected manually using split less mode.

The EOs constituents were identified by comparison of their linear retention indices and mass spectra with those in the computer library (NIST MS Library) and with literature data [20].

2.5. DPPH Assay (Free Radical Scavenging Activity)

For this test, the ability of hydrogen donating or radical scavenging was measured using the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl) [21]. The EOs dilutions were prepared in absolute ethanol, and then 1mL of each dilution was added to 1mL of a 200µM DPPH solution. The mixtures were incubated in dark for 30 min; afterwards the absorbance was measured at 517 nm against a blank. The inhibition percentage was calculated using the following formula:

$$I(\%) = \left(\frac{A_0 - A}{A_0} \right) \times 100$$

Where:

A_0 is the absorbance of the control reaction (containing all reagents except the test compound) and A is the absorbance of the test compound.

2.6. Phosphomolybdenum Assay

This assay is based on the reduction of Molybdate (VI) to Molybdate (V) by the antioxidant compounds and the formation of a green phosphate/Molybdate (V) complex at acidic pH with a maximum absorbance at 695 nm [22]. The EOs samples were diluted in absolute ethanol. An amount of 200 µL of each dilution was mixed with 2 mL of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The mixture was then incubated in a water bath set at 70 °C for 90 min [22]. The absorbance was determined at 695 nm. The results were expressed in terms of ascorbic acid equivalent AEAC.

2.7. Microbial Strains

The different samples of EOs were tested against eight bacteria: *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhi* (isolate), *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, Methicillin resistant strain of *S. aureus* ATCC 43300, *Bacillus cereus* ATCC 11779, *Enterococcus faecalis* ATCC 29212 ; a yeast: *Candida albicans* (isolate) and a filamentous fungi *Fusarium oxysporum* f. sp. *albedinis.* The fungal species *Fusarium oxysporum* special form *albedinis* was isolated from a fragment of date palm provided by the Regional Station of Plant Protection of Ghardaia (Algeria). The culture was then purified by performing a monospore culture.

2.8. Antimicrobial Tests

The antimicrobial activity of the different EO samples was screened by the disk diffusion test [23], and then the minimal inhibitory and bactericidal concentrations were determined using a dilution test [24]. The antifungal activity was tested using a direct contact test as described by El Ajjouri *et al.* [25].

3. RESULTS

3.1. Essential Oil Yield and Chemical Composition

The yields of the studied EOs were varying from moderate to low values. They were somehow important for *M. pulegium and T. vulgaris*. In the other hand, they were ranging from low to poor values for *T. algeriensis* (Table **1**).

The chemical compositions of the different EO samples are presented in Table **1**. For the EO of *T. vulgaris*, thirty two different compounds representing 96.47% of the total oil were identified, with proportions of monoterpene hydrocarbons and oxygenated monoterpenes equal to 52.98% and 39.14%, respectively. The major component of this oil was the γ-terpinene (25.70%) followed by thymol (20.83%), then *p*-cymene (20.04%). The oxygenated compounds were also present in lower amounts than the hydrocarbons: thymol methyl ether (7.82%), linalool (4.76%) and borneol (2.54%). The β-caryophyllene (2.28%) was the main sesquiterpene compound for this EO.

For the EO of pennyroyal (*M. pulegium*), twenty eight compounds were identified representing 76.38% of the total EO. The oil was mainly composed of pulegone (54.92%), which accounts for almost the majority of oxygenated monoterpenes. Monoterpene hydrocarbons represented only a small fraction of the EO content (2.67%).

For the case of *T. algeriensis*, forty four components were identified for the sample of Djelfa and thirty three components of that of Aflou (Laghouat), both accounting for 83.30 and 81.06% of the total EOs, respectively. Camphor was the predominant compound in the sample of Aflou (Laghouat) with a percentage of 17.68%, this content agrees with the results of Amarti *et al.* [26] and Zouari *et al.* [27] who have detected camphor as major compound for *T. algeriensis* from Morocco and Tunisia, respectively. The sample of Djelfa was predominantly composed of α-terpinenyle acetate (27.32%), a compound completely absent in the sample of Aflou. Camphor was also present, but with a lower content (10.77%) than recorded for the sample of Aflou (Laghouat). The eucalyptol was one of the major compounds in the EO sample of Aflou (10.04%), whereas it represented only 2.47% for the sample of Djelfa. The EO of Aflou sample showed a high content of monoterpene hydrocarbons (41.29%) compared to that of Djelfa (13.71%), with a predominance of camphene (8.73%), α-pinene (7.84%) and myrcene (6.95%). On the other hand, the two samples showed almost equal percentages of borneol (5.58 and 5.68%). Sesquiterpenes were minor compounds in the EOs of the two regions with a total percentage of 5.46 and 3.04% for Djelfa and Aflou respectively.

Table 1. Chemical composition of the essential oil samples: *Thymus vulgaris, Thymus algeriensis* and *Mentha pulegium*.

