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ARTICLE

GC-MS Profiling, *In Vitro* and *In Silico* Antibacterial and Antioxidant Potential of *Origanum elongatum* Essential Oil: Novel Source against Phytopathogenic Bacteria

Amine Batbat[1,2], Mohamed El Fadili[3], Naoufal El Hachlafi[4,*], Amine Elbouzidi[5,*], Chaymae Hmimen[6], Hiba Yahyaoui[2,7], Samir Jeddi[6], Abdellatif Benbouazza[2], Kawtar Fikri-Benbrahim[6], Mohamed Addi[5], Samiah Hamad Al-Mijalli[8], Khaoula Habbadi[2] and Hassane Greche[1]

[1]Laboratory of Applied Organic Chemistry, Faculty of Sciences and Techniques, Sidi Mohamed Ben Abdellah University, Imouzzer Road, Fez, 30000, Morocco
[2]Plant Protection Research Unit, National Institute of Agronomic Research, Regional Center of Agronomic Research of Meknes INRA-CRRA, Meknes, 50000, Morocco
[3]LIMAS Laboratory, Faculty of Sciences Dhar El Mahraz, Sidi Mohamed Ben Abdellah University, Fez, 30000, Morocco
[4]Faculty of Medicine and Pharmacy, Ibn Zohr University, Guelmim, 81000, Morocco
[5]Laboratoire d'Amélioration des Productions Agricoles, Biotechnologie et Environnement (LAPABE), Faculté des Sciences, Université Mohammed Premier, Oujda, 60000, Morocco
[6]Laboratory of Microbial Biotechnology and Bioactive Molecules, Faculty of Sciences and Technologies Faculty, Sidi Mohamed Ben Abdellah University, Imouzzer Road, Fez, P.O. Box 2202, Morocco
[7]Laboratory of Biotechnology and Bio-Resources Valorization, Faculty of Sciences, Moulay Ismail University, Meknes, 50000, Morocco
[8]Department of Biology, College of Sciences, Princess Nourah Bint Abdulrahman University, Riyadh, 11671, Saudi Arabia

*Corresponding Authors: Naoufal El Hachlafi. Email: naoufal.elhachlafi@usmba.ac.ma; Amine Elbouzidi. Email: amine.elbouzidi@ump.ac.ma

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ABSTRACT: This study highlights the regulatory potential antibacterial and antiradical of *Origanum elongatum* essential oil (EO), an endemic medicinal plant of Morocco used for its various properties. The chemical composition of the EO was characterized using gas chromatography-mass spectrometry (GC-MS). The antibacterial activity against different agricultural phytopathogens was determined by disc diffusion and microatmosphere methods, as well as by the determination of minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC), while the antioxidant activity was evaluated by DPPH and FRAP assays. To complement the experimental analyses, a molecular docking approach was used to predict and elucidate the mechanisms of action of the identified bioactive compounds, both for their antioxidant and antibacterial properties. The GC-MS analysis revealed a chemical composition dominated by the major compounds: *p-cymene*-2-ol (25.31%), thymol (23.88%), and γ-terpinene (19.26%). Furthermore, antibacterial analyses performed using different methodological approaches (disc diffusion, microatmosphere, MIC, and MBC) showed significant inhibitory activity against all phytopathogens tested. Moreover, *O. elongatum* EO exhibited interesting antioxidant ability with an IC_{50} value of 168.25 ± 1.14 µg/mL for DPPH assay and EC_{50} value of 164.22 ± 1.04 µg/mL for FRAP assay. Furthermore, in silico molecular docking demonstrated further insights into the interactions between the oil's active components and bacterial targets, supporting its mode of action. This in-depth characterization highlights the potential of *O. elongatum* EO as a natural alternative for the biocontrol of plant pathogens. It opens new perspectives for developing natural solutions to protect crops against plant diseases.

KEYWORDS: *Origanum elongatum*; medicinal plant; chemical composition; biological activity; antiradical activity; molecular docking

1 Introduction

Bacterial resistance represents a major challenge to human health and food production, highlighting the urgent need for the ongoing discovery of new natural antimicrobial compounds [1]. While chemical products are often effective and affordable, their residual toxicity and potential carcinogenic and teratogenic effects raise substantial concerns [2]. Consequently, there is growing attention in evaluating the antibacterial and antioxidant properties of natural substances for potential use as functional foods and therapeutic agents [3]. Essential oils (EOs) from aromatic and medicinal plants have demonstrated notable biological activity [4,5]. These oils, composed of a wide range of bioactive substances responsible for potent antimicrobial, anti-inflammatory, and antioxidant activities [6]. By targeting various microbial processes, EOs effectively prevent the emergence of resistant strains, thereby maintaining their efficacy as natural antimicrobial agents [7,8].

Within the Lamiaceae family, the genus *Origanum* includes species with strongly aromatic leaves and bioactive compounds [9]. *Origanum elongatum*, endemic to Morocco, thrives in temperate biomes at altitudes above 1000 m and is particularly rich in proteins, fibers, carbohydrates, and EOs [10]. The chemical composition of *O. elongatum*, which varies with genetic and environmental factors, includes bioactive substances such as thymol, carvacrol and *p*-cymene [11]. These constituents contribute to the plant's biological effects, including hepatoprotective, antioxidant, antiparasitic, antiviral, and antibacterial properties [12].

The practical applications of EOs are becoming increasingly of interest, particularly in the agricultural, food, and pharmaceutical sectors [13–15]. However, a number of studies have evidenced the potential utility of plant EOs in the development of biocontrol and food preservation products, due to their established antimicrobial and antioxidant properties [16,17].

Despite the effectiveness of *O. elongatum* in inhibiting various microorganisms [18,19], no studies have explored its effects against phytopathogens such as *Allorhizobium vitis*, *Agrobacterium tumefaciens*, and *Erwinia amylovora*. The defense against plant bacterial pathogens in agriculture predominantly relies on preventive treatments with copper-based chemicals [20]. While advantageous due to their high toxicity towards pathogens, low mammalian toxicity, cost-effectiveness, and prolonged stability, excessive use of copper compounds has led to soil accumulation and the selection of copper-resistant strains [21]. Therefore, finding alternatives or optimizing the use of copper is critical, and EOs present a promising, environmentally sustainable solution for plant and human health.

Allorhizobium vitis, the causative agent of Crown Gall in grapevines, is facilitated by the pathogen's presence in the soil and entry through wounds on the host plant [22]. Biological control methods, including the use of essential oils from *Origanum* and *Eucalyptus* species, have shown promise in managing this disease [23]. Similarly, *Agrobacterium tumefaciens*, another significant phytopathogen responsible for Crown Gall, induces tumor formation by transferring its T-DNA into host plants, with research focusing on various strains and mutants to inhibit tumor growth [24]. *Erwinia amylovora*, the agent behind fire blight in apple and pear trees, spreads through rain, insects, and pruning tools, with control measures primarily involving copper-based sprays and resistant cultivars [25].

The current exploration aims to assess the antibacterial and antioxidant effects of EO obtained from *O. elongatum* via *in vitro* and *in silico* experiments. This is achieved through the utilization of both experimental and computational docking techniques. To our knowledge, this work is the first to investigate the antibacterial activity and molecular docking patterns of *O. elongatum* EO against phytopathogenic bacteria, namely

Allorhizobium vitis, *Agrobacterium tumefaciens*, and *Erwinia amylovora*, offering valuable insights into its potential applications as biocontrol agents.

