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ORIGINAL ARTICLE

Thymus musilii Velen. as a promising source of potent bioactive compounds with its pharmacological properties: *In vitro* and *in silico* analysis

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Molecular docking

Abstract For the first time, we reported the phytochemical composition of the volatile oil from *Thymus musilii* Velen (*T. musilii*). The antioxidant and antimicrobial activities against various food-borne and clinical pathogenic microorganisms were also tested. The thyme oil was particularly rich in thymol (67.697 ± 0.938%), and thymyl acetate (12.993 ± 0.221%). The strongest antioxidant activity of the essential oil was registered with the tests: ABTS (IC_{50} = 5.6 × 10^{-4} mg/mL) and β-carotene/linoleic acid (IC_{50} = 3.2 × 10^{-3} mg/mL). This thymol-chemotype oil was active against all microorganisms tested with an inhibition growth zone ranging from 21.33 ± 1.52 mm for *Proteus mirabilis* (*P. mirabilis*) to 37.33 ± 1.15 mm for *Candida vaginalis* (*C. vaginalis*) strain. Overall, the tested oil exhibited bactericidal and fungicidal activities and only a small quantity of the tested essential oil was found to be sufficient for inhibiting the growth of the tested microorganisms. Furthermore, molecular docking results implies that, among the bioactive compounds, β-caryophyllene interacted strongly with the active site residues of TyrRS, GLMS and Gyrase enzymes and consequently support our *in vitro* results with the highest inhibition potential of this essential oil against tested pathogens, especially *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli (E. coli)*. Our results suggested that essential oil of *T. musiliii* exhibited strong biological activities with a promising source of various natural compounds.

1. Introduction

The use of medicinal plants as a source of therapy against various disorders have been practiced in Saudi Arabia since ages and many practices reported in the Prophetic Medicine are currently used in folk medicine in the Arabian Peninsula (Al-Essa et al., 1998). Among this category of plants, there are cultivated plants and others are spontaneous ones. These aromatic plants are grown as needed for their aerial parts (flowers, seeds, leaves, stems, bark) or their underground parts (bulbs, roots). Studies in the past have reported the presence of valuable medicinal plants from the different regions of Saudi Arabia (El-Tawil, 1983). However, the information of the indigenous medicinal plants of Saudi Arabia is scattered in a disorganized manner (Al-Asmari et al. 2014). Scientific studies have proven that these plants, including garlic, pomegranate, black seeds, costus, miswak, henna, ferns, Eucalyptus, ginger, and fenugreek are effective for treating human diseases (Noumi et al., 2017; Adnan, 2019; Reddy et al., 2020; Adnan et al., 2020). These species are exploited in human food, traditional medicine as well as for industrial purposes (agro-food, perfumery, cosmetics, pharmaceutical, etc.).

The mint family (Lamiaceae) is one of the largest and most distinctive families of flowering plants, with about 220 genera and almost 4000 species worldwide (Pirbalouti et al., 2015). This family has an almost cosmopolitan distribution. These plants are frequently aromatic in all parts and include many widely used culinary herbs, such as thyme. The genus *Thymus* L. belongs to the *Nepetoideae* subfamily of Lamiaceae family is a well-known aromatic herb and consists of about 330 species of herbaceous perennials and small shrubs in the world (Nickavar et al., 2005; Salehi et al., 2019).

The Mediterranean region can be described as the center of the genus (Cronquist, 1988; Morales, 2002; Jamzad, 2010). *Thymus* plants also includes many aromatic perennial and herbaceous plant that are cultivated in frequency due to their wide use in the food, cosmetic, and pharmaceutical industries (Nabavi et al., 2015). The genus *Thymus* is a taxonomically complex group of aromatic plants, traditionally used for medicinal purposes because of their antiseptic, antispasmodic and antitussive properties (Pina-Vaz et al., 2004, Nabavi et al., 2015). Previous chemical investigation on *Thymus* species have shown the presence of aromatic terpenes and terpenoids, flavonoids, and phenolic acid (Miri et al., 2002; Miguel et al., 2004; Ebrahimi et al., 2008; Tohidi et al., 2017). Thymol and carvacrol are the main phenolic compound of thyme oil. The major non-phenolic compounds were linalool and *p*-cymene (Piccaglia and Marotti, 1991).

Recent studies have shown that *Thymus* species have antibacterial, antifungal, and antioxidant activities (Bassam et al., 2004; Rahimmalek et al., 2009; Jordan et al., 2009). Gedikoğlu, et al. (2019) reported that the essential oil of thyme showed antimicrobial activity against *Bacillus cereus* NRRL (B3711), *Staphylococcus aureus* (ATCC 9144), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* ATCC (25922), *Salmonella enteritidis* (ATCC 13076) and *Salmonella typhimurium* (ATCC 14028). The anti-bacterial characteristic of *Thymus* spp. is due to the occurrence of thymol in this genus. This substance can be used as a disinfectant.

In Saudi Arabia, at least three species of *Thymus* (endemic and introduced) were identified: *T. bovei* Benth., *T. decussatus* Benth. and *T. musilii* Velen. In addition, *T. vulgaris* was largely cultivated in many regions of the kingdom. This species, *T. musilii* Velen. belongs to division: Tracheophyta, subdivision: Spermatophytina, Class: Magnoliopsida, Superorder: Asteranae, Order: Lamiales, Family: *Lamiaceae* Lindl., and Genus: *Thymus* L. It is distributed mainly in Iraq, Palestine, and Saudi Arabia (World Checklist of Selected Plant Families, 2010).

Growing to 30–70 cm tall by 40–60 cm wide, it is a bushy, woody-based evergreen subshrub with highly aromatic, green leaves and clusters of white flowers in early summer. Preferred the dry slopes, rocks and maquis, it was always found on clay or limestone soils. It has sessile leaves varying from elliptic to linear or diamond-shaped towards the apex. The flowers have a tube-like calyx and tubular corolla with a three lobed lower lip, and are united in spikes at the top of the branches (Fig. 1).

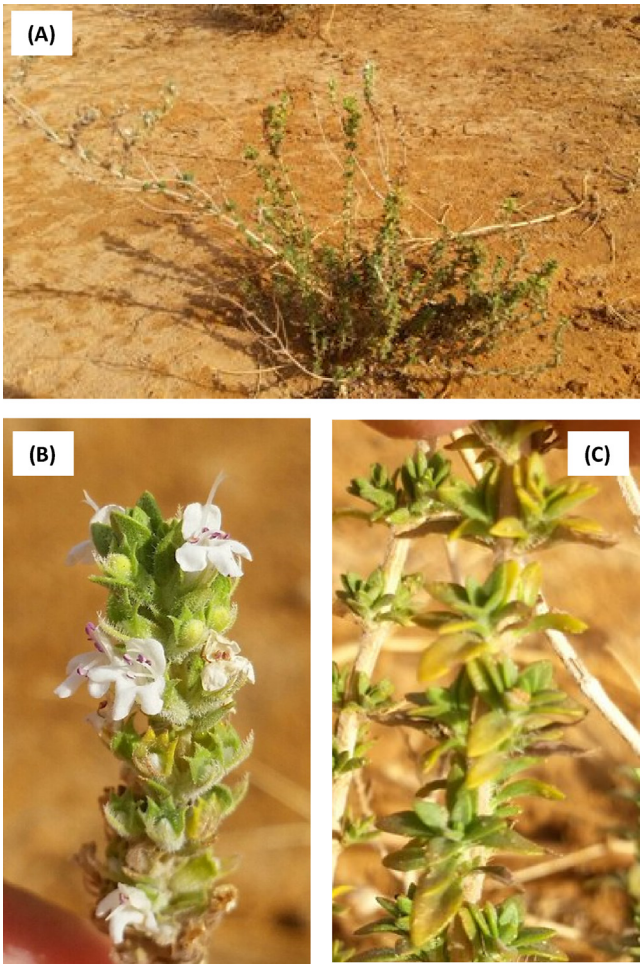


Fig. 1 *T. musilii* Velen specimen. (A): whole plant at flowering stage, (B): clusters of white flowers, (C): green leaves.

The roots are robust, and the fruit consists of a smooth, dark colored nutlet. In Bedouin population of Saudi Arabia, leaves and flowering tops of *T. musilii* were used as a garnish or added as a flavoring in cooking variety of foods, as well as in preparing infusion tea. An aromatic tea is made from the fresh or dried leaves. The leaves can be used either fresh or dried. If the leaves are to be dried, the plants should be harvested in early and late summer just before the flowers open and the leaves should be dried quickly.

The *in vitro* antimicrobial and antioxidant activities of the essential oil and extract of *T. vulgaris* have recently been reported. Al-Asmari et al. (2017) have studied the essential oil composition, whereas, Alharbi (2017) reported that the whole plant was used in traditional medicine to treat abdominal pain, and as anti-helminthic and carminative effects. Belonging this genus, *T. musilii* is a very interesting medicinal plant closely distributed on Arabian Peninsula, Iraq and Jordan landscapes (Batanouny and Sheikh, 1972; Govaerts, 2003). In the north of Saudi Arabia, it is locally used as an antiseptic traditional drug. This species has also been used for curing many bacterial and fungal diseases in traditional medicine in Saudi Arabia (survey, data not shown). In fact, it used by local Saudi population to cure many ailments. Leaves are used in treating respiratory diseases and the flowering tops are used as anti-helminthic, antiseptic and antispasmodic drug. However, antimicrobial and antioxidant properties of *T. musilii* Velen seem not to have been reported before.

To the best of our knowledge, this study is the first report on the biological properties of *T. musilii* Velen. The aim of this work was to investigate the chemical composition of the volatile oil obtained from the aerial parts of *T. musilii* cultivated under greenhouse conditions in Al-Gaad, Hail (Saudi Arabia) by using GC–MS technique. Additionally, the antioxidant and antimicrobial activities of the oil were assessed. To reach this objective, molecular docking studies of the bioactive compounds were also performed against tyrosyl-tRNA synthetase TyrRS from *S. aureus*, glucosamine 6-phosphate synthase (GLMS) from *E. coli* and Gyrase from *S. aureus* enzymes to better understand their mechanism of action.

2. Material and methods

2.1. Plant material sampling and essential oil extraction

The plant used in this study were collected in October 2019 from a nursery belonging to the Ministry of Agriculture in the region of Hail (Al-Gaad, Ha'il, Saudi Arabia). Dr. Ahmed Alghamdi, from the Department of Biology, Faculty of Science, University of Hail, Saudi Arabia identified the plant at the species level. A voucher specimen (AN 001) was deposited in the Department of Biology, University of Hail, Saudi Arabia. The volatile oil was collected using a clevenger-type apparatus after 3 h of hydro-distillation using 100 g from the aerial air-dried organs (flowering stage). The obtained oil was dried using anhydrous sodium sulfate and stored until use at −20 °C. The yield of extraction was calculated after three running cycle and expressed according to the dry weight.