Components	LRI[a]	LRI[b]	*Thymus vulgaris* (Djelfa)	*Thymus algeriensis*		*Mentha pulegium* (Djelfa)
				(Djelfa)	(Aflou-Laghouat)	
α-Thujene	921	924	1.10	0.05	0.26	0.05
α-Pinene	926	932	0.92	3.58	7.84	0.06
Camphene	938	946	1.08	2.75	8.73	0.02
Sabinene	961	969	0.04	0.37	–	0.09
β-Pinene	964	974	0.19	0.13	1.43	0.40
Myrcene	984	988	0.99	1.34	6.95	–
α-Phellandrene	995	1002	0.19	0.01	0.02	0.01
δ-Carene	1002	1008	0.07	0.01	–	0.04
α-Terpinene	1008	1014	1.93	0.20	–	–
***p*-Cymene**	1014	1020	**20.04**	0.97	0.69	–
Limonene	1019	1024	0.17	1.11	–	0.75
Eucalyptol	1021	1059	0.40	2.47	**10.04**	0.98
(*Z*)-*β*-Ocimene	1027	1032	–	0.03	5.23	–
***γ*-Terpinene**	1052	1054	**25.70**	0.32	–	0.27
Terpinolene	1077	1086	0.16	0.37	0.10	–
Linalool	1088	1095	4.76	1.46	1.34	0.43
β-Thujone	1103	1112	0.07	0.47	0.35	–
allo-Ocimène	1118	1128	–	0.02	–	0.05
Camphor	1123	1141	0.03	**10.77**	**17.68**	–
neo-isopulegol	1134	1167	–	-	-	1.02
Pinocarvone	1135	1160	–	2.99	1.65	–
Isopulegol	1142	1145	–	–	–	0.10
Borneol	1149	1165	2.54	5.58	5.68	1.06
Terpinen-4-ol	1162	1174	0.70	2.56	–	0.27
α-terpineol	1174	1186	0.28	3.12	1.25	–
Naphtalene	1186	1178	0.08	1.71	1.02	0.11
Thymol methyl ether	1218	1232	**7.82**	–	–	–
Nerol	1219	1227	–	0.63	0.96	–
Pulegone	1224	1233	–	–	–	**54.92**
Carvone	1234	1239	–	0.17	0.02	3.32
Linalyl acetate	1242	1248[c]	–	0.33	–	1.71
iso-Pulegyl acetate	1264	1275	–	–	–	6.50
Bornyl acetate	1268	1284	–	4.84	0.50	1.05
Thymol	1279	1289	**20.83**	0.03	0.04	–
Carvacrol	1294	1298	2.03	0.02	0.03	–
Eugenol	1330	1356	–	–	–	0.22

Table 1. contd…

Components	LRI[a]	LRI[b]	*Thymus vulgaris* (Djelfa)	*Thymus algeriensis*		*Mentha pulegium* (Djelfa)
				(Djelfa)	(Aflou-Laghouat)	
***α*-Terpinenyl acetate**	1341	1346	–	**27.32**	–	–
Neryl acetate	1350	1359	–	1.69	5.76	0.20
Geranyl acetate	1364	1379	–	0.42	0.45	–
β-Caryophyllene	1408	1417	2.28	0.12	0.22	1.78
Aromadendrene	1425	1439	0.10	0.16	0.05	–
allo-Aromadendrene	1445	1458	0.05	0.07	0.11	–
Germacrene D	1466	1484	0.03	0.25	0.01	0.57
Germacrene A	1496	1508	0.90	0.11	0.51	–
δ-Cadinene	1504	1522	–	0.08	–	–
(*Z*)-Nerolidol	1517	1531	–	0.04	0.05	–
Germacrene B	1544	1559	–	0.18	0.81	–
Ledol	1553	1602	0.30	2.72	0.42	–
Spathulenol	1555	1577	0.29	0.77	–	–
Caryophyllene oxide	1559	1582	0.40	0.96	0.86	0.40
Total Identified (%)			**96.47**	**83.30**	**81.06**	**76.38**
Monoterpene hydrocarbons			52.98	13.71	41.29	2.67
Oxygen-containing monoterpenes			39.14	64.13	36.73	70.96
Sesquiterpene Hydrocarbons			3.36	0.79	0.90	2.35
Oxygen-Containing Sesquiterpenes			0.99	4.67	2.14	0.40
Essential oil yield (% m/m)			1.34	0.56	0.27	1.47

[a] LRI: Linear Retention Indices determined experimentally on apolar column DB-5.
[b] LRI: Linear Retention Indices obtained from literature [20].
[c] LRI: Linear Retention Indices obtained from literature [61].

Table 2. Antioxidant acitivity of the essential oil samples of *Thymus vulgaris*, *Thymus algeriensis* and *Mentha pulegium* using DPPH free radical scavenging.

Essential Oil Sample	EC_{50} (mg/mL)
Thymus vulgaris	2.3 ± 0.1
Thymus algeriensis (Djelfa)	10.2 ± 0.9
Thymus algeriensis (Aflou)	> 45.0
Mentha pulegium	27.7± 2.4
Vitamin E	0.0207 ± 0.0007
Ascorbic acid	0.0078 ± 0.0001
Butylhydroxyanisole (BHA)	0.0011 ± 0.0003

3.2. Antioxidant Activity

3.2.1. DPPH Assay

Antioxidant activities of the different EO samples were evaluated using two complementary methods: the DPPH assay, and the phosphomolybdenum assay. The DPPH test measures the ability of the sample to provide protons, whereas the phosphomolybdenum assay measures the capacity of the sample to donate electrons.

