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Phytochemical Composition and Insight into Antibacterial Potential of *Origanum vulgare* Essential Oil from Saudi Arabia Using In Vitro and In Silico Approaches

Omkulthom Al kamaly [1], Ashwag S. Alanazi [1], Raffaele Conte [2] and Hamada Imtara [3,*]

[1] Department of Pharmaceutical Sciences, College of Pharmacy, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia
[2] Research Institute on Terrestrial Ecosystem (IRET), CNR, Via Pietro Castellino 111, 80131 Naples, Italy
[3] Faculty of Arts and Sciences, Arab American University Palestine, Jenin P.O. Box 240, Palestine
* Correspondence: hamada.tarayrah@gmail.com

Abstract: In Saudi Arabia, *Origanum vulgare* is widely disseminated. In the present work, we used GC-MS analysis to identify the components of *Origanum vulgare* essential oil. The disc diffusion assay was used to assess the essential oil's *in vitro* antibacterial efficacy against Gram-positive and Gram-negative *Staphylococcus aureus* and *Escherichia coli*. The antimicrobial efficacy against many microbial proteins, including tyrosyl-tRNA synthetase (TyrRS), DNA gyrase, and dihydrofolate reductase (DHFR), was further evaluated using molecular docking. Eighteen compounds were identified using GC-MS analysis, which constituted 99.81% of the total essential oil content. Terpinen-4-ol (34.3%), Beta-Terpineol (16.96%), 3-Epimoretenol (11.84%), and Alpha-Terpineol (3.86%) were the main substances identified. According to the antibacterial investigation, the inhibition zone against *Staphylococcus aureus* was 8 mm and 6 mm against *Escherichia coli*. High affinities were found between 3-Epimoretenol and tyrosyl-tRNA synthetase (TyrRS) and dihydrofolate reductase (DHFR) compared to positive controls (Clorobiocin, SCHEMBL2181345); the affinity values were −8.3 Kcal/mol and −9.2, respectively. The results of the present study indicate that *Origanum vulgare* essential oil can be used as a nutraceutical to treat infectious diseases.

Keywords: *Origanum vulgare*; essential oils; antimicrobial; gas chromatography; molecular docking

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1. Introduction

Pharmacological industries have created new antibiotics during the past three decades [1]. However, these medicines have not been able to stop the spread of many bacteria with the genetic capacity to spread and develop drug resistance [2]. Therefore, infections with these bacteria are linked to substantial morbidity and death, particularly in individuals who are immunocompromised [3,4].

As a result, interest in alternative and natural antibacterial agents is developing. Alternative agents, such as essential oils (EOs) extracted from fragrant plants, have been suggested [5]. Numerous papers in the literature [6,7] discuss the antibacterial activity of essential oils against a variety of microorganisms. The main mechanisms and sites of action of the different constituents of essential oils are: alteration of the cell wall, degradation of the cytoplasmic membrane, alteration of membrane proteins, leakage of cellular contents, coagulation of the cytoplasm and exhaustion of the force of proton motion [8,9]. In addition, The fundamental benefit of using these natural compounds is that, unlike prolonged use of synthetic antibiotics, they do not increase antibiotic resistance [10]. In addition to having antimicrobial qualities, essential oils and their constituents have also been demonstrated to have antiviral, antimycotic, antitoxic, antiparasitic, and insecticidal qualities, which may be connected to how these substances work in plants [11]. These activities are due to the fact that essential oils contain a complex mixture of volatile compounds that are

characterized by their low molecular weight and hydrophobicity. Among them are carbides (ex. limonene), alcohols (ex. geraniol), aldehydes (ex. cinnamaldehyde), ketones (ex. carvone), acids (ex. cinnamic acid), esters (ex. bornyl acetate), ethers (ex. 1,8-cineole), peroxides (ex. ascaridole), and phenols (ex. thymol and carvacrol) [12].

Origanum vulgare (oregano), one of numerous Origanum species from the Lamiaceae family are widely distributed. Oregano is a herbaceous or sub-woody plant at the base, 30 to 90 cm tall, with square stems bearing about forty branches with small, oval dark green leaves [13]. The inflorescences are spike-like and united in compound inflorescences. The calyx of oregano is tubular with five short teeth, bilabiate or not. The corolla is white, pink or purple [14]. The genus Origanum is mainly distributed around the Mediterranean basin. Of the Origanum species, 81% (35 out of 43 species) are found exclusively in the eastern Mediterranean, mainly in Turkey, Greece, and the Middle East. The species *Origanum vulgare* is also widely found in Eurasia and North Africa [15]. In this context, *Origanum vulgare* is one of the most common spices used in many foods. In addition, its oil and its compound are not limited to the use of aroma and flavor, but rather it has shown its effectiveness in many biological activities, including antioxidants (as a result of containing phenols, which play a role in neutralizing free radicals and also in the decomposition of peroxides) [16], antifungal [17], antibacterial [18], anticancer (explained by the potential of HE21 to inhibit the penetration of mutagens into cells; inactivating mutagens by trapping; directly capturing radicals produced by a mutagen or activating cellular antioxidant enzymes; to inhibit the metabolic conversion of cytochrome P450 by mutagens; or to activate the enzymatic process of detoxification of mutagens) [19], insecticidal [20], herbicidal [21], and nematicidal [22] properties. According to several studies, oregano essential oil has been shown to be analgesic, emmenagogue, decongestant, diaphoretic, vermicidal, antipyretic, anti-inflammatory, sedative, antimutagenic, larvicidal, laxative, nacritic, pectoral, tonic, and diuretic [23]. As a result, oregano essential oil is used in the composition of several pharmaceutical and parapharmaceutical preparations [24]. Its volatile oil contains up to 70% monoterpenoids (thymol and carvacrol) and sesquiterpenoids, dependent on geographic origin and plant developing stage [22,25].

