

Comparative chemical characterization and antibacterial evaluation of three Moroccan *Lamiaceae* essential oils against phytopathogenic bacteria

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ABSTRACT

Essential oils (EOs) obtained by hydrodistillation from *Origanum elongatum*, *Thymus vulgaris*, and *Lavandula stoechas* collected in Morocco's Fez–Meknes region were chemically characterized and tested against six phytopathogenic bacteria: *Erwinia amylovora*, *Pantoea ananatis*, *Pseudomonas viridiflava*, *Pseudomonas savastanoi*, *Allorhizobium vitis*, and *Pseudomonas marginalis*. Their constituents were identified by gas chromatography coupled with mass spectrometry (GC–MS). At the same time, the antibacterial activity was evaluated using disc diffusion, microatmosphere, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays. Thymol and carvacrol predominated in *O. elongatum* EO. The principal constituents of *L. stoechas* EO were linalool (25.76%) and camphor (21.09%), whereas thymol accounted for 47.79% of *T. vulgaris* EO. Disc diffusion and microatmosphere assays demonstrated the antibacterial effects of *O. elongatum* and *T. vulgaris* EOs. The lowest MICs were obtained for *O. elongatum* EO (0.15–0.30 mg/mL), followed by *T. vulgaris* (0.30–1.25 mg/mL), and *L. stoechas* (10–20 mg/mL). Molecular docking indicated favorable interactions between the identified constituents and bacterial target proteins, suggesting a molecular basis for the observed antibacterial effects. Overall, the present study suggests that *O. elongatum* and *T. vulgaris* EOs are promising plant-based candidates for further investigation in the management of bacterial plant diseases. However, their *in planta* efficacy, phytotoxicity, stability, and environmental safety require further evaluation before practical application.

Keywords: essential oils, phytopathogenic bacteria, antibacterial activity, GC-MS, molecular docking.

INTRODUCTION

Plant bacterial diseases pose a significant threat to global agricultural production, causing substantial losses in crop yield and quality

(Mulungu, 2024; Pantović-Golijan et al., 2025). Among the most economically important phytopathogenic bacteria are *Erwinia amylovora*, *Pantoea ananatis*, *Allorhizobium vitis*, and *Pseudomonas* species, including *Pseudomonas*

savastanoi, *Pseudomonas marginalis*, and *Pseudomonas viridiflava*, which infect a wide range of horticultural and woody crops (Mendes et al., 2024; Pantović-Golijan et al., 2025).

Management of bacterial plant diseases still relies primarily on copper-based bactericides and, in some countries, antibiotics (Mendes et al., 2024; Sharma et al., 2022). However, their extensive use has contributed to the emergence of resistant bacterial populations while raising concerns about environmental contamination and copper accumulation in agricultural soils (Koščak et al., 2024; Sharma et al., 2022). Beyond their environmental implications, pesticide applications warrant consideration of both worker exposure and possible effects on crop quality. When recommended safety measures were followed, inhalation and dermal risks during herbicide application remained within permissible limits (Novokhatska et al., 2024). Likewise, the protection schemes evaluated in sunflower did not significantly alter the fatty acid composition or the nutritional and biological value of the seeds (Hulai et al., 2022). Consequently, there is an increasing demand for sustainable and environmentally friendly alternatives for bacterial disease control (DuPont et al., 2024; Gupta et al., 2023).

As promising natural alternatives for the control of bacterial plant diseases, EOs from aromatic and medicinal plants have been reported as potential sources of active ingredients to manage plant pathogens by acting on membrane stability, enzymatic activities, and vital bacterial cellular processes (Gupta et al., 2023; Kesraoui et al., 2022). Their complex compositions, broad-spectrum antimicrobial action, and low environmental toxicity contribute to the increasing interest of using EOs as effective natural crop protection agents (Gupta et al., 2023; Pantović-Golijan et al., 2025).

Morocco possesses a rich diversity of aromatic and medicinal plants, many of which produce bioactive essential oils (EOs) with promising antimicrobial properties (Zrira, 2017). Recent studies have reported the antibacterial activity of *Thymus* and *Origanum* EOs against several phytopathogenic bacteria (Kosakowska et al., 2024; Yildiz et al., 2025). In addition, other EOs have also demonstrated promising activity against important plant pathogens (Dias et al., 2025). However, comparative studies evaluating Moroccan EOs from *Thymus vulgaris*, *Origanum elongatum*, and

Lavandula stoechas against multiple phytopathogenic bacteria remain limited.

Therefore, the present study intended to determine, by gas chromatography coupled with mass spectrometry (GC-MS), the chemical profile of Moroccan EOs from *T. vulgaris*, *O. elongatum*, and *L. stoechas*. It also assessed their antibacterial activity against six phytopathogenic bacteria through complementary *in vitro* assays and molecular docking. The scientific novelty of the present study therefore lies in comparing their chemical profiles and *in vitro* antibacterial performance under a common experimental framework, supplemented by exploratory molecular docking of selected constituents with bacterial protein targets.

MATERIALS AND METHODS

Sampling and preparation of botanical material

For the present study, plant specimens were collected from specific locations within the Fez-Meknes region of Morocco from February to July of 2023 (Table 1). For each species, all harvested plant material was pooled into a single batch ($n = 1$). The collected species were identified and confirmed by a botanist at INRA Meknes. The samples were harvested at full flowering, dried naturally in the shade, and then carefully stored in appropriate bags until hydrodistillation.

EOs extraction

The extraction of EOs was conducted by hydrodistillation method using a Clevenger-type apparatus. In summary, 200 g of dried plant material was immersed in 2 L of distilled water and subjected to hydrodistillation for a period of 3 h. For each plant species, one independent EO extraction was performed using a single batch of plant material ($n = 1$), and the extraction yield was determined once. Additional extraction runs, when needed, were performed using material from the same batch solely to obtain sufficient EO and were not treated or analyzed as independent extraction replicates. The oils obtained were collected and stored in amber glass bottles at a temperature of 4 °C until analysis.

Extraction yields (%) were calculated using Equation 1:

$$Y(\%) = \frac{V_{EO}}{m_{dry}} \times 100 \quad (1)$$

where: V_{EO} – volume of EO (mL); m_{dry} – mass of dried plant material (g).