The results of the tests of DPPH radical scavenging activities of the different samples on the EOs are shown in Table **2**. These results are expressed as EC_{50} values, that are

Table 3. Antioxidant acitivity of the essential oil samples of *Thymus vulgaris, Thymus algeriensis* and *Mentha pulegium* using Phosphomolybdenum assay.

Essential Oil	AEAC[a] (mg/mL)
Thymus vulgaris (Djelfa)	0.107 ± 0.007
Thymus algeriensis (Djelfa)	0.220 ± 0.022
Thymus algeriensis (Aflou)	0.148 ± 0.003
Mentha pulegium (Djelfa)	0.159 ± 0.002
Vitamin E	0.572 ± 0.023

[a]Antioxidant reducing power is expressed as *AEAC (Ascorbic acid Equivalent Antioxidant Power)*.

defined as the concentration of substrate that causes 50% loss of the concentration of the free radicals DPPH initially introduced; the higher the antioxidant activity, the lower is the value of EC_{50} [21]. In comparison with antioxidants of reference, the EO samples showed weak DPPH-radical-scavenging activities. Their activities can be classified as follows: *T. vulgaris*>*T. algeriensis* (Djelfa)>*M. pulegium*>*T. algeriensis* (Aflou-Laghouat). More effectively, *T. vulgaris* presented a very interesting EC_{50} value, and it could be considered active in comparison with the majority of common EOs.

3.2.2. Phosphomolybdenum Assay

The second test named Phosphomolybdenum test, measures the reducing power capacity in aqueous medium. Antioxidant reducing power is expressed as AEAC: *Ascorbic acid Equivalent Antioxidant Power*. By definition, AEAC is defined as the concentration of an antioxidant of reference (ascorbic acid in this case) in mg/mL, which gives the equivalent antioxidant power for a concentration of 1 mg/mL of the EO. High value of AEAC indicates high antioxidant capacity.

In comparison with vitamin E, the EOs showed moderate antioxidant capacities (Table **3**). The highest antioxidant capacity was observed for the EO of *T. algeriensis* (Djelfa); while the EO with the lowest activity was recorded for *T. vulgaris*. As a matter of fact, the EOs samples presented practically close values of antioxidant activities, and they were approximately 2 to 5 times lower than vitamin E; whereas, vitamin E presented almost two times lower antioxidant power than vitamin C.

3.3. Antimicrobial Activity

3.3.1. Disc Diffusion Test

The antibacterial activities of the EOs were also investigated. Inhibition zone diameters are includes in Table **4** for the four EO samples (values are average of three repetitions). The results showed a sensitivity of the microorganisms towards all the EOs, with a remarquable resistance for *Pseudomonas aeruginosa*. This resistance was observed by Cosentino *et al*. [28] for the EOs of *T. capitatus* and *T. herba barona*, Bouhdid *et al*. [29] for three species of *T.* genus and Hussain *et al*. [30] for six plants within the *Lamiaceae* family. Let's mention that comparisons of results of the founded antimicrobial activities with those of literature "Tables **4**(**c**) and **5**(**b**)" are not evident since the evaluations conditions are somehow different.

T. vulgaris EO sample had the strongest activity with the highest inhibition zone diameters, while the EOs of *T. algeriensis* and *M. pulegium* showed moderate activities.

In a diffusion test, the inhibition zone diameter for a component is determined by its antimicrobial activity, its solubility and capacity of diffusion in the media and by the characteristics of the microorganism itself [31]. Diffusion methods are likely appreciated because their simplicity and low cost, but these methods are not always reliable for the evaluation of antimicrobial activities of plant extracts, because the absence of inhibition zone doesn't mean that the tested compound is inactive, especially for compounds with low polarity that diffuse slowly in the culture media [32].

3.3.2. Minimal Inhibitory Concentration (MIC) and Bactericidal Concentrations (MBC) Tests

The determined MIC values were ranged from 0.127 mg/mL (*T. vulgaris* EO for *B. cereus* and *E. faecalis*) to higher than 4.5 mg/mL (for *Candida albicans* for the two samples of *T. algeriensis*). The same resistance was observed for *P. aeruginosa* towards all the EO samples (Table **5**).

T. vulgaris EO showed MICs values between 0.12 mg/mL (for the strains *B. cereus* and *E. faecalis*) and 1.01 mg/mL (for *K. pneumoniae* and *S. aureus*). The growth of MRSA, *S. typhi* and *E. coli* was inhibited at a concentration equal to 0.5 mg/mL. The resistance of *P. aeruginosa* was observed even with a concentration of 2.03 mg/mL. Imelouane *et al*. [33] found for this same species EO MIC values equal to 1.33 mg/mL for the strains *E. coli, S. aureus* and *S. epidermidis*. Kaloustian *et al*. [34] found MICs equal to 1 and 2 mg/mL for the strains *E. coli* and *S. aureus* respectively, while testing the EOs of *T. vulgaris* and *T. zygis*.

For the MBCs, *B. cereus* was the most sensitive strain, with MBC equal to 0.5 mg/mL. MRSA, *S. typhi, E. coli, S. aureus* and *E. faecalis* were inhibited at an oil concentration of 1.01 mg/mL. Many previous studies confirm the antimicrobial properties of EOs from *Thymus* genus [35-41], that is in correlation with our results. *T. algeriensis* EO showed a moderate to weak activity with more effectiveness on Gram positive bacteria (Table **6**), this weak activity has been also observed by Hazzit *et al*. [42] for two samples of *T. algeriensis*.

