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Research article

Comprehensive *in vitro* and *in silico* study of the antioxidant and antimicrobial attributes of chemically characterized essential oil derived from Moroccan *Thymus vulgaris*.

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Abstract

Recent studies have highlighted the bioactive and protective properties of phytochemicals found in essential oils (EOs), particularly in those derived from medicinal herbs. This study aimed to identify the phytochemical composition of *Thymus vulgaris* EO from Morocco and to evaluate its antioxidant and antimicrobial activities through both *in vitro* and *in silico* approaches. The EO composition was analyzed using GC-MS, revealing estragole (59.76%) as the primary component, followed by borneol (10.08%) and α-terpineol (6.81%). Antioxidant activity was assessed using FRAP and DPPH assays, resulting EC_{50} and IC_{50} values of 6.780 mg/mL and 0.058 mg/mL, respectively. Additionally, the EO demonstrated a substantial total antioxidant capacity of 240.30 mg AAE/g EO. The antimicrobial activity was evaluated against various bacterial and fungal strains using standard procedures. A considerable efficacy of *T. vulgaris* EO against all tested fungi was noted by inhibiting totally the growth of *A. flavus*, *A. niger* and *F. proliferatum* at 0.886 g/mL. Also, the tested EO inhibited the growth of *C. albicans* as well as Gram positive and negative bacteria with inhibition zones diameters ranging from 7.33 ± 0.58 to 56 ± 1.73 mm. The *in silico* antibacterial analysis revealed terpinen-4-ol as the most active molecule in the EO against *E. coli* Gyrase B (PDB ID: 3G7E), achieving a glide score of -5.987 kcal/mol. Regarding the antifungal activity, Δ-cadinene and thymol were identified as active phytocompounds targeting the sterol 14-α demethylase (CYP51) from *C. albicans* (PDB ID: 5FSA) and the β-1,4-endoglucanase from *A. niger* (PDB ID: 5I77), respectively, with glide scores of -7.376 and -5.551 kcal/mol. These findings highlight the promising potential of *T. vulgaris* EO for medical and industrial applications as a remedy against free radicals and resistant pathogenic microbes.

Keywords: *Thymus vulgaris*; Essential oil; Antimicrobial; Antioxidant; *In vitro*; *In silico*.

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1. Introduction

Microbial resistance, encompassing bacteria, fungi, viruses, and protozoa, represents a serious global health challenge. This issue largely stems from improper drug usage, which promotes the development of multidrug-resistant microorganisms and leads to increased mortality rates (Azad, 2024). Consequently, the search for anti-infective agents has become an essential priority (Cushnie and Lamb, 2011). Additionally, there has been a growing global interest in the conservation of food systems, driven by the rising economic burden associated with spoilage and contamination caused by oxidation and microbial pathogens (Rodriguez-Garcia et al., 2016). In parallel, oxidative stress has emerged as a significant concern in contemporary times, as it can negatively affect healthy cells by inducing their transformation into cancerous cells, driven by the accumulation of elevated levels of reactive oxygen species (ROS) (EL Abdali et al., 2023). While synthetic antioxidants, such as butylated hydroxytoluene (BHT), are widely used in the food industry for their antioxidative properties, they are linked to potential health risks, including liver damage and increased cancer risk (Djeridane et al., 2010). Nonetheless, these health challenges can be alleviated by using medicinal plants and their derivatives, which possess antioxidant and antimicrobial properties. These natural compounds present promising alternatives to synthetic antioxidants and conventional antibiotics (El Abdali et al., 2023b; Wang et al., 2023).

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In light of recent scientific advancements, the medicinal properties of aromatic plants have garnered significant attention due to their pharmacological activities, low toxicity, and economic efficiency (Chouhan et al., 2017; El Abdali et al., 2021). Among these natural resources, various bioactive molecules, such as flavonoids, phenolic acids, tannins, carotenoids, sterols, and terpenoids, have been identified and extensively studied (Berrougui et al., 2006; EL Abdali et al., 2023). A deeper understanding of the mechanistic pathways of these bioactive molecules in biological and pharmacological activities is now essential for their usage. In this context, *in silico* ligand-protein docking, employing computer-assisted drug design (CADD) methodologies, offers a valuable tool for identifying key binding sites and predicting the predominant binding modes of a ligand with a protein of known three-dimensional structure. This approach enables the development of structural hypotheses regarding how ligands inhibit their target proteins (Mali et al., 2022; Yu and MacKerell, 2017).

Northern Morocco, characterized by its Mediterranean climate, is abundant in medicinal and aromatic plants, many of which have been utilized in traditional medicine and local diets for centuries. Among these, *Thymus vulgaris* L., commonly known as thyme, stands out as a characteristic plant of the region. As a member of the Lamiaceae family, this medicinal and aromatic herb is notably rich in a variety of bioactive molecules, including volatile oils, phenolic acids, terpenoids, flavonoids, saponins, steroids, tannins, alkaloids, and polysaccharides. It occupies a significant place in traditional Moroccan medicine, where it has been widely employed to address a range of ailments, such as respiratory, digestive, and inflammatory disorders, as well as skin infections (Bellakhdar, 1997; Javed et al., 2013; Patil et al., 2021). The broad range of applications of *Thymus* varies depending on the region, medicinal purposes, the plant parts utilized, and the method of preparation (Patil et al., 2021). Similarly, the EO of *T. vulgaris*, which is rich in oxygenated terpenes such as thymol, borneol and carvacrol, has demonstrated a wide range of pharmacological properties (Halat et al., 2022; Mehani et al., 2024). Several works have shown the biological activities of *Thymus vulgaris*, like antimicrobial, anti-inflammatory, antioxidant, cytotoxic, antiviral, antineoplastic, antiseptic and anti-cancerous activities (Halat et al., 2022; Mehani et al., 2024; Patil et al., 2021). However, reports detailing the medicinal properties of EOs extracted from the northern Moroccan species, particularly their mechanisms of action in various biological activities, remain scarce. Therefore, this study aimed to assess, both *in vitro* and *in silico*, the antioxidant capacity and antimicrobial activity of EO derived from *T. vulgaris* (Immouzzer region of Morocco). Also, evaluating the EO against fungi and bacteria responsible for nosocomial infections and food spoilage, and identifying the chemical profile and active compounds.

2. Materials and Methods

2.1. Plant material

In the study, the aerial parts (leaves and flowers) of *Thymus vulgaris* L. were collected in the Immouzzer region of Morocco (33°38'11.6"N 4°57'37.4"W) in May 2022. The plant samples were identified by expert botanists using botanical references and flora catalogues. After identification, the samples were cleaned and then air-dried in a shaded area for 15 days to preserve their bioactive compounds before starting the extraction process.

2.2. EO extraction

A mass of 150 grams of dried *T. vulgaris* leaves was subjected to hydrodistillation for 3 hours using a Clevenger-type apparatus and 1000 mL of distilled water, following the protocol outlined in the European Pharmacopoeia (EDQM., 2004). The resulting essential oil (EO) was dehydrated with anhydrous sodium sulfate and subsequently stored under dark conditions at a temperature of 4-5 °C until further testing and analysis. The EO yield was calculated as a percentage (*v*/*w*) relative to the weight of the dried plant material (El Abdali et al., 2023a).

2.3. EO Chromatographic analysis

The chemical composition of *T. vulgaris* EO was analyzed using a gas chromatography system (TQ8040 NX, Shimadzu, Tokyo, Japan) coupled with a triple quadrupole tandem mass spectrometer (GC-MSMS). An apolar capillary RTxi-5 Sil MS column (30.00 m length, 0.25 mm internal diameter, and 0.25 µm film thickness) was utilized to separate the EO compounds. The source and interface temperatures were set at 200 °C and 280 °C, respectively. 1 µL of Helium was injected as the carrier gas. The oven temperature program began at 50 °C for 2 minutes, followed by two heating ramps: the first at a rate of 5 °C/min up to 160 °C, held for 2 minutes, and the second at a rate of 5 °C/min up to 280 °C, also held for 2 minutes. The injection was performed in split mode (with the split opening at 4 minutes), at an injection temperature of 250 °C and a pressure of 37.10 kPa. The chemical composition of the EO was expressed as a percentage of the total peak area. The phytochemical constituents were identified by comparing their retention indices with those available in the literature database (Adams, 2007).