The aim of the present study was to determine the main compounds in the essential oil of *Origanum vulgare* plant that was harvested from Saudi Arabia using GC-MS analysis and compare it with other studies, as well as investigate its antibacterial activity on antibiotic-resistant strains and clarify the antibacterial mechanism through in silico receptor-ligand docking study.

2. Materials and Methods

2.1. Chemicals

Mueller–Hinton agar and Mueller–Hinton broth were purchased from Biokar Diagnostics, Beauvais, France; dimethyl sulfoxide (DMSO) and Hexane were purchased from Sigma-Aldrich, Darmstadt, Germany.

2.2. Plant Material and Essential Oil Extraction

Origanum vulgare aerial portions were taken in March from Altaif city in Saudi Arabia's southern area during the spring season (2022). The plant was harvested, dried in the shade at room temperature, and powdered. The essential oil of *Origanum vulgare* plant was extracted using a Clevenger-type hydrodistillation equipment for 3 h (100 g plant material in 1 L water). The essential oil produced was stored at +4 °C until tested and evaluated.

2.3. Gas Chromatographic Analysis

The essential oil of *Origanum vulgare* plant was diluted with hexane at a ratio of 1:10 [26]. The GC-MS analysis to determine the volatiles compounds was performed at the research center of College of Pharmacy, King Saud University, Riyadh, by using a Clarus 500 PerkinElmer (30 m × 0.25 mm ID × 1 µm of capillary column) gas chromatograph equipped and coupled to a mass detector Turbo Mass Gold according to method described

by Khalil et al. [27]. The initial column temperature was programmed to 110 °C and maintained for 2 min. At an increment rate of 5 °C/min, the temperature was increased to 280 °C and kept for a period of 9 min. A volume of 1 µL of diluted oil was injected in temperature ensured as 250 °C with helium gas flow at rate 1 mL/min. The ionization voltage was 70 eV and the mass spectral scan range was set at 45–450 (*m/z*). By contrasting the mass spectra obtained with the mass spectra from the Adams Library [28] and the Wiley GC/MS Library (McLafferty and Stauffer, 1989) [29], the chemical makeup of the *Origanum vulgare* essential oil was determined.

2.4. Antimicrobial Activity Determination

2.4.1. Micro-Organism Tested

Gram-positive (*Staphylococcus aureus:* ATCC 25923) and Gram-negative (*Escherichia coli*: ATCC 25922) strains were used in this study. The strains are clinical isolates which are an important opportunistic pathogens in humans and isolated in Seattle, United States. The strains are commonly used in many studies as a quality control strains for antibiotics sensitivity and commercial products [30,31].

2.4.2. Preparation of Culture Media

Muller–Hinton agar medium (MH) was obtained by dissolving 38 g of Muller–Hinton agar medium in 1 L of distilled water (pH is 7.5 ± 0.2) and Muller–Hinton broth (21 g/L in distilled water). Each medium was sterilized in an autoclave at a temperature of 115 °C for 15 min.

2.4.3. Inoculum Preparation

Colonies were taken from 24 h cultures and suspended in sterile saline (0.9% NaCl). After vortexing for 15 s, the density of suspended was adjusted to a turbidity of 0.5 McFarland by using a spectrophotometer method. The final concentration of the inoculum was approximately 10^8 cfu/mL [32–34].

2.4.4. Disk Diffusion Assay

The antibacterial activity of Origanum vulgare essential oil was determined using the disc diffusion assay. A volume of 100 µL of suspensions (10^8 CFU/mL) in the growing phase was spread on Mueller–Hinton agar medium [35]. A volume of 20 µL of Origanum vulgare essential oil (dissolved in dimethyl sulfoxide (DMSO 2%); *v*:*v*) was added to 6 mm filter paper disk and placed on the inoculated Petri dishes. Then, the dishes were incubated at 37 °C for 24 h. The inhibition zone was measured to evaluate the antibacterial activity against the microorganism studied.

2.5. Molecular Docking

2.5.1. Preparation of the Ligand

From PubChem, we acquired the SDF format of beta terpineol (CID: 8748), terpinen-4-ol (CID: 11230), and 3-epimoretenol (CID: 604951). Using MyPol software, the SDF file was converted to PDB format and then to PDBQT by using AutoDock Tools v1.5.7. The nonpolar hydrogen atoms were combined, Gasteiger partial charges were added, and rotatable bonds were established for the finished ligand production.