3.4. Antifungal Activity

Inhibition growth percentages observed for the different EO samples against the filamentous fungi *Fusarium oxysporum* sp.f. *albedinis* are shown in Table **6**. All the EO tested exhibited inhibition effects against the tested fungi. The EO of *T. vulgaris* showed the strongest activity with a fungistatic and fungicide concentration equal to 1.753 mg/mL, and 3.438 mg/mL, respectively. While the EOs of *T. algeriensis* (Djelfa) and *M. pulegium* showed a moderate activity with MICs values equal to 8.963 mg/mL and 8.781 mg/mL, respectively. In another hand, these latest EOs did not exhibit any measured MFCs activity in the range of the concentration studied. These observed differences are proba-

Table 4a. Antimicrobial activity of essential oil samples of *Thymus vulgaris, Thymus algeriensis* and *Mentha pulegium* by disc-diffusion diameter inhibition zones.

Essential Oil Plant	*Thymus vulgaris*	*Thymus algeriensis*		*Mentha pulegium*
		Djelfa	Aflou	
Essential oil concentration (mg/disc)	2.23	2.32	2.23	2.27
	Inhibition Zone Diameters (mm)[a]			
Klebsiella pneumoniae	24,50 ± 2,12	16.00 ± 1.41	11.78 ± 2.27	10.33 ± 2.31
Pseudomonas aeruginosa	9.00 ± 0.00	7.83 ± 0.76	7.50 ± 0.71	9.17 ± 0.29
Salmonella typhi	24.00 ± 1.41	12.22 ± 0.70	16.67 ± 1.15	10.50 ± 0.71
Escherichia coli	31.17 ± 2.84	13.88 ± 1.24	14.33 ± 0.47	10.75 ± 0.35
Bacillus cereus	19.33 ± 2.52	17.54 ± 0.30	17.00 ± 0.00	11.33 ± 1.53
Staphylococcus aureus	22.33 ± 1.15	8.33 ± 0.58	9.00 ± 0.00	9.22 ± 1.07
MRSA	29.00 ± 1.00	14.17 ± 1.18	9.83 ± 1.44	9.33 ± 1.15
Enterococcus faecalis	15.33 ± 1.04	8.33 ± 0.58	9.00 ± 1.41	9.00 ± 1.41
Candida albicans	19.67 ± 1.53	10.33 ± 2.31	10.33 ± 1.53	7.50 ± 0.71

[a] three repetitions average ± SD

Table 4b. Antimicrobial activity of the controls used by disc-diffusion diameter inhibition zones.

	GM (10UI)	AMC (10 µg)	OX1 (1 µg)	E15 (15 µg)
	Inhibition Zone Diameters (mm)			
Klebsiella pneumoniae	9	9	0	10
Pseudomonas aeruginosa	21	0	0	8
Salmonella typhi	21	30	0	16
Escherichia coli	31	24	0	15
Bacillus cereus	31	28	18	33
Staphylococcus aureus	22	32	24	29
MRSA	12	22	16	0
Enterococcus faecalis	23	25	0	29

GM: Gentamicine ; UI: unité internationale ; AMC: Amoxicilline – acide clavulanique ; OX1: Oxacilline ;E15: Erythromycine.

Table 4c. Antimicrobial activity of essential oil samples of *Thymus vulgaris, Thymus algeriensis* and *Mentha pulegium* by disc-diffusion diameter inhibition zones (values from literature).

Essential Oil Plant		*Thymus vulgaris*				*Thymus algeriensis*			*Mentha pulegium*			
Country		**Morocco** [62]	**Morocco** [63]	**Libya** [64]	**Spain** [38]	**Algeria** [65]	**Tunisia** [66]	**Libya** [64]	**Iran** [54]	**Morocco** [67]	**Morocco** [68]	**Portugal** [69]
						Inhibition Zone Diameters (mm)[a]						
Microbial strains	***Klebsiella pneumoniae***	-	-	-	-	-	13.5	-	-	-	-	-
	Pseudomonas aeruginosa	-	-	-	-	-	14.5	-	-	-	-	-
	Salmonella typhi	-	-	-	36.0	-	15.0	-	-	-	-	9.3
	Escherichia coli	0.33;1.33	34.7	-	19.6;28.3	-	14.0	-		21.8;23.7	12.6	2.3
	Bacillus cereus	-	-	-	-	0.0-22.0	30.0	-	16.0	-	-	-
	Staphylococcus aureus	1.33	35.0		45.0	0.0-14.0	-	-	21.0	30.4	21.4	-
	Enterococcus faecalis	-	-	-	-	-	18.5	-	-	-	-	-
	Candida albicans	-	-	20.0;40.0	-	9.0-18.66	-	5.0;10.0	16.0	-	-	-

Table 5a. MIC and MBC for the essential oil samples of *Thymus vulgaris, Thymus algeriensis* and *Mentha pulegium*.