2.4. EO's in vitro antioxidant properties

2.4.1. Scavenging free DPPH radical test

The DPPH assay was conducted following a modified procedure described by Moattar et al., (2016). A solution was prepared by mixing 0.1 mL of thyme EO in methanol at various concentrations (0.1–100 mg/mL) with 0.75 mL of a 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution, also dissolved in methanol. The mixture was incubated for 30 minutes at room temperature. The absorbance of the resulting solution was measured at 517 nm and compared to that of a negative control, which contained methanol instead of the EO. The assay was repeated using butylated hydroxytoluene (BHT) as a standard reference antioxidant. The inhibition rate of the DPPH radical induced by the EO was then calculated based on this formula:

$$\text{DPPH inhibition (\%)} = [1 - (A/A_0)] \times 100 \quad (1)$$

A represents the absorbance of the sample solution, while A_0 denotes the absorbance of the negative control.

2.4.2. Ferric reducing assay

The ferric reducing antioxidant power (FRAP) assay was conducted according to the established protocol detailed by Moattar et al., (2016). The procedure involved the addition of 0.5 mL of a 1% potassium ferricyanide solution [$K_3Fe(CN)_6$] and 0.5 mL of a 0.2 M phosphate buffer solution (pH 6.6) to 0.1 mL of the studied EO at varying concentrations (0.1 to 25.0 mg/mL) dissolved in methanol. The mixture was then incubated for 20 minutes at 50°C in a water bath. Following incubation, 0.5 mL of 10% trichloroacetic acid was added to the mixture to induce acidification. Subsequently, 0.1 mL of 0.1% $FeCl_3$ and 0.5 mL of distilled water were added. The absorbance of the resulting solution was measured at 700 nm against a blank. The antioxidant activity was quantified and presented as the 50% effective concentration (EC_{50}), which represents the concentration required to achieve an absorbance of 0.5 nm. Additionally, the same procedure was applied to assess the antioxidant activity of conventional antioxidants BHT and quercetin.

2.4.3. Total antioxidant capacity assay

A reagent solution was prepared by combining 1 mL of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. To this mixture, 25 µL of EO was added. The reaction mixture was then incubated for 90 minutes at 95°C. After incubation, the absorbance was measured at 695 nm, following the procedure outlined by Maškovič et al., (2012).

The total antioxidant capacity was quantified using a calibration curve previously established with ascorbic acid and expressed as milligrams of ascorbic acid equivalent per gram of EO (mg AAE/g EO). The experiment was performed in triplicate to ensure accuracy and reproducibility of the results.

2.5. EO's antimicrobial assay

2.5.1 Microbial strains and growth medium

Eight bacterial strains were used to evaluate the antibacterial activity of *T. vulgaris* EO, including: *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* OP861484, *Streptococcus* sp CCMM/B24, *Escherichia coli* ATCC 25922, *Escherichia coli* ESBL, *Pseudomonas aeruginosa* ATCC 9721, carbapenem-resistant *Pseudomonas aeruginosa* 104 and *Klebsiella pneumoniae* CIPA22. The antifungal activity was evaluated against three Molds namely *Aspergillus flavus* MW741887, *Aspergillus niger* MTCC282, *Fusarium proliferatum* OP820542, as well as against the yeast *Candida albicans* ATCC1023.

S. aureus ATCC 29213, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 9721, carbapenem-resistant *P. aeruginosa,* and *E. coli* ESBL were kindly provided by Barguigua Abouddihaj, a Professor of Microbiology and Molecular Biology at Sultan Moulay Slimane University, Polydisciplinary Faculty of Beni Mellal. While, *B. subtilis* OP861484, *K. pneumoniae* CIP A22 and all the fungal strains were kindly provided by El Barnossi Azeddin, a Professor of Environmental Microbiology at Sultan Moulay Slimane University, Faculty of Sciences and Technology of Beni Mellal. Regarding *Streptococcus sp.* CCMM/B24, it was kindly provided by El Minaoui Fatima Ezahraa, a Doctor in Microbiology at Sultan Moulay Slimane University, Polydisciplinary Faculty of Beni Mellal.

The bacteria were maintained on Mueller Hinton (MH) agar medium at 37°C (hydrolysate of Casein 17.5 g/L, Meat Extract 2 g/L, Soluble Starch 1.5 g/L and Bacteriological Agar 17 g/L). Cultures for each fungal species were maintained in Potato Dextrose Agar (PDA) medium supplemented with tetracycline (Potato 200 g/L, Dextrose 20 g/L, Agar 15 g/L, Tetracycline 25 mg/L) at a concentration of 25 mg/L according to a protocol modified from (Anasa et al., 2019). The fungal and bacterial strains were subsequently stored at 4°C.

2.5.2. Antibacterial activity test

The evaluation of the antibacterial *in vitro* effect of *T. vulgaris* EO was conducted using the disk diffusion technique against the eight bacterial strains mentioned above (Weiss et al., 1996; Zhang et al., 2009). Thus, a volume of 15 µL from the EO was deposited on a 6 mm diameter disk in a dish containing the MH medium and previously spread with 100 µL of the bacterial suspension of one of the indicator strains (OD_{595nm} = 0.5). The dishes were incubated at 37°C for 24 hours after leaving for 2 hours at room temperature for pre-diffusion. After incubation, the diameters of the inhibition zones around the disks were measured in millimeters. This test was triplicate.

To ensure the validity of the tests, commercially available antibiotics were used as positive controls: ciprofloxacin 0.3% and tobramycin 0.3%. These antibiotics were tested individually against the same indicator bacterial strains. While the negative control corresponded to sterile distilled water.

2.5.3. Antifungal activity test against molds

The antifungal activity of *T. vulgaris* EO was evaluated against *A. flavus* MW741887, *A. niger* MTCC282 and *F. proliferatum* OP820542, according to the protocol of Foss et al., (2014) with some modifications. Thus, a 6 mm diameter disc of the indicator fungus taken from actively growing edge of one-week-old culture grown on solid PDA medium was placed in the center of the Petri dish and spaced 2 cm from a sterile 6 mm diameter disc.

Subsequently, a volume of 15 µL of the EO was deposited on the sterile disc. The plate was incubated at 25 °C for seven days. Pathogen grown on PDA without addition of the EO was used as a negative control while the positive controls corresponded to the activity of commercially available antifungals (Clotrimazole at 10 mg/mL and Pyrition at 10 mg/mL). This test was carried out in duplicate. The percent of mycelial growth inhibition was calculated using the following formula (Senhaji et al., 2014):

$$PI = (A - B/A)* 100 \quad (2)$$

Where: A = diameter of fungal colony in control, and B = diameter of fungal colony with plant EO.

2.5.4. Antifungal effect against Candida albicans

The antifungal activity of *T. vulgaris* EO against *C. albicans* was evaluated by the disk diffusion method according to protocols modified by Aouadhi et al., (2013) and Wanner et al., (2010). Thus, a volume of 100 µL of the fungal suspension at OD_{595}nm = 0.5 was spread uniformly on petri dishes containing PDA medium supplemented with tetracycline. Then, a volume of 15 µL of the EO was deposited on a sterile disk of 6 mm in diameter. The dish was incubated at 37 °C for 2 days. After incubation, the diameter of the inhibition zone around the disc was measured in millimeters. This test was repeated twice. The negative control corresponded to the same preparation free of EO.

2.6. Molecular docking of EO antibacterial and antifungal activities

The present study employed computational methods to investigate the antimicrobial potential of *T. vulgaris* EO. Specifically, the focus was on evaluating the inhibitory effects of the EO on *Escherichia coli* gyrase B, beta-1,4-endoglucanase from *Aspergillus niger* and sterol 14-alpha demethylase (CYP51) from *Candida albicans* enzymes which are key targets in the management of microbial infections.

2.6.1. Ligand preparation

For ligand preparation, an exhaustive collection of all phytocompounds identified in *T. vulgaris* EO via GC/MS analysis was compiled from the PubChem database in Structure Data File (SDF) format. Subsequently, a comprehensive pre-treatment process was applied to these ligands in preparation for docking simulations, using the LigPrep tool in the Schrödinger Software suite (v. 11.5). The OPLS3 force field was selected for this procedure, ensuring accurate molecular modeling. For each ligand, up to 32 stereoisomers were generated, and their ionization states were adjusted to reflect a physiological pH range of 7.00 ± 2.00. This rigorous preparation ensured that the ligands were suitable for subsequent docking calculations (Chebaibi et al., 2024; El Abdali et al., 2023b).