Gas chromatography–mass spectrometry analysis of essential oils

EO composition was determined using a Hewlett-Packard HP6890 gas chromatograph (Agilent Technologies) coupled to an HP 5973 mass-selective detector and fitted with a DB-5MS capillary column (30 m × 0.25 mm i.d.; 0.25 µm film thickness). The oven was initially maintained at 50 °C and then heated to 300 °C at 7 °C/min, while the injector was held at 290 °C. High-purity helium served as the carrier gas at 1 mL/min with a 60:1 split ratio. Mass spectra were acquired in electron-impact mode at 70 eV over an m/z range of 35–400. Before injection, 10 µL of each EO was diluted in 990 µL of hexane, and a 1 µL aliquot was analyzed. Each EO sample was analyzed once by GC–MS ($n = 1$). HP ChemStation software was used for data acquisition and processing. Kovats retention indices (RI) were established by analyzing a homologous series of n-alkanes (C8–C26; Sigma-Aldrich) under uniform chromatographic conditions. Compound identification relied on retention index values and mass spectral comparisons with published data (Adams, 2007) and automated library searches (NIST 98, Wiley 275, CNRS).

Bacterial strains

Six phytopathogenic bacterial strains were utilized in this study (Table 2). The strains have been procured from the collection at the Phyto-bacteriology and Biological Control Laboratory of INRA Meknes. A bacterial suspension intended for inoculation was prepared by harvesting two to three colonies from a freshly grown culture on YPGA medium (yeast extract, 5 g/L; peptone, 5 g/L; glucose, 10 g/L; agar, 15 g/L) and resuspending them in sterile saline solution (0.9% NaCl). Subsequently, the suspension was adjusted to a concentration of approximately 10^8 CFU/mL for disc diffusion and microatmosphere assays, and to 10^6 CFU/mL for the MIC assay.

Antibacterial activity

Disc diffusion assay

The antibacterial activity of the EOs was evaluated following the methodology previously outlined, with minor modifications (Aktepe et al., 2019). Briefly, a sterile 5 mm paper disc was imbibed with 2 µL of pure EO and positioned on YPGA medium previously inoculated with 100 µL of a bacterial suspension with a concentration of 10^8 CFU/mL. Sterile distilled water (SDW) and a paper disc impregnated with streptomycin (32 mg/L) were used as negative and positive controls, respectively. The diameter of the inhibition zone was recorded after incubation at 28 °C for 24 h. A positive control (streptomycin) was included in each assay ($n = 3$). Differences

Table 1. Geographic distribution and botanical description of plants studied

Common name	Scientific name	Order	Family	Sampling site	Plant part studied
Thyme	<i>Thymus vulgaris</i>	Lamiales	Lamiaceae	Sefrou	Aerial parts
Lavender	<i>Lavandula stoechas</i>	Lamiales	Lamiaceae	Bouiblane	Aerial parts
Oregano	<i>Origanum elongatum</i>	Lamiales	Lamiaceae	Bouiblane	Aerial parts

Table 2. Strains used in this study

Species	Origin	Reference
<i>Allorhizobium vitis</i>	Black raspberry in Hungary	(Habbadi et al., 2018)
<i>Pseudomonas savastanoi</i> pv. <i>savastanoi</i> (Pss)	Olive tree, Morocco	(Bouaichi et al., 2019)
<i>Pseudomonas marginalis</i>	Onion, Morocco	(Achbani et al., 2014)
<i>Erwinia amylovora</i>	Apple tree, Morocco	(Ameur et al., 2014)
<i>Pantoea ananatis</i>	Onion, Morocco	(Achbani et al., 2016)
<i>Pseudomonas viridiflava</i>	Onion, Morocco	(Sadik et al., 2015)

between each plant extract and the control, within each bacterial strain, were assessed by one-way ANOVA followed by Tukey's HSD test ($p < 0.05$).

Microatmosphere assay

The microatmospheric method was performed as previously described, with modifications (Battat et al., 2025). Bacterial strains (10^8 CFU/mL) were seeded on YPGA plates, and Whatman paper discs (5 mm) saturated with 8 μ L of EOs were attached to the lids of the Petri dishes. Following incubation at 28 °C for 24 hours, the inhibition zone diameters were measured. Each EO-strain combination was tested in triplicate using the same bacterial inoculum ($n = 3$ technical replicates).

Determination of MICs and MBCs

The MICs of EOs were determined by the dilution method (Clinical and Laboratory Standards Institute, 2018). All tests used YPG broth with 0.15% (w/v) bacteriological agar. Serial twofold dilutions of EOs from 20 to 0.038 mg/mL in 10% dimethyl sulfoxide (DMSO) were prepared in tubes, inoculated with bacteria at 10^6 CFU/mL, and brought to a final volume of 4 mL. The tubes were incubated at 28 °C for 24 h. Afterward, 50 μ L of 0.5 mg/mL resazurin was added (Sarker et al., 2007). The MIC was the lowest oil concentration at which resazurin remained blue after 2 h. Each EO concentration was tested in triplicate using the same bacterial inoculum ($n = 3$ technical replicates). Experiments were repeated three times. 50 μ L from each tube was plated on YPGA medium at concentrations $\geq$ MIC for MBC determination and incubated at 28 °C for 48 h. The three replicate tubes were subcultured separately for MBC determination ($n = 3$ technical replicates). The MBC was the lowest concentration with no growth after subculturing. The MBC/MIC ratio was calculated. An MBC/MIC ratio of ≤ 4 indicates bactericidal activity and >4 indicates bacteriostatic.

Molecular docking study

Molecular docking simulations were carried out to investigate the potential antibacterial activity of thymol, the major bioactive compound identified by GC-MS analysis of the EOs extracted from *T. vulgaris* and *O. elongatum*. The study aimed to evaluate the interaction of thymol with key bacterial target proteins from six pathogenic strains, thereby elucidating possible mechanisms of antibacterial action through structure-based

computational modeling (Er-rahmani et al., 2024; Jeddi et al., 2023). For this purpose, six target proteins were selected based on their biological importance and structural availability in the RCSB Protein Data Bank (PDB) (Ed-Dahmani et al., 2024). These included a lyase protein from *Pantoea ananatis* (PDB ID: 3T6C; resolution: 1.60 Å), a hydrolase protein from *P. viridiflava* (PDB ID: 6UAW; resolution: 1.50 Å), a transport protein from *A. vitis* (PDB ID: 8CI6; resolution: 1.20 Å), a hydrolase protein from *E. amylovora* (PDB ID: 4D74; resolution: 1.57 Å), an oxidoreductase protein from *P. savastanoi* (PDB ID: 4IV9; resolution: 1.95 Å), and an isomerase and lyase protein from *P. marginalis* (PDB ID: 1U1W; resolution: 1.35 Å). All protein structures were retrieved from the PDB and prepared using AutoDockTools 4.2 software (Ed-Dahmani et al., 2025). Preparation steps included the removal of co-crystallized ligands, water molecules, and non-essential heteroatoms. Polar hydrogens were added, and Gasteiger partial charges were assigned (El Fadili et al., 2025). When necessary, energy minimization was applied to relieve steric hindrance and optimize structural conformation.