Microbial Strains	*Thymus vulgaris*		*Thymus algeriensis*				*Mentha pulegium*	
			(Djelfa)		(Aflou-Laghouat)			
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
Klebsiella pneumoniae	1.016	2.032	2.114	> 4.227	2.030	> 4.059	2.070	2.070
Pseudomonas aeruginosa	> 2.032	> 2.032	> 4.227	> 4.227	> 4.059	> 4.059	> 2.485	> 2.485
Salmonella typhi	0.508	1.016	2.114	3.044	3.004	4.059	1.035	2.070
Escherichia coli	0.508	1.016	3.044	4.227	3.004	> 4.059	1.035	1.035
Bacillus cereus	0.127	0.508	0.264	0.528	1.015	1.015	1.035	2.485
Staphylococcus aureus	1.016	1.016	1.057	1.057	1.015	1.015	1.035	2.070
MRSA	0.508	1.016	0.528	3.044	1.015	2.030	1.035	2.070
Enterococcus faecalis	0.127	1.016	0.528	1.057	0.507	1.015	1.035	2.070
Candida albicans	0.564	1.129	4.697	> 4.697	4.510	4.510	1.150	2.301

***MIC:** Minimum Inhibitory Concentration; **MBC:**Minimum Bactericide Concentration.*

Table 5b. MIC (mg/mL) for the essential oil samples of *Thymus vulgaris, Thymus algeriensis* and *Mentha pulegium* (values from literature).

Plant		*Thymus vulgaris*				*Thymus algeriensis*		*Mentha pulegium*				
Country		Libya [64]	Spain [38]	Iran [70]	Morocco [63]	Tunisia [66]	Libya [64]	Morocco [71]	Iran [54]	Morocco [67]	Morocco [68]	Portugal [69]
Microbial strains	*Klebsiella pneumoniae*	-	-	-	-	6.0	-	-	-	-	-	-
	Pseudomonas aeruginosa	160.0	-	-	-	5.0	80.0	-	-	-	-	-
	Salmonella typhi	-	< 0.2	-	-	6.0	-	-	4.0	-	-	3.8
	Escherichia coli	-	0.5	-	9.3	6.0	-	2.0;4.0	4.0	4.0;2.0	1.0	3.2
	Bacillus cereus	-	-	-	-	1.0	-	-	1.0	-	-	-
	Staphylococcus aureus	80.0	< 0.2	0.05	10.7	-	20.0	2.0	0.5	2.0	1.0	-
	Enterococcus faecalis	80.0		0.05	-	3.0	80.0	-	-	-	-	-
	Candida albicans	-	-	-	-	-	-	-	2.0	-	-	-

bly due to the difference in the compositions between the EOs.

Soliman & Badiaa [43] tested the antifungal effect of EOs of three species of *Lamiaceae*: *T. vulgaris, M. viridis* and *Ocimum basilicum*. They revealed that the three oils have inhibitory activities against the tested fungal species (*Aspergillus flavus, A. parasiticus, A. ochraceus* and *Fusarium moniliforme*). For the same family Dambolena *et al.* [44] found that several samples of *Ocimum basilicum* and *Ocimum gratissimum* have an antifungal activity against *Fusarium verticillioides*. Ouraïni *et al*. [45] obtained significant antifungal activity for EOs of *T. saturejoides* and *M. pulegium*. The antifungal potency of the EO of thyme has also been shown by Yang & Clausen [46].

The results show that the extracts with high phenol concentration (*T. vulgaris*) have strong antifungal activities. Several *in vitro* and *in vivo* studies conducted by Figueiredo *et al*. [47] showed that the EOs containing phenolic structures are very active against a wide spectrum of pathogenic fungi. The mechanism of toxicity of the phenols towards fungi is based on the inactivation of fungal enzymes. Phenolic terpene also act by binding to the amino groups of the microbial membrane proteins causing the alteration of the permeability and leakage of intracellular components [25]. This effect was also mentioned by Pinto *et al.* [48] by studying the EO of *T. pulegioides* effect on the membrane of *Candida* and *Aspergillus*.

4. DISCUSSION

4.1. Essential Oil Yield and Chemical Composition

According to previous reports [49], several chemotypes of *T. vulgaris* were determined: geraniol, α-terpineol, sabinene hydrate (thuyanol), linalool, carvacrol, thymol and 1,8-cineole. Several species belonging to *Thymus* genera