2 Materials and Methods

2.1 GC-MS Analysis

The identification of chemical compounds and the determination of their quality in our OEEO sample was performed employing GC-MS analysis. The method that was adopted to analyze our sample was described by [26]. The analysis was done using a TRACE GC ULTRA coupled to the mass spectrometer (Polaris Q), with an electron initiation energy of 70 eV. A non-polar VB5 capillary was used. It measured 30 m long, 0.25 mm in diameter, and 0.25 um thick. The temperature parameters were carefully set: The injector at 250°C and the detector at 300°C; the oven was then programmed to increase from 40°C to 180°C at a speed of 4°C/min, then from 180°C to 300°C at a speed of 20°C/min. An injection of 0.5 µL of OEEO was performed without fractionation. The OE molecules were characterized by making comparisons of their kovats indices with those of n-alkanes series (C8 to C24) reported in the literature. The results obtained were compared with known references in order to determine the specific constituents of OEEO and evaluate their relative concentration. As a final step, the oil fractions were then carried out by comparing the MS fragmentation patterns of spectra with those reported by the NIST library data.

2.2 Antibacterial Activity

2.2.1 Bacteria

Three phytopathogens were used to determine the antibacterial effect of OEEO (*Erwinia amylovora*, *Agrobacterium tumefaciens* C58, *Allorhizobium vitis* S4). All the phytopathogens employed in this work were from the Laboratory of Phytopathology, Regional Centre of Agronomic Research of Meknes INRA-CRRA, Meknes. To make the bacterial solution for inoculation, two or three colonies were taken from a fresh culture on LPGA and then suspended in a sterile 0.9% NaCl solution. The final bacterial density was around 10^6 CFU/mL.

2.2.2 Disc Diffusion Assay

A preliminary antibacterial screening was done adopting the disc diffusion method, with certain modifications, according to a previously outlined methodology [27]. One plate of LB agar medium was inoculated with a solution of a bacterial culture. Next, 8 µL of OEEO was used to soak 6 mm sterile filter discs, which were then placed on each plate. Streptomycin (15 µg/disc) was served as a standard. The inhibition zone (IZ) was determined and expressed in millimeters after 24 h of incubation at 37°C. The mean ± SD of three different measurements is presented for each data set.

2.2.3 Micro-Atmospheric Assay

The micro-atmospheric technique described by Reference [28] with minor modifications was also adapted to indicate the antibacterial capacity of OEEO by gas contact. In short, after plating bacterial strains (10^8 CFU) on LB agar medium, Whatman discs (6 mm) soaked with 8 µL of OEEO were placed in each Petri dish lid. Inhibitory zone growth was measured by incubation for 24 h at 37°C.

2.2.4 MIC Assay

MIC of OEEO was examined using a technique that was reported in the literature, with some minor modifications [6]. A 96-well polypropylene microplate was used. In brief, 100 µL of double serial dilutions of OEEO (5% DMSO) and positive control (streptomycin) were added to each well in each row of the microplate. Then 10 µL of the bacterial suspension was calibrated against the 0.5 McFarland standard and 50 µL of double-strength LB broth medium was added to the microplates. The incubation period was 24 h at 37°C. A medium containing no bacterial suspension and 5% DMSO was used as a negative control of the experiment. A volume of 20 μL of resazurin was added to measure the development of the bacteria.

2.2.5 MBC Assay

The MBC tests were performed after MIC determination. 50 µL of each MIC tube was plated on LB agar plates specially prepared for this purpose. The plates were then incubated at 25°C to 35°C for 24 h. MIC at which no bacterial growth was observed was called the MBC. The MBC/MIC ratio was used to assess whether the effect was bacteriostatic or bactericidal [29].

2.3 Antioxidant Activity

2.3.1 DPPH Free Radical-Scavenging Assay

In the presence of a DPPH radical the antioxidant substances present in the EO can reduce the radical, resulting in a decrease in absorbance measured spectrophotometrically. In this study, the ability of OEEO to trap the DPPH radical was determined by the standard method described by [30] with a few modifications. Ascorbic acid and BHT were employed as standards.

2.3.2 Reducing Ferric Power Determination TEST FRAP

The test was carried out to investigate the ability of OEEO to convert Fe^{3+} into Fe^{2+}. The method used is a modified version described by Reference [31]. The BHT was used as a standard for the assay. The test was carried out in three independent experiments and the IC_{50} values were expressed as the mean ± SD.

2.4 Physicochemical and ADMET Pharmacokinetic Predictions

In the current study, two major compounds namely Thymol and *p-cymene*-2-ol extracted from *Origanum elongatum* essential oil with areas of 23.88% and 25.31%, respectively, were tested using *in silico* investigations, including their physicochemical and pharmacokinetic features of absorption, distribution, excretion, and toxicity (ADMET) [32], more than drug-likeness ability based on Lipinski rules of five, predictive model of Egan's boiled-egg, and oral bioavailability radars which were carried out with the assistance of Pkcsm and Swiss ADME servers [33]. In addition, a sum of ten molecular docking simulations was equally performed between both candidate ligands and all five targeted receptors including NADPH oxidase (2CDU.pdb) and 5-LOX Lipoxygenase (1N8Q.pdb) enzymes [34] to examine their antioxidant and anti-inflammatory effects. Subsequently, they were tested for their antimicrobial potential through three antibacterial proteins against *Erwinia amylovora* (4D74.pdb), *Agrobacterium tumefacien*s (5J9R.pdb), and *Allorhizobium vitis* (4WT7.pdb) pathogenic strains. The molecular docking processes were performed using Autodock software in which all five targeted proteins were prepared adding the Gasteiger charges and removing all water molecules and other suspended ligands [35,36]. At the same time, the produced intermolecular interactions were visualized in 2 and 3D using Discovery Studio software [37,38].

2.5 Statistical Analysis

The data analyses were done using Minitab Statistical Software 21. The statistical significance of the observed differences was assessed by employing a one-way analysis of variance (ANOVA) adopting Tukey's test.

3 Results and Discussion

3.1 Volatile Content

According to GC-MS analysis, the OEEO has a distinctive chemical profile (Table 1). Our OEEO is particularly rich in oxygenated monoterpenes, representing 51.45% of its total composition, and monoterpene hydrocarbons 42.65% (Table 2). Among the many compounds identified, *p-cymene*-2-ol stands out as the major component with a percentage of 25.31%. It was closely followed by thymol (23.88%). The third most abundant component is *γ*-terpinene which accounts for 19.26% of the total sample.

Table 1: Constituents of OEEO identified by GC-MS

No.	Compound	Formula	IR	Area
1	α-Thujene	$C_{10}H_{16}$	902	0.57
2	δ-Carene	$C_{10}H_{16}$	919	2.07
3	Morillol	$C_8H_{16}O$	969	0.48
4	β-myrcene	$C_{10}H_{16}$	958	1.43
5	γ-Terpinene	$C_{10}H_{16}$	998	19.26
6	*p*-cymene	$C_{10}H_{14}$	1042	13.12
7	Linalool	$C_{10}H_{18}O$	1082	2.48
8	L-4-terpineol	$C_{10}H_{18}O$	1137	0.71
9	endo-fenchol	$C_{10}H_{18}O$	1138	0.55
10	Isothymol methyl ether	$C_{11}H_{16}O$	1231	2.72
11	Thymol	$C_{10}H_{14}O$	1262	23.88
12	*p*-Ethylguaiacol	$C_{10}H_{14}O_2$	1416	0.51
13	caryophyllene oxide	$C_{15}H_{24}O$	1416	0.91
14	β-Caryophyllene	$C_{15}H_{24}$	1494	3.99
15	*p-cymene*-2-ol	$C_{10}H_{14}O$	1665	25.31
	Total (%)			97.99
	Yield (%)			5

Table 2: Chemical groups of OEEO

Chemical groups	Percentage
Monoterpene hydrocarbons	42.65%
Oxygenated monoterpenes	51.45%
Sesquiterpene hydrocarbons	4.9%
Oxygenated sesquiterpenes	–
Other	0.99