2.2. Characterization of the volatile oil

A Hewlett–Packard 6890 chromatograph equipped with a flame ionization detector (FID) and an electronic pressure control injector was used to study the chemical composition of the obtained volatile oil from *T. musilii* aerial parts. A gas chromatography apparatus coupled to mass spectrometry (GC–MS) on a gas chromatograph HP 7890 (II) and HP 5975 mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) with an electron impact ionization of 70 eV was used. An HP-5MS capillary column (Agilent Technologies, Hewlett-Packard, CA, USA; 30 m × 0.25 mm), with 0.25 m film thickness was used. Temperature was fixed to rise from 40 °C to 280 °C at a rate of 5 °C/min. The carrier gas was helium with a flow rate of 1.2 mL/min, a split ratio of 60:1, scan time and mass range of 1 s and 40–300 m/z, respectively. The identification of the bioactive components in *T. musilii* volatile oil was based on the calculated retention index (RI) relative to (C8–C22) n-alkanes and in comparison, with authentic compounds. Further identification of compounds was made by matching their recorded mass spectra with those stored in the Wiley/NBS mass spectral library of the GC–MS data system and

other published mass spectra (Adams, 2007) and data expressed as relative percentage of the total peak area as previously described by Essid et al. (2015) and Salem et al. (2018).

2.3. Antioxidant assays

2.3.1. DPPH radical–scavenging activity

The free radical-scavenging activity of the tested essential oil was measured using the protocol described by Chakraborty and Paulraj (2010) and Adnan et al. 2018. The ability to scavenge the DPPH radical was calculated using the following equation (Eq. (1)):

$$\text{DPPH scavenging activity}\,(\%) = (A_0 - A_1)\,/A_0 \times 100 \quad (1)$$

where

A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

The antioxidant activity was expressed as IC_{50} (mg/mL) which represented the extract concentrations scavenging 50% of DPPH radicals (Nishaa et al., 2012).

2.3.2. ABTS radical scavenging activity assay

The radical scavenging activity against ABTS radical cations was measured using the method of Chakraborty and Paulraj (2010). The inhibition percentage of ABTS radical was calculated using the following equation (Eq. (2)):

$$\text{ABTS scavenging activity}\,(\%) = (A_0 - -A_1)\,/A_0 \times 100 \quad (2)$$

where

A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

The antiradical activity was expressed as IC_{50} (mg/mL) which represented the extract concentrations scavenging 50% of ABTS radicals (Nishaa et al., 2012). A lower IC_{50} value represents a stronger ABTS scavenging capacity.

2.3.3. Reducing power capability assay

The reducing power was determined using the method of Bi et al. (2013). The extract concentration providing 0.5 of absorbance (IC_{50}) was calculated from the graph of absorbance at 700 nm against sample concentration (Barros et al., 2008). Ascorbic acid was used as a standard.

2.3.4. β-carotene/linoleic acid method

The β-carotene method was carried out according to Ikram et al. (2009). Antioxidant activity (inhibition percentage, PI %) was evaluated using the following equation (Eq. (3), Miraliakbari and Shahidi, 2008):

$$PI\% = A_{\beta-\text{carotene T120}}/A_{\beta-\text{carotene t0}} \times 100 \quad (3)$$

where

$A_{\beta\text{-carotene t0}}$ and $A_{\beta\text{-carotene T120}}$ refer to the corresponding absorbance values of the test sample, standard and control measured before and after incubation for 2 h, respectively.

All tests were performed in triplicate and ascorbic acid (standard) was used for comparison.

2.4. Screening of antimicrobial activities

The antimicrobial activity of the obtained essential oil was tested against four type strains namely *E. coli* ATCC 35218, *P. aeruginosa* ATCC 27853, *P. mirabilis* ATCC 29245, and *K. pneumoniae* ATCC 27736. Two clinical strains, *S. aureus* MDR (multidrug resistant bacteria), and *Enterobacter cloacae* (*E. cloacae*) were used. The antifungal activity was performed using *Candida albicans* (*C. albicans*) ATCC 10231, *Cryptococcus neoformans* (*C. neoformans*) ATCC 14116, *C. vaginalis* (clinical strain), and *Candida* sp. (clinical strain). Two fungal strains (*Aspergillus spp.*) were also tested: *A. fumigatus* ATCC 204305 and *A. niger*.

Two techniques were used to screen the antimicrobial effect of the obtained essential oil and its main component thymol purchased from Sigma Aldrich®, Germany. The disc diffusion assay was performed on Mueller-Hinton agar plates for all bacteria, Sabouraud chloramphenicol agar for yeasts, and Potato Dextrose agar for the *Aspergillus* strains. 10 mg of essential oil and thymol/6 mm-disc were tested in triplicate. Ampicillin and Amphotericin B were used as control. The minimal inhibitory concentration (MIC) and minimal bactericidal/fungicidal concentration (MBC/MFC) values were determined by using the microdilution assay as previously described by Snoussi et al. (2018). MBC/MIC ratio and MFC/MIC ratio were used to interpret the activity of the essential oil as described by Gatsing et al. (2009).

2.5. Molecular docking analysis of TyrRS, GLMS and Gyrase with phytochemicals of T. musilii

Crystal structures of tyrosyl-tRNA synthetase TyrRS from *S. aureus* (PDB: 1JIJ.pdb) (Qiu et al., 2001), glucosamine 6-phosphate synthase (GLMS) from *E. coli* (PDB: 1XFF.pdb) (Isupov et al., 1996), and Gyrase from *S. aureus* (PDB: 2XCT.pdb) (Bax et al., 2010) were fetched from Protein Data Bank (RCSBPDB). Following to the retrieval of crystal structures, LCMS identified phytochemicals 3-dimensional structures such as α-thujene, α-pinene, β-myrcene, α-terpinene, p-cymene, (1,8)-cineole, γ-terpinene, α-terpinolene, Borneol, Terpinen-4-ol, α-terpineol, 2-Isopropyl-5-methylanisole, Thymol, Carvacrol, Thymyl acetate, Carvacryl acetate, and β-caryophyllene were acquired from eminent database PubChem and converted to PDB format using Open Babel (O'Boyle et al., 2011). These seventeen compounds were then docked separately against the receptor structure (1JIJ, 1XFF and 2XCT) using molecular docking software Autodock 4.2.6 (Morris et al., 2009). Docking protocol was performed in a similar manner, which can be related to previous analyses (Sonawane and Barage, 2015; Parulekar and Sonawane, 2018). Apart from the grid centre and grid size, all other parameters used for docking with these seventeen compounds were kept same. For the preparation of the grid map using a grid box, Auto Grid (Morris et al., 2009) was used. The grid size was set to 126 × 126 × 126xyz points for TyrRS and gyrase receptors. For GLMS, grid size was set to 96 × 122 × 126 xyz points. Grid spacing was kept to

0.375 Å for all the receptors. The grid centre for TyrRS was designated at dimensions (x, y and z): −11.897, 17.862 and 91.741, for GLMS at (x, y and z): 1.979, 37.952 and 20.512, and for gyrase at (x, y and z): 7.841, 39.224 and 118.021. The grid box is cantered in such a way that it encloses the entire binding site of both the receptors and provides enough space for translation and rotation of ligands. The generated docked conformation was ranked by predicted binding energy and topmost binding energy docked conformation was analyzed using UCSF Chimera (Pettersen et al., 2004) for intermolecular hydrogen bonding of active site amino acid residues from the receptors with docked ligands.

2.6. Statistical analysis

The laboratory biological assays were conducted in triplicates for each sample. The IC_{50} of DPPH, ABTS, and β-carotene bleaching methods values were calculated by linear regression analysis. ANOVA and Duncan tests were performed with SPSS 16.0. The means of the test's values were also evaluated with the Least Significant Differences test at 0.05 significance level.

3. Results and discussion

3.1. Chemical composition of *T. musilii* Velen. essential oil

The air-dried aerial-parts of *T. musilii* yielded 2.736 ± 0.015% (v/w) essential oil on hydro-distillation. Seventeen components were identified in the obtained oil, belonging mainly to oxygenated monoterpenes (87.010 ± 0.279%) followed by monoterpenes hydrocarbons (11.013 ± 0.039%) and sesquiterpenes hydrocarbons (1.953 ± 0.005%). These data are summarized in Table 1. The chemical structure of the seventeen compounds identified in *T. musilii* essential oil were depicted in Fig. 2.

This essential oil can be defined as thymol/thymyl acetate chemotype (67.697/12.993%) as shown in the chromatogram (Fig. 3). Thymol (67.697 ± 0.938%), thymyl acetate (12.993 ± 0.221%), o-cymene (4.617 ± 0.119%), carvacrol (3.417 ± 0.105%), and γ-terpinene (2.633 ± 0.072).

Numerous studies have reported that oxygenated monoterpenes were the dominant family of compounds found in the *Thymus* genus essential oil (De Martino et al., 2009; Zarshenas and Krenn, 2015). The diversity of the composition of the volatile oil obtained from different species and subspecies belonging to the genus *thymus* can be explicated by endogenous (plant varieties, vegetative state, organ tested) and exogenous factors like climatic features, soil characteristics, and seasons (Tzakou et al., 1998; Cosentino et al., 1999; Pirbalouti et al., 2013a,b). It has also been reported that the frequency of irrigation and salicylic acid concentration can affect the yield and the content of essential oil obtained from *T. daenensis* Celak. and *T. vulgaris* L. (Khazaie et al., 2008, Pirbalouti et al., 2013c; Alavi-Samani et al., 2013). In addition, application of fertilizers increases the vegetative biomass, oil yield and diversity, and antioxidant activities of *T. daenensis* Celak. (Bistgani et al., 2018).

Thymol and carvacrol are the main phenolic compound of thyme oil. The major nonphenolic compounds were linalool and *p*-cymene (Piccaglia and Marotti, 1991). Thymol was the dominant phenolic compound detected in several *Thymus* species with different percentage as reported by Tohidi et al. (2019) including: *T. carmanicus* (40.8%), *T. daenensis* (20–80.4%), *T. eriocalyx* (5.3–66.34%), *T. fallax* (19.88–65.9%), *T. fedtschenkoi* (31.8%), *T. kotschyanus* (6.8–66.15%), *T. migricus* (55.6–79.74%), *T. pubescens* (37.9–63.5%),

Table 1 Chemical composition of *T. musilii* Velen. essential oil.