2.5.2. Preparation of the Receptors

The PDB file for each receptor was downloaded from the Protein Data Bank website. The X-ray crystal structures of the receptors were chosen due to their completeness, resolution, and suitability for our research objective. Table 1 gives details about the chosen receptors. To prepare the receptors in the form of PDBQT, Discovery Studio Visualizer v19.1.0 was used at the beginning, which removes water molecules and heteroatoms. After that AutoDock Tools v1.5.6 was used to add Gasteiger charges and polar hydrogen atoms.

Table 1. Description of the studied receptors.

Receptors	ID	Class
TyrRS	1jij	Ligase
DNA gyrase	1KZN	Isomerase
DHFR	3fyv	Oxidoreductase

TyrRS: tyrosyl-tRNA synthetase, DHFR: dihydrofolate reductase.

2.5.3. Docking Simulation

To run the docking simulations between receptors and molecules that used in this study, we used a AutoDock Vina 1.1.2 software.

2.5.4. Docking Validation Protocol

To examine the accuracy of the molecular docking algorithms, we compared the active sites experimentally produced by the X-ray diffraction method to the results of intermolecular interactions using the same previous methodology. Next, we applied the re-docking process, based on the overlay of the co-crystallized ligand bound to each target protein (coded 1JIJ, 1KZN, and 2FYV) on the docked co-crystallized ligand using the molecular docking simulation [36,37].

3. Results and Discussion

3.1. Phytochemical Analysis of Origanum vulgare

The *Origanum vulgare* essential oil was examined using a GC/MS system and the chromatogram results present in Figure 1.

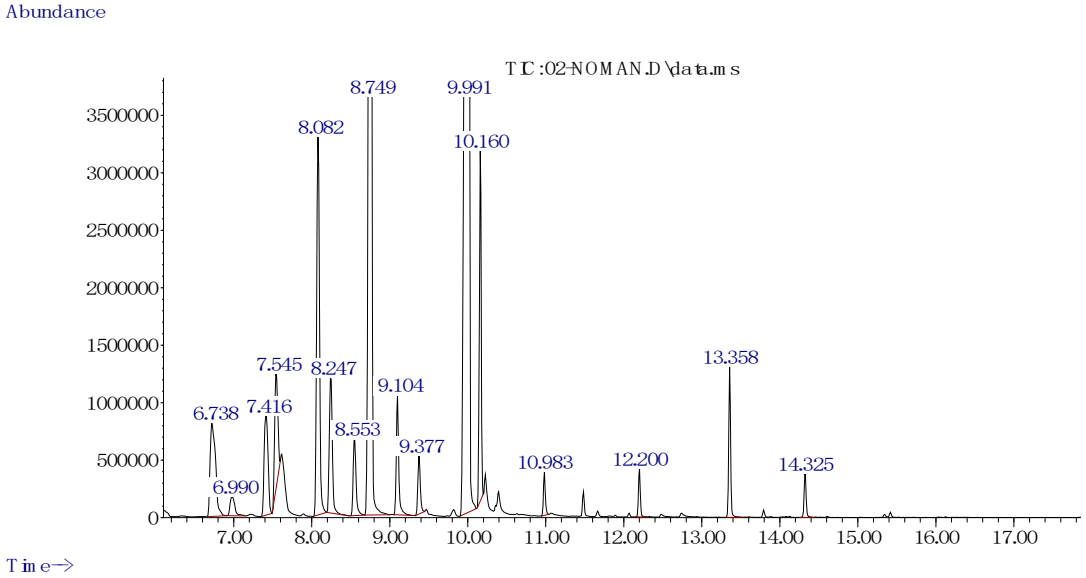

Figure 1. GC-MS chromatogram of *Origanum vulgare*.

The chemical composition of the essential oil of *Origanum vulgare* plant was analyzed by the gas chromatography–mass spectrometry (GC-MS) method. Table 2 and Figure 2 lists the elements along with their respective proportions (% of Area). A total of 18 compounds representing 99.98% of the total content were determined by GC/MS. The principal components of the essential oil of *Origanum vulgare* were terpinen-4-ol (43.32%), beta-terpineol (16.96%), 3-epimoretenol (11.84%), gamma-terpinen (6.35%), and alpha-terpieol (3.86%). These compounds constituted 82.33% of the total content, while the rest of the compounds constituted 17.65%. It should be noted that terpinen-4-ol was the higher content in this essential oil with an important concentration compared to the others. The results in the

present work are consistent with those conducted by Busatta et al., which showed that terpinen-4-ol is the main compound with a ratio of 21.43% [38]. However, Imtara et al. found that the main constituents in *Origanum vulgare* essential oil from Morocco were carvacrol (48.38%), thymol (26.55%), and γ-terpinene (7.90%) [26]. In the same country, Hayani et al. identified thymol (38.59%), carvacrol (26.65%), and o-cymene (14.33%) as the main compounds in this essential oil [39]. Similarly, *Origanum vulgare* essential oils from Italy are characterized by a high percentage of thymol, carvacrol, linalyl acetate, and γ-terpinene in greater quantities [40]. However, Portuguese researchers found that carvacrol, thymol, γ-terpinene, and β-fenchyl alcohol are considered the main molecules present in *Origanum vulgare* essential oil [41]. In 2018, a study conducted by Stešević et al. on *Origanum vulgare* essential oil from Montenegro showed that β-caryophyllene, linalyl acetate, and α-terpineol are the main compounds [42]. However, the variation seen in the percentages of the main components of the essential oils analyzed in this and several studies could be attributed to the origin of the plant, different environmental influences (geographical, seasonal and climatic conditions, sunlight, and salinity, as well as the effect of crop, and time of harvest), genetic background, and the extraction method of the plants [43–45]. As a result, the influence of these factors on biosynthetic pathways causes differences in the qualitative and quantitative terms of the characteristic majority chemicals, which result in the existence of several chemotypes distinguishing essential oils from various origins [46].

