



Food Science and Applied Biotechnology

e-ISSN: 2603-3380

Journal home page: www.ijfsab.com
<https://doi.org/10.30721/fsab2026.v9.i2>



Research Article

Chemical profiling, antioxidant, and antimicrobial potential of Algerian thyme, garlic, and lemon essential oils: *In vitro* and *in silico* evaluations

Khokha Alileche¹, Mohamed Nadjib Boukhatem², Sarah Balit³, Mourad Djeziri^{1,4}✉, Mohamed Abou-mustapha¹, Abdelkrim Ouali¹, Farid Chebrouk^{1,5}

¹Center for Scientific and Technical Research in Physicochemical Analysis (CRAPC), BP384, Bou-Ismaïl, 42004 Tipaza, Algeria

²Blida 1 University, Faculty of Natural and Life Sciences, Laboratory of Aromatic and Medicinal Plants, 09000 Blida, Algeria

³University of Bejaia, Faculty of Natural and Life Sciences, Applied Microbiology Laboratory, 06000 Bejaia, Algeria

⁴Research Laboratory of Food Technology, Faculty of Technology, M'hamed Bougara University of Boumerdes, Frantz Fanon City 35000, Boumerdes, Algeria

⁵University of Bejaia, Faculty of Natural and Life Sciences, Laboratory of Biomathematics, Biochemistry, Biophysics and Scientometrics (L3BS), 06000 Bejaia, Algeria

Abstract

This study evaluated the potential of essential oils (EOs) from *Citrus limon* L., *Allium sativum* L., and *Thymus vulgaris* L. as natural food preservatives by investigating their chemical composition, antioxidant and antimicrobial activities, and molecular interactions with microbial targets. Gas chromatography–mass spectrometry revealed distinct chemical profiles, with thyme and lemon oils dominated by monoterpenes (95.55% and 98.25%), particularly thymol (42.63%) and D-limonene (74.40%), respectively, whereas garlic oil was characterized by organosulfur compounds (83.16%), mainly diallyl trisulfide (35.94%) and diallyl disulfide (21.59%). In the DPPH assay, thyme oil exhibited the highest radical-scavenging activity (91%), followed by garlic (88%) and lemon (75%) oils, although all showed lower activity than vitamin C and BHT ($p < 0.05$). Thyme oil demonstrated the strongest antibacterial activity against the tested bacterial strains, particularly *Escherichia coli* and *Staphylococcus aureus*, with inhibition zones comparable to gentamicin. Garlic oil showed notable antifungal activity against *Candida albicans* and *Saccharomyces cerevisiae*, whereas lemon oil exhibited moderate antimicrobial effects. Molecular docking further indicated favorable interactions of thymol and diallyl trisulfide with *S. aureus* ParE and *C. albicans* CYP51, respectively. Overall, the findings highlight the complementary antimicrobial and antioxidant properties of these EOs and support their potential as natural preservatives for food applications.

Keywords

antimicrobial activity, drug-resistant pathogens, essential oils, molecular docking, natural preservatives

Abbreviations

ATCC – American Type Culture Collection; BHT – Butylated hydroxytoluene; DMSO – Dimethyl sulfoxide; DNA – Deoxyribonucleic acid; DPPH – 2,2-Diphenyl-1-picrylhydrazyl; EOs – Essential oils; GC-MS – Gas chromatography–mass spectrometry; GEO – Garlic essential oil; HCA – hierarchical cluster analysis; LEO – Lemon essential oil; MHA – Mueller–Hinton agar; PCA – principal component analysis; SDA – Sabouraud dextrose agar; TEO – thyme essential oil

✉ Corresponding author: Pr Mourad Djeziri, PhD, Natural Products and Food Sciences Division, Center for Scientific and Technical Research in Physicochemical Analysis (CRAPC), BP384, Bou-Ismaïl, 42004 Tipaza, Algeria; e-mail: m.djeziri@univ-boumerdes.dz

Article history:

Received 11 March 2026

Reviewed 31 July 2026

Accepted 31 August 2026

Available on-line 12 October 2026

<https://doi.org/10.30721/fsab2026.v9.i2.697>
 2026, UFT Academic publishing house, Plovdiv

Introduction

Essential oils (EOs) are complex mixtures of volatile, low-molecular-weight compounds produced by aromatic and medicinal plants and accumulated in specialized secretory structures. They are widely used in the food, pharmaceutical, and cosmetic industries because of their flavoring, aromatic, and biological properties (Güven et al. 2023). Their chemical composition is mainly characterized by monoterpenes, sesquiterpenes, phenylpropanoids, and other bioactive constituents that contribute to their antimicrobial and antioxidant activities (Sadgrove et al. 2022).

The increasing demand for safer and more natural food preservation strategies has stimulated interest in plant-derived EOs as potential alternatives to conventional preservatives. Their combined antimicrobial and antioxidant activities may help control microbial contamination and delay oxidative deterioration in food products (Diogo et al. 2025). Among the numerous EOs investigated for these applications, thyme (*Thymus vulgaris*), garlic (*Allium sativum*), and lemon (*Citrus limon*) oils are of particular interest because of their distinctive chemical compositions and biological properties. Thyme EO is generally rich in phenolic monoterpenes, particularly thymol and carvacrol, which contribute to its strong antimicrobial and antioxidant activities (Galovičová et al. 2021). Garlic EO is characterized by sulfur-containing compounds that are closely associated with its characteristic aroma and broad biological activities, including antimicrobial and antioxidant effects (Huang et al. 2023). Lemon EO, whose composition is typically dominated by limonene, has also attracted attention for its antimicrobial and antioxidant properties and its potential application in food systems (Haokip et al. 2023).

A comprehensive assessment of EO bioactivity requires the integration of complementary experimental and computational approaches. *In vitro* assays, such as the DPPH radical-scavenging method and disc diffusion assay, provide valuable information on antioxidant and antimicrobial activities but offer limited insight into the molecular mechanisms underlying these effects. Molecular docking can complement experimental investigations by predicting the interactions of bioactive compounds with relevant microbial target

proteins and identifying potential binding sites and interaction patterns (Dakpa et al. 2025).

In the present study, an integrated experimental and computational approach was applied to evaluate the bioactive potential of thyme, garlic, and lemon EOs. Their chemical compositions were characterized by gas chromatography–mass spectrometry (GC-MS), while antioxidant activity was assessed using the DPPH radical-scavenging assay and antimicrobial activity was evaluated by the disc diffusion method against selected bacterial and fungal strains. Molecular docking was subsequently performed to investigate the interactions of the major bioactive constituents, namely thymol, diallyl trisulfide, and D-limonene, with *Staphylococcus aureus* DNA topoisomerase IV (ParE) and *Candida albicans* sterol 14 α -demethylase (CYP51; PDB ID: 5FSA).

This integrated *in vitro* and *in silico* strategy provides complementary information on the chemical composition and biological activities of the selected EOs and contributes to understanding the potential mechanisms underlying the activity of their major constituents. The findings may support the development of EO-based natural preservation strategies and other applications requiring bioactive plant-derived compounds.

Materials and Methods

Plant material and essential oil extraction. Plant materials were collected in June in Tablat (Médéa, north-central Algeria; 800–900 m altitude), a Mediterranean region with continental influence and silty-clay to calcareous soils potentially affecting secondary metabolite accumulation. Peel *Citrus limon* fruits, *Allium sativum* bulbs, and *Thymus vulgaris* leaves were obtained from local farmers at commercial maturity, transported to the laboratory the same day under controlled conditions, and homogenized after removing damaged tissue.

Samples were transported to the laboratory in ventilated cardboard bags and stored at room temperature until extraction. Essential oils were obtained by hydro-distillation using a Clevenger-type apparatus, with triplicate extractions performed for each plant. Approximately 100 g of plant material was placed in a 2 L round-bottom flask containing 1 L of distilled water and connected to a 60 cm $\times$ 2 cm vertical condenser.

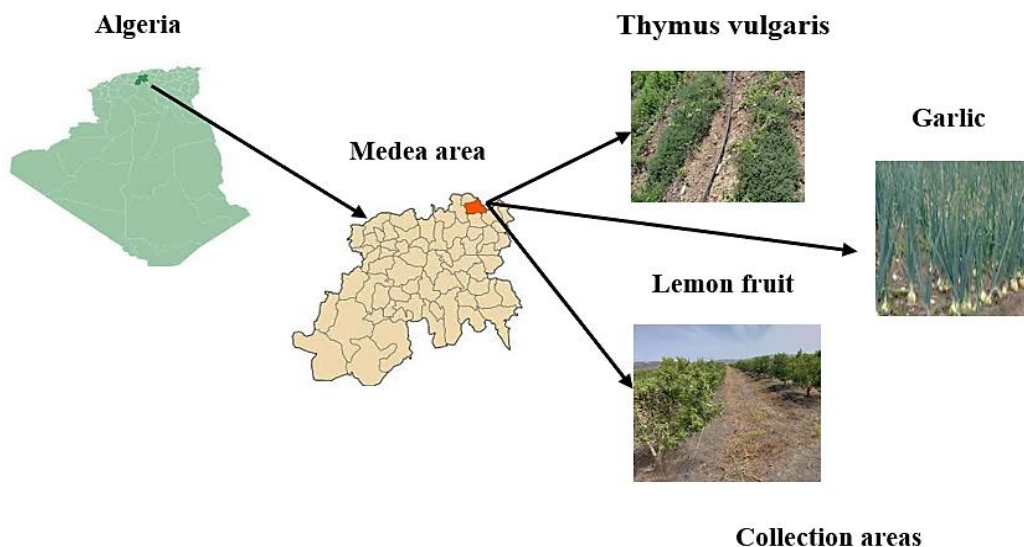


Figure 1. *Citrus limon* fruit, *Allium sativum* bulbs and *Thymus vulgaris* leaves collection areas

Distillation continued until no further oil was recovered. The oils were separated from water, dried over anhydrous sodium sulfate, and stored in amber vials at room temperature. EO yields were calculated using the following formula (1):

$$\text{Yield (\%)} = \frac{\text{Weight of collected essential oil}}{\text{Dry weight of extracted plant}} * 100 \quad (1)$$

Analysis of the chemical composition of selected essential oils. GC-MS analysis was performed using an Agilent HP 6890 gas chromatograph coupled to a 5973A quadrupole mass spectrometer. Separation was achieved on an HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm). A 1 μL sample was injected with a 1:80 split at 250°C. The oven temperature was programmed from 45°C (8 min) to 250°C at 2°C/min, held for 10 min. Helium (grade N 6.0) served as carrier gas at 0.6 mL.min⁻¹. MS source and quadrupole temperatures were 230°C and 150°C, respectively, with 70 eV ionization and mass scan range 55–550 m.z⁻¹. Volatile constituents were identified by comparing their Kovats retention indices (C8 - C28 n-alkanes) and their mass spectra with literature values and NIST 20 and Wiley 7N libraries (Babushok et al. 2011). Compound concentrations were expressed as relative percentage area using ChemStation software.