The active sites for each target were defined based on either the positions of co-crystallized native ligands or known conserved catalytic residues (El fadili et al., 2022; El fadili, Er-rajy, Abdalla, et al., 2023). Docking grid boxes were generated using a grid-point spacing of 0.375 Å and centered on the active-site region of each target protein. Molecular docking simulations were performed using AutoDock 4.2 with AutoDockTools (El fadili, Er-rajy, Imtara, et al., 2023; Nouioura et al., 2024). For each protein-ligand complex, the most favorable thymol binding pose was selected based on its predicted binding free energy (ΔG) and interaction geometry. Subsequently, protein-ligand interactions were analyzed using Discovery Studio Visualizer 2021 (Bouzammit et al., 2024), with particular attention to hydrogen bonds, hydrophobic interactions, and contacts with key active-site residues. These analyses were performed to characterize the nature and predicted strength of thymol binding to each bacterial protein target (Benkhaira et al., 2023).

Statistical analysis

All statistical analyses were performed using Minitab Statistical Software (version 21). Differences among groups were evaluated using a one-way analysis of variance (ANOVA), followed by

Tukey's multiple-comparison test for post hoc pairwise comparisons. A p -value of less than 0.05 was considered statistically significant.

RESULTS

Essential oil yield

The results of yield calculations from hydrodistillation extractions are shown in Figure 1. *T. vulgaris* showed the highest yield (2.069%), followed by *O. elongatum* (1.12%), and *L. stoechas* (1%). Considering these differences, the chemical composition was analyzed using the GC–MS method.

Chemical profile of EOs

The EOs obtained from *L. stoechas*, *T. vulgaris*, and *O. elongatum* were analyzed by GC–MS. Their major constituents and chemical-class distribution are summarized in Table 3, while the complete chemical profiles are provided in Supplementary Table S1.

L. stoechas EO primarily consisted of oxygenated monoterpenes, with linalool, camphor, borneol, and eucalyptol as major compounds. However, *T. vulgaris* oil was mostly composed of phenolic compounds and monoterpene hydrocarbons. The primary components were thymol, p-cymene, γ -terpinene, and carvacrol. *O. elongatum* oil showed a significant concentration of phenolic compounds and monoterpene hydrocarbons, with thymol, carvacrol, γ -terpinene, and p-cymene as the dominant constituents.

Antibacterial potential

Disc diffusion assay

The data in Table 4 show the zones of inhibition (mm) caused by various EOs against six phytopathogenic bacteria. One-way ANOVA revealed a significant treatment effect for all tested bacterial strains ($p < 0.05$), followed by Tukey's HSD post hoc test for pairwise comparisons. Clearly, the antibacterial activity varied significantly depending on the oil's origin. *T. vulgaris* EO exhibited notable activity, particularly against *A. vitis* (79 mm), *E. amylovora* (50.8 mm), and *Pss* (44.7 mm). *O. elongatum* EO demonstrated notable antimicrobial activity, with the greatest inhibition observed against *Pss* (61.6 mm), followed by *A. vitis* (56.4 mm) and *E. amylovora* (43.7 mm). By comparison, *L. stoechas* exhibited more modest activity, producing zones of inhibition measuring less than 22 mm. *O. elongatum* and *T. vulgaris* EOs produced significantly larger inhibition zones than the positive control for most tested bacteria. More specifically, *O. elongatum* EO differed significantly from the control for all six bacterial strains, whereas *T. vulgaris* EO differed significantly from the control for five strains, with no significant difference observed for *P. viridiflava*. By contrast, *L. stoechas* EOs did not differ significantly from the control against *E. amylovora*, *P. ananatis*, *P. viridiflava*, and *P. savastanoi*, but showed significantly greater inhibition against *A. vitis* and *P. marginalis* (Tukey's HSD, $p < 0.05$).

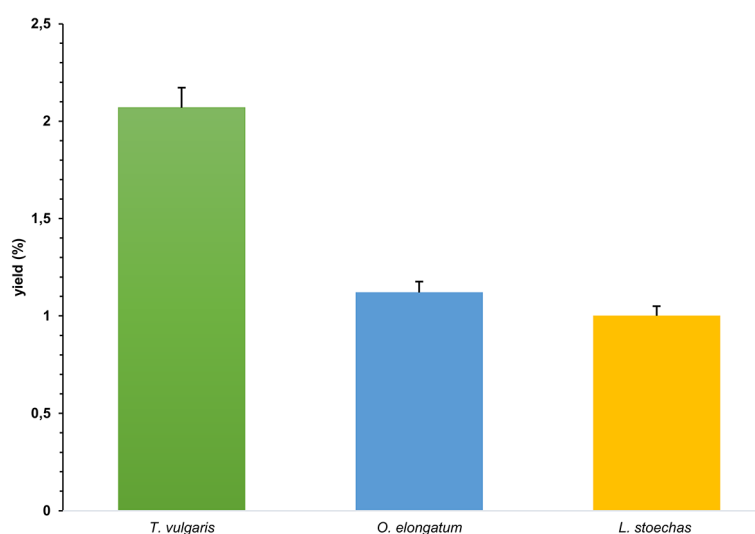


Figure 1. Yields of EOs obtained by hydrodistillation

Table 3. Major GC–MS constituents and chemical-class distribution of EOs from *L. stoechas*, *T. vulgaris*, and *O. elongatum*

No.	Major compound	<i>L. stoechas</i> (%)	<i>T. vulgaris</i> (%)	<i>O. elongatum</i> (%)
1	1,8-Cineole (Eucalyptol)	12.05	0.12	-
2	γ-Terpinene	0.17	8.19	9.11
3	Linalool	25.76	3.06	2.12
4	Camphor	21.09	0.05	-
5	Borneol	10.14	0.45	-
6	Carvacrol	1.06	5.03	21.71
7	p-Cymene	-	24.13	13.60
8	Thymol	-	47.79	29.54
Total monoterpene hydrocarbons		1.53	37.54	41.56
Total oxygenated monoterpenes		91.31	4.51	2.54
Total sesquiterpene hydrocarbons		1.66	52.82	51.25
Total oxygenated sesquiterpenes		2.04	2.15	-
Total phenols		1.31	0.80	3.22
Other compounds		2.03	0.44	-
Total identified (%)		99.88	98.26	98.57

Note: Major constituents were defined as $\geq 5\%$. Chemical classes were calculated from Table S1.