Table 2. The chemical composition of *Origanum vulgare* essential oil using GC-MS analysis.

#	Compound Name	Chemical Formula	Molecular Weight (g/mol)	RT (min)	Area %
1	Beta-Terpinene	$C_{10}H_{16}$	136.23	6.73	2.94
2	Beta-Myrcene	$C_{10}H_{16}$	136.23	6.99	0.53
3	2-Carene	$C_{10}H_{16}$	136.23	7.42	2.29
4	O-Cymene	$C_{10}H_{14}$	134.22	7.55	2.21
5	Gamma-Terpinene	$C_{10}H_{16}$	136.23	8.08	6.36
6	Terpinolene	$C_{10}H_{16}$	136.23	8.55	1.31
7	Beta-Terpineol	$C_{10}H_{18}O$	154.25	8.75	16.97
8	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans-	$C_{10}H_{18}O$	154.25	9.1	1.71
9	Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1α,2α,5β)-	$C_{10}H_{18}O$	154.25	9.38	0.85
10	Terpinen-4-ol	$C_{10}H_{18}O$	154.25	9.99	43.32
11	Alpha-Terpineol	$C_{10}H_{18}O$	154.25	10.16	3.86
12	Linalyl anthranilate	$C_{17}H_{23}NO_2$	273.37	10.98	0.48
13	Caryophyllene	$C_{15}H_{24}$	204.35	13.36	1.65
14	1,5-Heptadiene, 2,5-dimethyl-3-methylene-	$C_{10}H_{16}$	136.23	14.33	0.53
15	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	264.4	21.03	0.48
16	3-Epimoretenol	$C_{30}H_{50}O$	426.7	25.07	11.84
17	(8S,14) Cedran-diol,	$C_{15}H_{26}O$	238.4	27.93	1.43
18	Cycloeucalenyl acetate	$C_{32}H_{52}O_2$	468.8	28.33	1.22

RT: Retention time.

