Original Research Article

Antioxidant and antimicrobial activities of Thymus vulgaris L. *essential oil growing wild in Tunisia*

.

ABSTRACT

Chemical composition, antioxidant, and antimicrobial activities of Tunisian Thymus vulgaris essential oil (TVEO) were investigated. Results revealed that TVEO has as carvacrol (67.33%) chemotype, it was equipped with an important antioxidant capacity that is better (P<0.05) than synthetic antioxidants (BHT, BHA, Vitamin C and EDTA) except for superoxide anion test. A higher antimicrobial activity was also observed with IZ, MIC and MBC values of bacterial strains were ranged from 10.33±0.57 to 37.33±0.57mm; 0.019 to 0.078 mg/mL and 0.039 to 0.31 mg/mL respectively. But those of fungal strains were varied between 24.66±1-47.33±1.53 mm; 0.004-0.078 mg/mL and 0.019-0.15 mg/mL respectively.

Keywords: *Thymus vulgaris, essential oil, antioxidant, antibacterial, antifungal*

1. INTRODUCTION

Medicinal herbs and aromatic plants are considered an important reservoir of bioactive molecules widely used to treat various diseases [1-3]. They play a notable role in allelopathic communication and exhibited effective and significant biological activities [4-7]. Plant-extracted phytochemicals, and essential oils (EOs) have long been a source of therapeutic compounds, and for a long time, been recognized to displayed various biological effects [8-10]. Among potential new drug sources, aromatic plants rich in essential oils (EOs) have received great attention among scientists, and pharmaceuticals industry due to their economic viability, low toxicity, and their potential as alternatives to synthetic agents [11,12]. EOs are important due to their application as antioxidant agents against the phenomenon of oxidative that causing many health problems, like inflammations, cancer, neurodegeneration and cardiovascular diseases. In addition, plants can produce a large variety of secondary metabolites that affect the oxidative stability of EOs and have good antioxidant properties [13]. Potential antioxidants break down the radical chain reaction and act as radical scavengers. Additionally, the high potency of natural antimicrobials linked to their hydrophobic nature, allowed them the property to hamper the spread of multidrug-resistant (MDR) bacteria, which makes it imperative to identify new classes of antimicrobials and compounds that inhibit resistance mechanisms [14-16].

Currently, a strong consumer tendency to seek a more natural diet has prompted the research, development and application of new natural products with antimicrobial and antioxidant activities. EOs are in fact an attractive choice to replace synthetic preservatives which can provide flavouring and preservation [17]. A significant number of EOs have shown their significant effects in food packaging systems on the inhibition of bacterial growth and on oxidative stability [18,19]. On the other hand, EOs are known by their important in vitro antimicrobial and antioxidant activities [20-22], but less studies were shown the exploitation of their proprieties in seafood conservation, despite that they were classified in the GRAS

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substances list which it makes them to be used like a conservator. In consequence, the purpose of this study in its first phase was to provide the chemical composition by GC-MS and evaluate the in vitro antioxidant and antimicrobial activities of Tunisian *Thymus vulgaris* essential oil (TVEO).

2. MATERIAL AND METHODS

2.1. Plant material and extraction of essential oil

T. vulgaris plants were freshly collected from the mountainous region of Zaghouan (Tunisian locality) (upper semi-arid zone, latitude 36°26'N, longitude 10°46'E, Emberger's pluviothermic coefficient = 55.44, Altitude = 500 m, Rainfall = 400-500 mm/year). The specie was identified according to the flora of Tunisia. Arial part (stems, leaves and flowers) were dried at room temperature. The 100 g of aerial part were subjected to hydrodistillation for 3 hours with 500 ml distilled water using a Clevenger-type apparatus according to the European Pharmacopoeia. The distilled essential oil was dried over anhydrous sodium sulfate, filtered and stored at 4°C. Yield based on dried weight of the sample was calculated.

2.2. Essential oil analysis

2.2.1. Gas chromatography/mass spectrometry (GC/MS)

As described by Hajlaoui *et al.* [20], a Hewlett-Packard 5890 series II gas chromatograph equipped with HP-5MS capillary column (30m×0.25mm i.d., film thickness 0.25 µm; Hewlett-Packard) and connected to a flame ionization detector (FID).