Peak #	RI* on HP-5MS column	Compounds	Chemical formula	Percentage (Mean ± SD)
1	931	α-Thujene	$C_{10}H_{16}$	0.437 ± 0.015
2	939	α-Pinene	$C_{10}H_{16}$	0.303 ± 0.015
3	992	β-Myrcene	$C_{10}H_{16}$	0.710 ± 0.034
4	1018	α-Terpinene	$C_{10}H_{16}$	0.853 ± 0.028
5	1026	***p*-Cymene**	$C_{10}H_{14}$	**4.617 ± 0.119**
6	1033	1,8-Cineole	$C_{10}H_{18}O$	0.397 ± 0.005
7	1062	**γ-Terpinene**	$C_{10}H_{16}$	**2.633 ± 0.072**
8	1087	α-Terpinolene	$C_{10}H_{16}$	1.460 ± 0.081
9	1165	Borneol	$C_{10}H_{18}O$	0.763 ± 0.030
10	1174	Terpinen-4-ol	$C_{10}H_{18}O$	0.390 ± 0.017
11	1189	α-Terpineol	$C_{10}H_{18}O$	0.890 ± 0.036
12	1227	2-Isopropyl-5-methylanisole	$C_{11}H_{16}O$	0.080 ± 0.138
13	1290	**Thymol**	$C_{10}H_{14}O$	**67.697 ± 0.938**
14	1292	**Carvacrol**	$C_{10}H_{14}O$	**3.417 ± 0.105**
15	1356	**Thymyl acetate**	$C_{12}H_{16}O_2$	**12.993 ± 0.221**
16	1367	Carvacryl acetate	$C_{12}H_{16}O_2$	0.383 ± 0.015
17	1404	β-caryophyllene	$C_{15}H_{24}$	1.953 ± 0.102
Chemical classes				
Monoterpene hydrocarbons			**11.013 ± 0.039**	
Oxygenated monoterpenes			**87.010 ± 0.279**	
Sesquiterpenes hydrocarbons			**1.953 ± 0.005**	
Total compounds Identified (%)				**100**

RI: Retention index on a HP-5MS column. The data are expressed as mean ± SD (n = 3); SD: Standard Deviation.

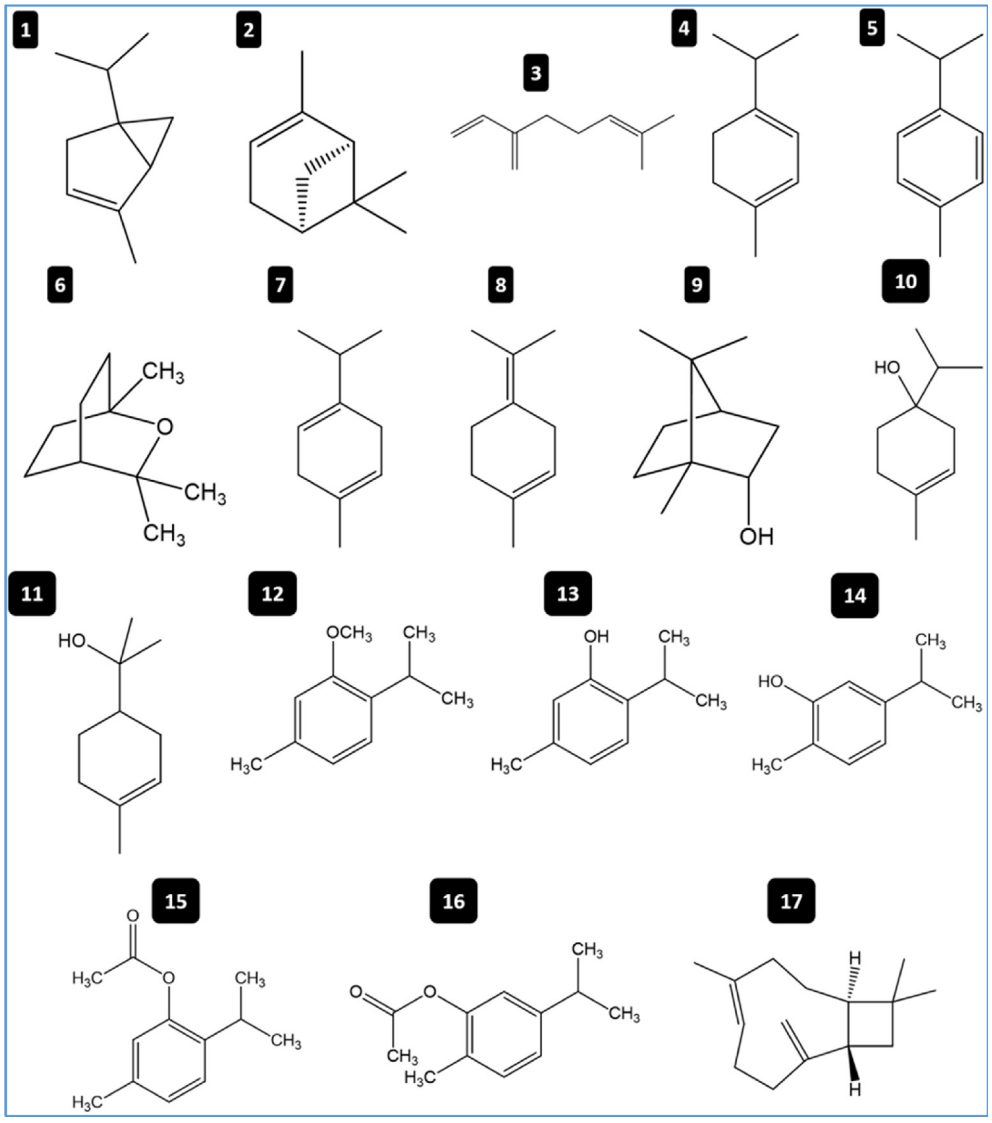


Fig. 2 Chemical structures of 17 bioactive molecules identified in *T. musilii* essential oil using GC–MS technique. Numbers in the figure correspond to the codes in Table 1.

T. serpyllum (52.45%), *T. transcaucasicus* (35.83–62.92%), and *T. trauveterri* (24.43–63.33%).

It has also been reported that thymol is the main phenolic compound in the essential oil of *T. cappadocicus* Boiss. (Albayrak and Aksoy, 2012), *T. pulegioides* (Pinto et al., 2006), *T. fontanesii* (Dob et al., 2006), *T. hyemalis* (Rota et al., 2008), *T. ciliatus* Desf. Benth. (Kabouche et al., 2009), *T. marschallianus* Willd (Cavar Zeljkovic et al., 2015), *T. pannonicus* (Pluhár et al., 2010), *T. vulgaris* (Asbaghian et al., 2011), *T. zygis* (Ballester-Costa et al., 2013), *T. numidicus* Poiret (Mina et al., 2014), *T. quinquecostatus* Celak. (Kim et al., 2014) and *T. lanceolatus* (Khadir et al., 2016a,b). More recently, Jan et al. (2020) reported that *T. afghanicus* harvested from the Himalayan-Afghanistan area was a thymol chemotype (27.7%).

In this study, thymyl acetate, which is formed after acetylation of thymol produced directly by terpene synthases (Keszei et al. 2008), was found to be the second phenolic compound in *T. musilii* oil (12.993%). This molecule has been reported in the essential oil of some *Thymus* species with different percentage including *T. longicaulis* (0–12.8%) and *T. pulegioides* L. (0.4–0.7%) from Italy (De Martino et al., 2009), *T. caespititius* Brot. from Portugal (11–15%, Mendes et al., 2013), *T. serpyllum* L. from Serbia (38.5%, Cancarevic et al., 2013), and *T. lanceolatus* from Algeria (0.006%; Khadir et al., 2016a,b).

3.2. Antioxidant activities of *T. musilii* essential oil

Because of the complex chemical compounds effect of the plants volatile oil, the antioxidant capacity of *T. musilii* essential oil is studied by four methods, DPPH, ABTS, FRAP and β-carotene bleaching methods in order to estimate the effectiveness of these compound diversity. Table 4 summarizes the free radicals scavenging activities of *T. musilii* essential oil and the commercialized standards, ascorbic acid and butylated hydroxyl-toluene (BHT). The IC_{50} of the essential oil and

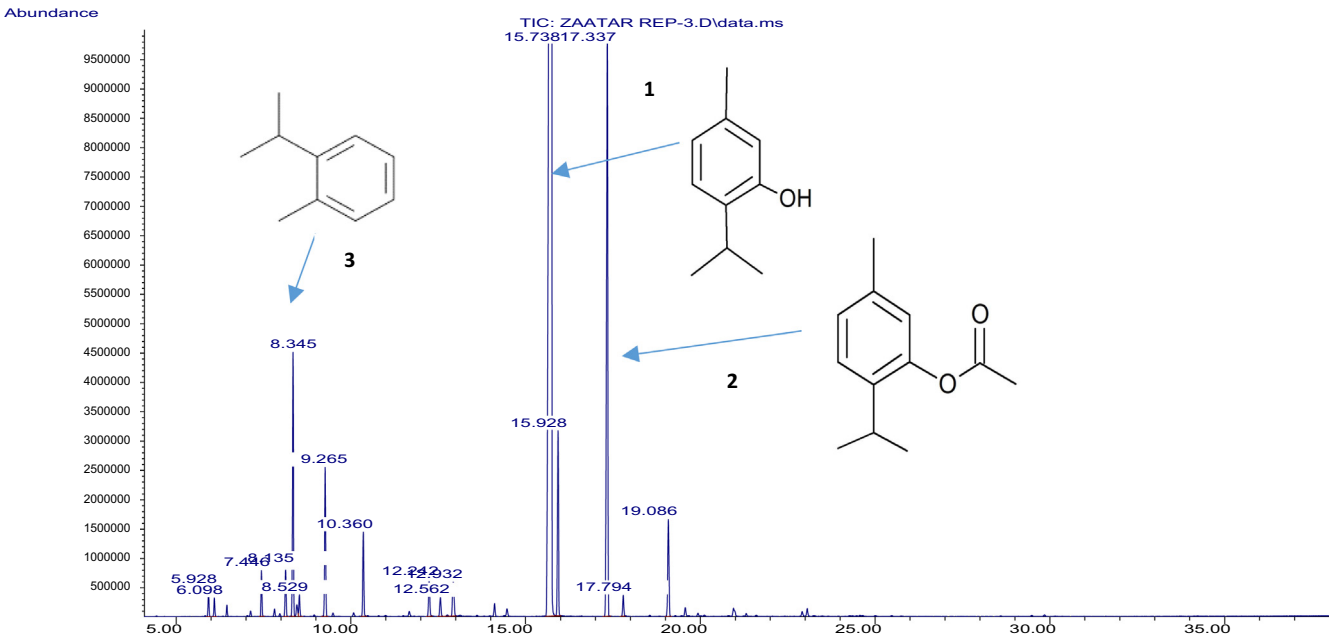


Fig. 3 Chromatogram obtained for *T. musilii* Velen essential oil. The main components identified are: 1 (Thymol), 2 (Thymol Acetate), and 3 (*o*-cymene).