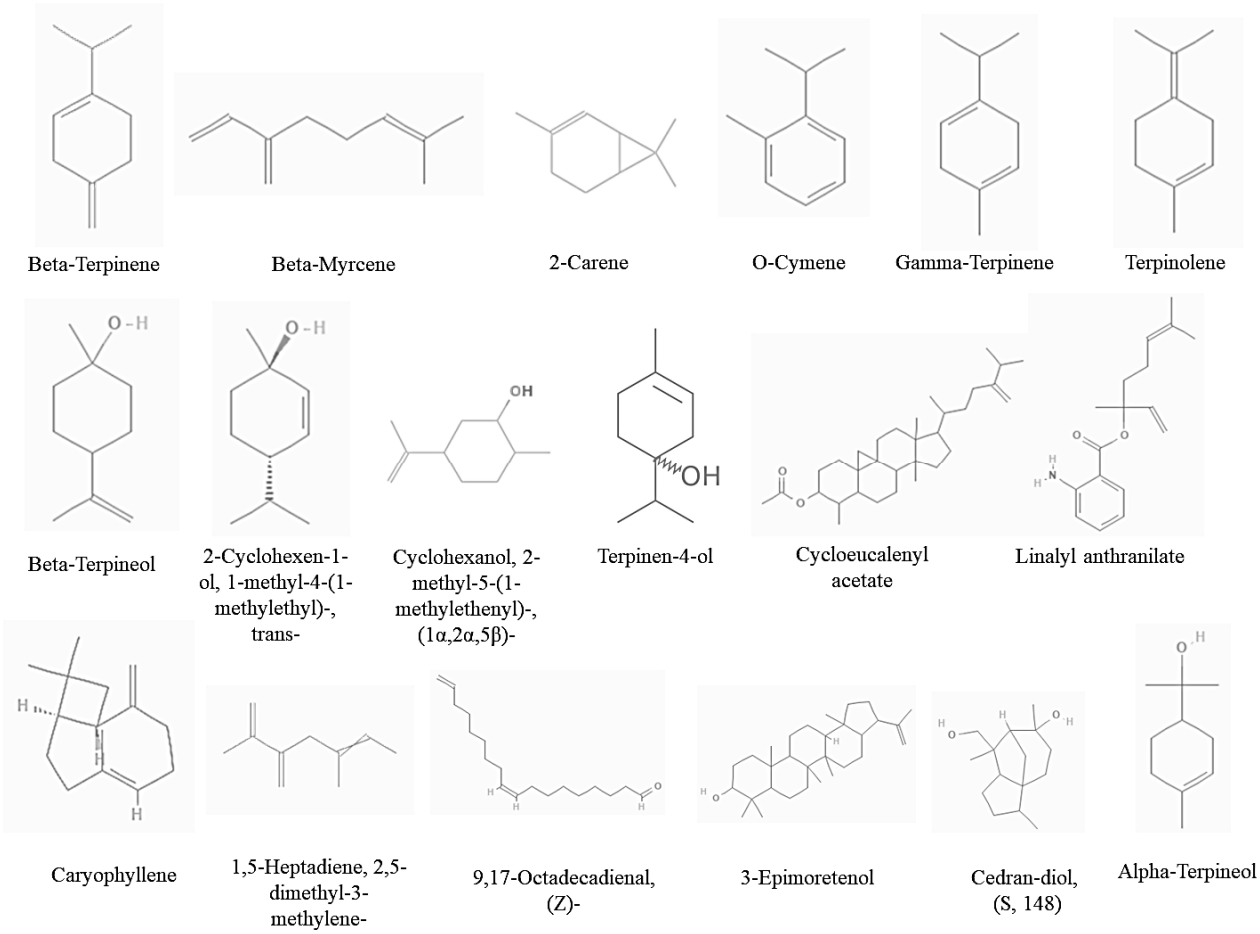

Figure 2. The chemical structures of compounds identified in *Origanum vulgare* essential oil.

3.2. Antimicrobial Activity

The disc diffusion method is acknowledged as a practical semi-quantitative approach for assessing the susceptibility of bacteria to various chemicals [38,47]. By examining the existence of inhibitory zone values, the antibacterial activity of *Origanum vulgare* essential oil against the pathogens taken into account in this investigation was determined. The results show that the greatest inhibition zones (8 mm) and the highest activity were seen against *Staphylococcus aureus* (Table 3). However, the essential oil antibacterial activity was effective against *Escherichia coli* as well (6 mm). These results support Gram-positive bacteria being more sensitive than Gram-negative bacteria [48]. Also shown in Table 3, it was observed that *Staphylococcus aureus* strain was resistant to two types of antibiotics (Penicillin G and Fusidic acid), while *Escherichia coli* strain was resistant to five antibiotics, namely, ciprofloxacin, trimethoprim/sulfamethoxazole, cefotaxime, amoxicillin, and cefuroxime. The essential oils are characterized mainly by their hydrophobicity. This allows their solubilization in the membranes, which causes a destabilization of the structure and an increase in the membrane permeability [49]. These changes lead to leakage of ions and intracellular compounds. Loss of cytoplasmic material results in cell bursting [50]. The mode of action of certain antibacterial molecules has been described in the literature. Chemical compounds of essential oils known for their antimicrobial effectiveness and broad spectrum are phenols (thymol, carvacrol, and eugenol), alcohols, (α-terpineol, terpinen-4-ol, and linalool), aldehydes, ketones, and, more rarely, carbides [51]. Terpinen-4-ol is the main compound identified in the present work and this compound is known for its antibacterial activity. This compound acts on many bacterial species, including Gram-positive and Gram-negative bacteria, and is highly effective against MRSA (Methicillin-resistant *Staphylococcus aureus*) infection [52]. In addition, according to Lambert et al., the combination of the main

active compounds would act synergistically by potentiating the antimicrobial action of the essential oil [53].

Table 3. Inhibition zone generated by *Origanum vulgare* essential oil on bacteria studied and list of antibiotics that bacteria have resisted.

	Escherichia coli	***Staphylococcus aureus***
Origanum vulgare essential oil	6 mm	8 mm
DMSO	-	-
Antibiotic resistant	CXM, AML, CTX, SXT, CIP	PG, FA

-: not active, mm: millimeter, DMSO: Dimethyl sulfoxide, PG: Penicillin G, FA: Fusidic acid, CXM: Cefuroxime, AML: Amoxicillin, CTX: Cefotaxime, SXT: Trimethoprim/sulfamethoxazole, CIP: Ciprofloxacin.

A study was carried out by Sahin et al. on 24 bacteria, including two types of *Staphylococcus aureus*, one of which was found to be resistant to the antibacterial activity of *Origanum vulgare* essential oil, and the other had a diameter of inhibition 10 mm, which is consistent with our results. Another study conducted by Busatta et al. on nine types of bacteria showed that all of these species are sensitive to the antibacterial effect of *Origanum vulgare* essential oil, with the exception of *Pseudomonas aeruginosa* [38]. In addition, the inhibition zone of *Origanum vulgare* essential oil in his study on *Escherichia coli* was 17.33 mm and on *Staphylococcus aureus* was 26.8 mm and these results are better than those in our study. The reason for this difference may be due to the fact that Origanum oil, in a Busatta et al. study, contained carvacrol and thymol compounds, which is known to antibacterial activity [54,55]. A study carried out on *Bacillus cereus* has elucidated the mode of action of carvacrol. This compound crosses the lipid bilayer and is localized between the chains of fatty acids. This deformation of the structure increases the membrane fluidity, leading to a modification of the passive permeability. A decrease in intracellular ATP was noted in bacteria exposed to carvacrol, as well as a decrease in membrane potential [8]. Concerning thymol mechanism action, a study showed that thymol alters membrane permeability, causes loss of intracellular substances, and interacts with intracellular sites [8]. However, it is important to note that several factors can affect the diameter of the inhibition zones of essential oils among them the diffusion and viscosity of the essential oils [8]. In 2018, Hamada et al. conducted a study on the synergistic effect of *Origanum vulgare* essential oil with different types of honey of different plant origin. The study showed that when mixing the oil with honey, it enhances the antibacterial activity of honey. At the same time, this mixture reduces the toxicity of essential oils [31].