2.6.2. Target protein preparation

For the preparation of proteins, three-dimensional crystal structures of the targeted enzymes including *Escherichia coli* gyrase B (PDB ID: 3G7E), beta-1,4-endoglucanase from *Aspergillus niger* (PDB ID: 5I77), and sterol 14-alpha demethylase (CYP51) from *Candida albicans* (PDB ID: 5FSA) were obtained, as outlined by Tourabi et al., (2023). These enzyme structures, retrieved in PDB format from the Protein Data Bank, were carefully processed using the Protein Preparation Wizard integrated into Schrödinger-Maestro (v. 11.5). The receptors resolutions were set at 2.20 Å, 1.80 Å, and 2.86 Å, for *E. coli* gyrase B (3G7E), beta-1,4-endoglucanase (5I77), and sterol 14-alpha demethylase (CYP51) (5FSA), respectively. The optimization procedure involved several steps: the addition of hydrogen atoms, assignment of bond orders, removal of water molecules, and determination of hydrogen bond networks. Additionally, the receptor atoms' potentials were adjusted, and energy minimization was performed using the OPLS3 force field (Amrati et al., 2023). Following optimization, the receptor grid was initialized through the grid generation module, where a specific ligand atom was selected, generating a default grid box. The ligands were then docked onto this grid box, derived from the target protein structure, using the Standard Precision method for docking simulations. This approach ensured accurate interaction modeling between the ligands and the enzyme active sites.

2.6.3. In silico test

The flexible ligand docking protocol was implemented using the Standard Precision mode of the Glide module in Schrödinger-Maestro software (v. 11.5). This docking procedure accounted for penalties applied to non-cis/trans amide bonds, ensuring a more realistic simulation of ligand flexibility. Key parameters were finely tuned: the partial charge threshold for ligand atoms was set at 0.15, and the Van der Waals scaling factor was adjusted to 0.80. The resulting docking scores were based on energy-minimized ligand conformations, with the most favorable docking conformation identified by the lowest Glide score (Beniaich et al., 2022; Kumar et al., 2022). This precise computational approach allowed for a detailed examination of the molecular interactions between the bioactive compounds found in *T. vulgaris* EO and the active sites of the targeted enzymes.

2.7. Statistical Analysis

In this study, GraphPad Prism 10 software of Microsoft (CA, USA) was employed for calculating mean values and standard deviations. For statistical analysis, one-way ANOVA was used, followed by Tukey's post-hoc test to compare differences between groups. Statistical significance was established at a threshold of $p < 0.05$.

3. Results and discussion

3.1. EO yield

The aerial parts of *T. vulgaris* collected from Immouzzer, Morocco, produced a clear yellow EO with a characteristic aroma, yielding 2.25% (*v/w*). EO yield from Thyme can vary widely depending on multiple factors. For instance, yields have been reported to range from 0.42% in Montenegro (Damjanović-Vratnica et al., 2015) to 1.8% in Southern Morocco (Labiad et al., 2022). Furthermore, the extraction of seven EOs extracted from Moroccan samples of *T. vulgaris*, *T. satureioides*, and *T. zygis* gave variable yields, ranging from 0.70 % to 4.12 % (Drioiche et al., 2022). Research indicates that both yield and composition of EO are influenced by various environmental and agronomic factors. Water stress, for example, can reduce herb fresh weight and EO yield, although it increases the concentration of the oil (Said-Al Ahl et al., 2019). In certain cases, drought conditions have been shown to boost EO yield by as much as 66% compared to normal irrigation conditions (Hassan et al., 2019). Additionally, other variables such as geographical location, harvest timing, plant parts utilized, drying methods, and extraction techniques can substantially affect EO yield

(Ben El Jilali et al., 2023; Houzi et al., 2024; Samara et al., 2023).

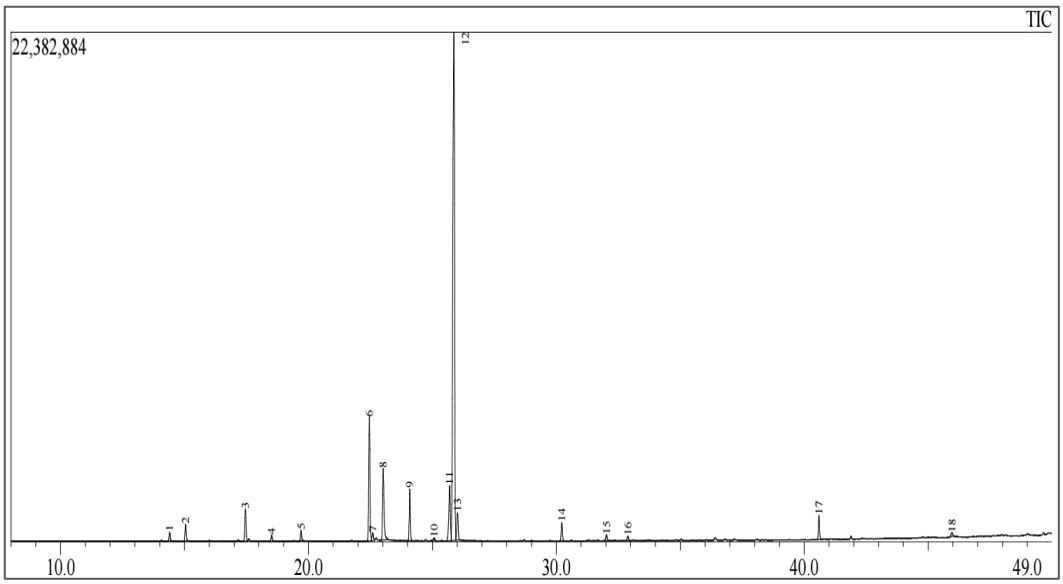

Figure 1: GC-MS chromatographic profile of *T. vulgaris* EO.

3.2. EO's phytochemical composition

The EO extracted from *T. vulgaris* was thoroughly analyzed to determine its chemical profile using gas chromatography-mass spectrometry (GC-MSMS). The resulting GC-MS spectrum is illustrated in Figure 1, while Table 1 detailed the identified components. Analysis revealed the presence of eighteen distinct phytocompounds in the thyme EO.

The major constituents included estragole (59.76%), followed by borneol (10.08%), α-terpineol (6.81%), and carvacrol (5.50%). Oxygenated monoterpenes were the predominant category, accounting for 91.26% of the EO composition, whereas sesquiterpenes contributed 2.41%, with other minor components making up less than 4% of the total profile. Considerable diversity in the chemical profile of *T. vulgaris* EO has been documented across various chemotypes examined globally.

Recent analysis of EO extracted from Iraqi *T. vulgaris* seeds revealed high levels of thymol (20.58%), carvacrol (12.20%), and estragole (11.10%) (Al-Assaf et al., 2023). Another investigation indicated that thyme EO from Montenegro was predominantly composed of geraniol (25.66%), geranyl acetate (20.34%), linalool (10.89%), and caryophyllene oxide (9.89%) (Damjanović-Vratnica et al., 2015).

Additionally, *T. vulgaris* oil from Nyons (France) was characterized by a high linalool content (76.2%) and linalyl acetate (14.3%), while the sample from Jablanicki, Serbia, was rich in geraniol (59.8%) and geranyl acetate (16.7%). The same study, reported that EO of the sample collected from Pomoravje District (Serbia) was primarily of the sabinene hydrate chemotype (cis-sabinene hydrate, 30.8%; trans-sabinene hydrate, 5.0%), while the EO from Richerenches area (France) was identified as the thymol chemotype (thymol, 47.1%; *p*-cymene, 20.1%) (Satyal et al., 2016). Furthermore, the primary constituent of *T. vulgaris* EO from southern Morocco was found to be carvacrol (27.31%) (Labiad et al., 2022).

The observed variations in the composition and concentration of EO compounds can likely be attributed to several influencing factors. These include specific selection criteria (such as age, maturity, health status, and parasite presence), preservation techniques employed before drying, the developmental stage of leaves, extraction methods, harvest timing, seasonal fluctuations, environmental conditions, and circadian biological rhythms (El Abdali et al., 2023b; Justus et al., 2018). The primary constituents of thyme EO possess notable bioactive properties, recognized for their antimicrobial, antioxidant, and anticancer activities. These attributes make thyme a widely favored ingredient in both culinary and medicinal applications (Halat et al., 2022; Patil et al., 2021).