2.3. Antioxidant activity

2.3.1. Scavenging ability on DPPH radical

$DPPH^{\cdot}$ quenching ability of essential oil was measured according to Felhi et al. [23]and Bakari et al. [24]. The antiradical activity was expressed as IC_{50} (µg/ml), the extract dose required to cause a 50% inhibition

2.3.2. Superoxide anion radical-scavenging activity

Superoxide anion scavenging activity was assessed using the method described by Duh *et al.* [25]. Evaluating the antioxidant activity was based on IC50.

2.3.3. Reducing power

The ability of the EO to reduce Fe^{3+} was assayed using the method described by Hajlaoui *et al.* [26].

2.3.4. Chelating effect on ferrous ions

The use of the ferrozine method assessed to evaluate in vitro chelating power. Indeed, free iron in the medium will be stabilized by ferrozine forming a complex

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ferrozine-Fe^{2+} purple through the same protocol as described by Felhi et al. [23]. and Bkari et al. [24].

2.3.5. β-Carotene-linoleic acid model system (β-CLAMS)

The β-CLAMS method by the peroxides generated during the oxidation of linoleic acid at elevated temperature. In this study the β-CLAMS was modified for the 96-well micro-plate reader as described by Mseddi et al. [27]. The results are expressed as IC_{50} values (µg/ml). All samples were prepared and analyzed in triplicate.

2.4. Antimicrobial activity

2.4.1. Microorganisms

In this study, the microorganisms tested belonging to 24 reference bacterial strains and 16 fungal strains that are presented respectively in tables 3 and 4. Bacterial strains are divided into 6 Gram-positive and 18 Gram-negative bacteria including 14 strains belonging to the genus *Vibrio*.

2.4.2. Disc-diffusion assay

Antimicrobial activity testing was done according to the protocol described by Vuddhakul *et al.* [28] and modified by Hajlaoui et *al*. [21] for *Vibrio* spp. strains. After incubation at 37°C for 18 to 24 h, the diameter of inhibition zone was measured with 1 mm flat rule and the diameters were interpreted according to the Committee of the French society of the antibiogram [29].

2.4.3. Micro-well determination of MIC, MBC and MFC

Minimal inhibition concentration (MIC), minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC) values were determined for all bacterial and fungal strains used in this study as described by Hajlaoui *et al.* [20].

2.5. Statistical analysis

All the experiments were conducted in triplicate and average values were calculated using the SPSS 26.0 statistics package for Windows. The differences in mean were calculated using the Duncan's multiple-range tests for means with 95% confidence limit ($P \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1. Essential oil composition

The GC–MS analysis revealed the identification of 23 compounds representing 98.22% of the total TVEO (Table 1 with the major constituents were carvacrol (67.33%) followed by β-phellandrene (7.10%), α-terpinolene (6.31%), β-caryophyllene (2.59%) and myrcene (2.34%). The oil was dominated by the monoterpene fraction (95.05%). In fact, the oxygen-

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containing monoterpene being the most representative group (72.15%), monoterpene hydrocarbons fraction in order of 22.88%, however sesquiterpenes fraction attained only 3.18% in the oil.

Table 1. Chemical composition, retention index (RI) and percentage composition of the TVEO.