Evaluation of the *in vitro* antioxidant activity of essential oils. Antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay with slight modifications of the reported protocol (Karagecili et al. 2023).

Briefly, 0.1 mL of EO extract was mixed with 3.9 mL of DPPH solution and vortexed for 30 sec. The mixture was incubated in the dark for 30 min, and absorbance was measured at 515 nm using a UV–Visible spectrophotometer: Shimadzu UV-1800 (Shimadzu, Japan). The percentage of DPPH radical inhibition (I%) was calculated using the following formula (2):

$$I(\%) = 1 - \frac{Abs_{sample}}{Abs_{control}} * 100 \quad (2)$$

Where Abs_{sample} represents the absorbance of the DPPH solution containing EO, BHT, or ascorbic acid, and $Abs_{control}$ corresponds to the absorbance of the DPPH solution without sample. All assays were performed in triplicate, and IC₅₀ values were determined from the dose–response curves.

Evaluation of the *in vitro* antibacterial activity. The *in vitro* antimicrobial activity of the essential oil (EO) was evaluated by the agar disc diffusion method according to Balouiri et al. (2016), with minor modifications. Reference strains from the American Type Culture Collection (ATCC)—*Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Candida albicans* (ATCC 10231), and *Saccharomyces cerevisiae* (ATCC 9763)—were tested. Mueller–Hinton agar (MHA) and Sabouraud dextrose agar (SDA) were inoculated with 100 μL of standardized bacterial and yeast suspensions, respectively. Sterile Whatman discs (6 mm) loaded

with 10 μL of EO solution (100 $\text{mg}\cdot\text{mL}^{-1}$) in DMSO; 1 $\text{mg}\cdot\text{disc}^{-1}$ were placed on the inoculated plates. After diffusion at 4°C for 1-2 h, plates were incubated at 37°C for 18-24 h (bacteria) or 30°C for 24-48 h (yeasts). Gentamicin (10 $\mu\text{g}\cdot\text{disc}^{-1}$) and fluconazole (25 $\mu\text{g}\cdot\text{disc}^{-1}$) were used as positive controls, and DMSO as the negative control. Inhibition zones were measured in mm using a digital caliper. All assays were performed in triplicate and reported as mean $\pm$ standard deviation.

In silico antimicrobial activity using computational methods

Protein targets preparation. For antibacterial analysis, the crystal structure of *Staphylococcus aureus* DNA topoisomerase IV subunit B (ParE) (PDB ID: 4URN) (Lu et al. 2014) was retrieved from the Protein Data Bank. This 24-kDa ParE domain, complexed with novobiocin, was resolved by X-ray diffraction at 2.3 Å resolution and belongs to the isomerase class (EC 5.99.1). This target was

selected because ParE contains the essential ATP-binding pocket required for bacterial DNA replication, making it a key site for developing novel agents to overcome antibiotic resistance. Prior to docking, heteroatoms, water molecules, and the co-crystallized ligand were removed. For antifungal evaluation, the crystal structure of *Candida albicans* sterol 14- α -demethylase (CYP51) complexed with posaconazole (PDB ID: 5FSA) was also obtained from the Protein Data Bank. CYP51 was chosen as it is the primary target of clinical azoles, playing a critical role in ergosterol biosynthesis and fungal cell membrane integrity. As it is the primary clinical target for azole antifungals, it serves as an ideal model for identifying novel antifungal candidates. This enzyme, involved in the fungal ergosterol biosynthesis pathway, was resolved at 2.86 Å by X-ray diffraction (Hargrove et al. 2017). Before docking, heteroatoms, ligands, and water molecules were removed, and polar hydrogen atoms were added to the protein.

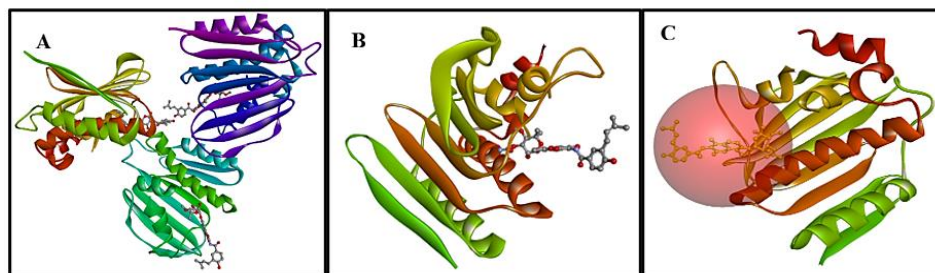


Figure 2. Three-Dimensional Structure of the Target Enzyme, DNA Topoisomerase IV Subunit B (ParE): A/ Overall quaternary structure of the ParE trimer, B/ Chain A, C/ Detailed view of the active site

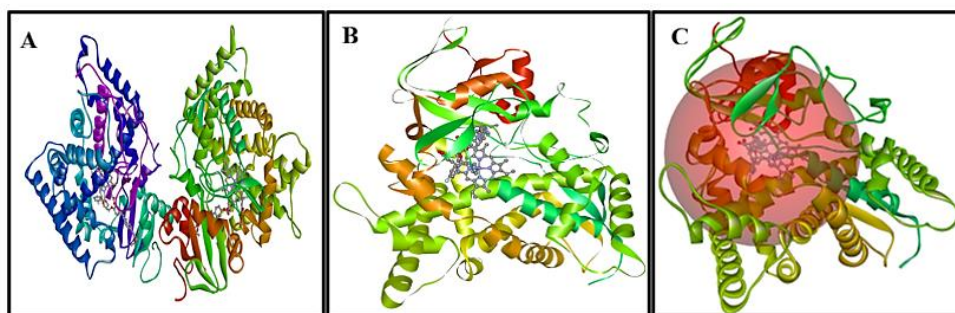


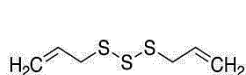
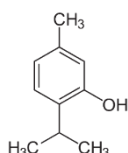
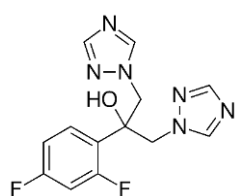
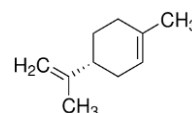
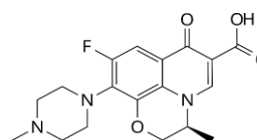
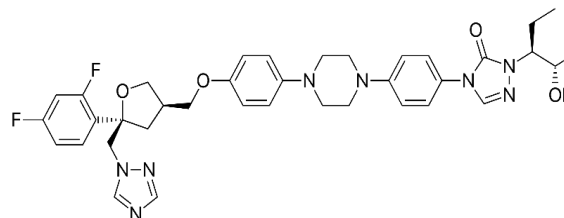
Figure 3. Crystal structure of sterol 14- α -demethylase (CYP51) from *Candida albicans* (PDB ID: 5FSA) complexed with the antifungal drug posaconazole. A/ Overall quaternary structure of the sterol 14- α -demethylase, B/ Chain A, C/ Detailed view of the active site

Ligand selection and preparation. Major essential oil terpenes - limonene, thymol, and diallyl trisulfide - were selected as natural ligands based on their reported antimicrobial properties. Reference drugs, including levofloxacin (antibacterial) and

posaconazole and fluconazole (antifungal), were used for comparison of binding affinities and interaction profiles. The 3D structures of all ligands (Table 1, Fig. 4) were retrieved from the PubChem database in SDF format.

Table 1. Chemical information and structural identifiers for compounds selected for molecular docking

Ligand	Compound ID	Molecular formula	Molecular weight(g.mol ⁻¹)	SMILES
Diallyl trisulfide	16315	C ₆ H ₁₀ S ₃	178	C=CCSSSCC=C
D-Limonene	440917	C ₁₀ H ₁₆	136	CC1=CC[C@H](CC1)C(=C)C
Thymol	6989	C ₁₀ H ₁₄ O	150	CC1=CC(=C(C=C1)C(C)C)O
Levofloxacin	149096	C ₁₈ H ₂₀ FN ₃ O ₄	361	C[C@H]1COC2=C3N1C=C(C(=O)C3=CC(=C2N4CCN(CC4)C)F)C(=O)O
Fluconazole	3365	C ₁₃ H ₁₂ F ₂ N ₆ O	306	C1=CC(=C(C=C1F)F)C(CN2C=NC=N2)(CN3C=NC=N3)O
Posaconazole	468595	C ₃₇ H ₄₂ F ₂ N ₈ O ₄	700	CC[C@H]([C@H](C)O)N1C(=O)N(C=N1)C2=CC=C(C=C2)N3CCN(CC3)C4=CC=C(C=C4)OC[C@H]5C[C@](O(C5))(CN6C=NC=N6)C7=C(C=C(C=C7)F)F

**Diallyl trisulfide****Thymol****Fluconazole****d-Limonene****Levofloxacin****Posaconazole****Figure 4.** Chemical structures of natural terpenes (diallyl trisulfide, d-limonene, and thymol) and the reference drugs (levofloxacin, posaconazole and fluconazole) used as ligands for docking.

Molecular docking simulation. For antibacterial activity, molecular docking was performed using the GPU-accelerated DockThor web server for flexible protein–ligand docking (Guedes et al. 2024). Genetic algorithm parameters were set to 1,000,000 evaluations, a population size of 750, and 24 independent runs, with other settings at default values. The active site was defined around the co-crystallized ligand using grid center coordinates $x =$

-31.949455 , $y = 7.203705$, $z = -4.681636$, with a $15 \times 15 \times 15 \text{ \AA}^3$ grid box and $0.25 \text{ \AA}$ spacing. Docking poses were ranked by predicted binding energy (kcal/mol), and the best-scoring pose was selected for interaction analysis. For antifungal activity, docking was performed using PyRx v0.8 with AutoDock Vina (Dallakyan and Olson 2015). Ligands were docked using grid center coordinates $x = 199.721$, $y = -2.058$, and $z = 36.447$, with grid

dimensions of $29.52 \times 25.00 \times 20.19 \text{ \AA}$ and an exhaustiveness of 8. The lowest-energy pose was retained for each ligand.