It is considerable to note that many compounds in thyme EO are known for their bioactive properties. Estragole, for example, a volatile terpenoid molecule, has been reported to exhibit various pharmacological effects, including antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory properties (Mahendra et al., 2023). Likewise, other compounds found in this EO, such carvacrol, borneol and α-terpineol are also recognized for exhibiting numerous pharmacological attributes and treating outcomes for people with skin diseases, foodborne illness, cancers and inflammatory disorders (Halat et al., 2022; Patil et al., 2021). The complex chemical composition of *T. vulgaris* EO highlights its potential as a rich source of bioactive substances, further emphasizing its importance in diverse applications within the fields of pharmacology and natural product research.

Table 1: Phytochemical composition of *T. vulgaris* EO.

	RT	RI	RI (Lit)	Compounds	Molecular formula	Content (%)
1	14.401	948	933	2-Pinene	$C_{10}H_{16}$	0.72
2	15.046	943	944	Camphene	$C_{10}H_{16}$	1.38
3	17.475	1042	1031	o-Cymene	$C_{10}H_{14}$	2.56
4	18.516	1053	1059	γ-Terpinene	$C_{10}H_{16}$	0.46
5	19.701	1082	1090	Linalool	$C_{10}H_{18}O$	0.81
6	22.458	1166	1169	Borneol	$C_{10}H_{18}O$	10.08
7	22.594	1173	1177	Terpinen-4-ol	$C_{10}H_{18}O$	0.72
8	23.016	1194	1199	α-Terpineol	$C_{10}H_{18}O$	6.81
9	24.089	1231	1224	Carvacrol methyl ether-	$C_{11}H_{16}O$	3.86
10	25.067	1202	1218	4-methoxy-Benzaldehyde	$C_8H_8O_2$	0.23
11	25.697	1262	1299	Carvacrol	$C_{10}H_{14}O$	5.50
12	25.868	1191	1196	Estragole	$C_{10}H_{12}O$	59.76
13	26.014	1293	1290	Thymol	$C_{10}H_{14}O$	2.46
14	30.223	1404	1408	Caryophyllene	$C_{15}H_{24}$	1.50
15	32.026	1398	1400	β-Longipinene	$C_{15}H_{24}$	0.54
16	32.886	1509	1513	Δ-Cadinene	$C_{15}H_{24}$	0.37
17	40.595	1840	1845	(E)-4-Methoxy-2-(prop-1-en-1-yl)phenyl 2-methylbutanoate	$C_{15}H_{20}O_3$	1.79
18	45.957	2577	2577	Isochiapin B	$C_{19}H_{22}O_6$	0.45
Monoterpenes (%)						91.26
Oxygenated Monoterpenes (%)						86.14
Sesquiterpenes (%)						2.41
Others (%)						6.33
Total (%)						100

RT = Retention time; RI = Retention Index; RI(Lit): Retention Index from literature.

3.3. In vitro EO antioxidant activity

Reactive oxygen species (ROS) can exert detrimental effects on cellular components and biological structures, contributing to oxidative stress (Circu and Aw, 2010; EL Abdali et al., 2023). Moreover, ROS contribute to the oxidation of fatty acids, a major factor in food spoilage, thus impacting food preservation and shelf life (El Abdali et al., 2023a). Numerous studies have explored the capacity of various EOs to neutralize reactive species and mitigate oxidative stress (Houzi et al., 2024; Leyva-López et al., 2017). In this study, the antioxidant potential of *T. vulgaris* EO was evaluated using a comprehensive *in vitro* approach that incorporated phosphomolybdenum total antioxidant capacity (TAC), ferric reducing antioxidant power (FRAP), and DPPH radical scavenging assays. The results obtained are presented in Table 2, as well as Figures 2 and 3. Based on our findings, both *T. vulgaris* EO and butylated hydroxytoluene (BHT) exhibited a dose-dependent increase in DPPH radical scavenging activity, as depicted in Figure 2. Furthermore, Table 2 data indicate that the IC_{50} (half maximal inhibitory concentration) value of *T. vulgaris* EO for DPPH radical scavenging was 0.058 ± 0.014 mg/mL. Statistical analysis via ANOVA demonstrated that the antioxidant potential of *T. vulgaris* EO was comparable to that of the reference antioxidant BHT, which recorded a higher IC_{50} value of 0.128 ± 0.032 mg/mL, with no statistically significant difference ($p > 0.05$).

The antioxidant capacity of the thyme EO was further evaluated using the FRAP assay, marking an EC_{50} value of 6.780 ± 0.080 mg/mL (Table 2). This outcome suggests that the EO is capable of reducing ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). However, its efficacy in this reduction process was statistically lower ($p < 0.05$) than that of the standard antioxidants BHT and quercetin, which had EC_{50} values of 0.359 ± 0.010 mg/mL and 0.035 ± 0.003 mg/mL, respectively. The total antioxidant capacity (TAC) of thyme EO was also assessed through the phosphomolybdenum assay, with results displayed in Figure 3. In this assay, molybdenum (Mo) in its hexavalent form (Mo VI), present as molybdate ions under acidic conditions, is reduced to the pentavalent form (Mo V) in the presence of antioxidants, forming a green phosphate/Mo(V) complex (Prieto et al., 1999). Based on the results (Table 2), the TAC of *T. vulgaris* EO, expressed in ascorbic acid equivalents (mg AAE/g EO), was 240.300 ± 27.080 mg/g. This was notably higher ($p < 0.05$) compared to conventional antioxidants, with BHT and quercetin displaying TAC values of 47.640 ± 1.310 mg/g and 28.350 ± 1.195 mg/g, respectively. Recent research further highlights that the antioxidant efficacy of plant extracts is primarily determined by the concentration of phenolic

compounds, the reactivity of these phenols toward chain-propagating peroxyl radicals, and the stability of the phenoxyl radicals formed during these reactions (Ćavar et al., 2013). EOs, including that of *T. vulgaris*, are known to contain an array of bioactive compounds. These phytochemicals serve multiple roles, such as scavenging free radicals, acting as reducing agents, inactivating pro-oxidants, and quenching reactive species (Singh and Maurya, 2024). Moreover, certain natural compounds, including EOs, may modulate the body's antioxidant defenses by upregulating antioxidant enzymes or downregulating enzymes that contribute to free radical production (Amorati et al., 2013). Considering all of this, our findings with those of other research, together, support the potential of thyme EO to mitigate and protect against the detrimental effects of free radicals. This protective capacity could play a role in addressing related issues, such as oxidative stress and the resulting challenges in food preservation and stability.

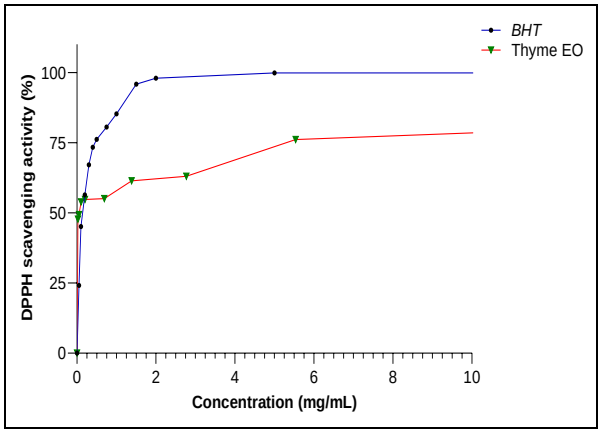

Figure 2: Anti-free DPPH radical scavenging activity of *T. vulgaris* EO and BHT at various concentrations.

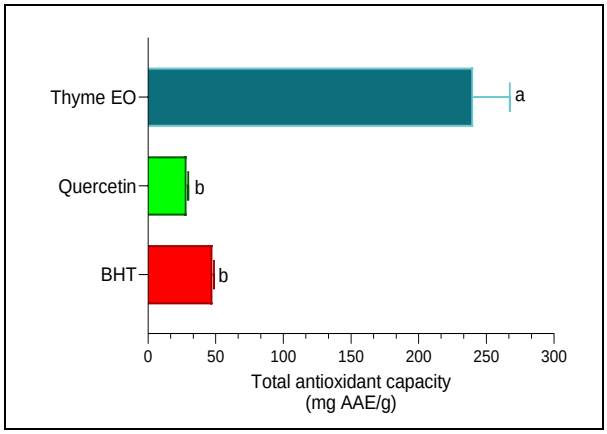

Figure 3: Total antioxidant capacity of *T. vulgaris* EO, BHT, and Quercetin. Bars marked with different letters indicate statistically significant differences at $p<0.05$.