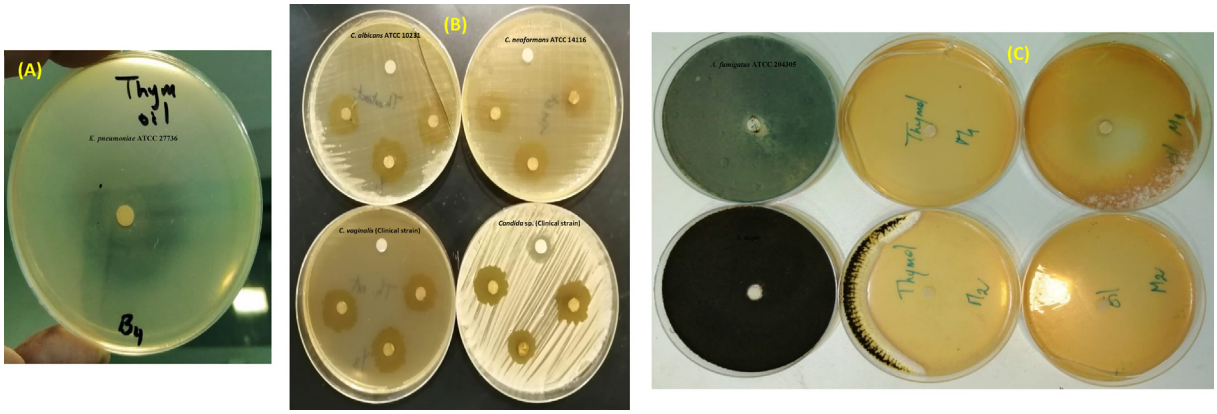


Fig. 4 Selected photos showing the antibacterial (A), anti-*Candida* spp. (B), anti-*Aspergillus* spp. (C) activity of the tested essential oil and its main component thymol.

the standards, which is the concentration required for scavenging half (50%) of the tested radicals, showed that ABTS and peroxyl radicals were strongly significantly inhibited by *T. musilii* (Table 4). Interestingly, *T. musilii* oil possess high antioxidant activities using ABTS (IC_{50} = 5.6 × 10^{-4} ± 2 × 10^{-5} mg/mL) and β-carotene bleaching (IC_{50} = 3.2 × 10^{-3} ±5 × 10^{-4} mg/mL) methods, followed by DPPH test (IC_{50} = 0.049 ± 1 × 10^{-4} mg/mL). This essential oil is significantly active on peroxyl radicals than the both tested standards (Table 4).

Literature review showed that no previous work was countered on *T. musilii* essential oil antioxidant capacity. However, several studies were conducted on the genus *Thymus* essential oils and on its antioxidant capacity (El-Bakkal et al., 2020; Goudjil et al., 2020). For instance, the anti-radicalar essential oils from the cultivated *T. carmanicus, T. kotschyanus*, *T. migricus*, and *T. vulgaris* collected, under various conditions, from Iran were studied by DPPH method (Tohidi et al., 2020). Under red, red-blue, blue, white and greenhouse light treatments, *T. carmanicus* (IC_{50} = 278; 259.2; 281; 467.4; 198.2 µg/mL), *T. kotschyanus* (IC_{50} = 621.8; 421.1; 304.6; 557.4; 384.7 µg/mL), *T. migricus* (IC_{50} = 358; 911.6; 176.8; 1274; 631.8 µg/mL), and *T. vulgaris* (IC_{50} = 560; 766; 400.6; 227.6; 314.3 µg/mL) inhibited DPPH radicals (Tohidi et al., 2020). *Thymus longicaulis* C. Presl subsp. *longicaulis* var. *longicaulis* essential oil collected from Turkey had strong radical inhibition percentage (IP = 87.69% at 0.4 mg/mL; 93.28% at 1 mg/mL; 94.15% at 2 mg/mL) using b-carotene–linoleic acid method. The same plant species possess moderate effect (IP = 28.17% at 0.1 mg/mL; 46.32% at 0.2 mg/mL, 63.26% at 0.5 mg/mL) using DPPH method, moderate effect using reducing power protocol (Absorbance = 0.128 at 0.2 mg/mL, 0.241 at 0.4 mg/mL, 0.550

Table 2 Growth inhibition zone, MIC and MBC values obtained for bacterial strains tested using disc diffusion and microdilution assays.

Code	Strain	*T. musilii* Velen essential oil				Main Compound (Thymol)				Ampicillin
		Mean ± SD* (mm)	MIC[a]	MBC[b]	MBC/MIC ratio	Mean ± SD (mm)	MIC	MBC	MBC/MIC ratio	Mean ± SD (mm)
B_1	*E. coli* ATCC 35218	35.33 ± 1.15[c]	12.5	50	4	12.66 ± 0.57[b]	3.125	6.25	2	7 ± 0[a]
B_2	*P. aeruginosa* ATCC 27853	35.33 ± 1.15[b]	12.5	100	>4	7 ± 0[a]	12.5	50	4	7.33 ± 0.57[a]
B_3	*Proteus mirabilis* ATCC 29245	21.33 ± 1.52[b]	12.5	25	2	6 ± 0[a]	3.125	6.25	2	6.33 ± 0.57[a]
B_4	*K. pneumoniae* ATCC 27736	36.33 ± 1.15[c]	12.5	25	2	9 ± 1[b]	3.125	6.25	2	6.66 ± 0.57[a]
B_9	*S. aureus* MDR (Clinical strain)	25.33 ± 1.15[b]	12.5	25	2	6 ± 0[a]	0.78	1.56	2	7.33 ± 0.57[a]
B_{10}	*E. cloacae* (Clinical strain)	31.00 ± 1.00[c]	3.125	6.25	2	8.66 ± 1.15[b]	0.39	0.78	2	6.66 ± 0.57[a]

*Inhibition zone around the discs impregnated with the essential oil (10 mg/disk) expressed as mean of three replicates (mm ± SD). SD: standard deviation. a: Minimal Inhibitory Concentration (mg/ml). b: Minimal Bactericidal Concentration (mg/ml). c: MBC/MIC ratio interpreted using the scheme of antimicrobial substances are considered as bacteriostatic agents when the ratio MBC/MIC > 4 and bactericidal agents when the ratio MBC/MIC≤4 (Gatsing et al., 2009). The letters (a–c) indicate a significant difference between the inhibition zones of essential oil, thymol and ampicillin against the tested bacteria according to the Duncan test ($p < 0.05$).

Table 3 Growth inhibition zone, MIC and MFC values obtained for fungal and yeast strains tested using disc diffusion and microdilution assays.

Code	Strain	*T. musilii* Velen essential oil				Main Compound (Thymol)				Amphotericin B (10 mg/ml)
		Mean ± SD* (mm)	MIC	MFC	MFC/MIC ratio	Mean ± SD* (mm)	MIC	MFC	MFC/MIC ratio	Mean ± SD* (mm)
Y_1	*C. albicans* ATCC 10231	34.00 ± 1.00[c]	6.25	25	4	13.66 ± 0.57[a]	12.5	100	8	22.66 ± 1.15[b]
Y_2	*C. neoformans* ATCC 14116	36.66 ± 1.15[c]	3.125	6.25	2	12 ± 1[a]	50	100	2	15.33 ± 0.57[b]
Y_3	*C. vaginalis* (Clinical strain)	37.33 ± 1.15[c]	6.25	12.5	2	12.66 ± 0.57[b]	25	100	4	6.66 ± 0.57[a]
Y_4	*Candida* sp. (Clinical strain)	37.33 ± 1.15[b]	6.25	12.5	2	11.66 ± 0.57[a]	25	100	4	12.33 ± 0.57[a]
M_1	*A. fumigatus* ATCC 204305	88.66 ± 1.15[c]	–	–	–	82.66 ± 2.31[b]	–	–	–	15.00 ± 1.00[a]
M_2	*A. niger*	87.33 ± 1.15[c]	–	–	–	74.33 ± 0.57[b]	–	–	–	6.00 ± 0.00[a]

[*] Inhibition zone around the discs impregnated with the essential oil (10 mg/disk) expressed as mean of three replicates (mm ± SD). SD: standard deviation.
[a] Minimal Inhibitory Concentration (mg/ml).
[b] Minimal Fungicidal Concentration (mg/ml).
[c] MBC/MIC ratio interpreted using the scheme of antimicrobial substances are considered as fungistatic agents when the ratio MFC/MIC > 4 and fungicidal agents when the ratio MFC/MIC ≤ 4 (Gatsing et al., 2009). The letters (a–c) indicate a significant difference between the inhibition zones of essential oil, thymol and amphotericin B against fungi according to the Duncan test ($p < 0.05$).

Table 4 Antioxidant activities of *T. musilii* essential oil against DPPH, ABTS, FRAP and β-carotene/linoleic acid scavenging tests as compared to ascorbic acid and BHT.

Essential oil and standards tested	Test System			
	DPPH IC_{50} (mg/mL)	ABTS IC_{50} (mg/mL)	β- carotene IC_{50} (mg/mL)	FRAP IC_{50} (mg/mL)
T. musilii Velen	$0.049 \pm 1 \times 10^{-4b}$	$5.6 \times 10^{-4} \pm 2 \times 10^{-5\ a}$	$3.2 \times 10^{-3} \pm 5 \times 10^{-4\ a}$	$>1^{c}$
BHT	$0.023 \pm 3 \times 10^{-4a}$	$0.018 \pm 4 \times 10^{-4b}$	$0.042 \pm 3.5 \times 10^{-3c}$	$0.05 \pm 3 \times 10^{-3\ a}$
Ascorbic Acid	$0.022 \pm 5 \times 10^{-4\ a}$	$0.021 \pm 1 \times 10^{-3b}$	$0.017 \pm 1 \times 10^{-3b}$	$0.09 \pm 7 \times 10^{-3b}$

BHT: Butylated hydroxytoluene. The letters (a–c) indicate a significant difference between the different antioxidant methods according to the Duncan test ($p < 0.05$).