N°	Compound	(RI) HP-5	%	Identification
1	α-Thujene	928	1,93	MS, RI
2	α-Pinene	935	1,12	MS, RI
3	Camphene	950	0,26	MS, RI
4	β-Pinene	978	0,73	MS, RI
5	Myrcene	991	2,34	MS, RI
6	α-Phellendrene	995	0,21	MS, RI
7	α-Terpinene	1006	1,62	MS, RI
8	p-Cymene	1015	0,14	MS, RI
9	β-Phellandrene	1027	7,10	MS, RI
10	γ-Terpinene	1031	1,15	MS, RI
11	Trans-sabinene hydrate	1047	0,09	MS, RI
12	α-Terpinolene	1061	6,31	MS, RI
13	Linalool	1089	0,21	MS, RI
14	Cis-sabinene hydrate	1100	1,92	MS, RI
15	Trans-p-Menth-2-en-1-ol	1148	0,34	MS, RI
16	Borneol	1169	0,71	MS, RI
17	α-Terpineol	1180	1,35	MS, RI
18	Transpiperitol	1198	0,21	MS, RI
19	Linalylacetate	1257	0,48	MS, RI
20	Carvacrol	1314	67,34	MS, RI
21	α-Copaene	1361	0,06	MS, RI
22	β-Caryophyllene	1427	2,59	MS, RI
23	α-Humulene	1446	0,05	MS, RI
Total identified		98,22		
Yield (g/100 g dry weight)		3,3		
Monoterpene hydrocarbons		22,88		
Oxygenated monoterpenes		72,15		
Sesquiterpene hydrocarbons		3,18		
Oxygenated sesquiterpenes		0		

The components and their percentages listed in order of their elution on apolar column (HP-5); MS: mass spectra; RI: retention index.

According to this study, the TVEO chemotype of this oil is carvacrol. Based on literature survey, different studies showed that TVEO from Tunisian provenances have a carvacrol chemotype ranged from 60 to 77% respectively for Monastir [30] and Sidi Bouzid [21] provenances. Also, this chemotype was defined in composition of *T. capitatus* EO harvested from Jendouba (interior north), Haouaria (littoral north) and Ain Tounine (littoral south) with respectively 66, 73 and 74% [31]. In Iran, Ardalan *et al.* [32] has identified twenty-nine components in TVEO of which representing 99.60%, 93.11%, and 97.54% of the oils of Estahban, Shiraz and greenhouse samples, respectively. The major constituents of Estahban sample were thymol (58.46%), γ-terpinene (15.06%), *p*-cymene (8.41%), carvacrol (2.07%) and terpinolene (2.05%). The major components of Shiraz sample were thymol (51.76%), *p*-cymene (11.04%), γ-terpinene (7.67%), terpinolene (2.89%) and carvacrol (2.78%). The major components of greenhouse sample were thymol (53.45%), *p*-cymene (12.37%), γ-terpinene (7.88%), terpinolene (3.12%) and carvacrol (2.76%).

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Generally, it appears that chemical composition of the EOs obtained from *Thymus* genus has been widely investigated. In fact, Ruiz-Navajas *et al.* [33] showed that main components of *T. moroderi* were camphor (26.74%), 1.8-cineol (24.99%), myrcene (5.63%) and α-pinene (4.35%) while in *T. piperella* the predominant compounds were carvacrol (31.92%), *p*-cymene (16.18%), γ-terpinene (10.11%) and α-terpineol (7.29%). On the other hand, Bagamboula, *et al.* [34] reported that no major differences observed on Eos from commercial thyme and those obtained by steam distillation of finely ground dried leaves. Only linalool and γ-terpinene were found in higher concentrations in the commercial oil. In another study the same authors investigated the oil of thyme and identified 28 compounds (92.6% of the total oil) and thymol (32.23%), γ-terpinene (21.19%) and *p*-cymene (20.27%) being the major constituents. Edoardo *et al.* [35] identified in thirty samples of EOs thyme collected in Italy 46 components covering more than 96% of the total composition. Eugenia *et al.*[36] analyzed the composition of the EO of *T. pulegioides* from Portugal where the oil was characterized by high amounts of thymol (26.0 %), carvacrol (21.0%) and its biogenetic precursor'sγ-terpinene (8.8%) and *p*-cymene (7.8%).

3.2. Antioxidant activity

3.2.1. DPPH radical scavenging activity

The effect of antioxidants on DPPH radical scavenging activity was thought to be due to their hydrogen-donating ability. Free radical scavenging activities of TVEO measured by DPPH assay were shown in Table 2. The oil was able to reduce the stable free radical DPPH with an IC_{50} value of 0.7±0.25μg/ml. This oil has a significant ability to neutralize the DPPH radial and therefore an important antioxidant activity significantly higher than the BHT standard used as positive control (11.5±0.62μg/ml).