Analysis of protein–ligand interactions. Docked complexes were analyzed to identify key amino acid residues involved in binding within the active sites of ParE and sterol 14- α -demethylase. Hydrogen bonds, hydrophobic contacts, and π – π stacking interactions were evaluated using BIOVIA Discovery Studio Visualizer (2021), Protein Plus (Fährrolfes et al. 2017), and LigPlot+ v. 2.2.9 (Laskowski and Swindells 2011). Reference drugs served as controls to validate the docking protocol. Comparative analyses assessed overlapping interacting residues, binding modes, and docking energies to evaluate the potential inhibitory capacity of terpenes and reference drugs.

Statistical analysis. Statistical analysis was performed according to standard one-way ANOVA methods, using the XLSTAT 2019 software, following by Fisher LSD test and a significance threshold of 5% were applied. A Principal Component Analysis (PCA) and hierarchical cluster analysis were conducted to highlight a group structure based on physicochemical parameters.

Results and Discussion

Variability in essential oil yields across plant species. The EO yields obtained by hydrodistillation for thyme, garlic, and lemon were within ranges commonly reported in the literature. The yield of TEO ($0.99 \pm 0.21\%$) is consistent with previously reported values (0.7 – 4.12%), which vary according to developmental stage, harvest time, and environmental conditions. The relatively low GEO yield ($0.15 \pm 0.03\%$) also agrees with published data, as sulfur-rich compounds exhibit lower volatility than terpenes. Mansouri et al. (2018) reported an average yield of $0.23 \pm 0.15\%$. Similarly, the yield in LEO ($0.43 \pm 0.09\%$) is below the expected range for citrus peel, although yields can vary between 0.87 per cent and 1.62%. Some studies attribute variations to genetic factors, others emphasize the dominant role of edaphoclimatic conditions, such as altitude, drought stress, and soil composition. Competing perspectives exist: some researchers argue that geographical origin is the primary determinant of chemical fingerprints, whereas others suggest that cultivation practices and harvest maturity exert equal or greater influence on

volatile profiles (Beise et al. 2024).

Chemical composition of thyme, garlic and lemon essential oils. GC-MS analysis of TEO identified 99.21% of the total compounds. Monoterpenes predominated (95.55%), whereas sesquiterpenes accounted for 3.29%. Thymol (42.63%) and carvacrol (20.25%) were the major constituents, together exceeding 62% and confirming a thymol–carvacrol chemotype. Other abundant compounds included γ -terpinene (20.43%), p-cymene (6.80%), α -terpinene (2.06%), and β -myrcene (0.90%) (Table 2). The minor sesquiterpene fraction was mainly represented by (E)-caryophyllene (1.44%), E- α -bisabolene (0.90%), and β -bisabolene (0.74%). Overall, TEO was characterized by a thymol- and carvacrol-rich profile (Table S2, supporting information).

Literature comparisons revealed marked geographic variation in TEO composition. Thymol-rich chemotypes have been reported in Romania, India, Italy, Brazil, and Algeria. Romanian oil contained thymol (47.59%), γ -terpinene (30.90%), and p-cymene (8.41%) (Benabed et al. 2018), while Indian samples showed thymol (46.2%), γ -terpinene (14.1%), and p-cymene (9.9%) (Marzieh and Sajedeh 2015). Similar profiles were reported in Italy (thymol 42.75%, γ -terpinene 18.01%, p-cymene 16.04%) (Hudaib et al. 2002) and Brazil (thymol 44.7%, p-cymene 18.6%, γ -terpinene 16.5%). Our sample therefore aligns with the thymol-rich chemotype. Conversely, French TEO exhibited a linalool chemotype, dominated by linalool (76.15%) and linalyl acetate (14.26%), whereas Serbian oil showed a geraniol chemotype, with geraniol (59.75%) and geranyl acetate (16.72%) as major constituents (Hudaib et al. 2002). These variations reflect the chemical plasticity of *T. vulgaris*, influenced by geographic origin, environmental conditions, harvest stage, and genetic factors, with implications for its potential applications. GC-MS studies have revealed substantial chemotypic diversity in TEO, strongly influenced by geographic origin (Satyal et al. 2016). French samples from Nyons exhibited a linalool chemotype dominated by linalool (76%) and linalyl acetate (14%), whereas those from Richerenches showed a thymol chemotype characterized by thymol (47%) and p-cymene (20%).

Table 2. Major constituents from thyme essential oil

Peak	Rt	KI	KI*	Compounds	Concentration (%)
1	13.13	990	990	β -Myrcene	0.90
2	14.73	1015	1017	α -Terpinene	2.06
3	15.33	1023	1024	p- Cymene	6.80
4	17.85	1059	1060	γ -Terpinene	20.43
5	34.76	1301	1290	Thymol	42.63
6	35.30	1309	1300	Carvacrol	20.25
7	42.20	1416	1419	(E)-caryophyllene	1.44
8	49.8	1541	1540	E- α -Bisabolene	0.90

In Serbia, geraniol (59% geraniol; 16% geranyl acetate) and sabinene hydrate chemotypes (30.8% cis-sabinene hydrate) have also been reported. These differences highlight the marked chemical variability of *T. vulgaris* populations, driven largely by geographic, genetic, and ecotypic factors that affect the relative abundance of monoterpenes, oxygenated monoterpenes, and phenolic compounds.

GC-MS analysis of GEO showed a predominance of organosulfur compounds (83.16%), which characterize garlic essential oil and contribute to its distinctive aroma and biological activities. Diallyl trisulfide (35.94%) was the major constituent, followed by diallyl disulfide (21.59%) and allyl

methyl trisulfide (6.79%), indicating a predominance of di- and trisulfide derivatives (Table 3). Other sulfur compounds included (E)-1-propenyl 2-propenyl disulfide (5.02%) and diallyl tetrasulfide (2.14%). Monoterpenes accounted for 8.05%, mainly d-limonene (7.31%), while sesquiterpenes represented 1.55%, primarily (Z)-caryophyllene. Overall, 93.14% of GEO constituents were identified (Table 2). The dominance of diallyl polysulfides, particularly diallyl trisulfide and diallyl disulfide, confirms the typical chemical profile of *A. sativum* essential oil and agrees with previous reports highlighting their contribution to its bioactivity (Huang et al. 2023).

Table 3. Major constituents from garlic oil

Peak	Rt	KI	KI*	Compounds	Concentration (%)
1	14.51	1028	1029	D-Limonene	7.31
2	18.94	1091	1088	Diallyl disulfide	21.59
3	19.50	1099	1097	(Z)-1-Propenyl 2-propenyl disulfide	1.99
4	20.01	1106	1103	(E)-1-Propenyl 2-propenyl disulfide	5.02
5	22.86	1146	1143	Allyl methyl trisulfide	6.79
6	28.26	1224	1217	2-Vinyl-1,3-dithi-4-ene	2.38
7	34.8	1316	1304	Diallyl trisulfide	35.94
8	49.2	1545	1538	Diallyl tetrasulfide	2.14

GC-MS analysis of LEO identified 39 volatile compounds, representing 99.51% of the total composition. Monoterpenes predominated (98.25%), while sesquiterpenes (0.91%) and other

compounds (0.35%) were minor. D-Limonene was the major constituent (74.40%) (Table 4), characteristic of cold-pressed and hydrodistilled LEO and largely associated with its biological

activities (Ganjitabar et al. 2023; Petretto et al. 2023). Other major monoterpenes included γ -terpinene (7.62%), β -pinene (2.77%), and α -terpineol (2.12%), while geranial (1.19%) and linalool (0.47%) occurred at lower levels. The minor sesquiterpene fraction was mainly composed of β -bisabolene (0.44%) and α -acoradiene (0.29%) (Table 4). Overall, the profile agrees with previous

reports, with variations attributable to environmental conditions, cultivar, extraction method, and plant maturity (Modica et al. 2024).

Table 5 shows some examples of chemotype variability among the three essential oils studied, according to their geographical area.

Table 4. Major constituents from lemon essential oil

Peak	Rt	KI	KI*	Compounds	Concentration (%)
1	9.57	929	936	α -Pinene	1.00
2	12.1	973	978	β -Pinene	2.77
3	13.1	990	990	β -Myrcene	1.60
4	16.12	1034	1029	D-Limonene	74.40
5	17.82	1059	1060	γ -Terpinene	7.62
6	19.73	1086	1088	Terpinolene	1.32
7	27.18	1191	1188	α -Terpineol	2.12
8	32.78	1272	1267	Geranial	1.19

(RT = Retention time; KI = Kovats indices relative to the homologous series of *n*-alkanes (C₈–C₂₈);

KI* = Kovats indices according to literature values.

Table 5: Geographical variability in the major constituents of thyme, garlic, and lemon essential oils

Essential oil	Country	Major components	References
Thyme (<i>Thymus vulgaris</i>)	Algeria	Thymol (47.53%), carvacrol (19.27%) and γ -terpinene (14.94%)	Benchallal et al. 2024
	France	Thymol (49.45-65.90%), o-cymene (7.15-23.08%) and carvacrol (4.5-6.12%)	Lanciu Dorofte et al. 2023
	Slovakia	Thymol (48.1%), <i>p</i> -cymene (11.7%), 1,8-cineole (6.7%)	Galovičová et al. 2021
	Morocco	carvacrol (59.70 %), thymol (16.5 %), and γ -terpinene (11.11 %)	Chahboun et al. 2024
Garlic (<i>Allium sativum</i>)	Italy	Allicin (50.9-62.2), diallyl disulfide (16.6-27.9)	Nazzaro et al. 2022
	Spain	Diallyl trisulfide (16.8–33.4%), diallyl disulfide (20.8–27.9%), allyl methyl trisulfide (14.5–19.2%), diallyl sulfide (1.9–9.5%) and allyl methyl disulfide (4.4–8.3%)	Beise et al. 2024
	Egypt	Diallyl trisulfide and diallyl disulfide (45.76 and 15.63%)	El-Sayed et al. 2017
	(Morocco)	Limonene (48.56-49.37), γ -terpinene (12.33-12.84), and α -pinene (2.45-2.81%)	El Aboubi et al. 2022
Lemon (<i>Citrus limon</i>)	(Nigeria)	Limonene (85.9), sabinene (3.9), myrcene (3.1)	Owolabi et al. 2018
	Slovakia	limonene (60.7%), β -pinene (12.6%), and γ -terpinene (10.3%)	Kačániová et al. 2024
	India	Limonene (55.40 %) and neral (10.39 %) trans-verbenol (6.43 %)	Paw et al. 2020

***In vitro* determination of antioxidant activity via DPPH radical scavenging.**

The DPPH assay showed a clear dose-dependent radical-scavenging activity for all tested EOs (Fig. 5). TEO exhibited the strongest activity, reaching 91% inhibition at 100 mg.mL⁻¹ and the lowest IC₅₀ (8.8 mg.mL⁻¹), likely due to its high thymol and carvacrol content and their strong hydrogen-donating capacity (Yildiz et al. 2021). Melkina et al. (2021) similarly reported a lower IC₅₀ for isolated thymol than for the whole EO, highlighting the contribution of phenolic compounds. These findings support TEO's potential as a natural antioxidant for food preservation and nutraceutical applications.