3.3. Docking Validation

The results presented in Figure 3 demonstrate that ASP195, ASP40, Tyr170, GLN174, ASP80, ASP177, and TYR36 amino acids residues are the active sites of 1JIJ.pdb protein in A-chain. Two of these intermolecular interactions are obtained computationally using molecular docking simulation as ASP40 and ASP195 amino acids residues. In addition, the result of superposition show that root mean square deviation (RMSD equal to 1.375 Å) is minimal and less the threshold 2 Å.

The results presented in Figure 4, show that ASN46, ASP73, and ARG136 amino acids residues, are the active sites of 1KZN.pdb protein in A-chain. ASN46 amino acid is one of these three intermolecular interactions, which is produced computationally using molecular docking simulation. In addition, the result of superposition shows that root mean square deviation (RMSD equal to 1.987 Å) is minimal and below the threshold 2 Å.

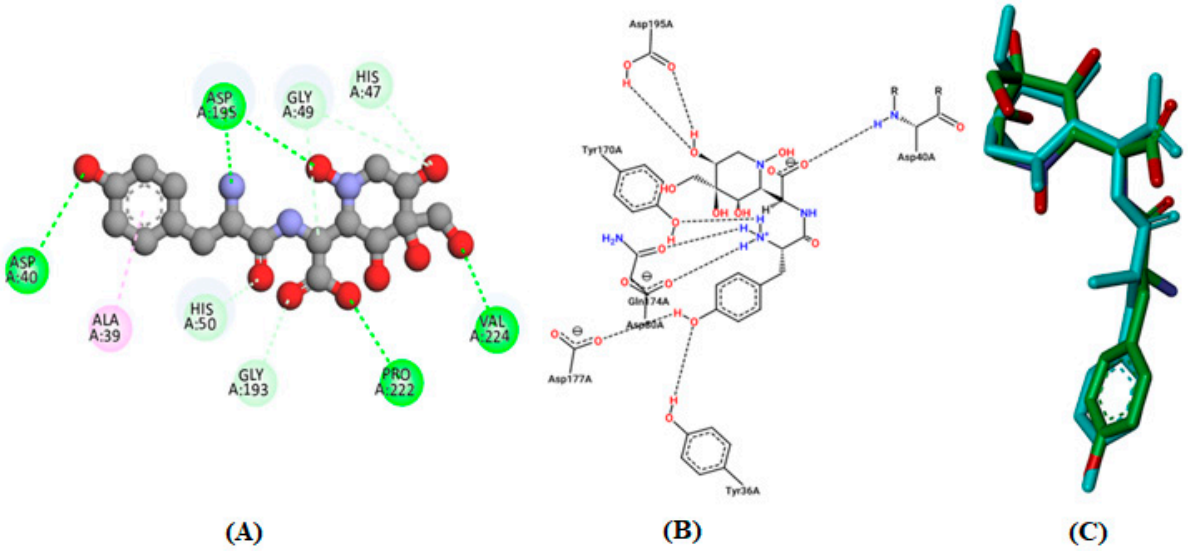

Figure 3. The results of intermolecular interaction between 1JIJ.pdb protein and co-crystallized ligand, which are produced computationally (**A**) and experimentally (**B**), and the result of superposition pose with RMSD of 1.375 Å (**C**).