This strong activity is comparable with those exhibited by various oils of Tunisian *Thymus* chemotypes [21]. The richness of this oil in oxygenated monoterpenes (72.15%) reinforce its antioxidative properties [21,22] and especially, the presence of carvacrol as major components in (67,33%) which may act as radical scavenging agent [21].

3.2.2. Superoxide anion radical-scavenging activity

As shown in Table 2, the Duncan statistically test revealed that activity of TVEO (IC_{50}=1.9 ± 0.3μg/mL) is found to be more effective than synthetic antioxidant BHT (IC_{50}=1.5 ± 0.2μg/mL). Moreover, TVEO capable of scavenging reactive species such as superoxide via a mechanism of electron/hydrogen donation and should be able to prevent oxidative damage of the major bio-molecules.

This important activity is strongly linked to the chemical composition of the oil and their wealth was mainly monoterpene compounds such as majority carvacrol, α-Terpinolene, γ-Terpinene, β-Phellandrene. In line with our findings, few studies reported that EOs containing phenolic compounds also have interesting antioxidant potentials [22]

3.2.3. Reducing power

Table 2 showed reductive potential of the studied oil whenever the measured value EC_{50}=0.28±0.02 μg/mL. The value showed a strong ferric ion reducing capacity more efficiently than positive controls BHT (EC_{50}=23±1μg/mL) and vitamin C (EC_{50}=37±2μg/ml). Bounatirou *et al.* [31] studying the reductive capacity of *T. capitatus* EO harvested from different Tunisian provenance showed that the extracted oils during the post-flowering stage

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had a reductive potential similar to BHA and BHT. This antioxidant activity is also attributed to the presence of natural antioxidants such as phenolic compounds [33].

3.2.4. Chelating effect on ferrous ions

As shown in Table 2, the TVEO has an important chelating ability (EC_{50}= 1.36 ± 0.3µg/mL). This ability is twenty times larger than the positive control EDTA (32.5 ± 1.32 µg/ml). In fact, several studies focus on *Thymus* genus essential oils showed that these oils have a stronger chelating power as compared to vitamin C, BHT and BHA [33,37]. Generally, the high ferrous ion chelating abilities of the EOs from *Thymus* genus would be beneficial in numerous fields such as food and pharmaceutical industry.

3.2.5.β-Carotene-linoleic acid model system

The obtained IC_{50} value (Table 2) of 12.2±0.65 µg/mL is more important than synthetic antioxidants with IC_{50}values in the range of 75±1 and 48±2.29 µg/mL respectively for the BHT and BHA. Thus, this important antioxidant activity of *Thymus* EO, estimated by the different tests, was in relation with chemical composition, which showed a predominance of phenolic compounds such as carvacrol [37,38].

Table 2. DPPH test (IC_{50}), superoxide anion radical-scavenging activity (IC_{50}), reducing power (EC_{50}), chelating power (EC_{50}), and β-carotene (IC_{50}) of TVEO, and authentic standards (BHT, BHA, EDTA and ascorbic acid). Values are in µg/mL.

	DPPH	$O_2^{\cdot-}$	RP	CP	β-carotene
TVEO	0.7 ^{b} ±0.25	1.9 ^{a} ±0.3	0.28 ^{c} ±0.02	1.36 ^{b} ±0.3	12.2 ^{c} ±0.65
BHT	11.5 ^{a} ±0.62	1.5 ^{a} ±0.2	23 ^{b} ±1		75 ^{a} ±1
BHA	-	-	-	-	48 ^{b} ±2.29
Vitamin C	-	-	37 ^{a} ±2	-	-
EDTA	-	-	-	32.5 ^{a} ±1.32	-

Means (three replicates) followed by least one same letter are not significantly different at P<0.05