GEO also showed considerable activity, with 88% maximum inhibition and an IC₅₀ of 10 mg.mL⁻¹. Its slower response at lower concentrations may reflect the higher doses required for sulfur-containing

compounds to achieve maximal activity. These results agree with reports of strong radical-scavenging activity in *Allium* EOs (Bajpai et al. 2015), although Herrera-Calderon et al. (2021) reported a much lower IC₅₀ for Peruvian GEO (124.60 ± 2.3 µg.mL⁻¹). Lazarova-Zdravkova et al. (2021) reported measurable radical-scavenging activity for clove essential-oil systems, highlighting the influence of essential-oil composition and formulation on antioxidant capacity.

LEO showed moderate activity, with 75% inhibition and an IC₅₀ of 18.6 mg.mL⁻¹, consistent with citrus EOs dominated by monoterpene hydrocarbons. All EOs were less potent than vitamin C (IC₅₀ = 5.8 mg.mL⁻¹). Overall, antioxidant activity ranked TEO > GEO > LEO, reflecting differences in their phytochemical profiles, particularly phenolic and sulfur-containing compounds.

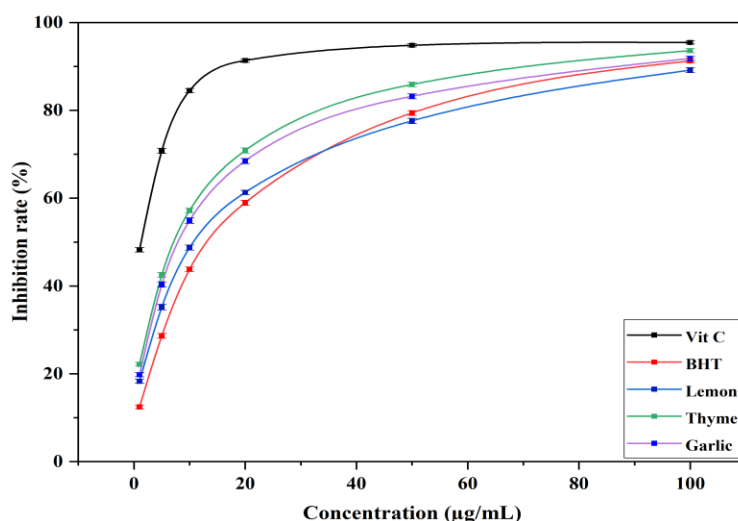


Figure 5. Antioxidant potential measured by DPPH (% Inhibition).

The antimicrobial properties of the three oil fractions.

The antimicrobial evaluation of EOs using the disc diffusion method revealed significant differences in their inhibitory effects against the tested bacterial and fungal strains (Table 6). Positive control: gentamicin (10 µg.disc⁻¹) for bacteria; fluconazole (25 µg.disc⁻¹) for yeasts. TEO demonstrated strong antimicrobial activity against the tested microorganisms, with its effectiveness varying according to the microbial strain. Against *E. coli*, TEO exhibited an inhibition zone (28.000 ± 0.794 mm) comparable to that of gentamicin (28.333 ± 0.306 mm), as indicated by the same statistical group. However, the inhibition

zones produced by TEO against *S. aureus* (26.400 ± 0.400 mm) and *B. subtilis* (22.467 ± 0.289 mm) were significantly lower than those of gentamicin (28.133 ± 0.379 and 28.000 ± 0.529 mm, respectively). These results indicate that, although TEO exhibited considerable antibacterial activity, its efficacy was comparable to gentamicin only against *E. coli*. This activity is likely related to phenolic monoterpenes, particularly thymol and carvacrol, known for broad-spectrum antimicrobial effects (Dong et al. 2024). GEO also showed strong activity, particularly against the fungal strains, with inhibition zones of 23.00 ± 0.624 and 20.433 ± 0.416 mm against *C. albicans* and *S. cerevisiae*,

respectively. However, these inhibition zones were substantially smaller than those observed with fluconazole (46 mm). GEO also produced an inhibition zone of 20.433 ± 0.153 mm against *S. aureus*. Its activity may be attributed to allyl sulfides that disrupt microbial membranes and metabolic pathways. The antibacterial potential observed for garlic essential oil is consistent with previous findings reported for *Allium sativum* extracts, which demonstrated antibacterial and antibiofilm effects against *Escherichia coli* (Haindongo et al. 2021).

TEO exhibited significantly stronger antifungal activity than GEO against both tested yeast strains. The inhibition zones produced by TEO were larger against *C. albicans* (30.67 mm) and *S. cerevisiae*

(28.37 mm) than those observed for GEO (23.00 and 20.43 mm, respectively), indicating a greater susceptibility of these fungal strains to TEO. LEO exhibited the weakest activity, with inhibition zones ranging from 10.80 ± 0.36 mm against *P. aeruginosa* to 15.47 ± 0.23 mm against *S. aureus*, consistent with its monoterpene-rich profile (Dorman and Deans 2000). Overall, TEO showed the strongest antibacterial activity, GEO the highest antifungal efficacy, and LEO the lowest antimicrobial potency, reflecting differences in chemical composition (Machado et al. 2014).

Multivariate Analysis (PCA and AHC). The results of the PCA analysis and the hierarchical cluster analysis are presented in Fig. 6 and Table 7.

Table 6. Inhibition zones (mm) of essential oils against microbial strains

Strain	Essential oils			Positive controls	
	<i>C. limon</i>	<i>A. sativum</i>	<i>T. vulgaris</i>	Gentamicin	Fluconazole
Inhibition zone diameter, mm					
<i>E. coli</i>	$11.223 \pm 0.434^{(c)}$	$14.333 \pm 0.493^{(b)}$	$28.000 \pm 0.794^{(a)}$	$28.333 \pm 0.306^{(a)}$	-
<i>P. aeruginosa</i>	$10.800 \pm 0.361^{(c)}$	$12.300 \pm 0.200^{(b)}$	$13.733 \pm 0.153^{(b)}$	$28.000 \pm 0.173^{(a)}$	-
<i>S. aureus</i>	$15.467 \pm 0.231^{(d)}$	$20.433 \pm 0.153^{(c)}$	$26.400 \pm 0.400^{(b)}$	$28.133 \pm 0.379^{(a)}$	-
<i>B. subtilis</i>	$14.467 \pm 0.379^{(d)}$	$18.533 \pm 0.321^{(c)}$	$22.467 \pm 0.289^{(b)}$	$28.000 \pm 0.529^{(a)}$	-
<i>C. albicans</i>	$13.700 \pm 0.265^{(d)}$	$23.000 \pm 0.624^{(c)}$	$30.667 \pm 0.252^{(b)}$	-	$46.000 \pm 0.500^{(a)}$
<i>S. cerevisiae</i>	$12.600 \pm 0.265^{(d)}$	$20.433 \pm 0.416^{(c)}$	$28.367 \pm 0.306^{(b)}$	-	$46.100 \pm 0.458^{(a)}$

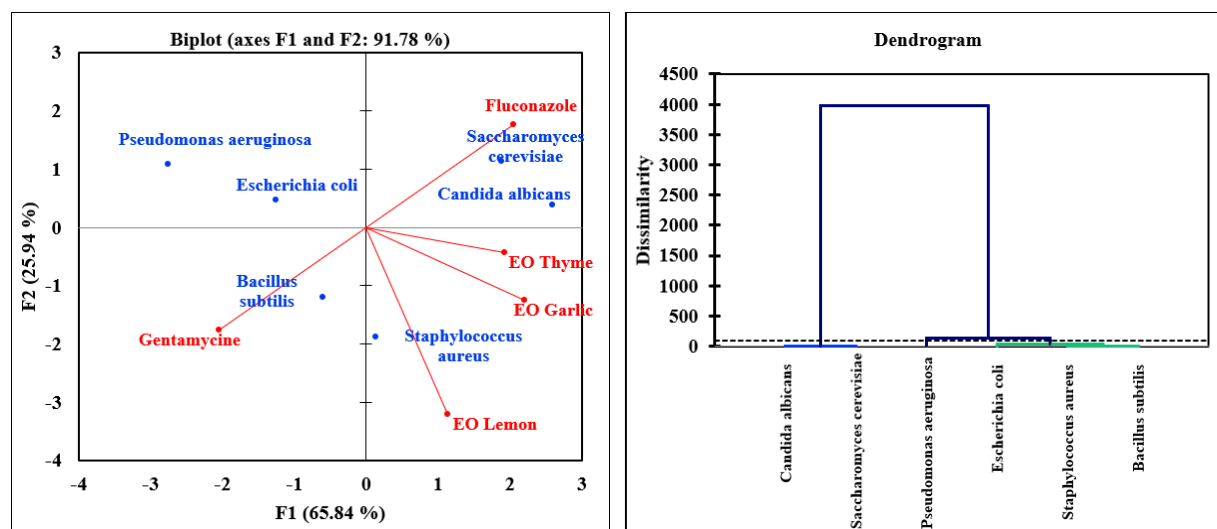


Figure 6. Observations graph, Dendrogram of different strain

Principal component analysis (PCA) explained 91.78% of the total variance (F1: 65.84%; F2: 25.94%), indicating strong discrimination and clear structuring of the antimicrobial response profiles. The correlation circle showed a marked co-projection of *Thymus vulgaris* and *Allium sativum* EOs on the positive F1 axis, indicating similar response patterns across the tested microorganisms.

Principal component analysis (PCA) explained 91.78% of the total variance (F1: 65.84%; F2: 25.94%), indicating strong discrimination and clear structuring of the antimicrobial response profiles. The correlation circle showed a marked co-projection of *Thymus vulgaris* and *Allium sativum* EOs on the positive F1 axis, indicating similar response patterns across the tested microorganisms.

Table 7. Hierarchical Bottom-up Classification of the different strain

Class	1	2	3
Objects	3	1	2
Sum of weights	3	1	2
Within-class variance	22.801	0.000	6.550
Minimum distance to centroid	3.282	0.000	1.810
Average distance to centroid	3.829	0.000	1.810
Maximum distance to centroid	4.869	0.000	1.810
	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>
	<i>Staphylococcus aureus</i>		<i>Saccharomyces cerevisiae</i>
	<i>Bacillus subtilis</i>		

Principal component analysis (PCA) explained 91.78% of the total variance (F1: 65.84%; F2: 25.94%), indicating strong discrimination and clear structuring of the antimicrobial response profiles. The correlation circle showed a marked co-projection of *Thymus vulgaris* and *Allium sativum* EOs on the positive F1 axis, indicating similar response patterns across the tested microorganisms. This proximity in the PCA reflects similarities in their observed antimicrobial activity profiles rather than evidence of a common mechanism of action. The antimicrobial effects of these EOs may nevertheless involve different mechanisms: thyme phenolic monoterpenes, mainly thymol and carvacrol, have been reported to affect cellular membranes and ergosterol-related processes in *Candida* spp., whereas garlic-derived allicin can interact with protein thiol groups, leading to enzymatic dysfunction and cellular destabilization (Huang et al. 2023).