A comparison of our results with other research on the OEEO reveals some interesting similarities and differences. For example, analysis by GC/MS showed that OEEO contains 27 compounds covering 99.08% of its constituents, with carvacrol 57.32% as the major compound, subsequently, *p*-cymene (14.70%) and *γ*-terpinene (10.0%) [39]. In another study, the OEEO from Moroccan Rif has reported 11 major constituents, with carvacrol (60.4%), *p*-cymene (14.0%) and *γ*-terpinene (9.4%) [11]. A study conducted in the same region identified 28 compounds in the OEEO, the most prevalent of which were carvacrol, thymol, and *p*-cymene [40]. Other studies have also found that the main compounds in the OEEO are carvacrol (63.0%), *γ*-terpinene (16.0%), and *p*-cymene (9.5%) [18]. An earlier study identified oxygenated compounds as the dominant component (65.14%), followed by hydrocarbon molecules (28.0%), with thymol representing the primary constituent at 63.4% [19]. Other work also noted that the major OEEO constituent is carvacrol (67.3%) [41]. Moussaoui, in 2013 had identified carvacrol (40.1%), thymol (14.2%), *p*-cymene (16.2%) and *γ*-terpinene (13.48%) as the major constituents of OEEO [42]. Similarly, GC-MS of OEEO seeds revealed a high chemical composition dominated by carvacrol (79.2%), with the presence of *p*-cymene (5.2%), *γ*-terpinene (3.7%), and linalool (2.4%) [43,44].

These comparisons show the diversity of the chemical profiles of OEEO while confirming the presence of some key compounds such as carvacrol, *γ*-terpinene, *p*-cymene, and thymol. However, contrary to what is generally expected in the literature, our OEEO does not contain carvacrol. Instead, it is rich in *p*-cymene, known as a precursor of the main monoterpenes such as carvacrol.

There are several possible explanations for this paradox. It is possible that the biosynthetic conditions or enzymes necessary to successfully convert *p*-cymene to carvacrol were not present or active at the time when our OEEO was formed [45]. In addition, ecological factors, genetic diversity, environmental variables, geographic location, and harvest season can all have an impact on chemical composition [40]. Indeed, the harvest site's latitude and altitude, temperature, solar radiation, and phylogenetic stage, all of which can modify the composition of EO [46]. However, different cultural practices can have an impact on how plants are grown and consequently, the quality and the quantity of the oil. Nevertheless, the plant's harvest season can have a big impact on the composition of its oils. In actuality, the oil content and composition can be significantly influenced by the age of the plant during harvest, it's possible that younger plants have distinct oil profiles from older ones [47]. Furthermore, differences in lighting and temperature throughout the year might impact oil synthesis, resulting in different compositions at different times of harvest. Seasons of harvest can also cause variations in the physiology and metabolism of plants, which can impact the properties of oil [48].

3.2 Antibacterial Activity

The results indicate significant variability in the sensitivity of the different bacteria to OEEO. *E. amylovora* was the most sensitive with a mean zone inhibition of 59 ± 3.1 mm. It was followed by *A. vitis* S4 with an inhibition zone (IZ) of 47 ± 2.8 mm. Finally, an IZ of 45 ± 1.9 mm was observed for *A. tumefaciens* C58.

The micro-atmosphere test was used to assess the effect of OEEO on bacterial growth in a closed atmosphere. The findings revealed that OEEO demonstrated notable antibacterial efficacy against all bacterial strains examined. *E. amylovora* showed the most sensitivity, with an average IZ of 29 mm. *A. vitis* S4 was the next most sensitive with an IZ of 23 mm. while *A. tumefaciens* C58 had an IZ of 20 mm (Table 3).

Table 3: Inhibition zone (mm) of tested bacteria against OEEO

Phytopathogen	Disc diffusion	Microatmosphere	Streptomycin
E. amylovora	59 ± 3.1^{c}	29 ± 2.8^{b}	12 ± 1.2^{a}
A. vitis S4	47 ± 2.8^{c}	23 ± 4.3^{b}	7 ± 2.3^{a}
A. tumefaciens C58	45 ± 1.9^{c}	20 ± 3.9^{b}	9 ± 1.02^{a}

Note: Data sharing with different letters indicates no significant differences (ANOVA test).

The objective of the MIC test was to ascertain the lowest concentration of OEEO that could be demonstrated to visibly inhibit the visible development of plant pathogen bacteria: *E. amylovora, A. tumefaciens* C58, *A. vitis* S4. The MIC of *E. amylovora*, was 62.5 µg/mL, that of *A. vitis* S4 was 125 µg/mL and for *A. tumefaciens* C58 the MIC was 150 µg/L. In particular, the MBC results showed that the minimum lethal concentration of OEEO for *E. amylovora* was 125 µg/mL. In contrast, the concentration required to kill *A. vitis* S4 was 125 µg/mL and for *A. tumefaciens* C58 it was 150 µg/mL. Consequently, OEEO has bactericidal activity against all phytopathogens tested, depending on her level of tolerance. MBC/MIC ratio was 2 against *E. amylovora* and 1 against *A. vitis* S4, and *A. tumefaciens* C58 (Table 4). The results of the positive controls demonstrated that the standard antibiotic exhibited MICs ranging between 65 to 130 µg/mL, contingent on the bacterial strains subjected to analysis. In comparison, the OEEO demonstrated remarkable antibacterial ability, particularly against *E. amylovora*, which is approximately 10 times more efficacious than the positive control. With regard to *A. vitis* S4 and *A. tumefaciens* C58, the MICs obtained were comparable to those of standard. Furthermore, the bactericidal effect observed for all the strains tested lends additional support to the hypothesis that this EO may serve as a valuable natural alternative to conventional antibacterials in the treatment of phytopathogenic bacterial infections.

Table 4: MIC, MBC (µg/mL) and the tolerance levels of phytopathogens on OEEO

Phytopathogens	OEEO		Streptomycin	Tolerance level	Effect
	MIC	MBC	MIC		
E. amylovora	62.5	125	65	2	Bactericidal
A. vitis S4	125	125	130	1	Bactericidal
A. tumefaciens C58	150	150	130	1	Bactericidal

The analysis of the antibacterial effect of OEEO on plant pathogens provides valuable insights into the potential for agricultural crop protection. The findings showed that our EO had very potent antibacterial capabilities against all bacteria tested, which could potentially be used in agriculture. The ability of OEEO to control the growth of *E. amylovora*, *A. vitis* S4, and *A. tumefaciens* C58 could lead to a major revolution in plant disease management methods. Based on a literature review of existing research, there is a wide collection of studies demonstrating the efficacy of OEEO against various pathogens [18,19,39]. However, no published work has been done on the effect of OEEO on the antibacterial ability of the three phytopathogenic bacteria investigated.