Our findings are consistent with previous studies that have also shown *T. vulgaris* EO to possess antioxidant properties (Aljabeili et al., 2018; Halat et al., 2022; Labiad et al., 2022; Patil et al., 2021). Numerous *in vitro* and *in vivo* methods have illustrated the antioxidant capacity of thyme EO across the world. Recent studies using *in vitro* assays have shown that *T. vulgaris* EO, particularly from Tiznit province in Morocco with carvacrol as the major compound (27.31%), demonstrates significant free radical scavenging capacity. This activity was measured through inhibition of the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$radicals, yielding IC_{50} and EC_{50} values of 8.13 ± 0.09 µg/mL and 7.39 ± 0.05 µg/mL, respectively (Labiad et al., 2022).

In the same study, EOs from *T. algeriensis* and *T. broussonetii* collected in other Moroccan regions showed comparable antioxidant effectiveness to that of *T. vulgaris*. Similarly, an analysis of *T. vulgaris* EO from Egypt, which had a high thymol content (41.04%), reported strong radical scavenging activity with values of 149.8 µmol TE/g and 192.4 µmol TE/g for $DPPH^{\bullet}$ and $ABTS^{\bullet+}$, respectively. This EO also achieved a 68.9% reduction in the β-carotene-linoleic acid bleaching assay and exhibited iron-chelation-related reducing power at 142.8 µmol AAE/g (Aljabeili et al., 2018). Likewise, in a study conducted in Spain, thyme EO rich in linalool (44.00%) and 4-terpineol (11.84%) showed notable antioxidant potential, with IC_{50} values for DPPH, FRAP, ferrous ion-chelating (FIC) ability, and ABTS radical cation scavenging activities recorded as 12.69, 13.29, and 6.46 mg/mL, respectively (Ballester-Costa et al., 2017). *In vivo*, thyme EO has been shown to potentially stimulate the body's endogenous antioxidant defenses by activating signaling pathways such as Nrf2/Keap1. This activation leads to the upregulation of antioxidant enzyme expression, thereby enhancing the body's ability to counteract oxidative stress (He et al., 2020). It is important to recognize that the antioxidant activity of EOs can vary significantly depending on their specific chemical composition. This composition, in turn, is influenced by various factors, including the plant's growth conditions, the extraction methods used, and the timing of the harvest (Tit and Bungau, 2023). EOs exhibit diverse biological and chemical properties, largely attributed to their complex composition of volatile and semi-volatile phytocompounds, which feature varying polarity and a range of functional groups.

These characteristics are known to differ depending on the test or method employed for analysis (El Abdali et al., 2023b). Extensive research on thyme EO has consistently associated its antioxidant capacity with its terpene-based constituents, including compounds such as estragole, borneol, carvacrol, and thymol. These monoterpenes demonstrate significant antioxidant potential, often functioning individually or synergistically to neutralize free radicals, as evidenced by numerous studies and experimental *in vivo* and *in vitro* findings (Halat et al., 2022; Horvathova et al., 2014; Mahendra et al., 2023; Mastelić et al., 2008; Patil et al., 2021). In practice, the antioxidant capacities of EOs are influenced by multiple pathways that affect oxidative stress, including free radical scavenging, regulation of antioxidant enzymes (e.g., superoxide dismutase), and modulation of pro-oxidative processes (Gonzalez-Burgos and Gomez-Serranillos, 2012; Leyva-López et al., 2017).

Table 2 : Antioxidant activities of *T. vulgaris* EO and standards.

	DPPH (IC_{50} mg/mL)	FRAP (EC_{50} mg/mL)	TAC (mg AAE/g EO)
Thyme EO	0.058 ± 0.014a	6.780 ± 0.080b	240.300 ± 27.080a
BHT	0.128 ± 0.032a	0.359 ± 0.010a	47.640 ± 1.310b
Quercetin	-	0.035 ± 0.003a	28.350 ± 1.195b

Table 3: Antibacterial effect of *T. vulgaris* EO and commercial antibiotics on the eight indicator bacteria in millimeters.

	Inhibition Diameters (mm)							
	SA	**STREP**	**BS**	**KP**	**EC**	**EC ESBL**	**PA**	**PA 104**
Thyme EO	16 ± 1	33 ± 1.73	56 ± 1.73	33.33 ±1.53	27 ± 1	16.33 ± 1.53	7.33 ± 0.58	7.33 ± 0.58
Ciprofloxacin (0.3%)	30 ± 0	50 ± 0	41.5 ± 0.5	16 ± 1	38 ± 0	17 ± 0	45 ± 0	11 ± 0
Tobramycin (0.3%)	28 ± 0	32 ± 0	40 ± 1	17.66 ± 1.52	26 ± 0	23 ± 0	28 ± 0	4 ± 0

SA: *Staphylococcus aureus* ATCC 29213; STREP: *Streptococcus* sp CCMM/B24; BS: *Bacillus subtilis* OP861484; KP: *Klebsiella pneumoniae* CIPA22; EC: *Escherichia coli* ATCC 25922; EC ESBL: *Escherichia coli* ESBL; PA: *Pseudomonas aeruginosa* ATCC 9721; PA 104: carbapenem-resistant *Pseudomonas aeruginosa* 104.

Table 4: Effect of the EO of *T. vulgaris* and conventional antifungals against different fungal strains.

	Thyme EO	Clotrimazole (10 mg/mL)	Pyrition (10 mg/mL)
	Diameter of fungal growth inhibition (mm)		
***Candida albicans* ATCC10231**	32.33 ± 1.15	32.66 ± 0.44	66 ± 1
	Mycelial growth inhibition (%)		
***Aspergillus flavus* MW741887**	100 %	54.11%	47.06%
***Aspergillus niger* MTCC282**	100 %	26.86%	81.34%
***Fusarium proliferatum* OP820542**	98%	80.46%	39.08%

3.4. EO's antimicrobial potential

Disk diffusion was performed to assess the antagonistic activity of *T. vulgaris* EO against indicator bacteria. After incubation, the antibacterial effect was detected by the observation of inhibition area, and the growth inhibition zone diameter (expressed in mm) was used to express the results obtained (Figure 4 and Table 3).

The percentage of mycelial growth inhibition exhibited by the studied EO was also calculated to assess the antifungal potential (Figure 5 and Table 4). According to the results obtained, *T. vulgaris* EO exhibited a significant inhibitory power against Gram-positive and Gram-negative indicator bacteria by showing inhibition halos with diameters ranging between 7.33 and 56 mm (Table 3 and Figure 4).

It should be noted that *Bacillus subtilis* OP861484 was the most sensitive bacterium to the antibacterial action of the EO while the least sensitive bacteria were *Pseudomonas aeruginosa* ATCC 9721 and carbapenem-resistant *Pseudomonas aeruginosa* 104. Regarding the antifungal effect, the studied EO at 0.886 g/mL completely inhibited the mycelial growth of *Aspergillus flavus* MW741887 and *Aspergillus niger* MTCC282 after 7 days of incubation at 25°C. Moreover, it inhibited 98% of the mycelial growth of *Fusarium proliferatum* OP820542.

These results exceeded largely those observed after treatment with conventional antifungals used. Similarly, the EO inhibited the growth of *Candida albicans* ATCC10231 by showing an inhibition halo of 32.33 ± 1.15 mm (Table 4 and Figure 5). *T. vulgaris* EO, widely known as thyme oil, has garnered growing attention in scientific research due to its potent antimicrobial properties.

Studies have shown that *T. vulgaris* EO exhibits significant antibacterial activity against both Gram-negative and Gram-positive pathogenic bacteria, as well as antifungal effects against a variety of fungal pathogens. These antimicrobial properties are often evaluated using agar disk diffusion and broth microdilution tests, which have consistently highlighted the oil's efficacy in inhibiting microbial growth.