In the biplot of observations (Figure 6), the co-localization of *Candida albicans* and *Staphylococcus aureus* in the F1+/F2– quadrant reflects shared sensitivity to these bioactive compounds, suggesting that common membrane targets may transcend taxonomic differences between yeasts and Gram-positive bacteria. This

inter-kingdom convergence highlights the central role of membrane disruption in the activity of phenolic-rich essential oils.

The distinct alignment of Fluconazole with yeasts in the factorial space provides an internal indicator of biological validity. By selectively inhibiting sterol 14 α -demethylase (CYP51), a key enzyme in ergosterol biosynthesis (Hargrove et al. 2017), this azole specifically targets fungal membranes, explaining its exclusive association with *C. albicans* and *Saccharomyces cerevisiae* in Fig. 6.

The ascending hierarchical classification (Fig. 6) and the class structure presented in Table 7 identified three main clusters: (i) a bacterial cluster comprising *E. coli*, *S. aureus*, and *B. subtilis*, which generally exhibited relatively high sensitivity to the tested essential oils, particularly TEO; (ii) *P. aeruginosa*, which formed a separate cluster and showed the lowest overall sensitivity to the tested essential oils; and (iii) a fungal cluster comprising *C. albicans* and *S. cerevisiae*, which displayed distinct susceptibility patterns compared with the bacterial strains. The clustering pattern observed among the microorganisms may be related to differences in their cell envelope structures and their interactions with the bioactive compounds present

in the essential oils. The comparatively lower sensitivity of *P. aeruginosa* may be partly associated with its Gram-negative outer membrane, which is rich in lipopolysaccharides and may act as a physicochemical barrier limiting the penetration of hydrophobic antimicrobial compounds (Pandey et al. 2017). However, this proposed mechanism should be considered as a possible explanation of the observed differential response and requires further experimental validation.

Citrus limon EO, positioned centrally on F2, showed moderate activity, likely due to its limonene-dominated composition, as this non-phenolic monoterpene generally exhibits lower antimicrobial efficacy than aromatic phenols (Liu et al. 2022).

Overall, the concordance between the factorial structure, dendrogram topology (Fig. 6), and class distribution (Table 7) indicates a high degree of statistical and biological consistency. These results suggest that thyme and garlic EOs exert preferential activity against fungal/yeast strains and Gram-positive bacteria, whereas Gram-negative bacteria exhibited a comparatively lower sensitivity under the experimental conditions used. This differential response may be related, at least in part, to

differences in cell envelope structure and permeability, although this hypothesis requires further experimental investigation.

In silico antibacterial activity of terpenes and reference antibiotic. Molecular docking has been increasingly used to investigate the possible interactions between bioactive compounds from essential oils and molecular targets associated with foodborne pathogens. Pranav et al. (2024) similarly investigated the molecular interactions of essential-oil constituents identified by GC-MS using a molecular docking approach.

Molecular docking studies were conducted to evaluate the interaction profiles and binding affinities of selected terpenes (limonene, diallyl trisulfide, and thymol) toward *Staphylococcus aureus* DNA topoisomerase IV subunit B (ParE), using levofloxacin as the reference inhibitor. The results (Table 8 and Fig. 7) provide insight into the inhibitory potential of these natural compounds compared with a clinically used antibiotic.

Docking scores showed that levofloxacin had the strongest affinity for the ParE active site (-7.614 kcal.mol $^{-1}$), followed by limonene (-7.108 kcal.mol $^{-1}$), diallyl trisulfide (-7.012 kcal.mol $^{-1}$), and thymol (-6.919 kcal.mol $^{-1}$).

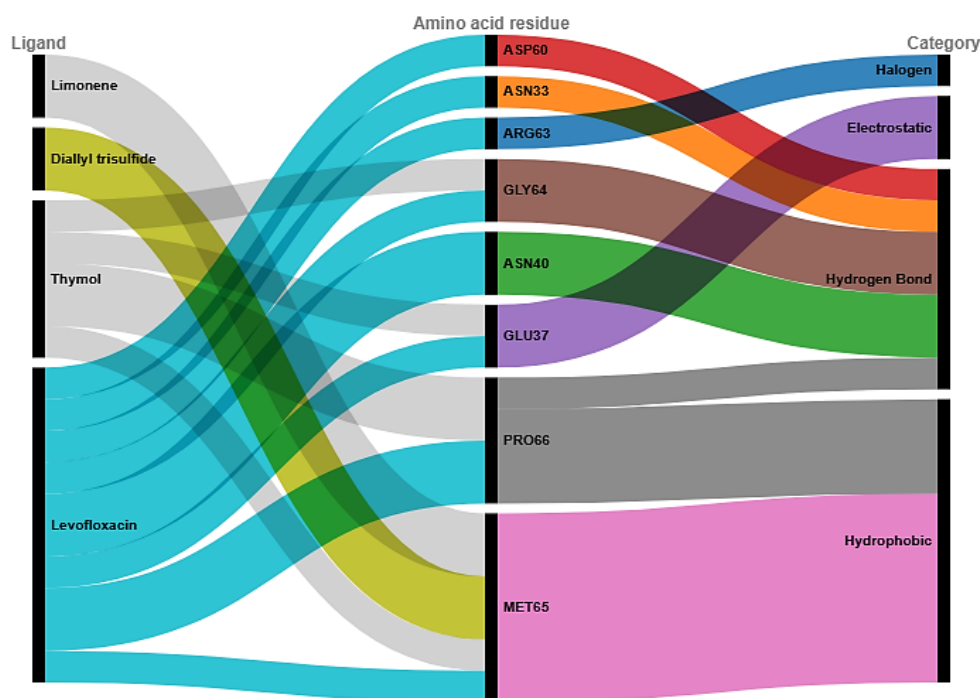


Figure 7. Molecular docking interactions of terpenes and levofloxacin with *Staphylococcus aureus* DNA Topoisomerase IV: Amino acid residues and interaction types

Table 8. Comparative molecular docking analysis of terpenes and levofloxacin targeting *Staphylococcus aureus* DNA topoisomerase IV (ParE, PDB ID: 4URN)

Ligand	Binding energy, kcal.mol ⁻¹	Amino acid residue	Distance (Å)	Category	Type
Levofloxacin	-7.614	ASP60	1,65142	Hydrogen Bond	Conventional Hydrogen Bond
		GLY64	2,68999	Hydrogen Bond	
		ASN40	2,86874	Hydrogen Bond	Carbon Hydrogen Bond
		ASN40	2,78967	Hydrogen Bond	
		ASN33	3,04271	Hydrogen Bond	
		ARG63	3,27144	Halogen	Halogen (Fluorine)
		GLU37	3,70146	Electrostatic	Pi-Anion
		PRO66	5,4463	Hydrophobic	Alkyl
		MET65	5,16411	Hydrophobic	Pi-Alkyl
		PRO66	5,10478	Hydrophobic	
Limonene	-7.108	MET65	5,0727	Hydrophobic	Alkyl
		MET65	5,47323	Hydrophobic	
Diallyl trisulfide	-7.012	MET65	4,58824	Hydrophobic	Alkyl
		MET65	4,81041	Hydrophobic	
Thymol	-6.919	GLY64	1,91908	Hydrogen Bond	Conventional Hydrogen Bond
		PRO66	3,25829	Hydrogen Bond	Carbon Hydrogen Bond
		GLU37	4,05585	Electrostatic	Pi-Anion
		PRO66	4,53555	Hydrophobic	Alkyl
		MET65	5,37071	Hydrophobic	Pi-Alkyl

Although slightly weaker than the reference drug, the negative docking energies indicate favorable interactions with the enzyme. Since ParE, a type II topoisomerase, plays a crucial role in DNA replication and segregation, its inhibition represents an important antibacterial strategy (Spencer and Panda 2023). Levofloxacin formed several hydrogen bonds and hydrophobic interactions within the active pocket, involving residues Asp60, Gly64, Asn33, and Asn40 (1.65–3.04 Å), along with a halogen interaction with Arg63 and a π -anion contact with Glu37. Additional hydrophobic contacts with Met65 and Pro66 further stabilized the complex. These interactions are consistent with the known mechanism of quinolones, which inhibit bacterial DNA replication (Hooper and Jacoby 2016).

In comparison, limonene and diallyl trisulfide mainly exhibited hydrophobic interactions, reflecting their lipophilic nature (Fig. 8). Both ligands interacted with Met65 within the hydrophobic region of the active pocket. Limonene formed two alkyl interactions at 5.07 and 5.47 Å, while diallyl trisulfide showed similar contacts at 4.58 and 4.81 Å, indicating that van der Waals and hydrophobic forces dominate their binding. Such

interactions may complement previously reported antimicrobial mechanisms of limonene, including membrane disruption and interference with cellular respiration (Han et al. 2021).

Thymol displayed a more complex interaction pattern involving both polar and hydrophobic contacts. It formed hydrogen bonds with Gly64 (1.91 Å) and Pro66 (3.25 Å), as well as a π -anion interaction with Glu37, alongside hydrophobic interactions with Met65 and Pro66. This balanced interaction profile may enhance thymol's adaptability within the binding cavity and contribute to its antibacterial activity.

As shown in Table 8, several residues, including Met65 was the only residue commonly involved in the binding interactions of all four ligands, whereas Gly64, Glu37, and Pro66 were additionally shared by levofloxacin and thymol. These findings indicate a partially conserved interaction region within the ParE binding site. The overlap between some of these residues and those interacting with levofloxacin suggests that certain terpenes may bind within a similar region of the target protein, although the interaction profiles varied among the tested compounds.