Table 4. Inhibition zone diameters (mm) of the positive control and the tested EOs against six phytopathogenic bacteria

Bacterial strain	Control	<i>L. stoechas</i> EO	<i>T. vulgaris</i> EO	<i>O. elongatum</i> EO
<i>E. amylovora</i>	6.00 $\pm$ 1.62 ^B	8.00 $\pm$ 2.47 ^B	50.80 $\pm$ 6.01 ^A	43.70 $\pm$ 2.91 ^A
<i>P. ananatis</i>	6.50 $\pm$ 0.92 ^B	8.00 $\pm$ 1.08 ^B	25.50 $\pm$ 6.26 ^A	29.30 $\pm$ 3.48 ^A
<i>P. viridiflava</i>	6.00 $\pm$ 0.75 ^B	7.20 $\pm$ 2.19 ^B	6.00 $\pm$ 0.96 ^B	29.30 $\pm$ 1.78 ^A
<i>Pss</i>	8.00 $\pm$ 0.91 ^c	12.30 $\pm$ 2.52 ^c	44.70 $\pm$ 7.23	61.60 $\pm$ 0.80 ^A
<i>A. vitis</i>	5.00 $\pm$ 0.68 ^D	21.20 $\pm$ 4.43 ^c	79.00 $\pm$ 0.15 ^A	56.40 $\pm$ 4.31 ^B
<i>P. marginalis</i>	6.00 $\pm$ 1.44 ^c	12.10 $\pm$ 0.91 ^B	18.30 $\pm$ 3.41 ^A	16.30 $\pm$ 1.97 ^{AB}

Note: Values are expressed as mean $\pm$ standard deviation (SD) from three independent replicates ($n = 3$). For each bacterial strain, treatments were compared using one-way ANOVA followed by Tukey's HSD post hoc test. Means sharing no superscript letter (A–D) differ significantly, whereas means sharing at least one letter do not ($p < 0.05$).

Microatmosphere assay

The microatmosphere test indicates variable antibacterial activity among the evaluated EOs. The EOs of *O. elongatum* and *T. vulgaris* exhibited notable zones of inhibition against *E. amylovora* (42.4 mm and 40 mm, respectively) and *P. ananatis* (31.1 mm and 30 mm, respectively) (Figure 2). These two EOs also showed moderate to weak activity when tested against other strains. On the other hand, *L. stoechas* demonstrated complete ineffectiveness in this assay, yielding no zone of inhibition for any bacterial strain. Moreover, it is noteworthy that streptomycin, employed as a reference antibiotic, exhibited no effect in this test.

Determination of MICs and MBCs

Analysis of the results revealed clear variations in the antibacterial activity of the EOs evaluated, depending on the EO and the target bacterial strain (Table 5). The EO of *O. elongatum* exhibited superior efficacy, with MIC and MBC values ranging from 0.15 to 0.3 mg/mL. The MBC/MIC ratio for each strain demonstrates a bactericidal effect. The EO of *T. vulgaris* exhibited significant activity, with bactericidal effects against *E. amylovora*, *P. ananatis*, and *A. vitis* ($\text{MBC/MIC} \leq 4$), while bacteriostatic effects were observed against the remaining strains. *L. stoechas* EO exhibited moderate antibacterial activity, with efficacy varying significantly

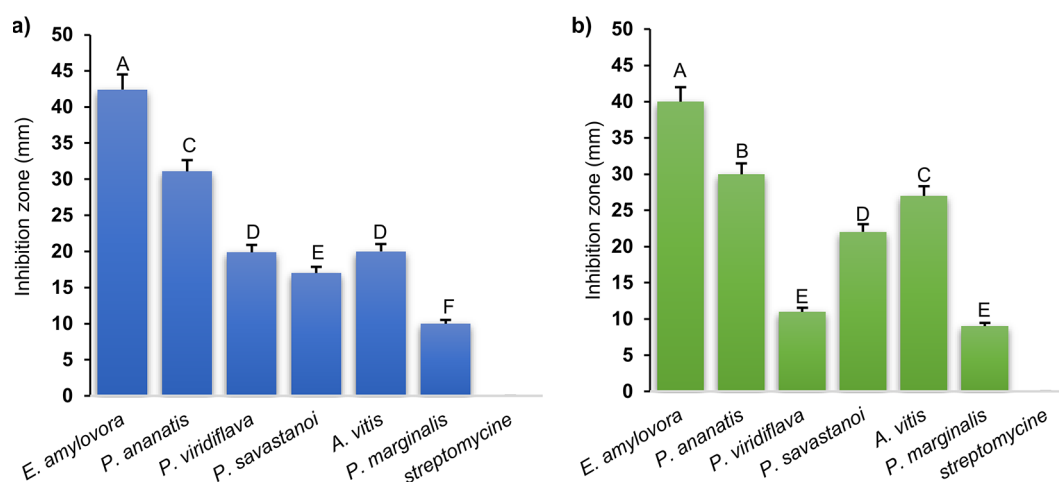


Figure 2. Inhibition zones induced by EOs in a microatmosphere assay against six phytopathogenic bacteria: (a) *O. elongatum* EO; (b) *T. vulgaris* EO. Values are expressed as mean $\pm$ standard deviation (SD) of three independent replicates ($n = 3$). Within each EO, inhibition zones among the six bacterial strains were compared using one-way ANOVA followed by Tukey's HSD post hoc test. Means followed by different letters (A–F) are significantly different ($p < 0.05$), whereas means sharing the same letter are not significantly different ($p \geq 0.05$).

based on the bacterial strain. It demonstrated bactericidal activity against *E. amylovora*, *P. ananatis*, and *A. vitis* ($\text{MBC/MIC} \leq 4$). For the other strains, it predominantly exhibited bacteriostatic effects ($\text{MBC/MIC} > 4$).

Molecular docking analysis

Molecular docking simulations were conducted to explore the binding affinity and interaction profile of thymol towards six selected protein targets involved in key physiological pathways of various pathogenic bacteria. These proteins were chosen to represent potential antibacterial targets from *P. ananatis*, *P. viridiflava*, *A. vitis*, *E. amylovora*, *P. savastanoi*, and *P. marginalis*. The

docking results, summarized in Table 6, revealed moderate to strong binding affinities, accompanied by specific molecular interactions that may explain the antibacterial potential of thymol towards the responsible pathogenic strains. Further analysis, as illustrated in Figure 3, provides two- and three-dimensional representations of the various types of intermolecular interactions formed during the ligand-protein complexation process.

First, thymol was docked to the lyase protein from *P. ananatis* (PDB ID: 3T6C), forming one conventional hydrogen bond with Glu275, one Pi-Pi T-shaped interaction with Phe41, two Pi-donor hydrogen bonds with Ser301 and His298, and more than three alkyl interactions involving Pro85, Val278, and Leu276 amino acid residues.