The results of our work confirm those already reported in the literature on the efficacy of EO in the biocontrol of the three phytopathogens tested. Significant antibacterial activity of *Origanum vulgare* EO has been reported in a study, with a MIC range between 7.8 and 625 µg/mL and a zone of inhibition of 15 ± 0.5 mm against *E. amylovora*. Furthermore, the EO of *Origanum compactum* showed an IZ of 40 mm, suggesting an

even stronger antibacterial activity against *E. amylovora* [49]. However, the EOs from *Foeniculum vulgare* and *Pimpinella anisum* also showed significant antibacterial activity with a MIC of 7.8 µg/mL and zones of inhibition of 36.33 and 26.31 mm respectively *vs. E. amylovora* [50]. Nevertheless, research conducted by [51] highlighted the potential of *Rosa damascene* EO against *E. amylovora*, providing a 45 mm zone of inhibition. The essential oil of *Satureja* L. on *E. amylovora*, also had a high antibacterial activity, MBC/MIC ranging from 0.09 and 0.18 µg/mL [52]. Furthermore, *Cinnamomum cassia* EO also reported an inhibition zone of 37.7 mm and an MBC of 1.04 µg/mL [53]. On the other hand, *A. vitis* has also shown sensitivity to the Eos. In a study by Habbadi and colleagues on *Origanum compactum* and *Thymus. Vulgaris* EOs *vs. A. vitis* S4 showed significant *in vitro* activity, with MICs of 156 and 312 µg/mL, respectively [54]. In parallel, a study of EOs from different plants reported percentage inhibitions ranging from 7.5% to 25.88% and MICs between 0.15 and 20 mg/mL [55]. In addition, the efficacy of four *Eucalyptus* EOs against *A.vitis* S4 has demonstrated inhibition percentages ranging from 13.67% to 20.50% and MICs from 20 to 40 mg/mL [56]. Moreover, *A. tumefaciens* has also exhibited notable sensitivity to essential oils effects as highlighted by many studies. However, Cuminum *cyminum* EO showed inhibitory activity with a MIC ranging from 170.2 to 7280 µg/mL. Similarly, *Thymus vulgaris* EO showed a MIC ranging from 75 to 1100 µg/mL, with an IZ measuring 11 mm [57]. *Mentha piperita* L EO was particularly effective against *A. tumefaciens*, with an IZ of 36 mm, and lowest MIC (0.02 mg/mL), and MBC of 0.39 mg/mL [58]. A study of five Eos against *A. tumefaciens* showed that *Thuja occidentalis* exhibited significant inhibition with an MIC of 400 mg/mL, closely followed by *Pelargonium graveolens* with an MIC of 540 mg/mL. In contrast, *Citrus sinensis, Myrtus communis*, and *Schinus terebinthifolius* Eos failed to inhibit *A. tumefaciens* development even at high concentrations (MIC > 1000 mg/mL) [59]. In addition, a study on Eos from Cinnamon, Thyme, and Oregano, provided the highest inhibition zones against *A. tumefaciens* with diameters of 44, 32.6, and 32.3 mm, respectively [49].

3.3 Antioxidant Activity

In the present work, the antioxidant's ability of OEEO to reduce ferric ions, and to scavenge DPPH free radical were determined. Our results indicate that OEEO has a significant anti-free DPPH radical potential, with an IC_{50} of 168.25 ± 1.14 µg/mL, clearly demonstrating its strong efficacy. In the FRAP assay, OEEO also exhibited remarkable antioxidant capacity with an EC_{50} value of 164.22 ± 1.04 µg/mL (Table 5). highlighting its ability to effectively reduce ferric ions. When compared to standards such as BHT and ascorbic acid, which have IC_{50} values of 63.21 ± 0.03 µg/mL and 41.32 ± 0.08 μg/mL, respectively, in the DPPH assay, OEEO shows effective antioxidant potency. Furthermore, in the FRAP assay, BHT had an EC_{50} of 89.44 ± 0.13 μg/mL, a value closely matched by OEEO, underscoring its potential as a potent natural antioxidant.

Table 5: Antioxidant potential of OEEO

	OEEO (µg/mL)	**BHT (µg/mL)**
DPPH (IC_{50})	168.25 ± 1.14^{b}	63.21 ± 0.03^{a}
FRAP (EC_{50})	164.22 ± 1.04^{b}	89.44 ± 0.13 ^{b}

Note: Data sharing with different letters designate no significant differences (ANOVA test).

Our results on the antioxidant activity of OEEO are in arrangement with those proved in the literature. In the study by Tagnaout and colleagues, OEEO was shown to have significant antiradical potential with an IC_{50} value of 2.855 ± 0.018 µg/mL in the DPPH assay and an EC_{50} value of 0.124 ± 0.013 µg/mL in the FRAP assay [39]. Another study revealed a significant antiradical activity with an EC_{50} value of 1.20 g extract/g

DPPH [11]. Many other researches have demonstrated the reducing power of EOs from the *Origanum* genus. A study on *Origanum majorana* showed very interesting results with an IC_{50} of 503.08 ± 0.06 µg/mL for the DPPH assay and an EC_{50} of 511.43 ± 0.61 µg/mL for FRAP [60]. Furthermore, a study on the antioxidant activity of Eos from *Origanum vulgare* at several phenological phases showed an IC_{50} of the DPPH radical ranging from 59 to 89 mg/L [61]. Another study found that the EO of *Origanum vulgare* obtained from the leaves, roots, and stems, exhibited significant antioxidant activity for each plant part. The IC_{50} values measured were 332 µg/mL for leaves, 357 µg/mL for roots, and 501 µg/mL for stems [62]. Similarly, in a previous study on *Origanum compactum* EO from Boulemane and Taounate regions was found to have strong antioxidant potential. In the case of Boulemane, the IC_{50} was 0.27 ± 0.01 mg/mL (DPPH) and 0.19 ± 0.03 mg/mL (FRAP), while in Taounate it was 37 µg/mL (DPPH) and 25 µg/mL (FRAP) [63].

Natural antioxidant compounds with and without phenolic characteristics contribute to oxidative inhibition through different mechanisms. Phenolic compounds, are known for their strong radical-scavenging potency, interact directly with free radicals, while non-phenolic and non-oxygenated compounds, such as terpenes and hydrocarbons, may exert their antioxidant effects by interrupting lipid peroxidation or stabilizing free radicals through hydrophobic interactions [64]. This study's comprehensive chemical profiling underscores the significant role of various constituents, beyond phenolic compounds, in the overall antioxidant activity of OEEO. Indeed, the powerful antioxidant potency of our OEEO is related to the high concentration of non-oxygenated substances, such as *p*-cymene, thymol, and terpenes, which have been clearly described in the published literature [45].

A significant correlation has been found between antioxidant properties and phenolic compound content, enabling them to act as reducing agents, as hydrogen donors, as singlet oxygen scavengers, as lipid peroxidation inhibitors, and as metal chelators [65]. Furthermore, thymol has been shown to have the highest DPPH radical scavenging activity [66]. However, *p*-cymene is characterized by its powerful antioxidant properties, playing an essential role in neutralizing free radicals and protecting cells from the harmful effects of oxidation [45]. Terpenes also have a strong antioxidant capacity. In particular, they prevent lipid peroxidation [67]. Overall, treatment strategies based on free radical scavenging antioxidants have demonstrated the potential to protect cells from delay, control, or ameliorate many complex diseases. Antioxidants may also be important in avoiding or reducing cellular injury and subsequent cellular alteration, including DNA mutations, lipid peroxidation in the cell membrane, and mitochondrion malfunction [68,69].

3.4 Physicochemical and ADMET Pharmacokinetic Predictions

As a result of *in silico* investigations applied to Thymol and *p-cymene*-2-ol as the main compounds extracted from OEEO, we noticed that both molecules labeled C11 and C15 were predicted with good physicochemical profiles meeting all five Lipinski rules, justified by molecular weight less than 500 g/mol threshold, molar refractivity index included in [40, 130] range, lipophilicity in solvent inferior to five, acceptors and donors of Hydrogen bond not exceed ten and five, respectively, as resulted in Table 6. Moreover, both chemical compounds were equally predicted with a desired ADME profile, explained by excellent human intestinal absorption (HIA of 93%), good levels of permeability to the central nervous system (CNS) and blood-brain barrier (BBB), any inhibitory effect on human cytochromes of 2C9, 2C19, 1A2, 2D6, and 3A4, except for Thymol (C11) which was predicted with positive impact to inhibit 1A2 cytochrome. In addition, according to the AMES toxicity test, both compounds were indicated as not toxic agents as shown in Table 7. Furthermore, the candidate's ligands were also predicted as CNS inhibitors as they are part of the yellow Egan's egg as displayed in Fig. 1, so they can cross the BB barrier with the highest possible probability. Then, the predictive model of bioavailability radars confirms that both ligands were predicted with good oral bioavailability as their associated radars are part of ideal zones as colored in pink (Fig. 2) [70]. Both molecules

were therefore evaluated with desirable physicochemical and pharmacokinetic profiles, providing significant similarities to drug candidates [71].