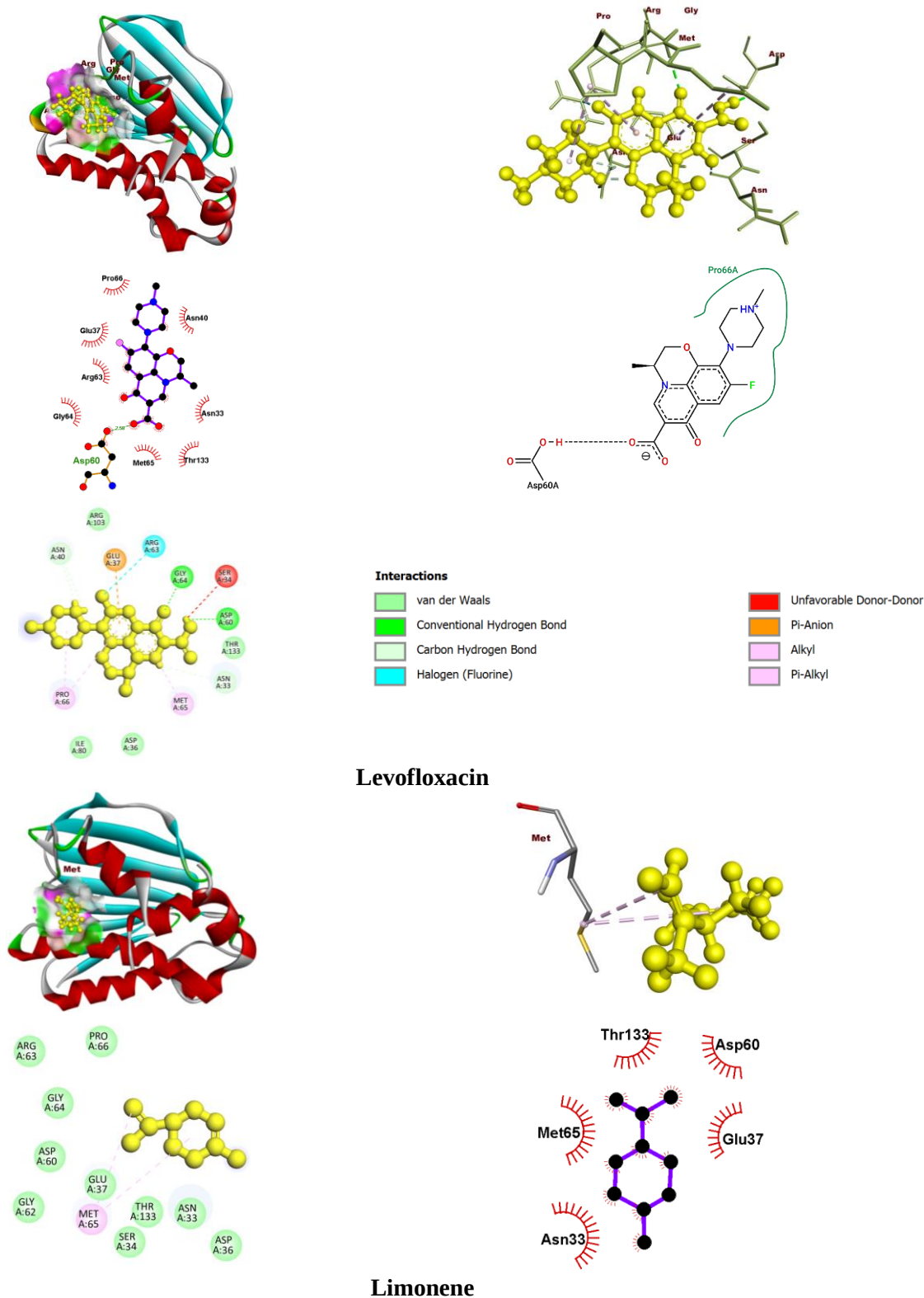


Figure 8. Visualization of the molecular interactions between ligands (Levofloxacin and limonene) and *Staphylococcus aureus* DNA topoisomerase IV subunit B (ParE, PDB ID: 4URN) generated using Discovery Studio, Protein Plus, and LigPlot+

Table 9. Docking scores and key binding interactions of antifungal drugs and terpenes with the crystal structure of *Candida albicans* sterol 14- α -demethylase (PDB ID: 5FSA)

Ligand	Binding energy, kcal.mol ⁻¹	Amino acid residue	Distance (Å)	Category	Type
Posaconazole	-11.7	GLY472	2.91438	Hydrogen Bond	Conventional Hydrogen Bond
		MET508:O	2.5106		
		SER507	2.73461		
		THR3111	2.50411	Hydrogen Bond	Carbon Hydrogen Bond
		MET508	3.57035		
		ILE471	3.65103	Halogen	Halogen (Fluorine)
		ILE304	3.7197	Hydrophobic	Pi-Sigma
		HIS377	3.66119		
		TYR118	4.0244	Hydrophobic	Pi-Pi Stacked
		PHE475	5.37379		
		PRO230	4.77227	Hydrophobic	Alkyl
		PHE233	4.56542		
		HIS377	3.85523	Hydrophobic	Pi-Alkyl
		PHE380	5.23765		
		ILE131	4.47316		
Fluconazole	-6.9	LEU376	5.24873	Hydrogen Bond	Conventional Hydrogen Bond
		PRO462	2.59794		
		PHE463	3.43906	Hydrogen Bond	Carbon Hydrogen Bond
		PRO462	3.30951	Halogen	Halogen (Fluorine)
		PRO375	5.07855	Hydrophobic	Pi-Alkyl
		ALA476	5.09205		
		THR3111	2.60161	Hydrogen Bond	Conventional Hydrogen Bond
Thymol	-6.2	ALA476	3.80652	Hydrophobic	Alkyl
		LEU370	4.26734		
		LEU480	5.1215		
		CYS470	4.66485		
		PHE463	5.07683	Hydrophobic	Pi-Alkyl
		LEU370	5.43806		
		PRO375	4.73983		
		ALA476	5.34033		
		LEU121	5.07266	Hydrophobic	Alkyl
		LEU376	4.78467		
d-Limonene	-6.0	MET508	5.34245		
		PRO230	4.89639		
		MET508	4.82436	Hydrophobic	Pi-Alkyl
		LEU376	4.53096		
		LEU121	4.99091		
		TYR118	4.16777		
		TYR118	3.57685	Hydrophobic	Pi-Alkyl
		PHE233	5.37326		
		PHE380	5.02386		
		PRO230	5.14729		
		TYR118	4.97851	Other Hydrophobic	Pi-Sulfur Alkyl
		PHE233	5.27957		
		PHE233	4.55992		
Diallyl trisulfide	-3.9	PHE233	3.76025	Hydrophobic	Pi-Alkyl
		PHE233	5.11873		
		HIS377	4.85836		
		PHE380	4.84302		
		PHE380	4.30159		

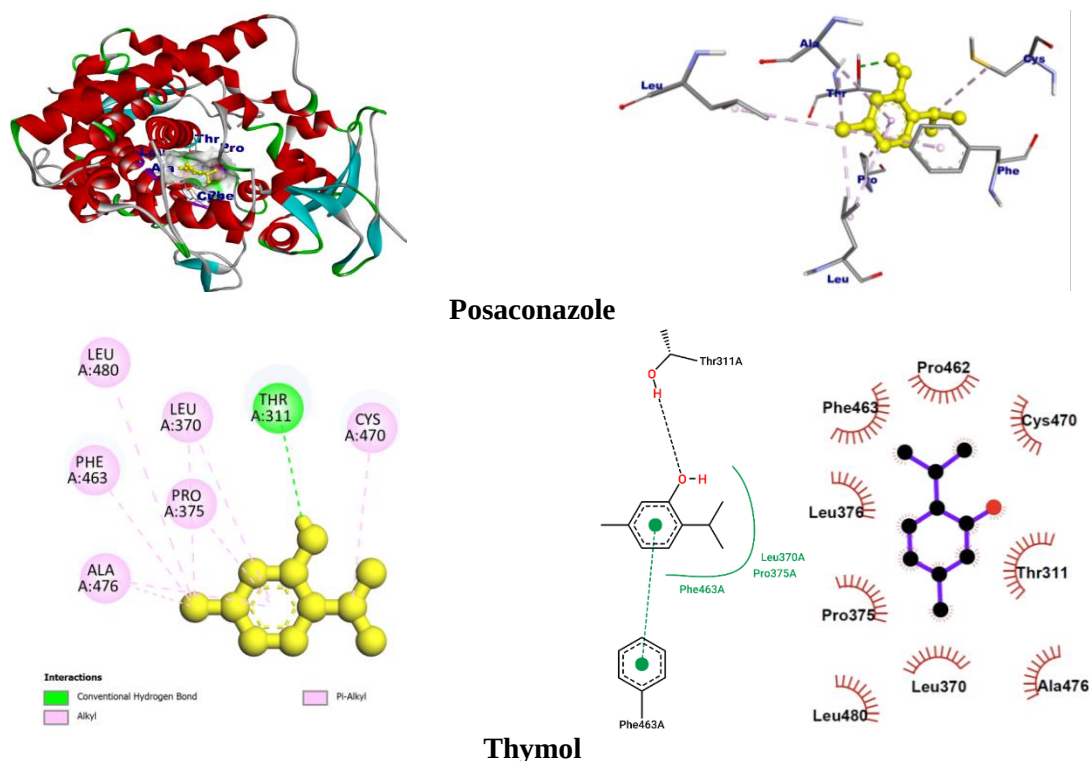


Figure 9. Molecular interactions between ligands and the active site of *C. albicans* sterol 14- α -demethylase (CYP51; PDB ID: 5FSA)

Furthermore, a detailed structural analysis elucidates the underlying rationale for the observed variations in docking scores between the natural ligands and the reference drug. While the superior binding affinity of levofloxacin is attributed to its extended molecular framework, which facilitates the formation of an extensive network of directional polar interactions, thymol exhibits a remarkably competitive affinity despite its significantly smaller molecular weight. The relatively small difference in predicted binding energy between levofloxacin ($-7.614 \text{ kcal.mol}^{-1}$) and thymol ($-6.919 \text{ kcal.mol}^{-1}$) suggests a comparable binding potential toward the target protein, although this result should be interpreted cautiously.

Overall, the docking results demonstrate that the studied terpenes can form predicted favorable binding poses with *S. aureus* DNA topoisomerase IV subunit B. Although their binding energies were slightly lower than that of levofloxacin, the interaction profiles support their potential as candidate compounds targeting of bacterial topoisomerases (Tse-Dinh 2009). Considering their natural origin and favorable lipophilicity, compounds such as thymol, limonene, and diallyl trisulfide may

represent promising scaffolds for the development of new antibacterial agents targeting topoisomerase IV.

In silico antifungal effect of terpenes and reference drugs. The docking results (Table 9) were interpreted based on binding energy values, interaction types, and residue involvement within the catalytic pocket. Lower binding energies indicate stronger binding affinity and potential inhibitory activity. The interaction profiles of terpenes were compared with those of fluconazole and posaconazole to evaluate their potential to act as competitive inhibitors of *C. albicans* sterol 14- α -demethylase (CYP51; PDB ID: 5FSA), a key enzyme in ergosterol biosynthesis (Torres et al. 2005).