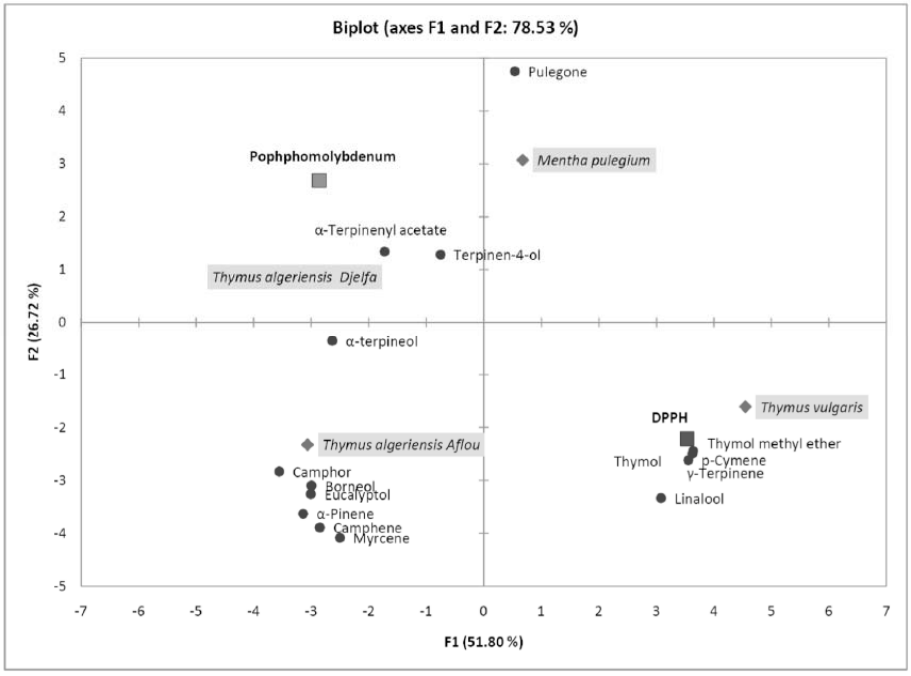

Fig. (1). Two dimensional plot on axes F1 and F2 of the essential oil samples end their antioxidant activities using Principal Component Analysis.

have similar compositions with a high content of phenolic terpenes (thymol or carvacrol) followed by a high percentage of *γ*-terpinene and *p*-cymene: *T. hyemalys* [50] *T. pulegioides* [48], *T. capitatus* and *T. herba-barona* [28].

Referring to literature, several major compounds were identified for the EO of this plant; previous studies confirm the predominance of pulegone as major compound: 73.40% on populations of Uruguay [51]; 85.40% on populations of Morocco [52]; while others have identified piperitone and piperitenone as the two dominant compounds [53, 54]. According to literature, three chemotypes have been established, pulegone type, piperitenone/piperitone type, and isomenthone / neoisomenthol type [55]. Beghidja *et al*. [56] studied the composition of several samples of pennyroyal from eastern Algeria, and claimed that these oils can be classified into two chemotypes: one of pulegone (pulegone with a percentage between 52 and 87%), and a new chemotype poor in pulegone and rich of nonoxygenated terpenic fractions (*α*-pinene, *α*-thujene, *β*-pinene, camphene, sabinene, *β*-phellandrene) and relatively high level of 1,8-cineol.

According to previous works which dealt with this plant from Algeria, it seems that there were some differences in the composition of the main compounds and which were related to the regions of collection [42, 57]. Indeed, Dob *et al*. identified for a sample of *T. algeriensis* (Medea - Algeria) the following major compounds: linalool (47.30%), thymol (29.20%) and *p*-cymene (6.80%) [57]; While Hazzit *et al*. have suggested the existence of two chemotypes (samples were collected from the Chrea National Park, and the Chlef regions); the first was characterized by terpinyl acetate (18.00%), *trans*-nerolidol (12.60%), *α*-pinene (11.10%), borneol (9.00%) and bornyl acetate (7.70%), while the second has presented the following major compounds: terpinen-4-ol (10.60%), camphor (10.10%), *p*-cymene (9.90%), *α*-pinene (6.50%) and 1,8-cineole (6.50%) [42]. Comparing the founding of these In a matter of fact, the difference in composition of the EOs of *T. algeriensis* might be due to several factors, such as climate, season of harvest, stage of development and even genetic profiles of the species [26, 50, 58-60].

4.2. Antioxidant Activity

The obtained results suggested that when the EOs of the studied plants exhibited high antioxidant activity in term of DPPH assay, they simultaneously exhibited low activity in term of phosphomolebdenum assay; this finding could be explained by the different reactions mechanisms involved in each assay and related to different active chemicals in each case.

4.2.1. DPPH Assay

The antioxidant activity of the EO of *T. vulgaris* is found to be four times more active than the EO of *T. algeriensis* (Djelfa), and twelve times more active than the EO of *M. pulegium*.

Since the results of the investigated antioxidant activity showed a large variation in the values of EC_{50} and which were depending on the nature of the studied plant, it was mandatory to try to find a correlation between the chemical composition of the EOs and their antioxidant activity power AAP ($=1/EC_{50}$) values. This was carried out by applying statistical methods involving Principal Component Analysis PCA (Fig. **1**). It was found that highest value of AAP (as it was the case of *T. vulgaris*) was correlated with the previously major identified components: *p*-cymene, thymol, linalool, thymol-ethyl-ether and *γ*-terpinene. At the opposite, *T. algeriensis* and *M. pulegium* presented the lowest activities, and where each correlated with their main EOs components, *i.e.* *α*-terpinenyl acetate and pulegone, respectively. Although, terpinen-4-ol and *α*-terpineol were minor compo-