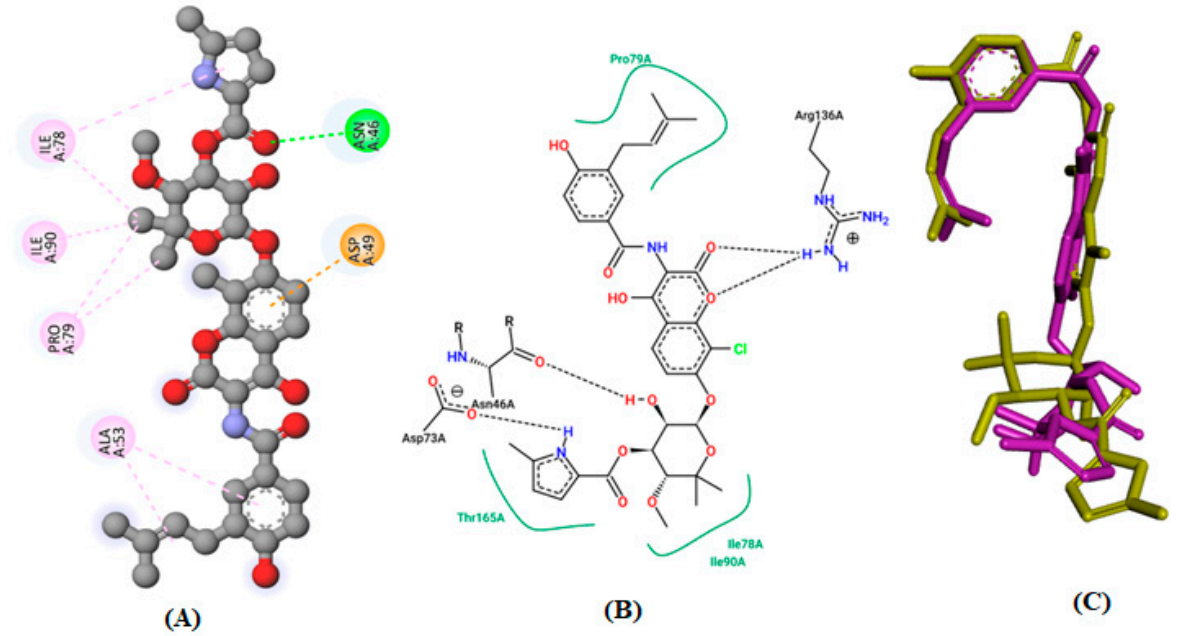

Figure 4. The results of intermolecular interaction between 1KZN.pdb protein and co-crystallized ligand, which are produced computationally (**A**) and experimentally (**B**), and the result of superposition pose with RMSD of 1.987 Å (**C**).

The results presented in Figure 5 show that LEU5, PHE92, and ASP27 amino acids residues are the active sites of 2FYV.pdb protein in X-chain. ASP27 and PH92 amino acids residues are two of these three intermolecular interactions, which are produced computationally using molecular docking simulation. In addition, the result of superposition show that root mean square deviation (RMSD equal to 1.948 Å) is minimal and below the threshold 2 Å. Consequently, the process of molecular docking is successfully validated.

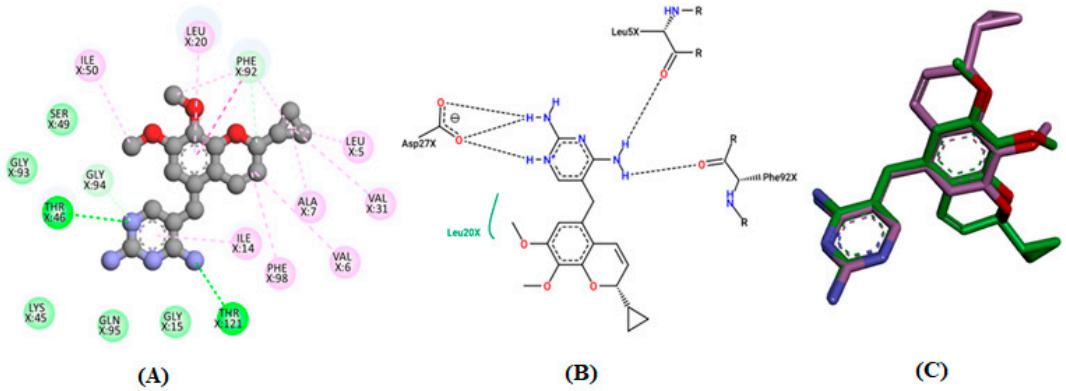

Figure 5. The results of intermolecular interaction between 1JIJ.pdb protein and co-crystallized ligand, which are produced computationally (**A**) and experimentally (**B**), and the result of superposition pose with RMSD of 1.948 Å (**C**).

3.4. Molecular Docking

Based on the results of the GC/MS, the major abundant compounds (Beta-terpineol, Terpinen-4-ol and 3-epimoretenol) were selected to clarify their role in antibacterial activity by conducting a molecular docking analysis between these compounds and the receptors that were selected based on several studies [56–58]. The results of affinities between the compounds and receptors are shown in the Table 4. In addition, chlorobiocin and SCHEMBL2181345 were used as positive controls to evaluate the outcomes of the molecules with receptors.

Table 4. Affinity results of the selected receptors and molecules.

	Affinities (Kcal/mol)		
	TyrRS	**DNA Gyrase**	**DHFR**
Beta-terpineol	−4.4	−6.1	−5.7
Terpinen-4-ol	No interaction	−6.6	−6.1
3-epimoretenol	−8.3	−7.4	−9.2
Clorobiocin	−8.2	−9.1	_
SCHEMBL2181345	_	_	−6.3

TyrRS: Tyrosyl-tRNA synthetase; DHFR: dihydrofolate reductase; _: no affinity.

The 3-epimoretenol and beta-terpineol compounds showed good affinities with the tyrosyl-tRNA synthetase (TyrRS) receptors with values of −8.3 Kcal/mol and −4.4 Kcal/mol, respectively, while there was no binding between terpinen-4-ol and the same receptor. (Figure 6). In addition, the value of the affinity between the positive control (Clorobiocin) and the tyrosyl-tRNA synthetase (TyrRS) was −8.2 Kcal/mol. This indicates that the interaction between the 3-epimoretenol compound and the tyrosyl-tRNA synthetase (TyrRS) receptor is almost similar to the interaction between the Clorobiocin and the tyrosyl-tRNA synthetase (TyrRS) receptor.