3.3 Antimicrobial activity

3.3.1. Antibacterial activity

The antibacterial activity of TVEO was assayed *in vitro* by following the diffusion in agar disc method using twenty-four bacteria associated with human pathogenic. As can be seen in Table 3, TVEO had an excellent inhibitory effect on all bacteria strains. Inhibition halos was ranged from 19±1mm (*E. feacalis* ATCC 29212) to 37.33±0.57mm (*B. cereus* ATCC 11778) for Gram positive bacteria and was ranged from 10.33±0.57 mm (*P. aeruginosa* ATCC 27853) to 35.66±0.57mm (*V. furnisii* ATCC 35016) for Gram negative bacteria (Figure 1) with higher potency than the commercial antibiotics, gentamicin and tetracycline against the major strains. As shown previously, the antibacterial activity of several oils obtained from

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other thyme varieties has been studied. Ruiz-Navajas *et al.* [33]tested antibacterial activity of two *Thymus* endemic species EOs collected from southeast of Spain: *T. moroderi* and *T. piperella* which had an inhibitory effect on 5 assayed bacteria, with inhibition halos ranged from 16.00 mm for *A. denitrificans* to 45.00 mm for *A. hydrophila.* Moreover, the study of Cetin *et al.* [39] focus on antibacterial activity of *T. sipyleus* subsp. *Sipyleus* var. *rosulans* EO from Turkey revealed a highest inhibitory effect on *Pseudomonas pseudoalkaligenes* (59 mm) and *S. aureus* (56 mm), followed by *B. subtilis, P. aeruginosa*, *S. pyogenes*, and *P. vulgaris* and a lowest inhibitory effect was marked on *Enterobacter cloacae*.

Table 3. Zones of growth inhibition (IZ mm±SD), minimal inhibition concentration (MIC mg/mL), minimal bactericidal concentration (MBC mg/mL) and ratio MBC/MIC showing antibacterial activity for TVEO against human pathogenic bacteria compared to that of positive standard antibiotic (Gentamycin, Tetracycline).

	TVEO				Antibiotic	
Bacteria species	**IZ[a]**	**MIC**	**MBC**	**MBC/MIC**	**IZ[b]**	**MIC**
***S. epidermidis* CIP106510**	28.66±0.57^{d}	0.019	0.078	4	21.33 ± 0.58	0.031
***S. aureus* ATCC25923**	25.66±1.15^{e}	0.019	0.039	2	32.67 ± 0.58	0.015
***M. luteus* NCIMB 8166**	28±0^{d}	0.039	0.078	2	27.67 ± 1.53	>0.003
***E. feacalis* ATCC 29212**	19±1^{g}	0.078	0.15	2	26 ± 1	0.007
***B. cereus* ATCC 11778**	37.33±0.57^{a}	0.019	0.078	4	26 ± 1	0.007
***B. cereus* ATCC 14579**	36.33±1.54^{ab}	0.039	0.078	2	28 ± 1	0.007
***E. coli* ATCC 35218**	24.66±0.57^{ef}	0.078	0.31	4	27.33±0.58	>0.003
***L. monocytogenes* ATCC19115**	30.33±0.57^{c}	0.039	0.15	4	37.67±0.58	0.015
***P. aeruginosa* ATCC 27853**	10.33±0.57^{k}	ND	ND	ND	21 ± 1	>0.078
***S. typhimurium* LT2 DT104**	19.66±0.57^{g}	0.078	0.15	2	30.33 ±0.58	>0.03
V. cholerae ATCC 9459	31±0.58^{c}	0.039	0.15	4	25±1	0.31
V. parahaemolyticus ATCC 17802	15±1^{l}	0.078	0.31	4	21±0	0.078
V. parahaemolyticus ATCC 43996	17.66±0.57^{h}	0.039	0.31	8	20±0	0.078
V. alginolyticus ATCC 33787	13±0^{j}	0.078	0.31	4	20±0	0.15
V. alginolyticus ATCC 17749	17.33±0.58^{h}	0.078	0.31	4	7±0	0.15
V. vulnificus ATCC 27562	20±0^{g}	0.039	0.15	4	13.33±0.57	0.31
V. harveyi ATCC 18293	16±0^{i}	0.078	0.31	4	18.33±0.58	0.078
V. proteolyticus ATCC 15338	25.33±0.57^{e}	0.019	0.15	8	20±1	0.078
V. furnisii ATCC 35016	35.66±0.57^{b}	0.019	0.078	4	20.33±0.57	ND
V. mimicus ATCC33653	24±0^{f}	0.039	0.15	4	20±0	ND
V .furnisii ATCC 33813	16±1^{l}	0.078	0.31	4	19±0	0.078
V. natrigens ATCC 14048	24±1^{f}	ND	ND	ND	21±0	ND
V. carhiaccae ATCC 35084	17.66±0.57^{h}	0.078	0.31	4	18.33±0.58	0.15
V. fluvialis ATCC 33809	19.33±1.15^{g}	0.078	0.31	4	18.33±0.57	0.31