The molecular docking analysis revealed a broad range of affinities. Posaconazole showed the strongest binding energy ($-11.7 \text{ kcal.mol}^{-1}$), consistent with its role as a potent azole antifungal, while fluconazole exhibited moderate affinity ($-6.9 \text{ kcal.mol}^{-1}$). The natural terpenes displayed slightly weaker binding energies, ranging from $-6.2 \text{ kcal.mol}^{-1}$ for thymol to $-3.9 \text{ kcal.mol}^{-1}$ for diallyl trisulfide. These results suggest that terpenes can interact with the CYP51 binding pocket through

moderate but favorable interactions. Such interactions may indicate a potential role in modulating the CYP51-mediated ergosterol biosynthetic pathway; however, this hypothesis requires experimental validation.

Posaconazole, the co-crystallized ligand, formed multiple hydrogen bonds with Gly472, Met508, Ser507, and Thr3111 (2.50–2.91 Å), ensuring stable anchoring near the heme catalytic center. Additional π - π stacking and hydrophobic interactions with Tyr118, Phe233, Phe380, Leu376, and Pro230 further stabilized the complex, explaining its strong binding affinity and effectiveness as a CYP51 inhibitor.

Among the tested terpenes, thymol exhibited the most favorable docking score ($-6.2 \text{ kcal.mol}^{-1}$). It formed a hydrogen bond with Thr3111 (2.60 Å), a residue also involved in posaconazole binding, suggesting partial occupation of the catalytic region (Fig. 9). Hydrophobic interactions further stabilized the complex, supporting the hypothesis that thymol may interfere with CYP51 activity. These findings agree with previous reports showing that thymol can inhibit ergosterol biosynthesis and disrupt membrane integrity in *C. albicans* (Kauser et al. 2024). Overall, the docking results provide a molecular explanation for the antifungal activity of essential oil constituents and highlight their potential as natural antifungal agents or adjuvants to azole drugs.

Conclusions

Essential oils from Algerian *Thymus vulgaris*, *Allium sativum*, and *Citrus limon* exhibited distinct chemical profiles and promising antioxidant and antimicrobial activities, which were associated with their major bioactive constituents, including thymol and carvacrol, organosulfur compounds, and limonene, respectively. Thyme EO showed the strongest antibacterial activity, whereas garlic EO exhibited notable antifungal activity, while lemon EO displayed comparatively moderate effects. The reduced susceptibility observed for *Pseudomonas aeruginosa* highlights the challenges associated with antimicrobial resistance and the variable response of microorganisms to essential oils. Overall, the findings support the potential of these EOs, particularly thyme and garlic oils, as candidates for further investigation in natural food preservation and other applications requiring plant-derived bioactive compounds. However, the present findings are

limited by the in vitro nature of the antimicrobial and antioxidant assays and the predictive nature of molecular docking. Further studies should therefore determine MIC/MBC or MFC values, evaluate EO efficacy, stability, safety, and sensory effects in real food matrices, and experimentally validate the molecular mechanisms suggested by docking analyses. Such investigations will be essential to establish the practical applicability of these Algerian essential oils and their major constituents.

Acknowledgements

We gratefully thank the Algerian Ministry of Higher Education and Scientific Research and the Algerian General Directorate of Scientific Research and Technological Development for the availability and the open doors of their research institutions. We wish to thank Dr Bachari Khaldoun from the National Center of Scientific Research in Physico-Chemical Analysis (CRAPC).

Author Contributions

Conceptualization and methodology, K.A. and F.C; formal analysis, K.A., M.D. and F.C.; resources, F.C and MN. B.; supervision, A.O.; project administration, K.A.; data analysis, M.D., F.C. and MN. B.; writing—original draft preparation, K.A., FC and S.B.; writing—review and editing, M.D. All authors have read and agreed to the published version of the manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

The study did not involve human or animal subjects.

Informed Consent Statement

Not applicable.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflict of interest.

References

- Babushok V.I., Linstrom P.J., Zenkevich I.G. Retention indices for frequently reported compounds of plant essential oils. *Journal of Physical and Chemical Reference Data*, 2011, 40(4): 043101. <https://doi.org/10.1063/1.3653552>
- Bajpai V.K., Sharma A., Kim S.H., Baek K.H. Chemical composition, antioxidant, lipid peroxidation inhibition and free radical scavenging activities of microwave-extracted essential oil from *Allium sativum*. *Journal of Essential Oil-Bearing Plants*, 2015, 18(2): 300-313. <https://doi.org/10.1080/0972060X.2013.764215>
- Balouiri M., Sadiki M., Ibensouda S.K. Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 2016, 6(2): 71-79. <https://doi.org/10.1016/j.jpha.2015.11.005>
- Beise D.C., Silva A.K.S., Guterres S.M., Zanatta C.B., Schneider K., Almeida A., Mazzuco V., Soldi C., Welter L.J., Stefenon V.M. Colorimetric, chemical, and genetic characterization of purple garlic in two producing regions in Brazil. *Pesquisa Agropecuária Brasileira*, 2024, 59(1): e03778. <https://doi.org/10.1590/S1678-3921.pab2024.v59.03778>
- Benabed K.H., Gourine N., Ouinten M., Bombarda I., Yousfi M. Chemical composition, antioxidant and antimicrobial activities of the essential oils of three Algerian Lamiaceae species. *Current Nutrition & Food Science*, 2017, 13(2): 97-109. <https://doi.org/10.2174/1573401313666170104105521>
- Benchallal F., Belkacemi H., Bellil Z., Chebrouk F., Touati A. *Thymus vulgaris* essential oil enclosed in β -cyclodextrin as drug delivery system: A promising alternative for antibiotic usage in combating *Salmonella*. *Proceedings of the Indian National Science Academy*, 2024, 90(4): 1095-1108. <https://doi.org/10.1007/s43538-024-00300-2>
- Chahboun N., Barrahi A., Koursaoui L., Mortada S., Fahsi K., Flouchi R., Karrouchi K., Faouzi M.E.A., El Faydy M., Abuelizz H.A., Thakur A., Fikri-Benbrahim K., Zarrouk A. Chemical composition, biological activities, and anti-corrosion performance of Moroccan essential oil *Thymus vulgaris* from the Oued Amlil region, Taza. *International Journal of Electrochemical Science*, 2024, 19(12): 100859. <https://doi.org/10.1016/j.ijeos.2024.100859>
- Dakpa G., Chiang Y.T., Lin L.Y., Tsao N.W., Wang C.H., Pérez-Sánchez H., Wang S.Y. Essential oil-derived compounds target core fatigue-related genes: A network pharmacology and molecular docking approach. *PLoS ONE*, 2025, 20(5): e0314125. <https://doi.org/10.1371/journal.pone.0314125>
- Dallakyan S., Olson A.J. Small-molecule library screening by docking with PyRx. In: *Chemical Biology* (J. Hempel, C. Williams, C. Hong Eds.). Methods in Molecular Biology, 1263. Humana Press, New York, 2015, pp. 243-250. https://doi.org/10.1007/978-1-4939-2269-7_19
- Diogo Gonçalves S., Koval I., Matos R.S., Caramelo A. Sage essential oil as a natural antigenotoxic agent: Comet assay findings and pharmacological relevance. *Applied Biosciences*, 2025, 4(4): 54. <https://doi.org/10.3390/applbiosci4040054>
- Dong H., You Y., Wang N., Wang M., Song T., He Y., Mei L. Development of amphipathic derivatives of thymol and carvacrol as potent broad-spectrum antibacterial agents. *European Journal of Medicinal Chemistry*, 2024, 276(10): 116716. <https://doi.org/10.1016/j.ejmech.2024.116716>
- Dorman H.D.M., Deans S.G. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *Journal of Applied Microbiology*, 2000, 88(2): 308-316. <https://doi.org/10.1046/j.1365-2672.2000.00969.x>
- El Aboubi M., Ben Hdech D., Bikri S., Benayad A., El Magri A., Aboussaleh Y., Aouane E.M. Chemical composition of essential oils of *Citrus limon* peel from three Moroccan regions and their antioxidant, anti-inflammatory, antidiabetic and dermatoprotective properties. *Journal of Herbmed Pharmacology*, 2023, 12(1): 118-127. <https://doi.org/10.34172/jhp.2023.11>
- El-Sayed H.S., Chizzola R., Ramadan A.A., Edris A.E. Chemical composition and antimicrobial activity of garlic essential oils evaluated in organic solvent, emulsifying, and self-microemulsifying water-based delivery systems. *Food Chemistry*, 2017, 221: 196-204. <https://doi.org/10.1016/j.foodchem.2016.10.052>
- Fährrolfes R., Bietz S., Flachsenberg F., Meyder A., Nittinger E., Otto T., Rarey M. ProteinsPlus: A web portal for structure analysis of macromolecules. *Nucleic Acids Research*, 2017, 45(W1): W337-W343. <https://doi.org/10.1093/nar/gkx333>
- Galovičová L., Borotová P., Valková V., Vukovic N.L., Vukic M., Štefániková J., Bobková M., Celá P., Kačániová M. *Thymus vulgaris* essential oil and its biological activity. *Plants*, 2021, 10(9): 1959. <https://doi.org/10.3390/plants10091959>
- Ganjitabar H., Hadidi R., Garcia G.A., Nahon L., Powis I. Analysis of the volatile monoterpene composition of citrus essential oils by photoelectron spectroscopy employing continuously monitored dynamic headspace sampling. *Analyst*, 2023, 148(24): 6228-6240. <https://doi.org/10.1039/D3AN01448G>
- Guedes I.A., da Silva M.M.P., Galheigo M., Krempser E., de Magalhães C.S., Barbosa H.J.C., Dardenne L.E. DockThor-VS: A free platform for receptor-ligand virtual screening. *Journal of Molecular*