For instance, EO derived from *T. vulgaris* cultivated in Romania demonstrated strong antimicrobial effects against seven common foodborne pathogens, including *S. aureus*, *E. coli*, *S. typhimurium*, *P. aeruginosa*, *K. pneumoniae*, *E. faecalis*, and *C. albicans*.

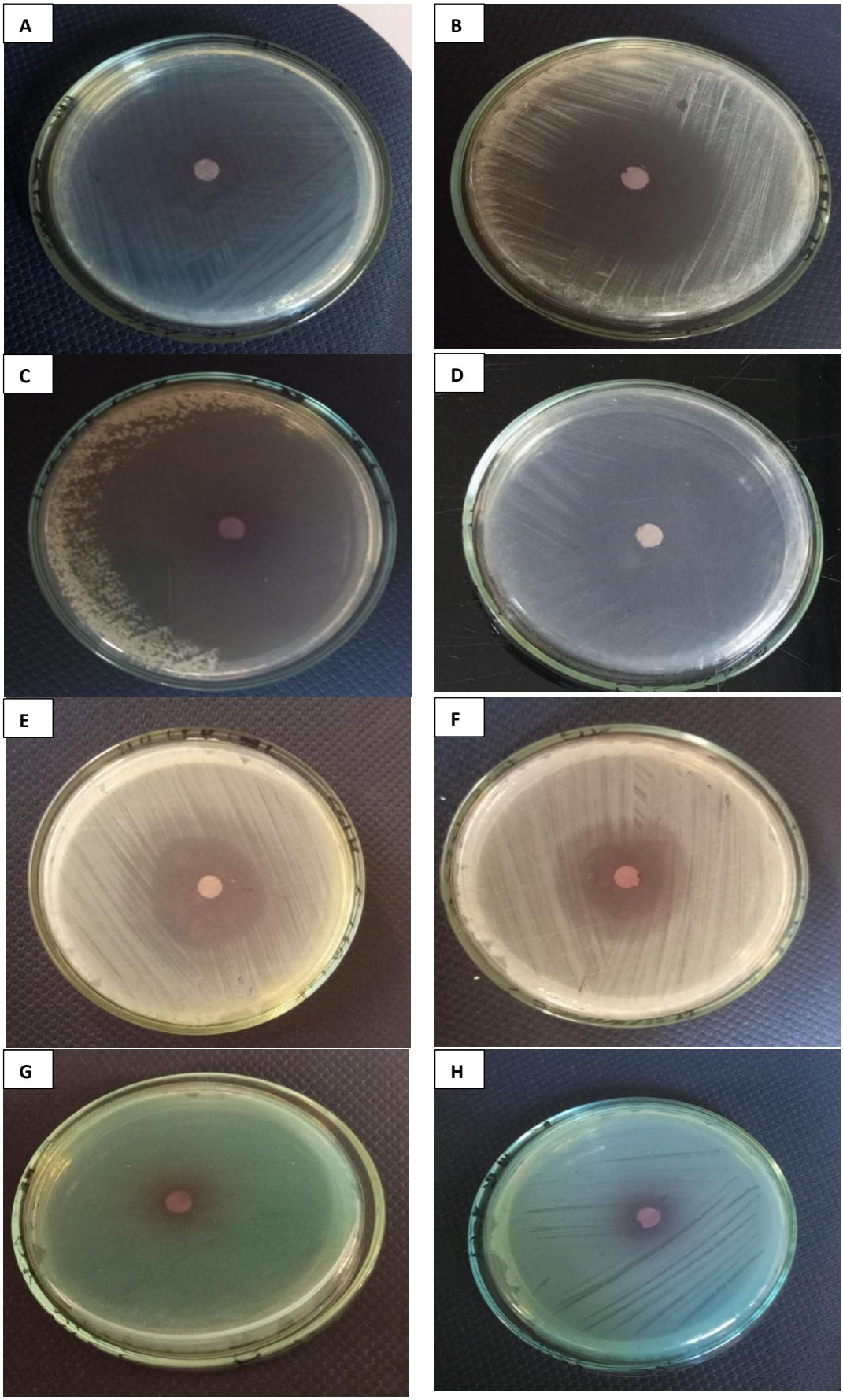

Figure 4: Inhibition zone of *T. vulgaris* EO against *S. aureus* ATCC 29213 (A), *Streptococcus* sp CCMM/B24 (B), *B. subtilis* OP861484 (C), *K. pneumoniae* CIPA22 (D), *E. coli* ATCC 25922 (E), *E. coli* ESBL (F), *P. aeruginosa* ATCC 9721 (G), and carbapenem-resistant *P. aeruginosa* 104 (H).

The inhibition zones, measured for oil concentrations ranging from 5 to 20 µL, varied between 8.99 and 34.99 mm, with major bioactive constituents such as thymol, *p*-cymene, and γ-terpinene contributing to its efficacy (Borugă et al., 2014). Further research has highlighted the oil's effectiveness against microbes associated with foodborne illnesses. For example, the antibacterial properties of *T. vulgaris* EO were observed against pathogens like *K. pneumoniae*, *P. aeruginosa*, and *S. saprophyticus*, with reported minimum inhibitory concentrations (MICs) between 64 and 512 µg/mL and minimum bactericidal concentrations (MBCs) ranging from 256 to 1024 µg/mL (Diniz et al., 2023).

Additionally, *T. vulgaris* EO sourced from Saudi Arabia demonstrated antifungal activity against seven pathogenic fungi, including *C. albicans*, *C. glabrata*, *C. kefyr*, *C. parapsilosis*, *A. flavus*, *A. niger*, and *Fusarium* sp. The MIC and MFC values for this oil ranged from 2.5 to 10 mg/mL, indicating its strong fungicidal potential (Al-Shahrani et al., 2017). Similarly, the antifungal activity of Iraqi *T. vulgaris* EO was evaluated against *C. albicans*, revealing MIC and MFC values of 0.015 mg/mL and 0.031 mg/mL, respectively. The study also noted that higher concentrations of the oil significantly enhanced antifungal effectiveness compared to triple antibiotic paste used for comparison (Sadoon Abd and Al. Haidar, 2024).

The biological activities of EOs are predominantly attributed to their major chemical constituents (Chouhan et al., 2017). For instance, estragole, which constitutes a significant proportion (59.76%) of our EO analyzed in this study, has been reported to exhibit considerable antimicrobial activity against various bacterial and fungal strains. These include both Gram-positive and Gram-negative bacteria, as well as fungal pathogens such as *C. albicans*, *A. flavus*, and *M. canis* (Fontenelle et al., 2011; Liang et al., 2023; Mahendra et al., 2023).

Borneol was also recognized for its diverse antibacterial and antifungal attributes (Sulaiman et al., 2022; Yang et al., 2020). Similarly, thymol, another key bioactive compound, has demonstrated pronounced antifungal activity, particularly against *C. albicans* and *M. canis* (Fontenelle et al., 2011). Moreover, carvacrol, a monoterpenoid phenol predominantly present in the EO under investigation, has shown remarkable antibacterial and antifungal properties (Niu et al., 2020; Wijesundara et al., 2021).

The varying antimicrobial effects of *T. vulgaris* EO on fungal and bacterial strains are attributed to the diverse mechanisms of action of its active compounds. These mechanisms are closely linked to the complex chemical composition of thyme EO and the specific pathogens targeted.