Table 6: Prediction of the physicochemical characteristics of for thymol and *p-cymene*-2-ol

OEEO molecules	Physico-chemical characteristics					Lipinski's five rules
	MW	MR index	Log P	HBA	HBD	(No/Yes)
Rule	≤500 (g/mol)	130 ≥ MR index ≥ 40	<5	≤10	<5	
Thymol	150.22	48.01	2.32	1	1	Yes
p-cymene-2-ol	150.22	48.01	2.24	1	1	Yes

Table 7: Prediction of the ADME-Toxicity pharmacokinetic characteristics for thymol and *p-cymene*-2-ol

	A	D		M							E	T
OEEO molecules	HIA	BBBP	CNS permeability	Substrate	Inhibitor						Total clearance	AMES test of toxicity
				Cytochromes								
				2D-6	3A-4	1A-2	2C-19	2C-9	2D-6	3A-4		
	(% Absorbed)	(Log BB)	(Log PS)	(No/Yes)							Numeric (Log ml/min/kg)	(No/Yes)
Thymol	93.24	0.366	−1.349	No	No	Yes	No	No	No	No	0.259	No
p-cymene-2-ol	93.694	0.427	−1.576	No	No	No	No	No	No	No	0.219	No

Note: A: Absorption; D: Distribution; M: Metabolism; E: Excretion; T: Toxicity.

3.5 Molecular Docking Simulations

The docking simulation revealed that Thymol and *p-cymene*-2-ol ligands were docked for the first time to NADPH oxidase protein encoded in the PDB by 2CDU with the lowest binding energies (BE) of −6.24 and −6.26 kcal/mol respectively, producing several intermolecular interactions, including one Hydrogen bond fixed to Asp282 active site, more than two active sits detected through two Alkyl and Pi-Alkyl bonds fixed with Ala10 and Ala300 AA residues as resulted in Fig. 3. The same substances were secondly docked to 5-LOX Lipoxygenase enzyme encoded by 1N8Q.pdb with BE of −5.86 and −6.13 kcal/mol respectively, in which various intermolecular interactions such as Sigma bond detected towards His523 and Pi-sulfur bond fixed to Lys545 amino acid residues could justify the anti-inflammatory activities of both docked ligands as presented in Fig. 4. In the next stage, the candidate's ligands were equally complexed to the antibacterial protein of *Erwinia amylovora* pathogenic strain (4D74.pdb) with BE of −6.27 and −6.31 kcal/mol, respectively, demonstrating two common interactions of Pi-Sulfur bond type detected with Cys9 AA residue more than one common interaction of Pi-Anion type established with Asp115 amino acid residue, more than Alky, Pi-Alkyl, and Pi-Donor Hydrogen bonds detected with Arg115, Cys14, Tyr117, and Glu11 AA residues, as presented in Fig. 5. Moreover, Thymol and *p-cymene*-2-ol compounds were also complexed to the antibacterial protein of *Agrobacterium tumefaciens* pathogenic strain encoded by 5J9R.pdb with BE of −5.61 and −5.37 kcal/mol, respectively, in which three Hydrogen bonds were created towards Il191, Gly193, and Asp85 AA residues in A chain as displayed in Fig. 6. Finally, the same major compounds were complexed to the antibacterial protein from *Allorhizobium vitis* pathogenic strain (4WT7.pdb) with BE of −5.03 and −5.08 kcal/mol, respectively, in which the Thymol compound interacted with the targeted receptor forming

one Hydrogen bond with Gln290 active site in A chain, while *p-cymene*-2-ol compound complexed to the same protein creating two Hydrogen bonds detected towards Arg297 and Glu294 AA residues as resulted in Fig. 7.

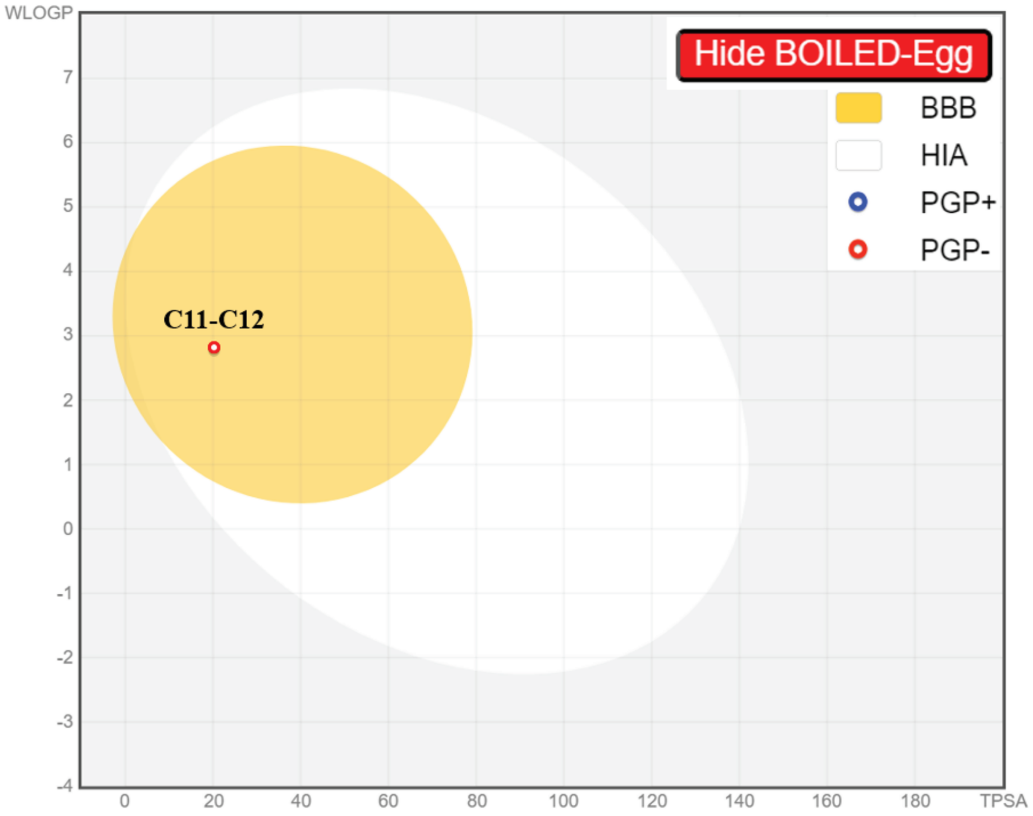

Figure 1: Egan's boild egg model for thymol and *p-cymene*-2-ol

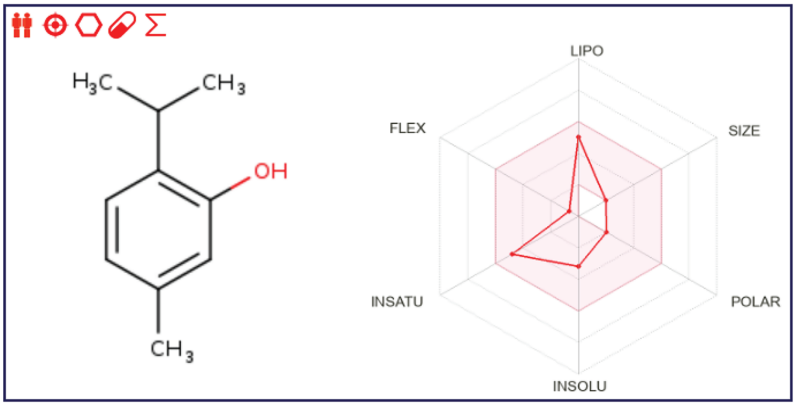

Figure 2: (Continued)