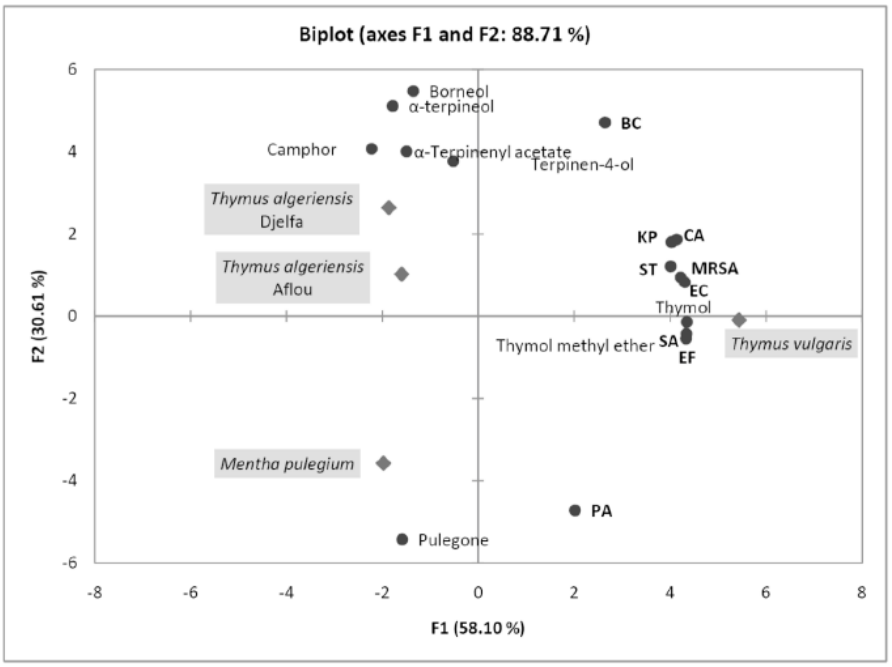

Fig. (2). Two dimensional plot on axes F1 and F2 of the essential oil samples end their antimicrobial activity (disk diffusion test) using Principal Component Analysis.

nents for *T. algeriensis* and *M. pulegium*, their relatively high percentages was also correlated with low antioxidant activity. Finally, *T. algeriensis* from Aflou, had presented practically no activity, and its EO was correlated with the presence of simultaneously higher percentages of *α*-pinene, camphene, myrcene, eucalyptol, camphor and borneol.

4.2.2. Phosphomolybdenum Assay

First, let's mention that vitamin E (antioxidant of reference) which was used for comparison is almost 2 times less active than ascorbic acid (Table **3**). Second, the values of the antioxidant power for this assay were completely different to those found using DPPH assay. This time, *T. algeriensis* (Aflou) and *M. pulegium* presented almost similar activities and were the most active EOs (6 to 7 times less active than ascorbic acid). At the opposite, *T. vulgaris* EO exhibited the lowest antioxidant power (almost 10 times less active than ascorbic acid).

It was found that highest reduction power of the EOs of *T. algeriensis* (Djelfa) is highly correlated with *α*-terpinenyl acetate, terpinen-4-ol and *α*-terpineol. In another hand both *T. algeriensis* (Aflou) and *M. pulegium* with practically close reduction powers were correlated positively with different components: *α*-pinene, camphene, myrcene, eucalyptol, camphor and borneol for *T. algeriensis* (Aflou); and only pulegone for *M. pulegium*. At the contrary, *T. vulgaris* with the lowest reduction power was related to higher percentages of *p*-cymene, thymol, linalool, thymol-ethyl-ether and *γ*-terpinene.

4.3. Antimicrobial Activity

According to Fig. **2**, the *T. vulgaris* was found to be the most active EO and exhibited important resistance against most of studied bacteria: *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Klebsiella pneumoniae* and *Candida albicans*. According to PCA analysis, this activity is due mainly to presence of thymol and thymol-methyl-ether. Alternatively, the activity against *Bacillus cereus* was correlated with high percentages of camphor, borneol, *α*-terpinenyl acetate, terpinen-4-ol and *α*-terpineol. Finally, the relatively slight improvement of activity of *M. pulegium* EO against *Pseudomonas aeruginosa* is probably due to the presence of the high percentage of pulegone. As a result, and based on the PCA, it seems that pulegone does not contribute to any practical antimicrobial activity, since high percentage of pulegone imply slight improvement of this activity.

4.3.1. Disc Diffusion Test

The analysis of the simultaneous values of MIC and MIB with the chemical composition of the EOs against different bacteria using PCA revealed that the most active EOs plants were those of *T. algeriensis* (Djelfa & Aflou) (Fig. **3**). This strong activity was in strong correlation with several chemicals: eucalyptol, camphor, myrcene, *α*-pinene, camphene, *α*-terpineol, *α*-terpinenyl acetate and terpinen-4-ol. For *M. pulegium* where its EO presented relatively moderate activity (MBC) against *Bacillus cereus*, *Staphylococcus aureus* and *Enterococcus faecalis*; this moderate activity is believed to be correlated to high content of pulegone.

4.3.2. Antifungal Activity

It was found that the EOs were exhibiting high MIC and at the same time presenting low or undetected activities in term of MIB values.