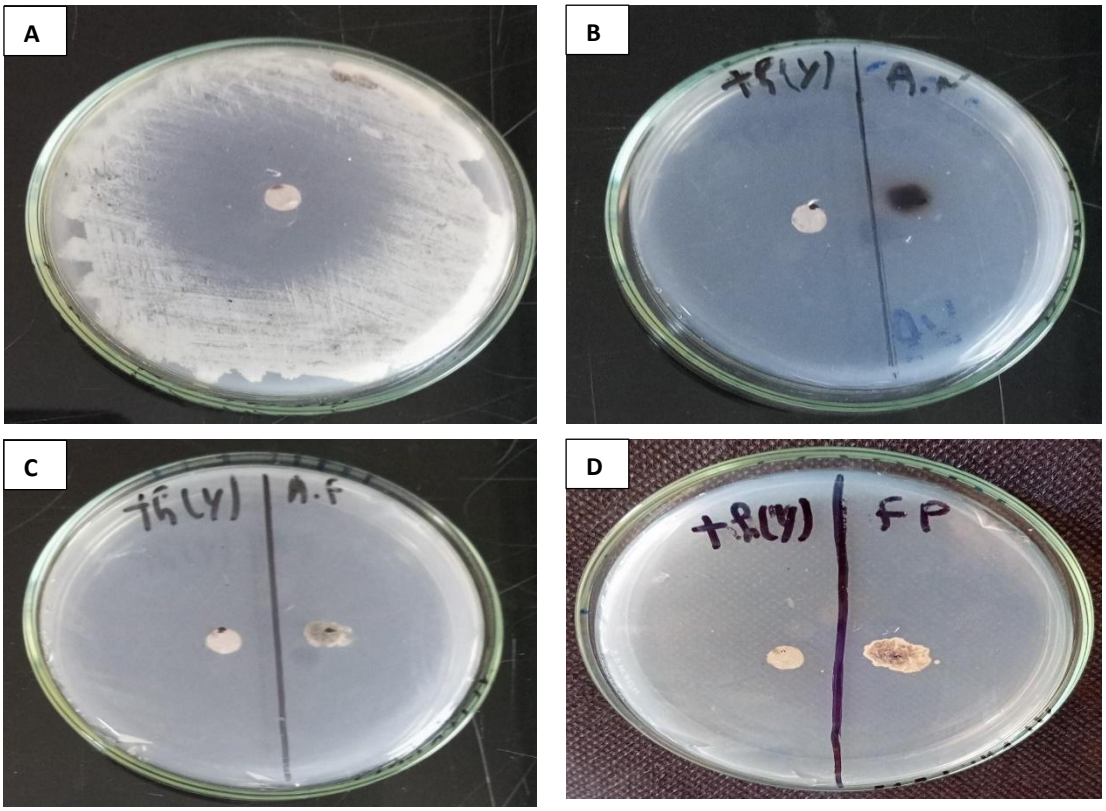

Figure 5: Antifungal effect of *T. vulgaris* EO against *C. albicans* ATCC10231 (A), *A. niger* MTCC282 (B), *A. flavus* MW741887 (C) and *F. proliferatum* OP820542 (D).

For instance, estragole, which is a predominant constituent of the thyme EO analyzed in this study, has been shown to exhibit potent antifungal activity against *A. flavus*. This activity is mediated by its ability to inhibit fungal growth and aflatoxin biosynthesis through the modulation of intracellular redox homeostasis (Liang et al., 2023). Similarly, thymol has been reported to target ergosterol in fungal cell membranes, disrupting cell integrity and function (Kowalczyk et al., 2020). Furthermore, thymol has been shown to alter the citrate metabolic pathway and various enzymes involved in ATP synthesis. Its intracellular activity disrupts essential energy-generating mechanisms, reducing the bacterium's capacity to recover (Pasqua et al., 2010). Carvacrol, a structural isomer of thymol, also demonstrates remarkable antibacterial potential. Studies indicate that carvacrol targets both the outer and cytoplasmic membranes, inducing passive ion transport across the cytoplasmic membrane. This compound has also been shown to compromise the outer membrane integrity of Gram-negative bacteria (Storia et al., 2011). As a result, the assignment of antimicrobial activity to individual active molecules is challenging due to the chemical complexity of the EO and the variability in the efficacy of its components against different microbial targets.

Table 5: Docking results of ligands (compounds of *T. vulgaris* EO) in the targeted enzymatic receptors.

Ligands	Glide score (kcal/mol)		
	E. coli gyrase B (PDB :3G7E)	**Sterol 14-alpha demethylase (CYP51) (PDB ID: 5FSA)**	**Beta-1,4-endoglucanase (PDB ID: 5I77)**
(E)-4-Methoxy-2-(prop-1-en-1-yl)phenyl 2-methylbutanoate	-5.839	-5.895	-3.152
2-Pinene	-4.368	-4.959	-4.301
4-methoxy-Benzaldehyde	-5.937	-6.196	-5.008
α-Terpineol	-4.856	-5.921	-4.192
β-Longipinene	-5.161	-7.137	-5.41
Borneol	-5.603	-6.303	-5.285
Camphene	-4.905	-5.203	
Carvacrol	-5.573	-6.791	-5.321
Carvacrol methyl ether	-5.725	-6.235	-4.341
Caryophyllene	-4.368	-7.025	
Δ-Cadinene	-5.745	-7.376	
Estragole	-4.286	-4.908	-2.949
γ-Terpinene	-5.538	-5.094	-4.243
Linalool	-3.188	-3.673	-2.721
o-Cymene	-5.286	-6.346	-4.601
Terpinen-4-ol	-5.987	-6.14	-4.769
Thymol	-5.783	-6.526	-5.551

The antimicrobial efficacy of EOs against pathogenic microbes is often linked to the lipophilic nature of their monoterpenes. These phytochemicals disrupt the cytoplasmic membrane of microorganisms, reducing its permeability to protons and larger ions. This disruption not only compromises the membrane's integrity but also interferes with its essential roles as a protective barrier, an enzymatic platform, and an energy converter. Despite these insights, the precise mechanisms underlying the antimicrobial properties of monoterpenes remain insufficiently understood (Stringaro et al., 2014). Overall, the findings from this study, alongside existing literature, substantiate the potent antimicrobial activity of *T. vulgaris* EO against a broad spectrum of pathogenic bacteria and fungi. This highlights its potential as a natural alternative to combat antibiotic resistance and underscores its value as a promising candidate for addressing global antimicrobial challenges.

3.5. In silico molecular docking of EO's antibacterial and antifungal activities

Molecular docking has become an essential tool in contemporary research, leveraging computer-aided drug design (CADD) techniques to analyze and predict interactions between various compounds (ligands), whether stable or volatile, such as EOs, and molecular targets linked to diverse biological functions (Mali et al., 2022). Molecular docking, in particular, enables the calculation of binding affinity energies for ligand-protein complexes and the identification of active sites within three-dimensional structures. This approach supports the generation of *in silico* hypotheses regarding the mechanisms of action of numerous bioactive molecules (Mali et al., 2022; Yu and MacKerell, 2017).

The antibacterial and antifungal effects of EOs derived from *T. vulgaris* species have been observed and proved against a

wide range of bacteria and fungi (Aljabeili et al., 2018; Mehani et al., 2024; Sakkas and Papadopoulou, 2017). The observed antimicrobial properties of *T. vulgaris* plants are believed to be linked, in part, to the phytochemical compounds found in its EOs, such as estragole, borneol, thymol, carvacrol, α-terpineol and others compounds which are mostly found in the EO under investigation (Halat et al., 2022; Mahendra et al., 2023; Patil et al., 2021). Within this context, the focus of our research was to computationally (*in silico*) analyze and elucidate the mechanisms of action of *T. vulgaris* EO compounds in inhibiting the enzymatic activities related to the vitality of the studied microbes, aiming to validate and support the experimental findings related to the observed antimicrobial activity.

Escherichia coli gyrase B is an enzyme involved in the topoisomerization of *E. coli* DNA, and also the activation of ATPase activity, which makes it an important enzymatic target for antibiotics and studies on DNA dynamics (Brino et al., 2000; Vanden Broeck et al., 2019). Otherwise, the beta-1,4-endoglucanase produced by *Aspergillus niger* possesses enzymatic properties that facilitate the decomposition of polysaccharides, playing a crucial role in the processing of lignocellulosic biomass (de Vries and Visser, 2001). Furthermore, the sterol 14-alpha demethylase (CYP51) of *Candida albicans* is a key enzyme in the biosynthesis of sterols, playing a crucial role in the conversion of lanosterol into ergosterol, an essential component of the cell membrane of fungi. This enzyme is also an important target for azole antifungals, which inhibit its activity to treat fungal infections (Hargrove et al., 2017). These microbial enzymatic complexes serve as critical targets for the development of anti-infective drugs. Consequently, their inhibition represents a promising therapeutic strategy for managing microbial infections.

In silico evaluation of the antibacterial activity of *T. vulgaris* EO revealed that terpinen-4-ol, 4-methoxy-benzaldehyde, and (E)-4-methoxy-2-(prop-1-en-1-yl)phenyl 2-methylbutanoate were the most active EO's phytocompounds against *E. coli* Gyrase B (PDB ID: 3G7E), achieving glide scores of -5.987, -5.937, and -5.839 kcal/mol, respectively (Table 5). Moreover, the 2D and 3D docking visualizations of *T. vulgaris* EO show that terpinen-4-ol established a single hydrogen bond with residue GLU 50 in the active site of *E. coli* gyrase B (Figure 6A and 7A).