Table 5. EOs antibacterial activity against phytopathogenic bacteria strains tested (mg/mL)

Bacteria	<i>L. stoechas</i>				<i>T. vulgaris</i>				<i>O. elongatum</i>			
	MIC	MBC	MBC/MIC	Effect	MIC	MBC	MBC/MIC	Effect	MIC	MBC	MBC/MIC	Effect
<i>E. amylovora</i>	20	80	4	+	1.25	5	4	+	0.3	0.3	1	+
<i>P. ananatis</i>	20	80	4	+	1.25	5	4	+	0.15	0.3	2	+
<i>P. viridiflava</i>	20	100	5	-	0.6	2.5	4.16	-	0.3	0.3	1	+
<i>Pss</i>	20	100	5	-	0.6	2.5	4.16	-	0.15	0.6	4	+
<i>A. vitis</i>	10	40	4	+	0.3	0.3	1	+	0.15	0.6	4	+
<i>P. marginalis</i>	10	50	5	-	0.6	2.5	4.16	-	0.3	0.3	1	+

Note: MIC and MBC were measured in triplicate ($n = 3$). All three measurements were identical under each experimental condition ($\text{SD} = 0$). Since no variation was observed, ANOVA was not performed, and F-statistics, df, p-values, Tukey's HSD tests, and 95% confidence intervals were not reported. The symbol “+” indicates a bactericidal effect ($\text{MBC/MIC} \leq 4$), whereas “-” denotes a bacteriostatic effect ($\text{MBC/MIC} > 4$).

Table 6. Molecular docking results of thymol with selected antibacterial proteins, including protein classification, key intermolecular interactions, and binding energies (kcal/mol)

Thymol complexed to	3T6C.pdb	6UAW.pdb	8CI6.pdb	4D74.pdb	4IV9.pdb	1U1W.pdb
Identification and Classification	Lyase protein from <i>P. ananatis</i> (R of 1.60 Å)	Hydrolase protein from <i>P. viridiflava</i> (R of 1.50 Å)	Transport protein from <i>A. vitis</i> (R of 1.20 Å)	Hydrolase protein from <i>E. amylovora</i> (R of 1.57 Å)	Oxidoreductase protein from <i>P. savastanoi</i> (R of 1.95 Å)	Isomerase and lyase protein from <i>P. marginalis</i> (R of 1.35 Å)
Grid boxes	X = 85.123 Y = 24.35 Z = 13.402	X = 34.206 Y = 14.144 Z = 20.211	X = 17.442 Y = -1.285 Z = -19.616	X = 19.896 Y = -21.609 Z = -10.532	X = 122.5 Y = 29.668 Z = -73.245	X = 7.284 Y = 30.962 Z = 24.809
Produced intermolecular interactions	Glu275 Phe41 His298 Ser301 Pro85 Val278 Leu276	Trp87 Gln120 Trp61 Phe256	Tyr145 Trp418 Trp178 Trp423	Cys14 Ile13 Tyr117 Ile10 Cys9	Phe476 Trp466 Gly95 Arg77 Cys511	Asp208 Asn42 Ser44 Val205 Pro19 Ala209 Arg244
Binding energies (Kcal/mol)	-6.35	-4.97	-5.32	-6.38	-6.93	-5.27

Second, the compound was docked to the hydrolase protein from *P. viridiflava* (PDB ID: 6UAW), revealing two hydrogen bonds with Gln120 and Trp87, one carbon–hydrogen bond with Trp61, and one alkyl interaction with Phe256. Third, thymol was complexed with the transport protein from *A. vitis* (PDB ID: 8CI6), forming one hydrogen bond with Tyr145, one Pi-Pi T-shaped interaction with Trp178, and two alkyl interactions with Trp418 and Trp423. Fourth, thymol was docked to the hydrolase protein from *E. amylovora* (PDB ID: 4D74), establishing two hydrogen bonds with Cys14 and Ile13, one Pi-Pi stacked interaction with Tyr117, one Pi-donor hydrogen bond with Gly11, as well as additional alkyl and Pi-alkyl bonds with Cys9 and Ile10. Fifth, docking to the oxidoreductase protein from *P. savastanoi* (PDB ID: 4IV9) revealed one Pi-Pi T-shaped interaction with Phe476, one carbon–hydrogen bond with Trp466, one Pi-donor hydrogen bond with Gly95, along with alkyl and Pi-alkyl interactions involving Cys511 and Arg77. Finally, the candidate molecule was docked to the isomerase and lyase protein from *P. marginalis* (PDB ID: 1U1W), exhibiting key intermolecular interactions, including one conventional hydrogen bond with Asn42, one Pi-anion interaction with Asp208, one Pi-donor hydrogen bond with Ser44, and various alkyl and Pi-alkyl bonds within chain A.

For comparison, thymol demonstrated consistent binding patterns across all six targets, involving key residues near or within the known active or functional regions of the proteins, as shown in Figure 4. In particular, residues such as Glu275

for 3T6C.pdb, Trp87 and Gln120 for 6UAW.pdb, Tyr145 and Trp418 for 8CI6.pdb, Cys14 for 4D74.pdb, Phe476 for 4IV9.pdb, and Asp208 for 1U1W.pdb, which demonstrates that docking poses of thymol occupied well-defined binding pockets, suggesting favorable conformations and possible inhibitory potential.

To ensure the reliability of the docking results, the docking procedure was further validated by re-docking the co-crystallized ligand into the active sites of the target proteins. The comparison of the experimental and predicted ligand conformations yielded the following RMSD values: 0.887 Å (3T6C); 0.943 Å (6UAW); 1.023 Å (8CI6); 0.341 Å (4D74); 0.876 Å (4IV9); and 0.764 Å (1U1W). RMSD values obtained in the analysis were lower than the generally accepted value of 2.0 Å, which indicates that the docking procedure accurately reproduced the crystallographic binding poses, contributing to the reliability of the prediction of the binding modes of thymol. The superposition of the initial native ligand conformation and the conformations gained after the re-docking process is presented in Figure 4.

DISCUSSION

Essential oil yield

The yield of *O. elongatum* collected from Bouiblane was lower than the previously reported value of 1.76% and the range of 3.4–3.7% (Drionche et al., 2024; Ramzi et al., 2017). However,