Table 5 Literature review of some *Thymus* species thymol-chemotype and microorganisms used for the antimicrobial activities.

Thymus species	Origin	Main Components	Bacteria and Fungi tested	Reference
T. vulgaris L.	Yemen	Thymol (51.34%), *p*-cymene (18.35%), ß-caryophyllene (4.26%).	*B. subtilis*, *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *E. coli*, *Mycobacterium smegmatis*, *C. albicans* and *C. vaginalis*.	Al Maqtari et al., 2011
	Romania	Thymol (47.59%), γ-terpinene (30.90%) and *p*-cymene (8.41%).	*S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *S. Typhimurium* ATCC 14028, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 13882, *E. faecalis* ATCC 29212 and *C. albicans* ATCC 10231	Borugă et al., 2014
	Balkan Peninsula	Thymol (49.1%), *p*-Cymene (20%), carvacrol (3.5%), α-thujene (1.9%), α-pinene (1.2%), ß-mycrene (1.3%), *trans*-ß-ocimene (1.4%), γ-Terpinene (4.2%), borneol (1.7%), terpinene-4-ol (2%), ß-caryophyllene (3.7%), δ-cadinene (2.3%).	*C. albicans* ATCC 10234, *C. glabrata*, *C. krusei*, *C. tropicalis* ATCC 750, *P. aeruginosa*, *E. faecalis*, *S. sanguinis*, *S. salivarius*, *S. mutans*, *L. acidophilus*, *S. aureus*.	Nikolic et al., 2014
	Italy	Thymol (46.2–67.5%), caryophyllene oxide (2.2–7.3%), geranyl propanoate (0–2.2%), linalool (0.3–2.7%), *trans*-myrtanol (0–2.3%), citronellyl formate (0–2.5%), ethyl-2-octynoate (0–1.8%).	*S. aureus* ATCC 25923, *E. faecalis* ATTC 29212, *B. cereus* ATCC 1177, *B. subtilis* ATCC 6633, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. epidermidis* ATCC 12228, *K. pneumoniae* ATCC 10031, *S. typhi* Ty2 ATCC 19430 and *P. vulgaris* ATCC 13315.	Mancini et al., 2015
	France	Thymol (47.06%), *p*-cymene (20.07%), γ-terpinene (9.03%), linalool (5.00%), carvacrol (3.24%).	*C. albicans* ATCC 18804, *Cryptococcus neoformans* 24067 (serotype D or var. *neoformans*), *Aspergillus niger* ATCC 16888.	Satyal et al., 2016
	Republic of Moldova	Thymol (55.44 ± 0.62%), m-Cymene (11.88 ± 0.32%), γ-Terpinene (5.74 ± 0.20%), o-Cymen-5-ol (5.14 ± 0.19%), ß-caryophyllene (1.53 ± 0.07%), Terpinen-4-ol (1.04 ± 0.04%), 2-Carene (1.04 ± 0.04%).	*A. flavus* MUCL 19006	Aprotosoaie et al., 2019
T. longicaulis C. Presl	Italy	Thymyl acetate (0–12.8%), t-Cadinol (0.3–9.2%), *p*-cymene (0.4–9.0%), ß-caryophyllene (2.2–5.7%), γ-terpinene (0.9–5.5%), Germacrene D (5.3%), thymol (6.4–9.3%), thymol methyl ether (0.8–5.5%), carvacrol (0–12.8%), Carvacryl acetate (0–13.6%).	*S. aureus* ATTC 25923, *S. faecalis* ATTC 29212, *B. subtilis* ATCC 6633, *B. cereus* PCI 213, *P. mirabilis* ATCC 12453, *E. coli* ATCC 25922, *S. typhi* Ty2 ATCC 19430, *P. aeruginosa* (ATCC 27853).	De Martino et al., 2009
	Balkan Peninsula	Thymol (46.3%), δ-3-Carene (1.6%), *p*-Cymene (9.4%), γ-terpinene (16.2%), linalool (1.4%), borneol (2.2%), thymyl methyl ether (11.4%), β-Caryophyllene (2.1%), carvacrol (1.4%).	*H. influenzae*, *N. meningitidis*, S. aureus, *S. pneumoniae*, *S. pyogenes*, *C. albicans*	Vladimir-Knežević et al., 2012
T. pulegioides	Italy	Thymol (21.8–26.3%), *p*-cymene (17.6–	*S. aureus* ATTC 25923, *S. faecalis* ATTC	De Martino et al.,

Table 5 (*continued*)

Thymus species	Origin	Main Components	Bacteria and Fungi tested	Reference
L.		19.9%), linalool (4.7–5.6%), *ß*-caryophyllene (5.9–7.5%), thymol methyl ether (6.0–10.8%), carvacrol (3.1–4.7%).	29212, *B. subtilis* ATCC 6633, *B. cereus* PCI 213, *P. mirabilis* ATCC 12453, *E. coli* ATCC 25922, *S. typhi* Ty2 ATCC 19,430 and *P. aeruginosa* ATCC 27853.	2009
T. daenensis Celak.	Iran	Thymol (3.8–78.3%), *ρ*-cymene (2.7–11.6%), caryophyllene (2.1–5.6%), methyl carvacrol (2.9–4.9%), *g*-terpinene (2.5–12.9%), geraniol (0–3.4%), α-humulene (0–3.2%), carvacrol (2–15.2%), γ-terpinene (3.9–12.9%), aromadendrene (0–3.9%), carvacrol methyl ether (3.4–4.27%), δ-terpinene (0–4.3%).	*L. monocytogenes, S. aureus, S. iniae, E. coli, P. aeruginosa, K. pneumonia, H. pylori, A. niger, A. fumigatus, C. albicans* and *S. cerevisiae.*	Zarshenas and Krenn, 2015
	Iran	α-pinene (0.51%), 1,8-cineole (0.58%), γ-terpinene (5.74%), linalool (0.52%), thymol (74.32%), carvacrol (4.31%), *trans*-caryophyllene (3.56%), caryophyllene oxide (0.42%).	*C. albicans* vaginal, *E. coli* O157:H7; *B. cereus, L. monocytogenes* and *S. aureus.*	Pirbalouti et al., 2009 Pirbalouti et al., 2010 Pirbalouti et al., 2014
T. capitatus L.	Algeria	Thymol (51.22%), carvacrol (12.59%), γ-terpinene (10.3%), *trans*-13-octadecenoic acid (9.04%), linalool (2.29%), caryophyllene (2.01%), pentadecanoic acid (1.92%), α-terpinene (1.78%), ß-myrcene (1.49%), caryophyllene oxide (1.21%).	*E. coli, S. typhi, S. aureus, S. pneumoniae, Cladosporium herbarum, Alternaria infectoria, A. ochraceus,* and *Trichophyton* sp.	Goudjil et al., 2020
	Tunisia	Thymol (69.95–81.49%), α-cubebene (0–3.44%), *β*-ocimene (3.09–3.16%), carvacrol (0–2.56%), α-terpinene (2.25–3.83%).	*E. coli* ATCC 8739, *S. typhimurium* NCTC 6017, *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, *A. hydrophila*, *L. monocytogenes* ATCC 7644, *B. cereus*, *A. flavus*, *A. niger* and *C. albicans.*	Aouadhi et al., 2013
		Thymol (89.06%), *p*-cimene (5.04%), γ-terpinene (3.19%).	*S. aureus* CIP7625, *L. monocytogenes* Scott A 724, *E. coli* ATCC 10536, *K. pneumoniae* CIP8291, *S. cerevisiae* ATCC 4226, *C. albicans* IPA 200, *M. ramamnianus* ATCC 9314, *A. westerdijkiae* NRRL 3174.	Mkaddem et al., 2010
T. cappadocicus Boiss.	Turkey	Thymol (70.82%), cymene (9.52%), *g*-terpinene (9.27%).	*A. hydrophila, E. coli, M. morganii, K. pneumoniae, P. mirabilis, P. aeruginosa, S. typhimurium, Y. enterocolitica, B. brevis, B. cereus, B. subtilis, L. monocytogenes, S. aureus, C. albicans* and *S. cerevisiae.*	Albayrak and Aksoy, 2012
T. striatus	Balkan Peninsula	Thymol (59.5%), γ-terpinene (11.6%), *p*-cymene (6.4%), carvacrol-methyl ether (5.9%), carvacrol (4.9%), α-terpinene (3.3%), E-caryophyllene (2.3%).	*A. alternata, A. niger, A. ochraceus, A. versicolor, A. flavus, A. terreus, C. cladosporioides, P. funiculosum, P. helianthi, T. viride, T. mentagrophytes, M. canis,* and *E. floccosum*	Couladis et al., 2004
T. algeriensis Boiss. and Reut	Balkan Peninsula	Thymol (36%), carvacrol (14%), α-pinene (1.1%), *β*-mycrene (2.3%), *p*-cymene (6.3%), *β*-bisabolene (4%0, α-terpinene (1.6%), γ-terpinene (4.8%), linalool (1.3%), camphor (1.1%), caryophyllene oxide (1%).	*C. albicans* ATCC 10234, *C. glabrata*, *C. krusei*, *C. tropicalis* ATCC 750, *P. aeruginosa*, *E. faecalis*, *S. sanguinis*, *S. salivarus*, *S. mutans*, *L. acidophilus*, *S. aureus.*	Nikolic et al., 2014
T. numidicus Poiret	Algeria	Thymol (40.40%), carvacrol (13.37%), thymol methyl ether (8.30%), *β*-myrcene (2.37%), *p*-cymene (7.18%), γ-terpinene (6.41%), linalool (4.06%), *β*-caryophyllene (2.48%), *β*-bisabolene (3.26%).	*S. aureus* ATCC 25923, *E. coli*, *P. aeruginosa* ATCC 27853, *C. albicans.*	Messara et al., 2016
T. zygis	Spain	α-pinene (36.8 ± 1.7–93.9 ± 4.8 mM), myrcene (32.7 ± 0.6–145.6 ± 6.4 mM), α-terpinene (14.6 ± 0.5–102.1 ± 5.5 mM), *p*-cymene (705.7 ± 22.9–1212.8 ± 13.0 mM), γ-terpinene (448.5 ± 22.4–1462.8 ± 38.2 mM), linalool (223.6 ± 2.8–386.8 ± 13.6 mM), terpinen-4-ol (8.9 ± 0.3–45.7 ± 0.3 mM), thymol (1923.2 ± 27.5–	*S. aureus* ATCC 6538, *E. coli* ATCC 8739, *P. aeruginosa* ATCC 9027, *C. albicans* ATCC 10231.	Cutillas et al., 2018