ND: not determined; SD: Standard deviation; IZ[a]: Inhibition zone in diameter (mm) around the discs (6mm) impregnated with 10 µl of essential oil; IZ[b]: Inhibition zone in diameter (mm) of Gent= Gentamycin (10 µg/disc) and Tet= Tetracycline (30µg/disc) were used as positive reference standards antibiotic discs; MBC/MIC: approximate values.

The majority tested strains showed greater sensitivity against TVEO. In fact, for the Gram-positive bacteria, MIC values were ranged from 0.019 to 0.078 mg/mL for studied oil, while MBC values were ranged from 0.039 to 0.15 mg/mL. Concerning Gram negative bacteria including *Vibrio* strains, MIC and MBC values were ranged respectively from 0.019 to 0.078 mg/mL and from 0.078 to 0.31 mg/mL. This sensitivity decreases specifically in *Vibrio* spp. In fact, values of MIC and MBC recorded in this genus were higher in comparison with other strains (0.078 and 0.31mg/mL). Among *Vibrio* spp. strains, *V. furnissi* ATCC 153338 proved the most sensitivity against the oil with MIC= to 0.019 mg/mL and MBC= 0.078 mg/mL. While *V. alginolyticus* ATCC 33787 was the most resistant strain with MIC and MBC values

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respectively of 0.078 and 0.31 mg/mL. In addition, the oil has similar activity against *V. cholerae* ATCC 9459, *V. parahaemolyticus* ATCC 17802 and *V. mimicus* ATCC33653 strains.

In comparison with literature data, our results showed similarities. In fact, Snoussi *et al.* [40] demonstrated that TVEO (local market from Mahdia, Tunisia) exhibited a high range of anti-*Vibrio* spp. strains, especially against food-borne pathogen *Vibrio parahaemolyticus* with a MIC and MBC values were interestingly low (MIC 0.078-0.156 mg/mL and MBC >0.31-1.25 mg/mL). These authors were also reported that this important activity was related to chemical composition of thyme oil rich in carvacrol (60.27%), γ-terpinene (11.20%), *p*-cymene (7.58%) and bornyl acetate (4.93%). Furthermore, anti-*Vibrio alginolyticus* activity of TVEO (from Sidi Bouzid, Tunisia) was tested by Hajlaoui *et al.* [21].

Several studies evaluate thyme oil antimicrobial activity harvested in different Mediterranean regions. For example, the study of Fadli *et al*. (2012) [41] shows that the two oils of *T. maroccanus* and *T. broussonetii* harvested from Essaouira region (South-West of Morocco) and from Ourika valley (High Atlas of Morocco) respectively showed in interesting antibacterial activity. The two essential oils inhibited Gram-negative bacteria (*E. coli*, *E. cloacae*, *K. pneumoniae*) at concentrations ranging from 0.342 to 0.685 mg/mL. In contrast, Gram-positive bacteria were inhibited with MICs ranging from 0.086 mg/mL to 0.342 mg/mL. TVEO collected from the cultivated fields of the Botanical Gardens, University of Agriculture; Faisalabad, Pakistan showed an important antibacterial activity with a MIC ranged from 0.07 to 1.25 mg/mL [41].

From our study, the ratio MBC/MIC values obtained are ≤ 4 (Table 3) for majority of tested strains. This result indicates a bactericidal effect of this oil. Whereas, TVEO has a bacteriostatic effect only against *V. parahaemolyticus* ATCC43996 and *V. proteolyticus* ATCC 15338. This study indicates that TVEO exhibited a significant antibacterial activity against all tested bacteria which can be explained by the richness of this oil on oxygen monoterpene group (72.15%). Several studies have shown the importance of the fraction oil in inhibiting microorganism's expansion [21,41].