The results of PCA showed (Fig. **4**), that the highest MIC values were correlated for different sets of EOs components for both *T. algeriensis* (Djelfa) and *M. pulegium*. Moreover, for almost the same components observed previously with disc diffusion test, the high MIC value for *M. pulegium* was attributed to the high composition of pulegone, but for *T.*

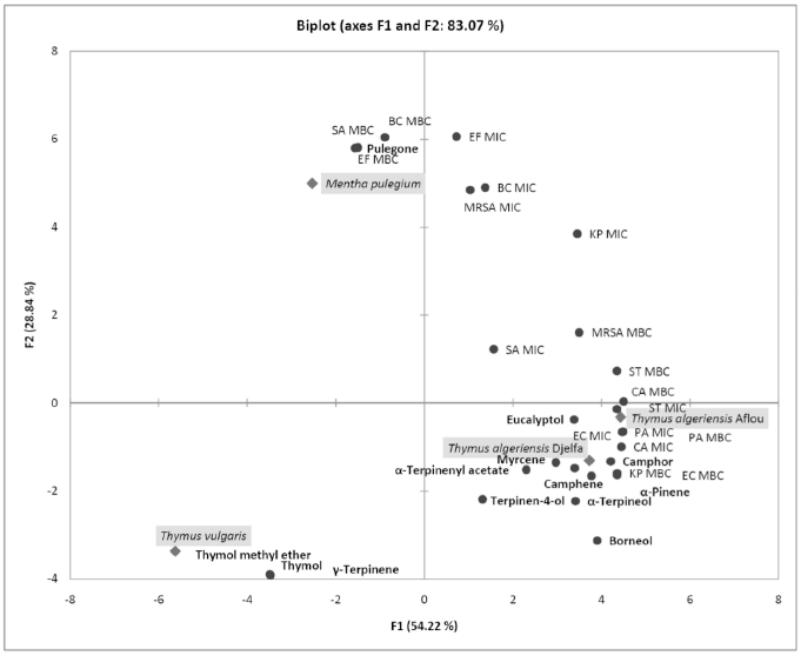

Fig. (3). Two dimensional plot on axes F1 and F2 of the essential oil samples end their antimicrobial activity (minimal inhibitory and bactericidal concentrations) using Principal Component Analysis.

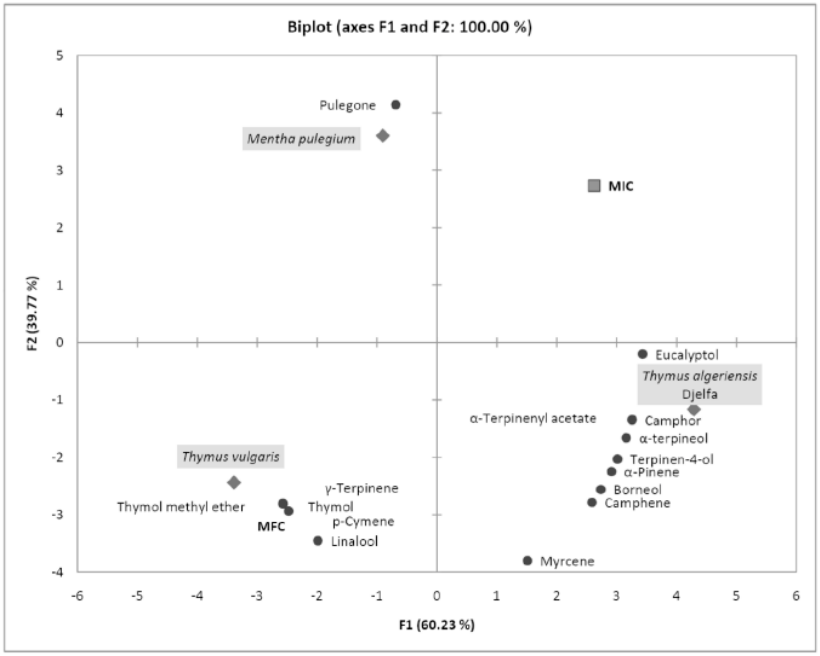

Fig. (4). Two dimensional plot on axes F1 and F2 of the essential oil samples end their antifungal activity using Principal Component Analysis.

algeriensis (Djelfa) it was due to the presence of eucalyptol, *α*-terpinenyl acetate, terpinen-4-ol, *α*-terpineol, *α*-pinene, borneol and camphene. The EO of *T. vulgaris* with the lowest value of MIC was correlated with the following components: thymol-ethyl-ether, *γ*-terpinene, thymol, *p*-cymene and linalool. At the opposite, the EO of this later plant presented a good MFC value in comparison with the rest of the studied plants, in which they did not offer any measurable activity in the range of the studied concentrations. This high activity is due to the presence of thymol-ethyl-ether, *γ*-terpinene, thymol, *p*-cymene and linalool.

CONCLUSION

As a conclusion, the EOs tested in this study showed moderate to strong antioxidant and antimicrobial activities. Almost all EOs exhibited a radical scavenging activity (DPPH assay), with different values of EC_{50}. *T. vulgaris* EO had the lowest value of EC_{50} which makes it the strongest EO among the tested samples. For antimicrobial activity, all the samples had inhibited the growth of the tested microbial strains, with the strongest antibacterial and antifungal activity attributed to *T. vulgaris* EO. The study of the relation

between the chemical composition of the EOs and the antioxidant or the antimicrobial activity revealed the presence of different strong correlations with some major identified compounds in each case of study. It could be concluded that, thyme, *T. vulgaris* EO were effective as antioxidant activity due, in part, to the presence of several compounds, like thymol, thymol-methyl-ether, linalool and carverol, in their chemical compositions. They could be used as flavoring agents and good sources of antioxidants in making food with healthy benefits and good sensory acceptability. Further studies are required to collect more data about the toxicity of these EOs.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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