(*continued on next page*)

Table 5 (*continued*)

Thymus species	Origin	Main Components	Bacteria and Fungi tested	Reference
		3636.2 ± 15.2 mM), carvacrol (34.3 ± 1.3–112.9 ± 2.5 mM), E-β-caryophyllene (24.1 ± 0.3–50.4 ± 1.0 mM)		
T. serpillum L.	Balkan Peninsula	Thymol (38.5%), carvacrol (4.7%), α-pinene (2%), camphene (2.4%), γ-terpinene (7.2%), linalool (2.4%), borneol (6%), thymol methyl ether (3.8%), thymol acetate (2.8%).	*C. albicans* ATCC 10234, *C. glabrata*, *C. krusei*, *C. tropicalis* ATCC 750, *P. aeruginosa*, *E. faecalis*, *S. sanguinis*, *S. salivarius*, *S. mutans*, *L. acidophilus*, *S. aureus*.	Nikolic et al., 2014
T. lanceolatus	Algeria	Thymol (69.61%), γ-terpinene (8.38%), *p*-cymene (5.07%), carvacrol (3.57%), α-terpinene (1.31%), linalool (1.01%), β-mycrene (1.72%), α-thujene (1.07%), α-pinene (0.73%), d-limonene (0.62%), β-pinene (0.43%).	*S. aureus* ATCC 29213, *S. epidermidis* ATCC 14990, *S. capitis* ATCC 35661, *S. pyogenes* ATCC 12344, *S. agalactiae* ATCC 27956, *Bacillus subtilis* ATCC 6051, *P. fluorescens* ATCC 13525, *S. typhimurium* ATCC 14028, *S. flexneri* ATCC 700930, *E. coli* ATCC 25922, *A. fumigatus* ATCC 1022, *Geotrichum candidum* ATCC 12784, *S. racemosum* ATCC 14831, *C. albicans* (ATCC 90028).	Khadir et al., 2016a, b
T. linearis Benth.	India	Thymol (54.9%), γ-terpinene (16.6%), *p*-cymene (5.2%), α-thymol methyl ether (3.2%), terpinene (2.6%), thymyl acetate (2.8%), β-bisabolene (2.3%), (E)-caryophyllene (2.0%), myrcene (1.8%), α-thujene (1.6%), carvacrol (1.5%), borneol (1.1%).	*S. aureus* MRSA 33591, *S. epidermidis* MRSE 51625, *S. aureus* MRSA (BAA-44), *S. aureus* MTCC-96, *S. epidermidis* MTCC-435, *E. faecalis* MTCC-439, *C. albicans* ATCC 14053, *C. tropicalis* ATCC 2013180, *C. glabrata* ATCC-15126.	Kumar et al., 2020
T. kotschyanus	Iran	α-pinene (5.49–12.72%), β-Myrcene (0.80–1.51%), α-terpinene (1.62–1.80%), *p*-cymene (0–21.35%), m-cymene (0–8.87%), 1,8-cineole (4.57–4.79%), γ-terpinene (4.00–8.01%), 4-terpineol (0–2.19%), α-terpineol (0.92–1.08%), thymol methyl ether (2.10–2.44%), carvacrol methyl ether (0–4.14%), thymol (29.96–47.48%), carvacrol (0.62–3.79%), β-bourbonene (0.15–3.30%), caryophyllene (1.27–2.92%).	*E. faecalis* ATCC 29212, *S. aureus* ATCC 25952, *S. aureus* ATCC 33591, *S. aureus* ATCC 29213, *S. sanguis* PTCC 1449, *E. aerogenes* ATCC 13048, *K. pneumoniae* ATCC 700603, *P. mirabilis* ATCC 43071, *E. coli* O157:H7	Mobaiyen et al., 2017
T. eigii	Turkey	Thymol (24.77%), carvacrol (14.00%), *p*-cymene (10.91%), γ-terpinene (6.53%), borneol (6.48%), caryophyllene (3.92%), α-pinene (2.03%), α-thujene (2.34%), β-myrcene (2.68%), α-terpinene (2.28%), 1-octen-3-ol (2.94%), 17 *trans*-sabinene hydrate (2.19%), 4-terpineol (2.55%), (-)-caryophyllene oxide (2.01%).	*E. faecalis* ATCC 29212, *E. casseliflavus* ATCC 700327, *S. aureus* ATCC 29213, *S. aureus* ATCC BAA 977, *E. hormaechei* ATCC 700323, *K. pneumoniae* ATCC 700603, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *C. parapsilosis* ATCC 22019, *C. albicans* ATCC 14053.	Ulukanli et al., 2018.
T. willdenowii Boiss & Reut	Morocco	Thymol (35.5–47.3%), *p*-cymene (13.9–23.8%), γ-terpinene (8.9–20.3%), carvacrol (3–5.6%), linalool (3–3.5%), camphor (0.9–3.7%), borneol (0.7–4.7%).	*E. coli* ATCC 25922, *P. mirabilis* ATCC 35659, *B. cereus* ATCC 10876, *C. albicans* ATCC 10231, *A. brasilliensis* ATCC 16404.	Ouknin et al., 2019
***T. musilii* Velen**	Saudi Arabia	Thymol (67.69 ± 0.93%), thymyl acetate (12.99 ± 0.22%), *p*-cymene (4.61 ± 0.11%), Carvacrol (3.41 ± 0.10%), γ-terpinene (2.63 ± 0.07%).	*E. coli* ATCC 35218, *P. aeruginosa* ATCC 27853, *P. mirabilis* ATCC 29245, *K. pneumoniae* ATCC 27736, *S. aureus* MDR, *E. cloacae*, *C. albicans* ATCC 10231, *Cryptococcus neoformans* ATCC 14116, *C. vaginalis*, *Candida* sp., *A. fumigatus* ATCC 204305 and *A. niger*.	**This study**

Table 6 Literature review of the antimicrobial activity of the main component identified in *T. musilii* Velen essential oil: thymol.

Strains Tested	MIC	MBC/MFC	Reference
Bacillus cereus	327.581 ppm	–	Falcone et al. 2005
Bacillus subtilis	422.332 ppm	–	
Bacillus licheniformis	422.811 ppm	–	
Lactobacillus curvatus	723.45 ppm	–	
Lactobacillus plantarum	941.01 ppm	–	
Candida lusitaniae	307.901 ppm	–	
Pichia subpelliculosa	422.781 ppm	–	
Saccharomyces cerevisiae	337.761 ppm	–	
Staphylococcus aureus ATCC 68380	0.31 mg/mL	–	Tombetta et al., 2005
Escherichia coli ATCC 15221	5.00 mg/mL	–	
Candida albicans ATCC 10231	0.16 µl/mL	0.32 µl/mL	Pinto et al., 2006
Candida guilliermondii MAT23	0.16 µl/mL	0.16 µl/mL	
Candida parapsilosis ATCC 90018	0.32 µl/mL	0.32 µl/mL	
Candida krusei ATCC 6258	0.16 µl/mL	0.32 µl/mL	
Candida tropicalis ATCC 13803	0.16 µl/mL	0.32 µl/mL	
Candida albicans	0.16 µl/mL	0.32 µl/mL	
Candida tropicalis	0.16 µl/mL	0.32 µl/mL	
Candida glabrata	(0.16–0.32) µl/mL	0.32 µl/mL	
Candida krusei	0.16 µl/mL	0.32 µl/mL	
Trichophyton rubrum	0.16 µl/mL	0.16 µl/mL	
Trichophyton mentagrophyte	0.16 µl/mL	0.32 µl/mL	
Epidermophyton floccosum	0.16 µl/mL	0.16 µl/mL	
Microsporum gypseum	0.16 µl/mL	0.32 µl/mL	
Microsporum canis	0.08 µl/mL	0.16 µl/mL	
Aspergillus niger ATCC 16404	0.16 µl/mL	0.64 µl/mL	
Aspergillus niger CECT 2574	0.16 µl/mL	0.64 µl/mL	
Aspergillus fumigatus CECT 2071	0.16 µl/mL	0.64 µl/mL	
Aspergillus fumigatus ATCC 46645	0.16 µl/mL	0.64 µl/mL	
Aspergillus flavus	0.32 µl/mL	0.64 µl/mL	
Aspergillus niger	0.16 µl/mL	0.64 µl/mL	
Aspergillus fumigatus	0.16 µl/mL	0.64 µl/mL	
Salmonella typhimurium SGI1	2.5 mM	–	Palaniappan and Holley, 2010
Escherichia coli N00-666	2.5 mM	–	
Staphylococcus aureus blaZ+	2.5 mM	–	
Streptococcus pyogenes ermB+	0.31 mM	–	
Escherichia coli O157:H7	500–1000 µg/mL	1000–2000 µg/mL	Rivas et al., 2010
Escherichia coli O26	1000 µg/mL	1000 µg/mL	
Escherichia coli O111	1000 µg/mL	2000 µg/mL	
Escherichia coli O103	1000 µg/mL	1000 µg/mL	
Escherichia coli O145	1000 µg/mL	>2000 µg/mL	
Salmonella Typhimurium	2000 µg/mL	2000 µg/mL	
Listeria monocytogenes	1000 µg/mL	1000 µg/mL	
Hafnia alvei	500 µg/mL	500 µg/mL	
Staphylococcus aureus	500 µg/mL	500 µg/mL	
Lactobacillus sakei	1000 µg/mL	2000 µg/mL	
Pseudomonas putida	1000 µg/mL	2000 µg/mL	
Bacillus thermosphacta	–	250 µg/mL	
Streptococcus mutans MTCC 890	125 µg/mL	–	Mathela et al., 2010
Staphylococcus aureus MTCC 96	62.5 µg/mL	–	
Bacillus subtilis MTCC 121	125 µg/mL	–	
Staphylococcus epidermidis MTCC 435	125 µg/mL	–	
Escherichia coli MTCC 723	250 µg/mL	–	
Escherichia coli	< 0.019–0.039 mg/mL	–	Pirbalouti et al., 2011
Pseudomonas aeruginosa	<0.019–0.039 mg/mL	–	
Staphylococcus aureus	< 0.019–156 mg/mL		
Bacillus cereus	< 0.019–0.156 mg/mL		
Micrococcus luteus	1250 µg/mL		Hernández-Hernández et al., 2014
Phytophthora infestans	400.26 µl/l	–	Ben and Hamada, 2014
Phytophthora ultimum	263 µl/l	–	