Fadli *et al*. [41] studying the correlation between the chemical composition of *T. maroccanus* and *T. broussonetii* EOs and their antimicrobial effect showed that the activity level could be attributed to the presence of high concentrations of carvacrol.

3.3.2. Antifungal activity

The inhibitory effects of the EO isolated from *T. vulgaris* on the growth of 16 pathogenic fungal species are shown in Table 4. Results revealed an important inhibitory effect of the oil against tested fungi. In fact, obtained values of IZ, MIC and MFC were respectively ranged from 24.33±1.53 to 48.33±1.53 mm (figure 1), 0.004 to 0.078mg/mL and >0.009 to 0.15mg/mL. These values showed that the oil exhibit a more important antifungal activity than synthetic antifungal amphotericin-B. IZ, MIC and MFC obtained values of amphotericin-B were ranged from 11-24±0.57 mm, 0.009-0.078mg/mL and 0.019-0.31mg/mL, respectively. Similarly, Fadli *et al*. [41] showed that anticandidal activity of *T. maroccanus* and *T. broussonetii* EOs was lower than amphotericin-B and fluconazol. In fact, IZ values are respectively 44.5±0.35, 38.5±0.70, 22.5±0.70 and 16.5±0.70 mm. Indeed, MIC values are 0.25 mg/mL for *T. maroccanus* and *T. broussonetii* oils and 16 mg/mL for amphotericin-B and fluconazole. Majority of previous studies showed that *Thymus* genus Eos have a great antifungal potential, thanks to their wealth of oxygenated monoterpene and particularly phenol compounds such as carvacrol [21,41].

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Table 4. Zones of growth inhibition (IZ mm±SD), minimal inhibition concentration (MIC mg/mL), minimal fungicidal concentration (MFC mg/mL) and ratio MFC/MIC showing antifungal activity for TVEO against human pathogenic fungal compared to that of positive standard antifungal (Amphotericin B).

	TVEO				Antifungal (Amp B)			
Fungal species	IZ[a]	MIC	MFC	MFC/ MIC	IZ[b]	MIC	MFC	MFC/ MIC
Yeast strains								
***C. albicans*ATCC 90028**	48.33±1.53[a]	0.019	>0.078	4	11±0	0.078	0.31	4
***C. glabrata*ATCC 90030**	47.33±1.53[ab]	0.009	0.019	2	14.33±0.57	0.009	0.078	9
***C. parapsilosis*ATCC 22019**	37±1[e]	0.009	0.039	4	10.33±0.57	0.039	0.078	2
***C. krusei*ATCC 6258**	46.33±0.53[ab]	0.004	>0.009	2	12±0	0.009	0.019	2
C. tropicalis	43.33±2.51[cd]	0.004	0.019	5	24±0	0.019	0.039	2
C. glabrata	37.33±0.57[e]	0.039	0.078	2	22±1	0.039	0.15	4
C. albicans	42.66±1.15[cd]	0.019	0.039	2	20 ±0	0.078	0.15	2
C. Parapsilosis	45±0[bc]	0.009	0.039	4	23±0	0.078	0.15	2
C. sake	43.33±1.58[cd]	0.009	0.019	2	23±0	0.039	0.078	2
C. kefyr	27.00±2.00[f]	0.078	0.15	2	22.33±0.57	0.078	0.15	2
C. holmii	24.33±1.53[g]	0.039	0.15	4	22 ± 0	0.039	0.15	4
Saccharomyces cerevisae	41.33±2.52[d]	0.009	0.019	2	18±0	0.009	0.039	4
Dermatophytic strains								
Trichophyton violaceum	47±0[ab]	0.009	0.039	4	19.33±0.57	0.078	0.31	4
Trichophyton rubrum	41.33±1.58[d]	0.009	0.078	9	24 ± 0.57	0.039	0.15	4
Trichophyton mentagrophytes	43.33±2.51[cd]	0.009	0.039	4	22 ± 0	0.078	0.15	2
Microsporum canis	24±1[g]	0.039	0.15	4	21 ± 0	0.039	0.078	2

SD: Standard deviation; IZ[a]: Inhibition zone in diameter (mm) around the discs (6mm) impregnated with 10 µl of essential oil; IZ[b]: Inhibition zone in diameter (mm) of Amp B= Amphotericin B (20 µg/disc) used as positive reference standards antifungal disc; MFC/ MIC: approximate values.