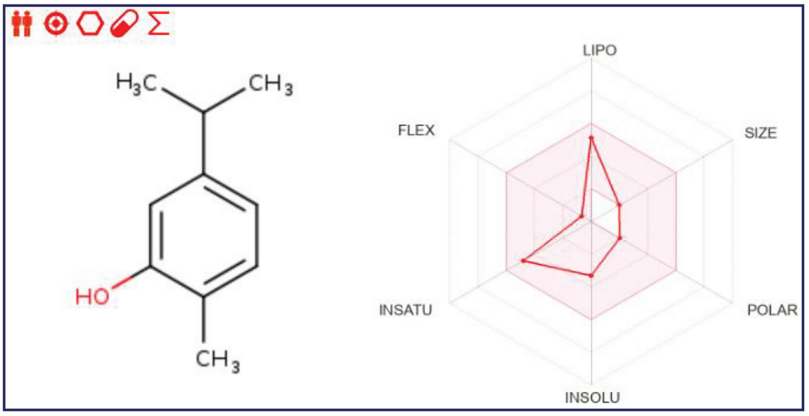

Figure 2: Bioavailability radars for thymol and *p-cymene*-2-ol, respectively

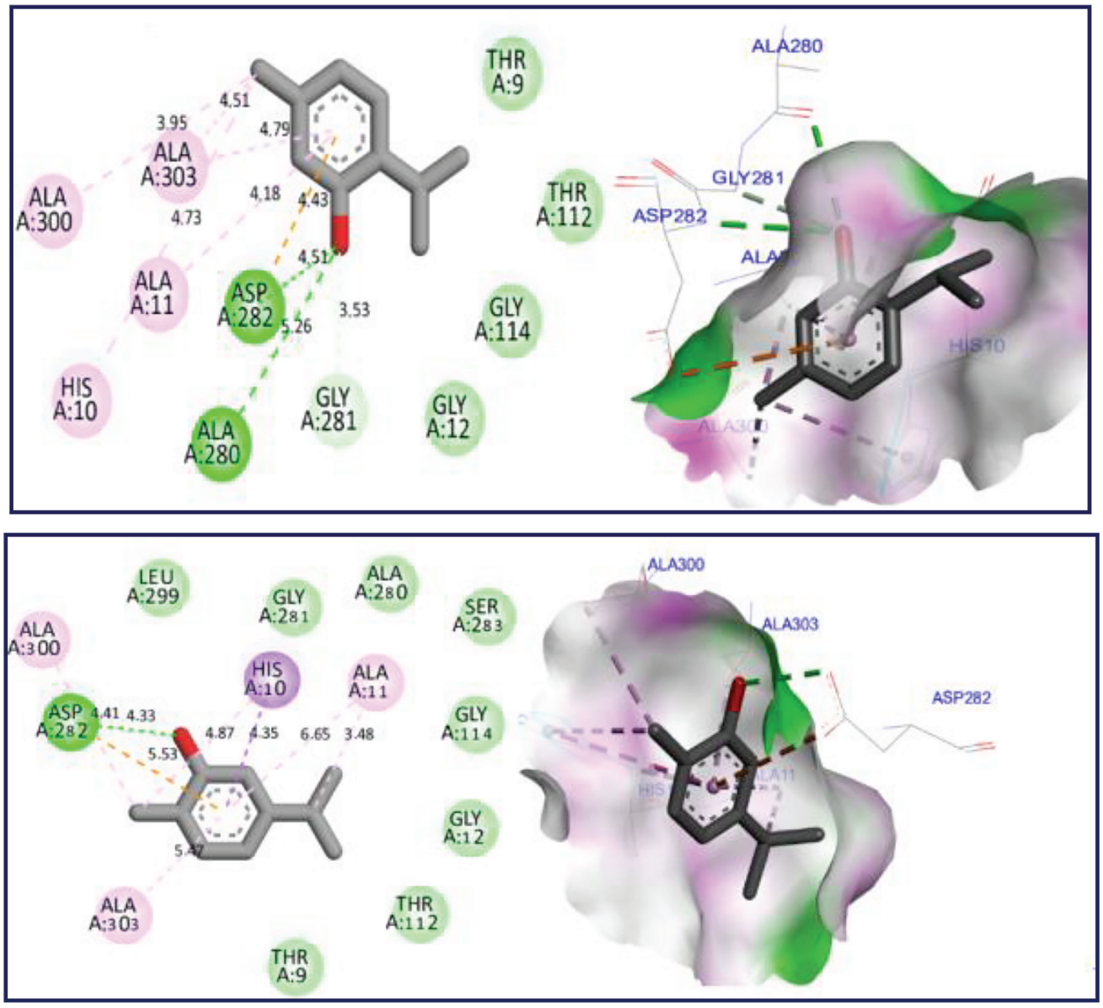

Figure 3: 2D and 3D views of molecular docking interactions against NADPH oxidase protein (2CDU.pdb) for thymol and *p-cymene*-2-ol, respectively