(*continued on next page*)

Table 6 (*continued*)

Strains Tested	MIC	MBC/MFC	Reference
Botrytis cinerea	> 600 µl/l	–	
Rhizoctonia solani	64.56 µl/l	–	
Aspergillus niger	100 mg/mL	–	Abbaszadeh et al., 2014
Aspergillus fumigatus	150 mg/mL	–	
Aspergillus flavus	100 mg/mL	–	
Aspergillus ochraceus	100 mg/mL	–	
Alternaria alternata	100 mg/mL	–	
Botrytis cinerea	100 mg/mL	–	
Cladosporium spp.	100 mg/mL	–	
Penicillium citrinum	100 mg/mL	–	
Penicillium chrysogenum	100 mg/mL	–	
Fusarium oxysporum	100 mg/mL	–	
Rhizoctonia oryzae	100 mg/mL	–	
Escherichia coli	187.5 µg/mL	375 µg/mL	Du et al., 2015
Clostridium perfringens	375 µg/mL	750 µg/mL	
Salmonella Typhimurium	375 µg/mL	750 µg/mL	
Salmonella Enteritidis	750 µg/mL	1500 µg/mL	
Salmonella Pullorum	375 µg/mL	750 µg/mL	
Lactobacillus acidophilus	1500 µg/mL	3000 µg/mL	
Lactobacillus reuteri	1500 µg/mL	3000 µg/mL	
Lactobacillus salivarius	1500 µg/mL	3000 µg/mL	
Pythium insidiosum	160–320 µg/mL		Jesus et al., 2015
Helicobacter pylori	0.043 ± 0.024 µl/mL		Falsafi et al., 2015
Mycobacterium tubercolosis	0.75 µg/mL		Andrade-Ochoa et al., 2015
Mycobacterium bovis	2.02 µg/mL		
Candida albicans	39 µg/mL	–	De Castro et al., 2015
Candida krusei	39 µg/mL	–	
Candida tropicalis	78 µg/mL	–	
Aspergillus flavus CGMCC 32890	80 µg/mL	–	Shen et al., 2016
Bacillus cereus	0.007 mg/mL	–	Guimarães et al., 2019
Salmonella Typhimurium	0.003 mg/mL	0.12 mg/mL	
Escherichia coli	0.007 mg/mL	0.12 mg/mL	
Staphylococcus aureus	0.007 mg/mL	0.12 mg/mL	
Cronobacter sakazakii lv27	0.05%	–	Berthold-Pluta et al., 2019
Cronobacter malonaticus lv31	0.05%	–	
Cronobacter muytjensii s50	0.05%	–	
Cronobacter *turicensis* lv53	0.05%	–	
Cronobacter condimenti s37	0.05%	–	
Escherichia coli ATCC 35218	3.125 mg/mL	6.25 mg/mL	**This study**
Pseudomonas aeruginosa ATCC 27853	12.5 mg/mL	50 mg/mL	
Proteus mirabilis ATCC 29245	3.125 mg/mL	6.25 mg/mL	
Klebsiella pneumoniae ATCC 27736	3.125 mg/mL	6.25 mg/mL	
Staphylococcus aureus MDR	0.78 mg/mL	1.56 mg/mL	
Enterobacter cloacae	0.39 mg/mL	0.78 mg/mL	
Candida albicans ATCC 10231	12.5 mg/mL	100 mg/mL	
Cryptococcus neoformans ATCC 14116	50 mg/mL	100 mg/mL	
Candida vaginalis (Clinical strain)	25 mg/mL	100 mg/mL	
Candida sp. (Clinical strain)	25 mg/mL	100 mg/mL	

at 1 mg/mL), and it had no chelating activity till 1 mg/mL (Sarikurkcu et al., 2010).

Compared to the previous studies, *T. musilii* essential oil in the present study exhibited a strong antioxidant effect. This activity can be explained by the chemical composition classes, monoterpene hydrocarbons (11.01%) and oxygenated monoterpenes (87.01%), of the volatile oil. Most researchers revealed the antiradical effect of monoterpenes (Badawy et al., 2019; Wojtunik-Kulesza et al., 2019). The antioxidant capacity of thymol (IC_{50} = 31.426 mg/mL), β-cymene (IC_{50} = 916.89 mg/mL), α-terpineol (IC_{50} = 480.56 mg/mL), myrcene (IC_{50} = 22.136 mg/mL), α-pinene (IC_{50} = 880.74 mg/mL) were evaluated using N,N-dimethyl-1,4-phenylene diamine (DMPD) reagent (Badawy et al., 2019).

Other study focused on the antioxidant of α-terpinene (IC_{50} = 0.6 and 7.5 mM) and γ-terpinene (IC_{50} = 2.8 and 30.0 mM) using ABTS and DPPH methods, respectively (Li and Liu, 2009). Previous work demonstrated that γ-terpinene

Table 7 Binding affinities of top-rated pose of ligand-receptor complex. Binding affinity measured in kcal/mol.

Compounds	1XFF	1JIJ	2XCT
α-Thujene	−4.5	−5.5	−4.9
α-Pinene	−4.4	−5.6	−4.5
β-Myrcene	−3.3	−5.1	−4
α-Terpinene	−4.5	−6	−4.9
p-Cymene	−4.3	−5.7	−5
1,8-Cineole	−4.8	−5.1	−4.8
γ-Terpinene	−4.5	−6	−4.9
α-Terpinolene	−4.4	−5.9	−5.3
Borneol	−4.9	−5.4	−4.8
Terpinen-4-ol	−4.6	−5.8	−5
α-Terpineol	−4.9	−6.1	−5.1
2-Isopropyl-5-methylanisole	−4.4	−4.9	−5.1
Thymol	−4.5	−5.9	−5.4
Carvacrol	−5.2	−6.3	−5.4
Thymyl acetate	−5.1	−6.1	−4.8
Carvacryl acetate	−5	−6.1	−5.6
β-Caryophyllene	**−5.4**	**−6.8**	**−6.2**

1XFF: glucosamine 6-phosphate synthase (GLMS) from *E. coli,* 1JIJ: tyrosyl-tRNA synthetase TyrRS from *S. aureus,* 2XCT: Gyrase from *S. aureus*.

(IC_{50} = 15.5 mg/mL) inhibited DPPH radicals (Sonboli et al., 2005). This antioxidant assay may be related to a high area of thymol (67.7%). Several studies confirmed the strong *in vitro* and *in vivo* biological effect of thymol (Abd El-Naby et al., 2020; Arafa et al., 2020; Jafari et al., 2020). The registered effect may referred to the major compound, thymol (67.7%), and/or to the synergism between main and minor compounds of the essential oil (Ciesla et al., 2016). The antioxidant activities were studied, in literature, towards the whole essential oils, to single compounds and as well as to combination (Graßmann, 2005; Tohidi et al., 2020).

3.3. Antimicrobial activities of *T. musilii* essential oil

The antibacterial activity of *T. musilii* essential oil was tested against six bacteria, four yeasts and two fungal strains using both disc diffusion (Fig. 4) and microdilution assays. Obtained results showed that, the tested bacteria were resistant to ampicillin with a mean diameter of growth inhibition zone ranging from 6.33 ± 0.57 mm to 7.33 ± 0.57 mm. In addition, the mean diameter of growth inhibition zones ranged from 21.33 ± 1.52 mm for *P. mirabilis* to 36.33 ± 1.15 mm for *K. pneumoniae*. The clinical strain *S. aureus* MDR, resistant to ampicillin, was susceptible to the oil tested (25.33 ± 1.15 mm). Small quantities of oil (12.5 mg/mL) can inhibit the growth of all tested bacteria, except for *E. cloacae* (MIC value = 3. 125 mg/mL). MBCs values were ranging from 6.25 mg/mL (*E. cloacae*) to 100 mg/mL for *P. aeruginosa*. As compared to the single bioactive molecule, thymol, *T. musilii* essential oil exhibited bactericidal activity for all tested bacteria with MBC/MIC ratio inferior to 4 except for *P. aeruginosa* (MBC/MIC ratio = 8). All these data are summarized in Table 2. Using the literature review, high antimicrobial activity of *Thymus* species (chemotype thymol) was recorded against a large collection of bacterial and fungal species (Table 5).

Similar results were obtained with the yeast and fungi strains tested. Interestingly, high diameter of inhibition zone was recorded for the two clinical yeast strains: *Candida* sp. (37.33 ± 1.15 mm), and *C. vaginalis* (37.33 ± 1.15 mm). The MIC and MFC values were 6.25 mg/mL and 12.5 mg/mL, respectively for both strains. Using the MFC/MIC ratio scheme proposed by Gatsing et al. (2009), *T. musilii* seems to be more effective than thymol on the four tested yeast strains as they have the lowest ratio ranging from 2 to 4. It is important to highlight also that the tested (thymol/thymyl acetate) chemotype oil was very active on the two *Aspergillus* strains with mean inhibition zone about 88.66 ± 1.15 mm for *A. fumigatus* to 87.33 ± 1.15 mm for *A. niger*. All these data are summarized in Table 3.