Regarding antifungal activity, Δ-cadinene, β-longipinene, and caryophyllene found to be the most active molecules against the active site of sterol 14-alpha demethylase (CYP51) from *Candida albicans* (PDB ID: 5FSA), with glide scores of -7.376, -7.137, and -7.025 kcal/mol, correspondingly (Table 5). Δ-Cadinene, the most active compound, exhibited no bond formation within the active site of sterol 14-alpha demethylase (CYP51) (Figures 6B and 7B). Against the beta-1,4-endoglucanase from *Aspergillus niger* (PDB ID: 5I77), thymol, borneol, and carvacrol demonstrated considerable inhibitory activity, and marking glide scores of -5.551, -5.410, and -5.321 kcal/mol, correspondingly (Table 5). The interaction of thymol with the enzyme's active site involved the formation of two hydrogen bonds, specifically with residues SER 196 and TRP 201 (Figures 6C and 7C).

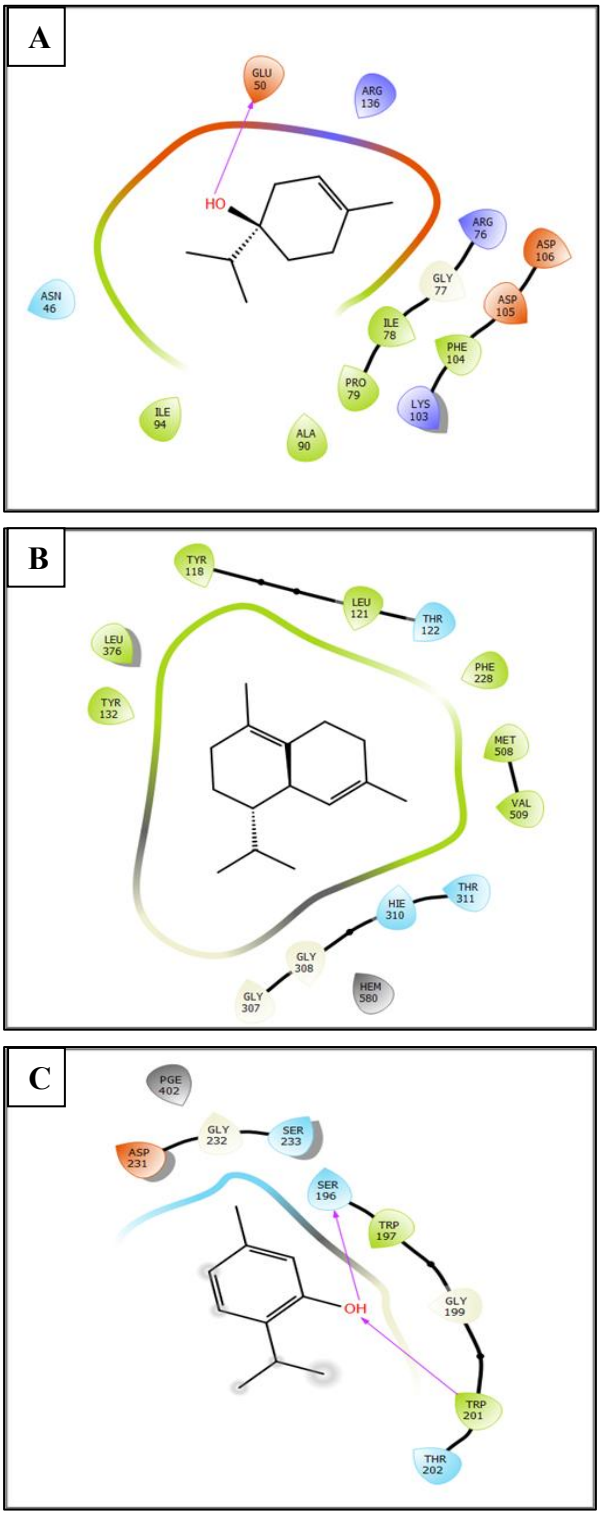

Figure 6: The 2D viewer of ligands (EO compounds) interactions with the enzyme's active site. A: Interactions of terpinen-4-ol with the active site of *E. coli* gyrase B: Interactions of Δ-Cadinene with the active site of sterol 14-alpha demethylase (CYP51) from *C. albicans*; C: Interactions of thymol with the active site of beta-1,4-endoglucanase from *A. niger*.

The *in silico* findings provide a detailed explanation and further validation of the experimentally observed antibacterial and antifungal properties attributed to certain

phytochemical compounds present in the EO derived from *T. vulgaris*, as previously documented. Furthermore, these results shed light on the potential mechanisms underlying the inhibition of microbial enzymatic activity by specific compounds found in the analyzed EO.

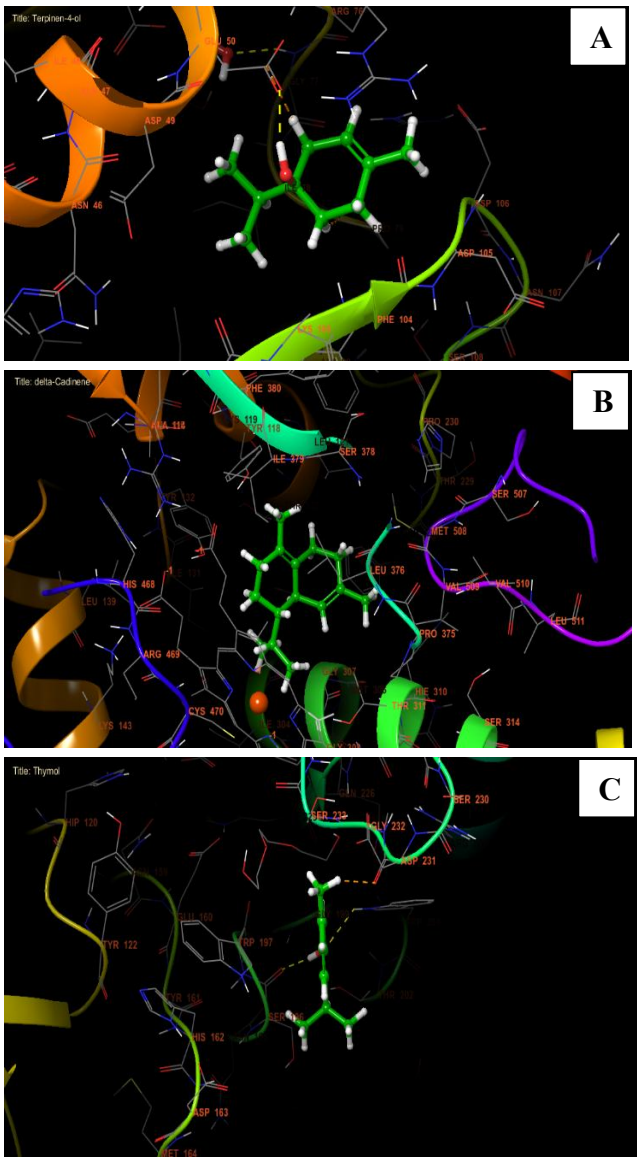

Figure 7. The 3D viewer of ligands (EO compounds) interactions with the enzyme's active site. A: Interactions of terpinen-4-ol with the active site of *E. coli* gyrase B: Interactions of Δ-Cadinene with the active site of sterol 14-alpha demethylase (CYP51) from *C. albicans*; C: Interactions of thymol with the active site of beta-1,4-endoglucanase from *A. niger*.

4. Conclusion

In conclusion, the results obtained indicate that the EO extracted from Moroccan *Thymus vulgaris* exhibits significant antioxidant and antimicrobial properties. These findings highlight the potential effectiveness of this essential oil as a natural and potent therapeutic agent in mitigating the effects of free radicals and addressing the increasing challenge of microbial resistance to pathogenic microorganisms. Computational studies supported the experimental results. Therefore, this essential oil may offer benefits in managing oxidative stress-related disorders and food spoilage issues; however, further validation, particularly through *in vivo* studies, is necessary to confirm these findings. The observed bioactivities are partly attributed to the presence of monoterpene phytochemicals in the EO under investigation.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data availability statement

Data will be available upon request from the corresponding author.

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