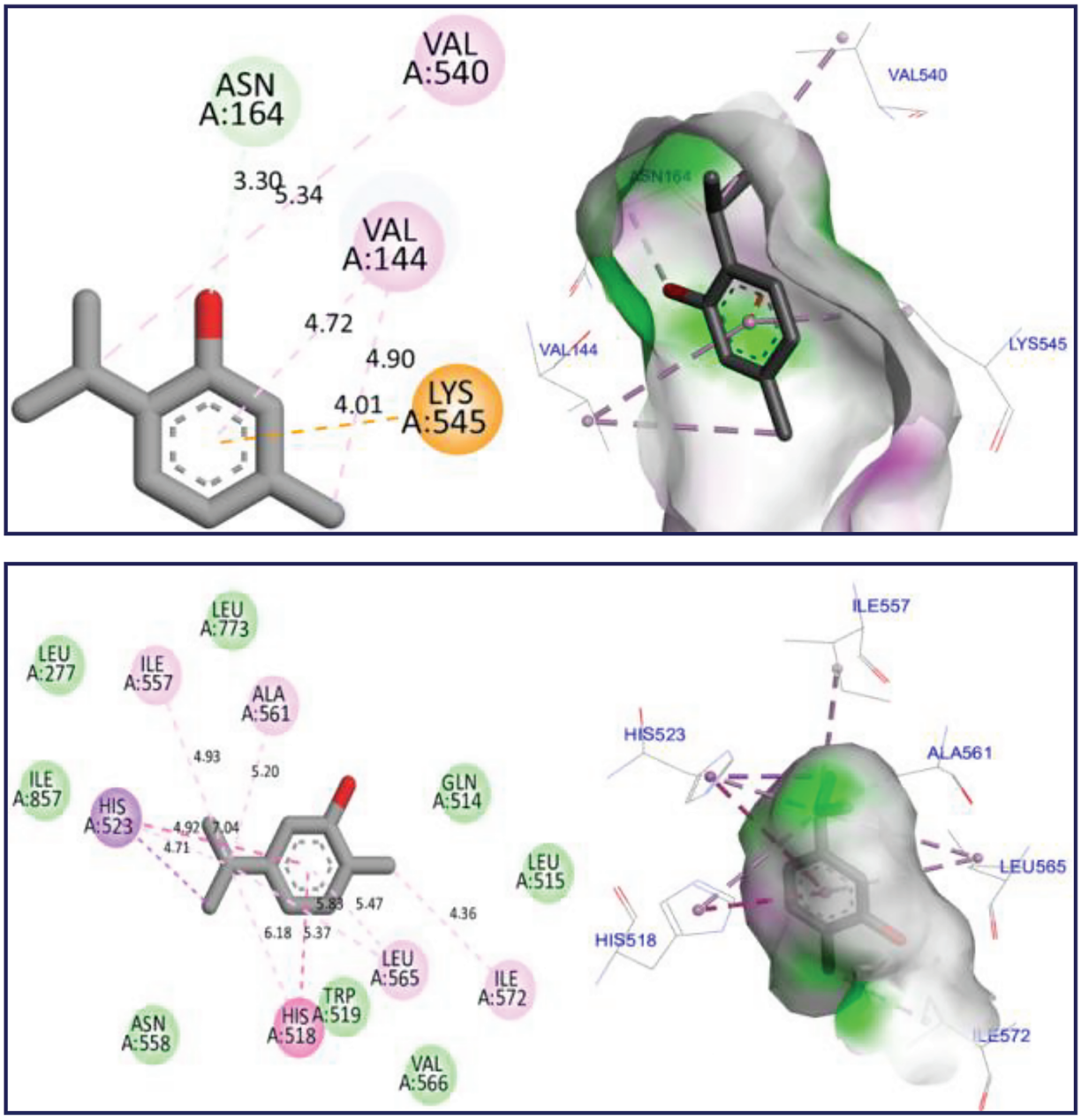

Figure 4: 2D and 3D views of molecular docking interactions against 5-LOX Lipoxygenase enzyme (1N8Q.pdb) for thymol and *p-cymene*-2-ol, respectively

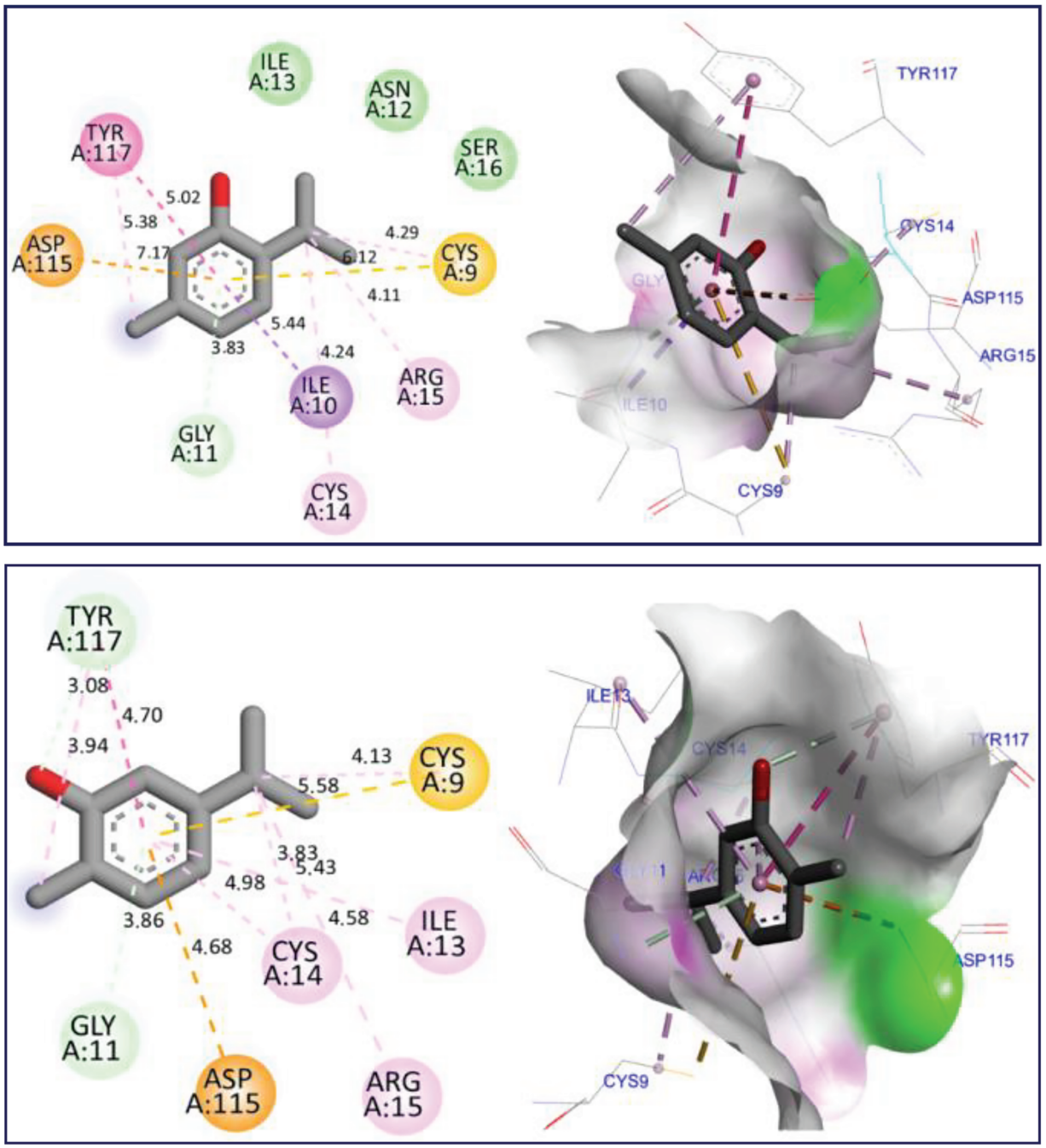

Figure 5: 2D and 3D views of molecular docking interactions against *Erwinia amylovora* pathogenic strain (4D74.pdb) for major compounds thymol and *p-cymene*-2-ol, respectively