- Biology, 2024, 436(17): 168548.
<https://doi.org/10.1016/j.jmb.2024.168548>
- Güven L., Behbudbayli U., Ertürk A., Hancı H., Yılmaz B., Kaya Y., Gülçin İ. Determination of antioxidant, antimicrobial, anticholinesterase, antityrosinase, antidiabetic and antiglaucoma activities of essential oils from three different *Thymus* species and their chemical characterization by GC-MS analysis. *Journal of Essential Oil-Bearing Plants*, 2023, 26(6): 1424-1446.
<https://doi.org/10.1080/0972060X.2023.2288276>
- Haindongo N.K.N., Anyogu A., Ekwebelem O.C., Anumudu C., Onyeaka H. Antibacterial and antibiofilm effects of garlic (*Allium sativum*), ginger (*Zingiber officinale*) and mint (*Mentha piperita*) on *Escherichia coli* biofilms. *Food Science and Applied Biotechnology*, 2021, 4(2): 166-176.
<https://doi.org/10.30721/fsab2021.v4.i2.146>
- Han Y., Chen W., Sun Z. Antimicrobial activity and mechanism of limonene against *Staphylococcus aureus*. *Journal of Food Safety*, 2021, 41(5): e12918. <https://doi.org/10.1111/jfs.12918>
- Haokip S.W., Sheikh K.A., Das S., Devi O.B., Singh Y.D., Wangchu L., Heisnam P. Unraveling physicochemical profiles and bioactivities of citrus peel essential oils: A comprehensive review. *European Food Research and Technology*, 2023, 249(11): 2821-2834. <https://doi.org/10.1007/s00217-023-04353-0>
- Hargrove T.Y., Friggeri L., Wawrzak Z., Qi A., Hoekstra W.J., Schotzinger R.J., Lepesheva G.I. Structural analyses of *Candida albicans* sterol 14 α -demethylase complexed with azole drugs address the molecular basis of azole-mediated inhibition of fungal sterol biosynthesis. *Journal of Biological Chemistry*, 2017, 292(16): 6728-6743.
<https://doi.org/10.1074/jbc.M117.778308>
- Herrera-Calderon O., Chacaltana-Ramos L.J., Huayanca-Gutiérrez I.C., Algarni M.A., Alqarni M., Batiha G.E.S. Chemical constituents, in vitro antioxidant activity and in silico study on NADPH oxidase of *Allium sativum* L. (garlic) essential oil. *Antioxidants*, 2021, 10(11): 1844.
<https://doi.org/10.3390/antiox10111844>
- Hooper D.C., Jacoby G.A. Topoisomerase inhibitors: Fluoroquinolone mechanisms of action and resistance. *Cold Spring Harbor Perspectives in Medicine*, 2016, 6(9): a025320.
<https://doi.org/10.1101/cshperspect.a025320>
- Huang L., Liu Z., Wang J., Fu J., Jia Y., Ji L., Wang T. Bioactivity and health effects of garlic essential oil: A review. *Food Science & Nutrition*, 2023, 11(6): 2450-2470. <https://doi.org/10.1002/fsn3.3253>
- Hudaib M., Speroni E., Di Pietra A.M., Cavrini V. GC-MS evaluation of thyme (*Thymus vulgaris* L.) oil composition and variations during the vegetative cycle. *Journal of Pharmaceutical and Biomedical Analysis*, 2002, 29(4): 691-700.
[https://doi.org/10.1016/S0731-7085\(02\)00119-X](https://doi.org/10.1016/S0731-7085(02)00119-X)
- Karagecili H., Yılmaz M.A., Ertürk A., Kiziltas H., Güven L., Alwaseel S.H., Gülçin İ. Comprehensive metabolite profiling of Berdav propolis using LC-MS/MS: Determination of antioxidant, anticholinergic, antiglaucoma, and antidiabetic effects. *Molecules*, 2023, 28(4): 1739.
<https://doi.org/10.3390/molecules28041739>
- Kačániová M., Čmiková N., Vukovic N.L., Verešová A., Bianchi A., Garzoli S., Ben Saad R., Ben Hsouna A., Ban Z., Vukic M.D. Citrus limon essential oil: Chemical composition and selected biological properties focusing on the antimicrobial (in vitro, in situ), antibiofilm, insecticidal activity and preservative effect against *Salmonella enterica* inoculated in carrot. *Plants*, 2024, 13(4): 524.
<https://doi.org/10.3390/plants13040524>
- Kausar S., Raj N., Ahmedi S., Manzoor N. Mechanistic insight into the membrane-disrupting properties of thymol in *Candida* species. *Microbe*, 2024, 2(3): 100045.
<https://doi.org/10.1016/j.microb.2024.100045>
- Lanciu Dorofte A., Dima C., Ceoromila A., Botezatu A., Dinica R., Bleoanca I., Borda D. Controlled release of β -CD-encapsulated thyme essential oil from whey protein edible packaging. *Coatings*, 2023, 13(3): 508.
<https://doi.org/10.3390/coatings13030508>
- Laskowski R.A., Swindells M.B. LigPlot+: Multiple ligand-protein interaction diagrams for drug discovery. *Journal of Chemical Information and Modeling*, 2011, 51(10): 2778-2786.
<https://doi.org/10.1021/ci200227u>
- Lazarova-Zdravkova N., Tsanova D., Stoyanova Y., Chilev C., Georgieva N., Peshev D. Study of the biological activity of essential oil-water mixtures. *Food Science and Applied Biotechnology*, 2021, 4(1): 48-56.
<https://doi.org/10.30721/fsab2021.v4.i1.130>
- Liu S., Li S., Ho C.T. Dietary bioactives and essential oils of lemon and lime fruits. *Food Science and Human Wellness*, 2022, 11(4): 753-764.
<https://doi.org/10.1016/j.fshw.2022.03.001>
- Lu J., Patel S., Sharma N., Soisson S.M., Kishii R., Takei M., Singh S.B. Structures of kidelomycin bound to *Staphylococcus aureus* GyrB and ParE show a novel U-shaped binding mode. *ACS Chemical Biology*, 2014, 9(9): 2023-2031.
<https://doi.org/10.1021/cb5001197>
- Machado T.F., Pereira R.D.C.A., Batista V.C.V. Seasonal variability of the antimicrobial activity of the essential oil of *Lippia alba*. *Revista Ciência Agronômica*, 2014, 45(3): 515-519.
<https://doi.org/10.1590/S1806-66902014000300011>

- Mansouri N., Aoun L., Dalichaouche N., Hadri D. Yields, chemical composition and antimicrobial activity of two Algerian essential oils against forty avian multidrug-resistant *Escherichia coli* strains. *Veterinary World*, 2018, 11(11): 1539-1550. <https://doi.org/10.14202/vetworld.2018.1539-1550>
- Melkina O.E., Plyuta V.A., Khmel I.A., Zavilgelsky G.B. The mode of action of cyclic monoterpenes (-)-limonene and (+)-alpha-pinene on bacterial cells. *Biomolecules*, 2021, 11(6): 806. <https://doi.org/10.3390/biom11060806>
- Modica G., Strano T., Napoli E., Seminara S., Aguilar-Hernández M., Legua P., Continella A. Qualitative traits and peel essential oil profiles of 24 Italian and international lemon varieties. *Food Bioscience*, 2024, 59(6): 103881. <https://doi.org/10.1016/j.fbio.2024.103881>
- Nazzaro F., Polito F., Amato G., Caputo L., Francolino R., D'Acerno A., Fratianni F., Candido V., Coppola R., De Feo V. Chemical composition of essential oils of bulbs and aerial parts of two cultivars of *Allium sativum* and their antibiofilm activity against food and nosocomial pathogens. *Antibiotics*, 2022, 11(6): 724. <https://doi.org/10.3390/antibiotics11060724>
- Negahban M., Saeedfar S. Essential oil composition of *Thymus vulgaris* L. *Russian Journal of Biological Research*, 2015, 3(1): 35-38. <https://doi.org/10.13187/ejbr.2015.3.35>
- Owolabi M.S., Avoseh O.N., Ogunwande I.A., Setzer W.N., Ogungbo R., Ogundajo A.L., Lawal O.A., Flamini G. Chemical composition of *Citrus limon* (L.) Osbeck growing in southwestern Nigeria: Essential oil chemotypes of both peel and leaf of lemon. *American Journal of Essential Oils and Natural Products*, 2018, 6(4): 36-40. Available at: <https://www.essencejournal.com/archives/2018/vol6 issue4/PartA/6-3-19-398.pdf>
- Pandey A.K., Kumar P., Singh P., Tripathi N.N., Bajpai V.K. Essential oils: Sources of antimicrobials and food preservatives. *Frontiers in Microbiology*, 2017, 7(1): 2161. <https://doi.org/10.3389/fmicb.2016.02161>
- Paw M., Begum T., Gogoi R., Pandey S.K., Lal M. Chemical composition of *Citrus limon* L. Burmf peel essential oil from North East India. *Journal of Essential Oil-Bearing Plants*, 2020, 23(2): 337-344. <https://doi.org/10.1080/0972060X.2020.1757514>
- Petretto G.L., Vacca G., Addis R., Pintore G., Nieddu M., Piras F., Rosa A. Waste *Citrus limon* leaves as a source of essential oil rich in limonene and citral: Chemical characterization, antimicrobial and antioxidant properties, and effects on cancer cell viability. *Antioxidants*, 2023, 12(6): 1238. <https://doi.org/10.3390/antiox12061238>
- Pranav R.J., Kumar S.S.K., Rangaraj P.R., Sangapillai K., Ragunathan R. Exploring the therapeutic potential of cinnamon and clove essential oils for mitigating foodborne diseases: Bioactivity and molecular interactions. *Food Science and Applied Biotechnology*, 2024, 7(1): 122-132. <https://doi.org/10.30721/fsab2024.v7.i1.318>
- Sadgrove N.J., Padilla-González G.F., Phumthum M. Fundamental chemistry of essential oils and volatile organic compounds, methods of analysis and authentication. *Plants*, 2022, 11(6): 789. <https://doi.org/10.3390/plants11060789>
- Satyap P., Murray B.L., McFeeters R.L., Setzer W.N. Essential oil characterization of *Thymus vulgaris* from various geographical locations. *Foods*, 2016, 5(4): 70. <https://doi.org/10.3390/foods5040070>
- Spencer A.C., Panda S.S. DNA gyrase as a target for quinolones. *Biomedicines*, 2023, 11(2): 371. <https://doi.org/10.3390/biomedicines11020371>
- Torres H.A., Hachem R.Y., Chemaly R.F., Kontoyiannis D.P., Raad I.I. Posaconazole: A broad-spectrum triazole antifungal. *The Lancet Infectious Diseases*, 2005, 5(12): 775-785. [https://doi.org/10.1016/S1473-3099\(05\)70297-8](https://doi.org/10.1016/S1473-3099(05)70297-8)
- Tse-Dinh Y.C. Bacterial topoisomerase I as a target for discovery of antibacterial compounds. *Nucleic Acids Research*, 2009, 37(3): 731-737. <https://doi.org/10.1093/nar/gkn936>
- Yildiz S., Turan S., Kiralan M., Ramadan M.F. Antioxidant properties of thymol, carvacrol, and thymoquinone and their efficiencies on the stabilization of refined and stripped corn oils. *Journal of Food Measurement and Characterization*, 2021, 15(1): 621-632. <https://doi.org/10.1007/s11694-020-00665-0>