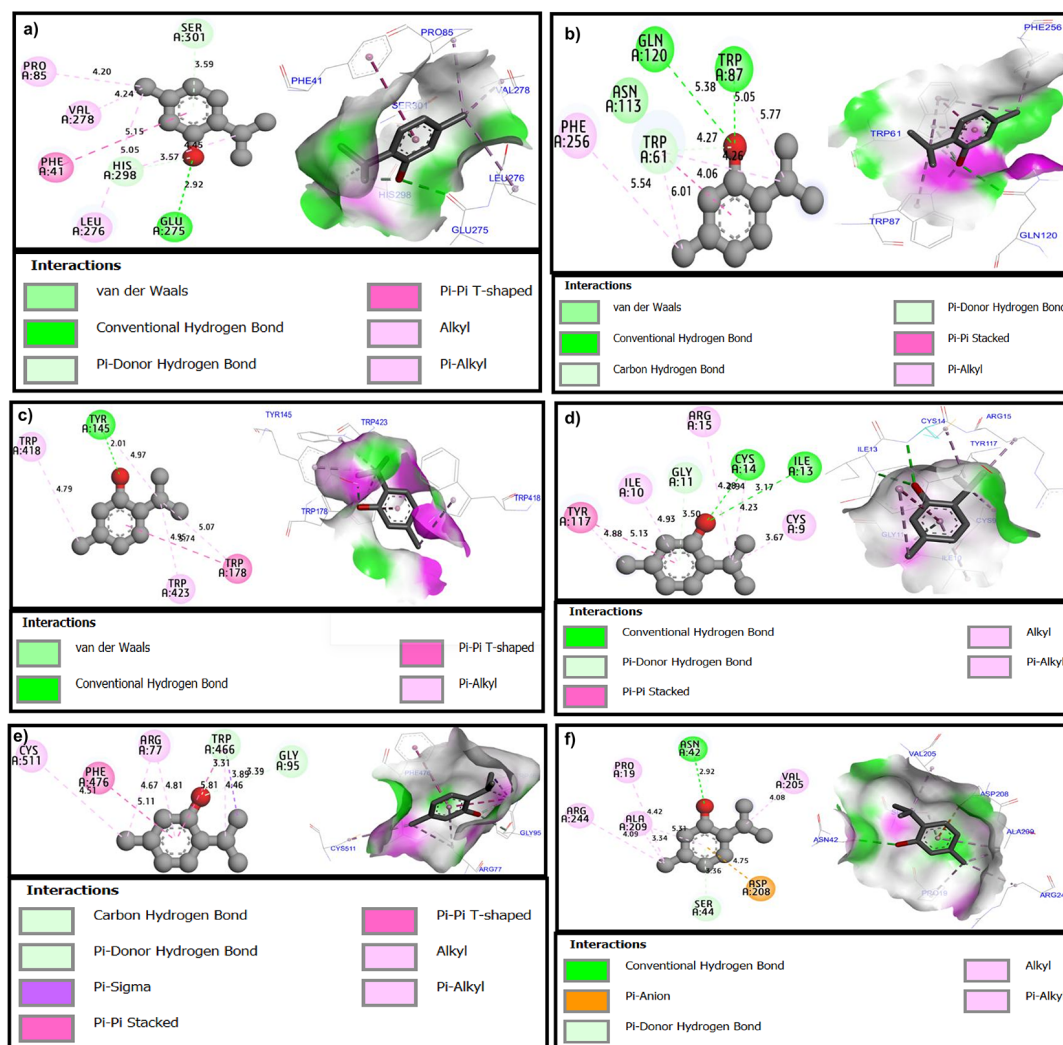


Figure 3. Predicted chemical interactions in 2D and 3D views of thymol in complex with six bacterial target proteins (3T6C.pdb, 6UAW.pdb, 8CI6.pdb, 4D74.pdb, 4IV9.pdb, and 1U1W.pdb) against *P. ananatis* (a), *P. viridiflava* (b), *A. vitis* (c), *E. amylovora* (d), *Pss* (e), and *P. marginalis* (f) pathogenic strains, respectively

it remained within the reported range of 0.81–3.12% (Bakha et al., 2020).

The yield of *T. vulgaris* collected from Sefrou was slightly higher than the previously reported range of 1.0–1.8% (El Hattabi et al., 2016; Labiad et al., 2022). Nevertheless, a higher yield of 3.6% has been documented at the full-bloom stage, indicating that phenological stage may considerably influence EO production (Zantar et al., 2015).

The yield of *L. stoechas* collected from Bouiblanc was lower than the previously reported range of 2–3.4% (Bachiri et al., 2018; Laqhaili et al., 2023). However, comparable values have been documented in other studies (Karan, 2018; Radi et al., 2024).

These variations may be associated with environmental conditions, developmental stage, plant material preparation, and extraction conditions,

which influence EO biosynthesis and accumulation (Mansinhos et al., 2024; Mugao, 2024).

Chemical profile of EOs

The linalool- and camphor-dominant profile of *L. stoechas* differed from previously described profiles dominated by camphor (48.1%) and fenchone (30.5%) or by camphor (47.67%), fenchone (20.78%), and 1,8-cineole (12.17%) (Karan, 2018; Soulaïmani et al., 2025). A distinct Moroccan profile dominated by camphor (24.35%), cubenol (15.54%), and fenchone (9.58%) has also been documented (Radi et al., 2024). Together, these profiles highlight the considerable chemical variability of this species. Its comparatively moderate antibacterial activity may be partly associated with