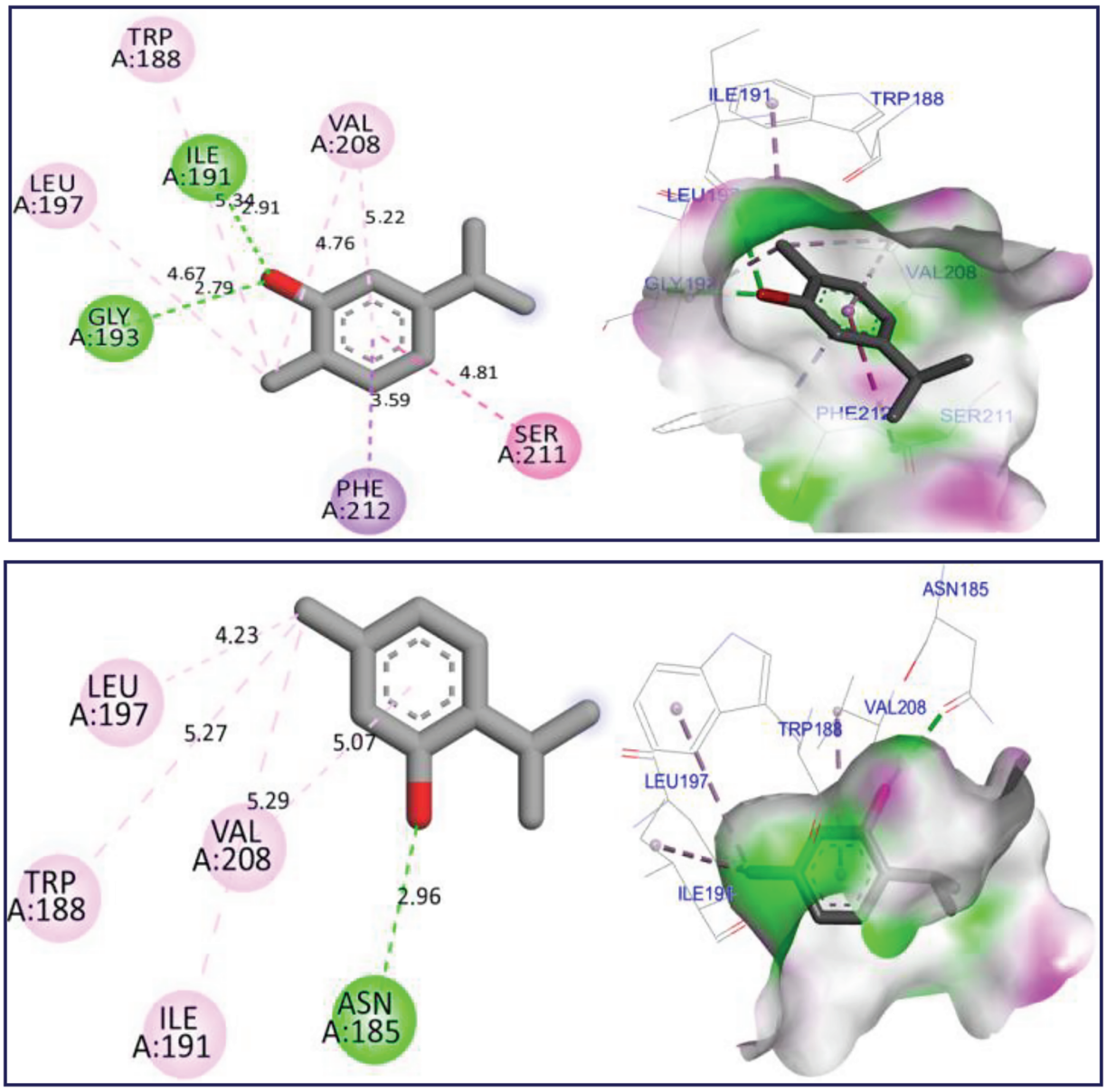

Figure 6: 2D and 3D views of molecular docking interactions against *Agrobacterium tumefaciens* (5J9R.pdb) pathogenic strain for thymol and *p-cymene*-2-ol, respectively

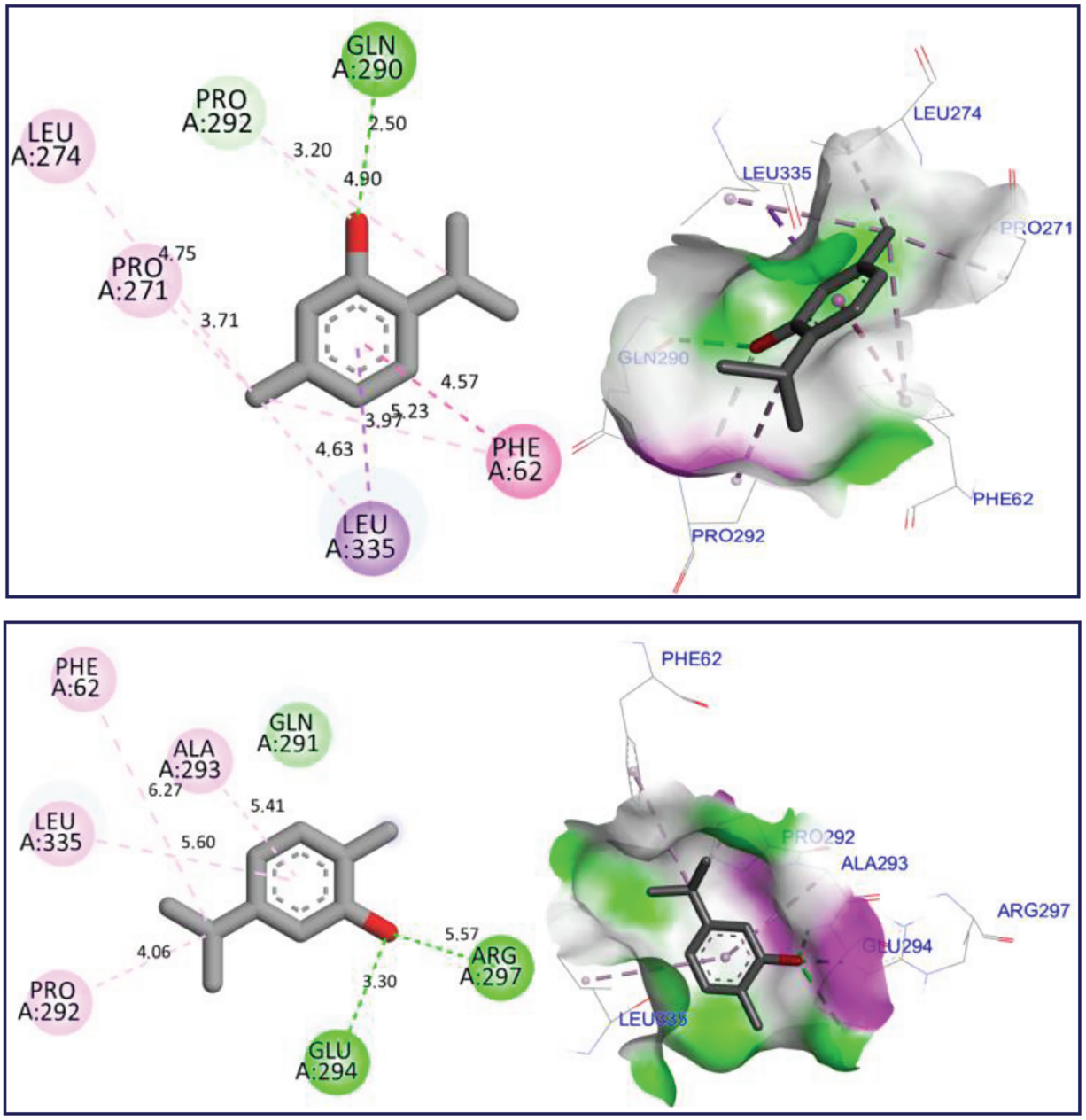

Figure 7: 2D and 3D views of molecular docking interactions against *Allorhizobium vitis* pathogenic strain (4WT7.pdb) for thymol and *p-cymene*-2-ol, respectively

4 Conclusion

O. elongatum EO has shown to be a novel and potent natural source of active substances with significant antibacterial and antioxidant properties. Through GC-MS analysis, a diverse array of chemical constituents, including phenolic compounds and monoterpenes, were identified in *O. elongatum* EO, with several known for their remarkable antibacterial and antioxidant properties. *In vitro* assays demonstrated strong antibacterial efficacy against various phytopathogenic bacteria such as *Erwinia amylovora*, *Agrobacterium tumefaciens*, and *Allorhizobium vitis*, as well as robust antioxidant properties. These dual activities suggest a

possible correlation, as the antioxidant capacity may contribute to the oil's antibacterial efficacy by mitigating oxidative stress in bacterial cells.

Furthermore, *in silico* molecular docking provided further insights into the interactions between the oil's active components and bacterial targets, supporting its mode of action.

The combination of experimental and computational results suggests that *O. elongatum* EO could be a valuable natural agent in the fight against phytopathogens, offering a sustainable and eco-friendly solution for agricultural applications. Our results pave the way for the practical implementation of *O. elongatum* EO as an eco-friendly biocontrol agent in agricultural systems. Its potent antibacterial activity against phytopathogenic bacteria positions it as a viable alternative to synthetic antimicrobial drugs, which are often accompanied by environmental degradation and the emergence of resistant pathogens. By integrating this EO into crop management practices, farmers can enhance disease control while minimizing harmful chemical residues in the soil and water. Moreover, its antioxidant potential contributes to plant health and stress resilience, further supporting sustainable agriculture. This nature-based solution not only endorses ecological balance but also strengthens agricultural productivity and resilience, addressing key challenges in modern farming. Further studies, including field trials and toxicity assessments, are recommended to fully explore its practical applicability.

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