

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

ABSTRACT

The aim of this work is to investigate the chemical composition, antioxidant, and antimicrobial activities of Tunisian *Thymus vulgaris* essential oil (TVEO). DPPH, superoxide anion, reducing power, chelating effect on ferrous ions and β -Carotene assays have been employed to determine the antioxidant potential of TVEO. In contrast, 24 reference bacterial strains and 16 fungal strains have been used for the assessment of the antimicrobial activity. 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 ± 0.57 mm; 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. In summary, the obtained data makes TVEO as a good and suitable candidate for its use in food and pharmaceutical purposes.

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

1. INTRODUCTION

Medicinal herbs and aromatic plants are considered as 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 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. Therefore, it is urgent to identify new classes of antimicrobials 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, inhibition of bacterial growth and oxidative stability [18,19]. On the other hand, EOs are known for their important *in vitro* antimicrobial and antioxidant activities [20-22], but less studies were shown the exploitation of their proprieties in seafood conservation. In consequence, the purpose of this study is 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 by Dr. Zouhair Noumi, University of Sfax, Tunisia (Voucher No: H2TV/300). Aerial 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: quenching ability of essential oil was measured according to the previously our work [23,24]. The antiradical activity was expressed as IC₅₀ (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 as described previously [25]. Evaluating the antioxidant activity was based on IC₅₀.

2.3.3. Reducing power

The ability of the EO to reduce Fe³⁺ was assayed as cited previously [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 ferrozine-Fe²⁺ purple through the same protocol as done by our team [23,24].

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 elsewhere [27]. The results are expressed as IC₅₀ values (μ g/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 previously [28,21] 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 as done previously [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-

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	<i>p</i> -Cymene	1015	0,14	MS, RI
9	β -Phellandrene	1027	7,10	MS, RI
10	γ -Terpinene	1031	1,15	MS, RI
11	<i>Trans</i> -sabinene hydrate	1047	0,09	MS, RI
12	α -Terpinolene	1061	6,31	MS, RI
13	Linalool	1089	0,21	MS, RI
14	<i>Cis</i> -sabinene hydrate	1100	1,92	MS, RI
15	<i>Trans</i> - <i>p</i> -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, for the same EO, some authors reported the identification of twenty-nine components representing 99.60%, 93.11%, and 97.54% of the oils of Estahban, Shiraz and greenhouse samples, respectively [32]. 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%).

Generally, it appears that chemical composition of the EOs obtained from *Thymus* genus has been widely investigated. In contrast, the 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%) [33]. On the other hand, it has been 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. In another study, the authors identified in thirty samples of EOs thyme collected in Italy, 46 components covering more than 96% of the total composition [35]. Also, analyzing the composition of the EO of *T. pulegioides* from Portugal, the authors showed that the oil was characterized by high amounts of thymol (26.0 %), carvacrol (21.0%) and its biogenetic precursor γ -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 $0.7 \pm 0.25 \mu\text{g/ml}$. This oil has a significant ability to neutralize the DPPH radical and therefore an important antioxidant activity significantly higher than the BHT standard used as positive control ($11.5 \pm 0.62 \mu\text{g/ml}$).

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 ($IC_{50}=1.9 \pm 0.3 \mu\text{g/mL}$) is found to be more effective than synthetic antioxidant BHT ($IC_{50}=1.5 \pm 0.2 \mu\text{g/mL}$). 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 $EC_{50}=0.28 \pm 0.02 \mu\text{g/mL}$. The value showed a strong ferric ion reducing capacity more efficiently than positive controls BHT ($EC_{50}=23 \pm 1 \mu\text{g/mL}$) and vitamin C ($EC_{50}=37 \pm 2 \mu\text{g/ml}$). Studying the reductive capacity of *T. capitatus* EO harvested from different Tunisian provenance showed that the extracted oils during the post-flowering stage had a reductive

potential similar to BHA and BHT [33]. 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 ($EC_{50} = 1.36 \pm 0.3 \mu\text{g/mL}$). This ability is twenty times larger than the positive control EDTA ($32.5 \pm 1.32 \mu\text{g/ml}$). 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 $12.2 \pm 0.65 \mu\text{g/mL}$ is more important than synthetic antioxidants with IC_{50} values in the range of 75 ± 1 and $48 \pm 2.29 \mu\text{g/mL}$ 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 $\mu\text{g/mL}$.

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

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 $19 \pm 1 \text{mm}$ (*E. faecalis* ATCC 29212) to $37.33 \pm 0.57 \text{mm}$ (*B. cereus* ATCC 11778) for Gram positive bacteria and was ranged from $10.33 \pm 0.57 \text{mm}$ (*P. aeruginosa* ATCC 27853) to $35.66 \pm 0.57 \text{mm}$ (*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

other thyme varieties has been studied. Moreover, 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).