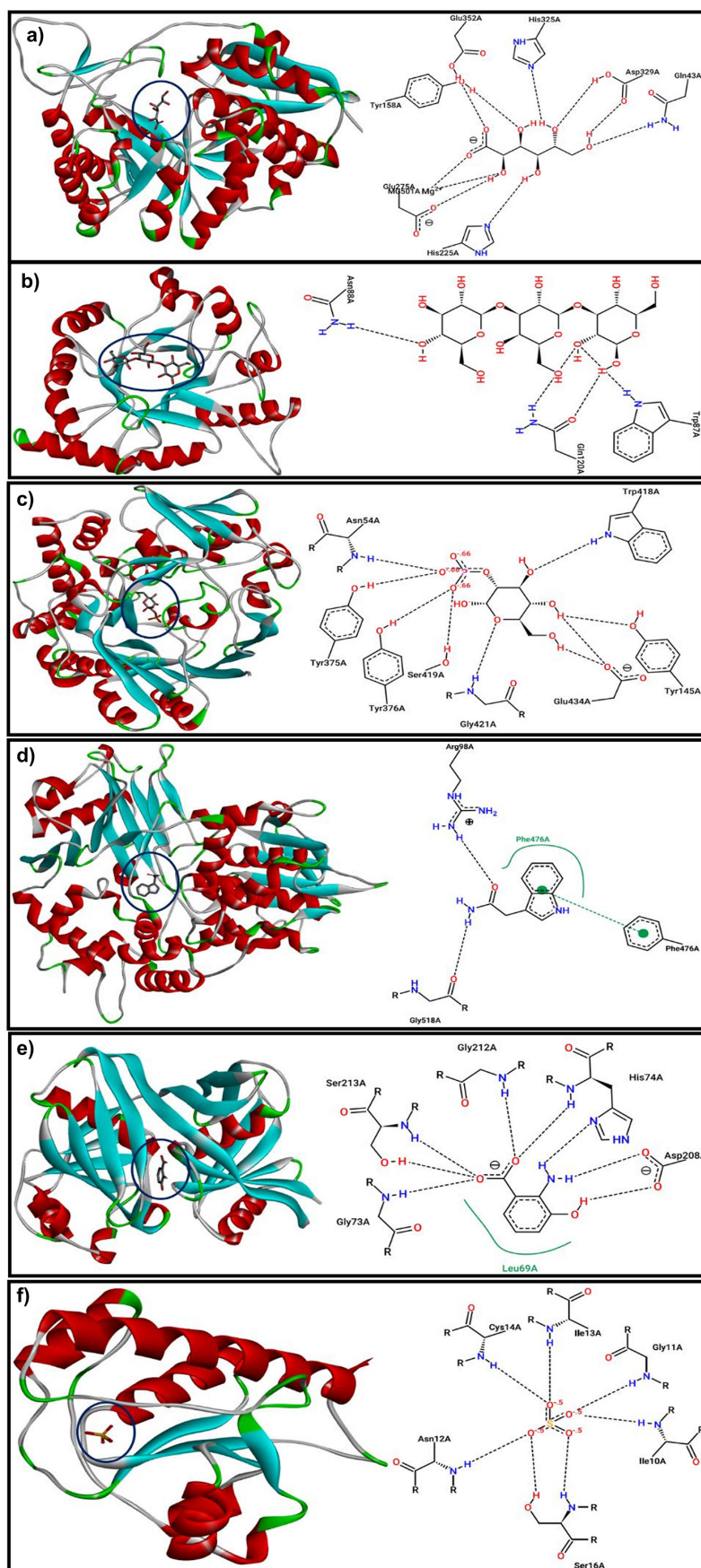


Figure 4. Active sites of six bacterial target proteins (3T6C.pdb, 6UAW.pdb, 8CI6.pdb, 4D74.pdb, 4IV9.pdb, and 1U1W.pdb) from *P. ananatis* (a), *P. viridiflava* (b), *A. vitis* (c), *E. amylovora* (d), *P. savastanoi* (e), and *P. marginalis* (f) pathogenic strains, respectively

its limited phenolic content compared with thymol- and carvacrol-rich EOs (Kosakowska et al., 2024).

The thymol-dominant profile of *T. vulgaris* falls within the broad ranges reported for thymol (0.9–71.2%) and carvacrol (2.3–87.5%), reflecting considerable chemotypic variability (Raal et al., 2024). Its strong antibacterial activity may be related to the predominance of phenolic compounds, particularly thymol and carvacrol, which can disrupt bacterial cytoplasmic membranes (Kosakowska et al., 2024; Zamuner et al., 2023).

The thymol- and carvacrol-rich profile of *O. elongatum* contrasted with carvacrol-dominant profiles containing 57.32% and 60.42% carvacrol (Oualili et al., 2019; Tagnaout et al., 2023). Variations related to plant development have also been documented, with thymol and carvacrol contents ranging from 13.0% to 29.54% and from 20.25% to 37.26%, respectively, across three phenological stages (Batbat et al., 2026). Its phenolic profile may contribute to its pronounced antibacterial activity, consistent with the activity of other phenol-rich *Origanum* EOs (Chroho et al., 2024; Kosakowska et al., 2024).

In addition to phenological stage, differences in EO composition may result from genetic, geographical, climatic, and agronomic factors, as well as harvest period and extraction conditions (El Hachlafi et al., 2024).

Antibacterial potential

Disc diffusion

The pronounced antibacterial activity of *T. vulgaris* EO may be related to its phenolic profile, particularly the predominance of thymol and carvacrol, which can disrupt bacterial membranes and increase their permeability (Zamuner et al., 2023). Its activity against *Pss* exceeded the previously reported range of 16.5–25.4 mm for undiluted *T. vulgaris* EO (Koščák et al., 2025).

Similarly, the strong activity of *O. elongatum* may be associated with its high proportion of thymol and carvacrol, which are known to affect bacterial membrane integrity (Zamuner et al., 2023). Its activity against *E. amylovora* was lower than the previously reported value of 59 ± 3.1 mm, whereas its activity against *A. vitis* was higher than the reported value of 47 ± 2.8 mm (Batbat et al., 2025). Against *Pss*, *O. elongatum* EO produced a significantly larger inhibition zone than *T. vulgaris* EO (Tukey's HSD, $p < 0.05$). This

difference may depend on bacterial susceptibility and the chemical profile of each EO. *L. stoechas* showed only moderate activity. Its low phenolic content, together with the predominance of oxygenated monoterpenes, may partly explain this weaker effect. Despite its overall lower activity, *L. stoechas* EO produced significantly greater inhibition than the control against *A. vitis* and *P. marginalis* (Tukey's HSD, $p < 0.05$). An inhibition zone of 18 ± 1 mm against *E. amylovora* has previously been reported for a fenchone-dominant chemotype (Loukhaoukha et al., 2018). The predominance of linalool and camphor in the present EO, compared with the previously reported fenchone-dominant profile, suggests that chemotype differences may influence antibacterial effectiveness (El Hachlafi et al., 2024; Loukhaoukha et al., 2018).

Nevertheless, comparisons of inhibition zones should be interpreted cautiously because EO volume, disc diameter, inoculum density, culture medium, incubation conditions, bacterial strain, and compound diffusibility may vary among studies.

Microatmosphere assay

The strong vapor-phase activity of *O. elongatum* and *T. vulgaris* may be associated with their high thymol and carvacrol contents, as these compounds retain antibacterial activity after volatilization (Cid-Pérez et al., 2024). The greater susceptibility of *E. amylovora* compared with the other tested bacteria suggests that vapor-phase activity is bacterial species-dependent (Pinto et al., 2024).

The activity of *O. elongatum* against *E. amylovora* was higher than the previously reported inhibition zone of 29 ± 2.8 mm obtained by microatmosphere (Batbat et al., 2025). This difference may result from variations in EO composition, bacterial strain, applied volume, headspace volume, and exposure conditions (Pinto et al., 2024). The activity of *T. vulgaris* is also qualitatively consistent with its previously demonstrated antibacterial effectiveness in the vapor phase (Antih et al., 2021). Antibacterial effects of *Origanum* and *T. vulgaris* EOs against *A. vitis* have also been documented, with inhibition rates of 49.83% and 30.25%, respectively (Habbadi et al., 2018). More recently, *O. vulgare* EO vapor was shown to inhibit *P. ananatis* and *P. marginalis*, providing independent qualitative evidence of the susceptibility of these bacteria to volatile oregano constituents (Shu et al., 2025). Nevertheless, direct quantitative comparisons

with the present results are not appropriate because the studies differed in *Origanum* species, EO dose, headspace volume, exposure conditions, and response metrics.

In contrast, the inactivity of *L. stoechas* may be related to its low phenolic content or an insufficient concentration of active constituents in the headspace (Antih et al., 2021; Cid-Pérez et al., 2024). The absence of activity with streptomycin is consistent with the principle of the microatmosphere assay, which requires the antimicrobial agent to volatilize and migrate through the headspace; therefore, this result should not be interpreted as bacterial resistance (Pinto et al., 2024).