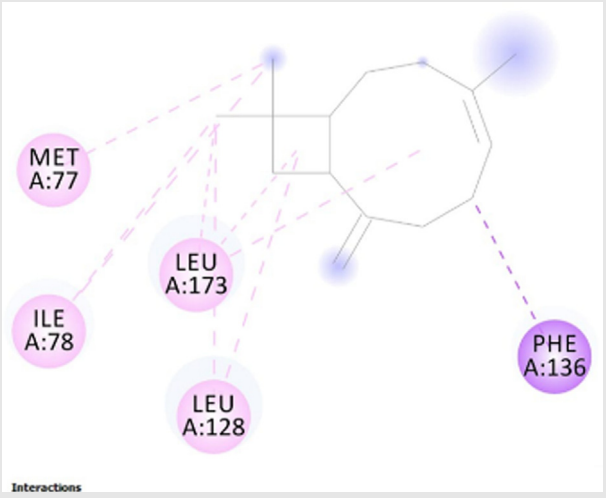
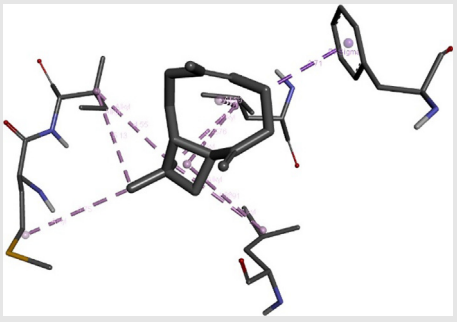
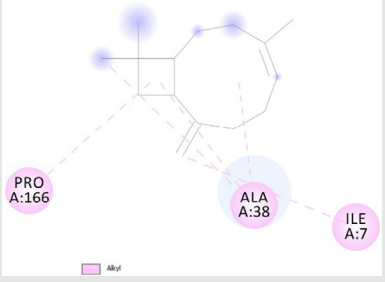
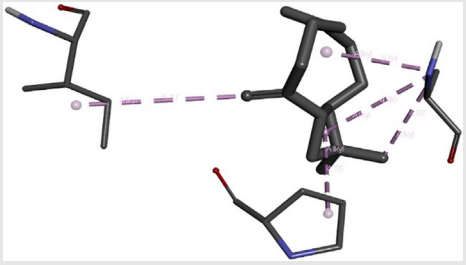
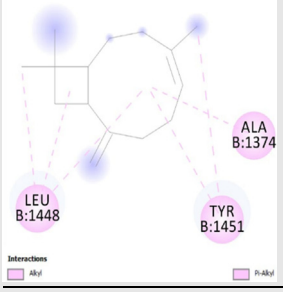
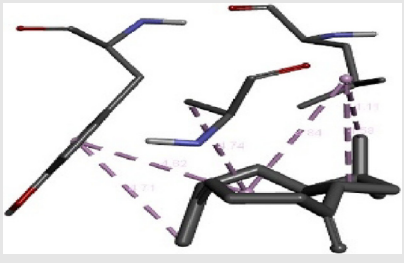
Using the disc diffusion test, Vladimir-Knežević and colleagues (2012) reported similar results with *T. longicaulis* species (Chemotype thymol, 46.3%) tested against *Haemophilus influenzae* (IZ = 42 mm), *Neisseria meningitidis* (IZ = 53 mm), *S. aureus* (IZ = 35 mm), *S. pneumoniae* (IZ = 43 mm), and *S. pyogens* (IZ = 41 mm). Additionally, Bozin et al. (2006) reported that *T. vulgaris* essential oil (chemotype thymol) was active against a wide range of Gram-positive and Gram-negative bacteria, including the same species tested in our study. In fact, the highest growth inhibition zones were recorded for *Micrococcus flavus* (IZ = 48.2 mm), *S. epidermidis* (IZ = 48 mm), *S. aureus* (IZ = 26.2 mm), *B. subtilis* (IZ = 40.6 mm), *E. coli* (IZ = 29.4 mm), and *P. aeruginosa* (IZ = 12 mm).

Previous reports have noticed the anti-*C. albicans* activity of different species belonging to the *Thymus* genus. In fact, Pinto et al. (2006) reported a significant activity of *T. pulegioides* oil (thymol 26%/carvacrol 21% chemotype) against *Candida*, *Aspergillus* and dermatophyte species explained by the alteration in the cytoplasmic membrane and ergosterol content.

In addition, Pirbalouti et al. (2009) founded that *T. daenensis* Celak. essential oil effectively inhibits the growth of vaginal *C. albicans* strains at high concentration (50–55 µl). The same oil was active against *E. coli* O157:H7, *B. cereus*, *L. monocytogenes,* and *C. albicans* with a diameter of growth inhibition zone and MIC values about (7 mm/ > 10 $mg{\cdot}mL^{-1}$, 25 mm/0.625 $mg{\cdot}mL^{-1}$, 16 mm/2.5 $mg{\cdot}mL^{-1}$, and 19 mm/ < 0.039 $mg{\cdot}mL^{-1}$ respectively (Pirbalouti et al., 2010). Thymol-rich chemotype of *T. daenensis* Celak essential oil can inhibit the growth of *S. aureus* isolated from milk with MIC and MBC values about 62 µg/mL and 630 µg/mL, respectively (Pirbalouti et al., 2014). Couladis et al. (2004) reported the high activity of *T. striatus* (Chemotype thymol, 59.5%) against a large collection of *Aspegillus*, *Cladosporium*, *Penicillium*, *Trichoderma*, *Tricophyton*, *Microsporum*, and *Epidermophyton* strains with MICs values ranging from 0.5 to 2 µl. In 2014, Nikolic and colleagues reported that *T. serpyllum* (Thymol, 38.5%) was active against four *Candida* species (*C. albicans*, *C. tropicalis*, *C. glabrata*, and *C. krusei*) with MICs values ranging from 01. to 0.2 µl. More recently, Satyal et al. (2016) demonstrated that *T. vulgaris* essential oils inhibit the growth of *C. neoformans* var. *neoformans*, and *C. albicans* with MICs values about (313/156) $\mu g{\cdot}mL^{-1}$, and (1250/625) $\mu g{\cdot}mL^{-1}$, respectively for linalool and geraniol chemotypes.

A brief literature review summarized the antimicrobial activity of thymol against a large collection of bacteria, yeast and fungi (Table 6). High activity of the *Thymus* plant species can be associated to the dominance of thymol with different percentage. In fact, this molecule is known to exhibit antimicrobial, antioxidant, immunological, anti-inflammatory, anti-

Table 8 Interacting active site residues of receptors with natural bio-compounds.

2D interactions, Receptor Ligand Interactions, Distance in Angstroms	3D interaction Receptor–Ligand
Receptor – Ligand: 1JIJ – β-Caryophyllene (MET77) S—S (Ligand) Alkyl interaction: 4.75 A°; (ILE78) C—C (Ligand) Alkyl interaction: 4.55 A°; (ILE78) C—C (Ligand) Alkyl interaction: 5.13 A°; (LEU128) C—C (Ligand) Alkyl interaction: 4.63 A°; (LEU128) C—C (Ligand) Pi-Alkyl interaction 4.91A °;(PHE136) phenyl ring—C (Ligand) Pi-sigma interaction: 3.71 A°; (LEU173) C—C (Ligand) Alkyl interaction: 3.85 A°; (LEU173) C—C (Ligand) Pi-Alkyl interaction: 4.69 A °; (LEU173) C—C (Ligand) Pi-Alkyl interaction: 4.76 A°.	**Receptor – Ligand: 1JIJ – β-Caryophyllene**
	
Receptor – Ligand: 1XFF – β-Caryophyllene (ILE7) CC—CH (Ligand): 5.37 A°; (ALA38) C—Phenyl ring (Ligand) Pi-Alkyl interaction: 3.64 A°; (ALA38) C—Alkyl ring (Ligand) Pi-Alkyl interaction: 4.40 A°; (ALA38) C—C (Ligand) Alkyl interaction: 4.07 A°; (PRO166) phenyl ring—Alkyl ring (Ligand) Pi-Alkyl interaction: 4.76 A°.	**Receptor – Ligand: 1XFF – β-Caryophyllene**
	
Receptor – Ligand: 2XCT – β-Caryophyllene (ALA1374)C—phenyl ring (Ligand) – Pi-alkyl interaction: 4.74 A°; (LEU1448) C—phenyl ring (Ligand)–Pi-alkyl interaction: 4.84 A°; (LEU1448) C—alkyl ring (Ligand)–Alkyl interaction: 4.58 A°; (LEU1448) C—C (Ligand)–Alkyl interaction: 4.11 A°; (TYR1451) phenyl ring—C (Ligand) Pi-alkyl interaction: 4.71 A°; (TYR1451) phenyl ring—phenyl ring (Ligand) Pi-alkyl interaction 4.82 A°.	**Receptor – Ligand: 2XCT – β-Caryophyllene**
	

cancer, and cardiovascular protection properties (Nagoor et al., 2017; D'agostino et al., 2019). This terpenoid molecule inhibits the hyphal production in *Fusarium graminearum* (Gao et al., 2016), decreases the membrane permeability leading to the loss of cytoplasmic membrane integrity and loss of electrolytes in *C. albicans* species by binding to ergosterol (De Castro et al., 2015), and inhibits the telomerase activity in *S. cerevisiae* species (Darvishi et al., 2013). It has been demonstrated that thymol can kill Methicillin-resistant *S. aureus* strain by increasing the formation of reactive oxygen species (Li et al., 2014).

3.4. Molecular docking analysis

In order to correlate the binding of isolated *Thymus* bioactive molecules with its biological activities, the main compounds were docked to the active site of TyrRS, GLMS and Gyrase, respectively to demonstrate their potential inhibition against *S. aureus* and *E. coli* pathogens. The binding affinities of top-rated pose of different ligand-receptor complex (Table 4) revealed that among all tested bioactive compounds, the best binding affinity was found with β-caryophyllene-enzymes with values of −5.4 kcal/mol, −6.8 kcal/mol and −6.2 kcal/mol, respectively for β-caryophyllene-TyrRS, β-caryophyllene-GLMS and β-caryophyllene-Gyrase, suggesting its highest binding efficiency and therefore was selected for further investigation.

To get insight into the mechanism of TyrRS, GLMS and Gyrase inhibition by β-caryophyllene, we elucidate their molecular interaction mode in the active site residues of receptors. The outcomes compiled in Table 8 showed that β-caryophyllene-TyrRS complex was mainly stabilized by Alkyl interactions with Met77, Ile78 and Leu128, Pi-Alkyl interactions with Leu128 and Leu173 and Pi-sigma interactions with Phe 136 residues. Alkyl and Pi-Alkyl interactions were also formed between β-caryophyllene and GLMS residues of Ile7, Ala38 and Pro166. However, the amino acid residues involved in stabilizing the complex caryophyllene-Gyrase are Ala1374 (Pi-Alkyl), Leu1448 (Pi-Alkyl and Alkyl) and Tyr1451 (Pi-Alkyl). As shown, Phe136 and Leu173 of TyrRS from *S. aureus*, Ala38 from Gyrase in *S. aureus* and Leu1448 from GLMS in *E. coli* formed stronger Pi-Sigma, Alkyl and Pi-Alkyl interactions with the natural bioactive compounds (Tables 7 and 8) and therefore, could possibly inhibit the activity of enzyme resulting in the neutralization of their virulence.

4. Conclusion

In the present study, the antioxidant and the antimicrobial assays of the essential oil from *T. musilii* were evaluated. The obtained findings suggest that this cultivated species can constitute a good source of antioxidant, antibacterial and antifungal compounds, namely, thymol. Nevertheless, these biological results deserve further deep *in vivo* studies in order to use this plant as possible bio-source in food and pharmaceutical industries. Molecular docking results together with the findings of *in-vitro* antimicrobial potency suggest that *T. musilii* essential oil is a potent inhibitor of *S. aureus* and *E. coli* and subsequently lead to novel discovery of plant-based therapeutic products.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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