Bacteria species	TVEO				Antibiotic	
	IZ ^a	MIC	MBC	MBC/MIC	IZ ^b	MIC
<i>S. epidermidis</i> CIP106510	28.66±0.57 ^d	0.019	0.078	4	21.33 ± 0.58	0.031
<i>S. aureus</i> ATCC25923	25.66±1.15 ^e	0.019	0.039	2	32.67 ± 0.58	0.015
<i>M. luteus</i> NCIMB 8166	28±0 ^d	0.039	0.078	2	27.67 ± 1.53	>0.003
<i>E. faecalis</i> ATCC 29212	19±1 ^g	0.078	0.15	2	26 ± 1	0.007
<i>B. cereus</i> ATCC 11778	37.33±0.57 ^a	0.019	0.078	4	26 ± 1	0.007
<i>B. cereus</i> ATCC 14579	36.33±1.54 ^{ab}	0.039	0.078	2	28 ± 1	0.007
<i>E. coli</i> ATCC 35218	24.66±0.57 ^{ef}	0.078	0.31	4	27.33±0.58	>0.003
<i>L. monocytogenes</i> ATCC19115	30.33±0.57 ^c	0.039	0.15	4	37.67±0.58	0.015
<i>P. aeruginosa</i> ATCC 27853	10.33±0.57 ^k	ND	ND	ND	21 ± 1	>0.078
<i>S. typhimurium</i> LT2 DT104	19.66±0.57 ^g	0.078	0.15	2	30.33 ±0.58	>0.03
<i>V. cholerae</i> ATCC 9459	31±0.58 ^c	0.039	0.15	4	25±1	0.31
<i>V. parahaemolyticus</i> ATCC 17802	15±1 ⁱ	0.078	0.31	4	21±0	0.078
<i>V. parahaemolyticus</i> ATCC 43996	17.66±0.57 ^h	0.039	0.31	8	20±0	0.078
<i>V. alginolyticus</i> ATCC 33787	13±0 ^j	0.078	0.31	4	20±0	0.15
<i>V. alginolyticus</i> ATCC 17749	17.33±0.58 ^h	0.078	0.31	4	7±0	0.15
<i>V. vulnificus</i> ATCC 27562	20±0 ^g	0.039	0.15	4	13.33±0.57	0.31
<i>V. harveyi</i> ATCC 18293	16±0 ⁱ	0.078	0.31	4	18.33±0.58	0.078
<i>V. proteolyticus</i> ATCC 15338	25.33±0.57 ^e	0.019	0.15	8	20±1	0.078
<i>V. furnisii</i> ATCC 35016	35.66±0.57 ^b	0.019	0.078	4	20.33±0.57	ND
<i>V. mimicus</i> ATCC33653	24±0 ^f	0.039	0.15	4	20±0	ND
<i>V. furnisii</i> ATCC 33813	16±1 ⁱ	0.078	0.31	4	19±0	0.078
<i>V. natrigens</i> ATCC 14048	24±1 ^t	ND	ND	ND	21±0	ND
<i>V. carhiaccaae</i> ATCC 35084	17.66±0.57 ^h	0.078	0.31	4	18.33±0.58	0.15
<i>V. fluvialis</i> 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. furnisii* 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 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, it has been 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) [40]. 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).

Several studies evaluate thyme oil antimicrobial activity harvested in different Mediterranean regions. For example, one of them 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 an 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].

Studying the correlation between the chemical composition of *T. maroccanus* and *T. broussonetii* EOs and their antimicrobial effect, it was affirmed that the activity level could be attributed to the presence of high concentrations of carvacrol [41].

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 \pm 1.53 to 48.33 \pm 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 \pm 0.57 mm, 0.009-0.078mg/mL and 0.019-0.31mg/mL, respectively. Similarly, it has been demonstrated that the anticandidal activity of *T. maroccanus* and *T. broussonetii* EOs was lower than amphotericin-B and fluconazole [41]. In fact, IZ values are respectively 44.5 \pm 0.35, 38.5 \pm 0.70, 22.5 \pm 0.70 and 16.5 \pm 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].

Table 4. Zones of growth inhibition (IZ mm \pm 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).

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

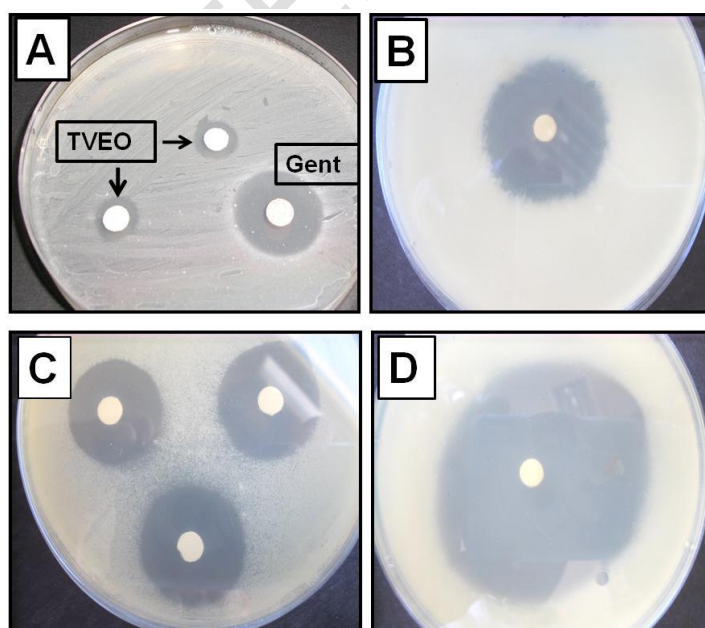


Fig. 1. Agar plate pictures representing the range of inhibition zone resulted after using TVEO for bacteria strains (A: *P. aeruginosa* ATCC 27853 and B: *B. cereus* ATCC 11778) and for fungual strains (C: *Microsporum canis* and D: *C. albicans* ATCC 90028).

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). 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 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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