Previous greenhouse and field studies have shown that thyme EO can reduce bacterial populations and disease development in infected seeds, whereas the effectiveness of oregano EO may be less consistent and depend on the botanical species, chemotype, applied concentration, and experimental conditions (Sotelo et al., 2021, 2025).

Overall, *O. elongatum* and *T. vulgaris* appear promising for vapor-phase applications. However, formulation, exposure conditions, phytotoxicity, and *in planta* efficacy require further investigation because strong *in vitro* activity does not necessarily ensure effective plant protection (Giovanardi et al., 2025; Mendes et al., 2024).

Determination of MICs and MBCs

The high antibacterial potential of *O. elongatum* EO is supported by previously reported MIC and MBC values of 62.5 and 125 µg/mL, respectively, against *E. amylovora* (Batbat et al., 2025). Carvacrol-rich *O. compactum* EO also exhibited MIC and MBC values of 0.03% and 0.06% (v/v), respectively, against this pathogen, although direct numerical comparison is inappropriate because the units, botanical species, and chemotype differed (Proto et al., 2022).

The antibacterial activity of *T. vulgaris* EO against *A. vitis* was comparable to the previously reported MIC range of 0.3–0.312 mg/mL (Habbadi et al., 2018, 2021). Its activity against *Pss* was also consistent with the reported MIC of 1.25 mg/mL (Koščák et al., 2025). Differences among thyme EOs may be related to their chemotypes, as thymol-rich oils generally exhibit lower MIC and MBC values than thujanol-rich oils (Proto et al., 2022).

In contrast, the lower antibacterial activity of *L. stoechas* EO against *A. vitis* agrees with a

previously reported MIC of 10 mg/mL (Habbadi et al., 2021). Substantially lower MIC and MBC values of 2.5 and 5 µg/mL, respectively, have nevertheless been documented against *E. amylovora* for a fenchone-rich chemotype (Loukhaoukha et al., 2018). Such difference could be explained by a variability in chemotype, location, plant stage at the time of collection and laboratory conditions used (El Hachlafi et al., 2024). Direct comparisons among studies should be interpreted cautiously because the units, inoculum density, solvents, incubation conditions, and endpoint determination may differ (Proto et al., 2022). Taken as a whole, the antibacterial potential of the investigated EOs appears to be associated with their chemical profiles, particularly their thymol and carvacrol contents, which can affect bacterial membrane integrity (Zamuner et al., 2023).

Molecular docking analysis

The predicted interaction was favourable in thymol for three proteins, in particular 4IV9, 4D74 and 3T6C. The observed prediction supports a possible molecular mechanism complementary to the reported antimicrobial effect of thyme EOs (*T. vulgaris*, *O. elongatum*), which are rich in thymol. The interaction with 4D74 is relevant since it represents AmsI, the tyrosine protein phosphatase controlling amylovoran synthesis, a key virulence determinant in *E. amylovora* (Salomone-Stagni et al., 2016). Tryptophan 2-monooxygenase is also present as 4IV9, which is a key enzyme in the synthesis of indole-3-acetic acid and gall formation of *P. savastanoi* (Gaweska et al., 2013).

There is substantial evidence that thymol favourably binds to microbial enzyme-binding sites, as has been shown through molecular docking and dynamics simulation (Cruz et al., 2022). For the same *E. amylovora* target, 4D74, a thymol binding energy of −6.27 kcal/mol has previously been reported, providing directly comparable evidence (Batbat et al., 2025).

The positioning of thymol within the predicted binding pockets and its interactions with several amino acid residues may contribute to complex stabilization. However, these interactions alone do not demonstrate enzyme inhibition. In addition, the comparison of docking scores for distinct protein targets or docking protocol implementations should be done cautiously since docking scores vary according to scoring function and simulation conditions (Agu et al., 2023).

Therefore, the correlation suggested between affinity and activity against bacteria is speculative and must be verified by simulations of molecular dynamics and enzyme inhibition assays.

Limitations

Although the results are encouraging, the study was restricted to *in vitro* antibacterial assays and molecular docking predictions. Although the results are promising, they are based only on *in vitro* antibacterial tests and molecular docking predictions. The geographical coverage was limited as well. Aerial plant parts were collected at just two Moroccan sites, Sefrou and Bouiblane, so the findings may not reflect the activity of plants growing in other regions or under different environmental conditions. The extraction procedure presents another limitation. For each species, the EO came from one batch of plant material, meaning that only one independent extraction was carried out ($n = 1$). Consequently, between-extraction variability in EO yield and chemical composition could not be estimated. Although the antibacterial assays included technical replicates and repeated experiments, these evaluated only assay-level variability and did not account for variability associated with independent plant batches or extraction procedures. Future studies should therefore include multiple independently collected batches of plant material and independent extractions to establish the reproducibility of EO yield, chemical composition, and antibacterial activity. Greenhouse-based *in planta* experiments using appropriate host-pathogen systems are needed to confirm disease suppression, effective doses, and reproducibility under realistic conditions. Further experimental validation of the predicted protein-ligand interactions is also required. Moreover, the volatility, limited environmental stability, potential phytotoxicity, and variable composition of EOs remain important constraints on their development as biopesticides, highlighting the need for formulation optimization, dose standardization, and greenhouse and field validation (Aqeel et al., 2023; Assadpour et al., 2024). If their efficacy and plant compatibility are confirmed, the phenol-rich EOs of *T. vulgaris* and *O. elongatum* could be developed into formulations or vapor-phase treatments for crop protection, following formulation optimization and field validation.

CONCLUSIONS

The EOs tested possess diverse antibacterial activity and chemical profiles. The phenol-rich EOs of *T. vulgaris* and *O. elongatum*, particularly their thymol and carvacrol contents, showed stronger antibacterial activity than *L. stoechas* EO. *O. elongatum* exhibited bactericidal activity against all tested strains, whereas *T. vulgaris* showed strain-dependent bactericidal and bacteriostatic effects. The scientific novelty of this study lies in the standardized side-by-side comparison of the chemical composition and *in vitro* antibacterial performance of these three Moroccan *Lamiaceae* EOs against the same panel of six phytopathogenic bacteria. Molecular docking results showed favorable interactions between the predicted target proteins and the extracted constituents that suggest a possible molecular mechanism for their experimental activities. Overall, these findings support the potential of *T. vulgaris* and *O. elongatum* EOs as candidates for further investigation in plant disease management. However, greenhouse-based *in planta* validation, together with assessments of EO stability, phytotoxicity, formulation, dosage, application conditions, and environmental fate, is required before their practical use in crop protection can be considered.

Acknowledgements

The authors thank the Phytobacteriology and Biological Control Laboratory, Regional Center of Agricultural Research of Meknes (INRA, Morocco), and the Laboratory of Natural Resources and Environment (LRNE), Faculty of Multidisciplinary Studies of Taza, for providing facilities and technical support for this study.

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