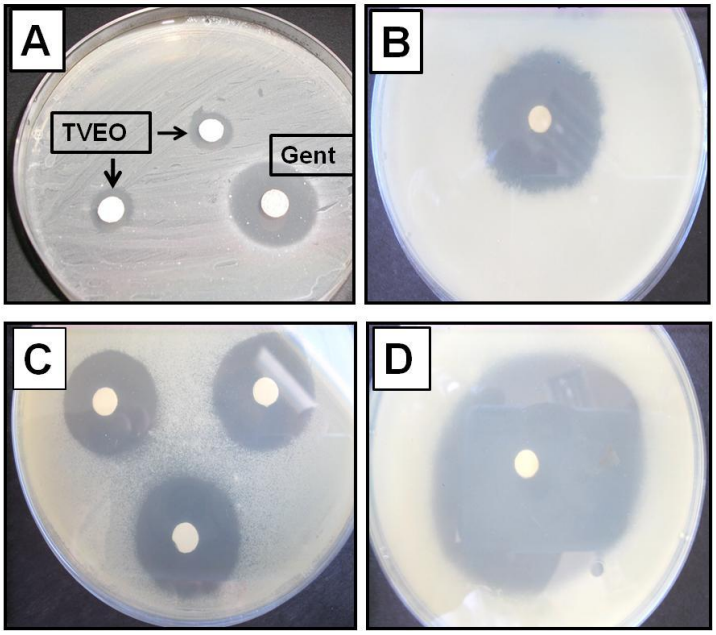

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Fig. 1. Agar plate pictures representing the range of inhibition zone resulted after using TVEO for bacteria strains (A: *P. aeruginosa* ATCC 27853 and B: *B. cereus* ATCC 11778) and for fungual strains (C:*Microsporumcanis*and D:*C. albicans*ATCC 90028).

According to this study, fungal strains (yeasts and moulds) were more sensitive than bacteria ($Gram^+$ and $Gram^-$) against TVEO. Indeed, the values of IZ, MIC and MFC were lowest for fungi (table 3 and table 4). This result was confirmed by several studies. In fact, Cosentino *et al.* [43] study of Sardinian (Italy) *Thymus* Eos showed values of MFC (225-450 µg/mL) less important than MBC (225-900 µg/mL). In addition, other studies have confirmed the sensitivity of fungal tested strains by other oils. The antimicrobial activity of cumin EO showed effectively fungi sensitivity by MIC and MFC values which were ranged between 0.009-0.078 mg/mL and 0.019 to 0.31 mg/mL, in front MIC and MBC values for bacteria which ranged between 0.039 to 0.31 mg/mL and 0.31 to 1.25 mg/mL [21].

In most tested strains, MFC/MIC Ratio values showed that studied TVEO have a fungicidal effect because they were ≤ 4 (Table 4). In exception, TVEO has a fungistatic effect against *Candida tropicalis* and *Trichophyton rubrum* (MFC/MIC equal to 5 and 9, respectively). These results highlighted the potential utilization of thyme oil in dermatomycoses, which are common infections caused by filamentous fungi and by some yeasts that can be severe in immunocompromised patients.

4. CONCLUSION

The present investigation showed that TVEO is characterized by the abundance of carvacrol (67.33%). For all antioxidant tests activities, this EO showed a more important activities comparing to standards synthetic antioxidant. The studied EO showed high antibacterial and antifungal activities against a wide range of microorganisms known to cause serious infections. Antibacterial activity of this EO seems to be more efficient against *Vibrio* strains, but antifungal ones have a fungicidal effect for the majority of fungal strains. This might be related to its chemical profile.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an

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avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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