Among the tested compounds, 3-epimoretenol had the best affinity value with DNA gyrase receptor (−7.4 Kcal/mol), but it was less than that between the DNA gyrase receptor and the Clorobiocin (−9.1 Kcal/mol). Beta-terpineol had the lowest affinity value, which was −6.1 Kcal/mol (Figure 7). While the value of the affinity between terpinen-4-ol and DNA gyrase receptor is −6.6 Kcal/mol.

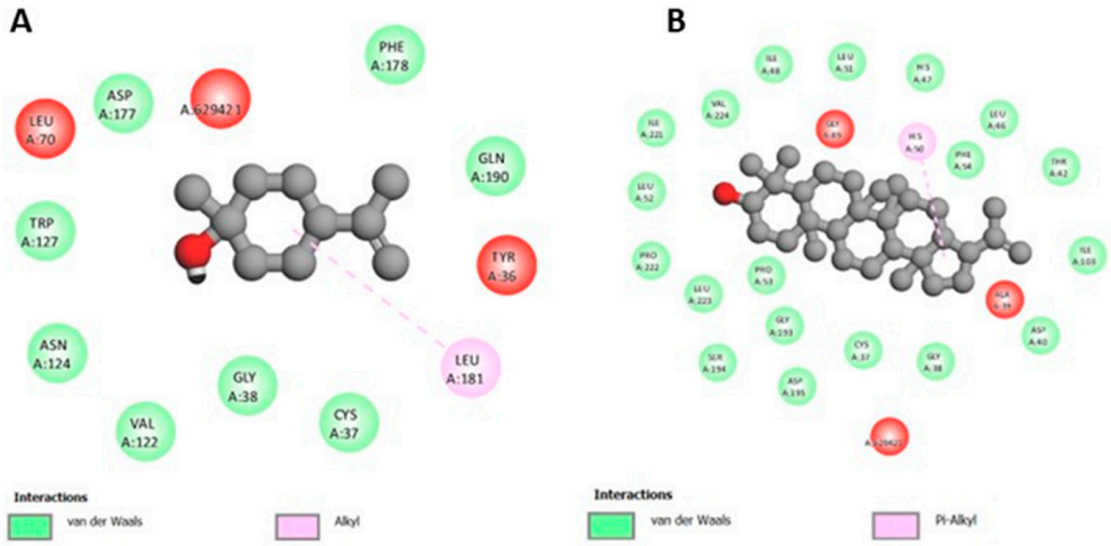

Figure 6. The interaction between tyrosyl-tRNA synthetase (TyrRS) and tested compounds. (**A**): Beta-terpineol; (**B**): 3-epimoretenol.

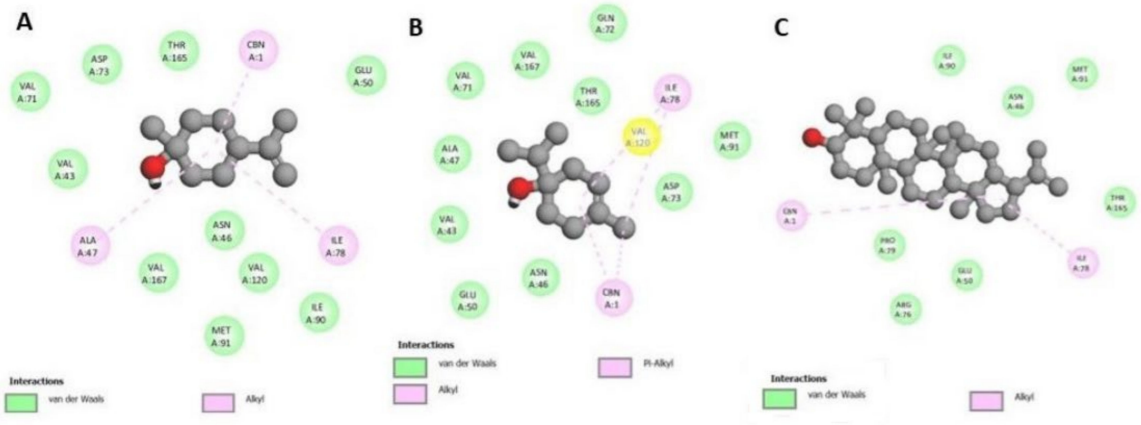

Figure 7. The interaction between DNA gyrase and tested compounds. (**A**): Beta-terpineol; (**B**): Terpinen-4-ol (**C**): 3-epimoretenol.

With regard to the dihydrofolate reductase (DHFR) receptor, it interacted with the three tested compounds; the highest value was for the 3-epimoretenol compound with a value of −9.2 Kcal/mol (Figure 8), followed by the terpinen-4-ol and beta-terpineol compounds with values of −6.1 Kcal/mol and −5.7 Kcal/mol, respectively. The value of the affinity between the SCHEMBL2181345 and the dihydrofolate reductase (DHFR) receptor was −6.3 Kcal/mol, which is less than that found with 3-epimoretenol. This indicates that the interaction between the 3-epimoretenol compound and the dihydrofolate reductase (DHFR) receptor is better than the interaction between the SCHEMBL2181345 and the dihydrofolate reductase (DHFR) receptor.

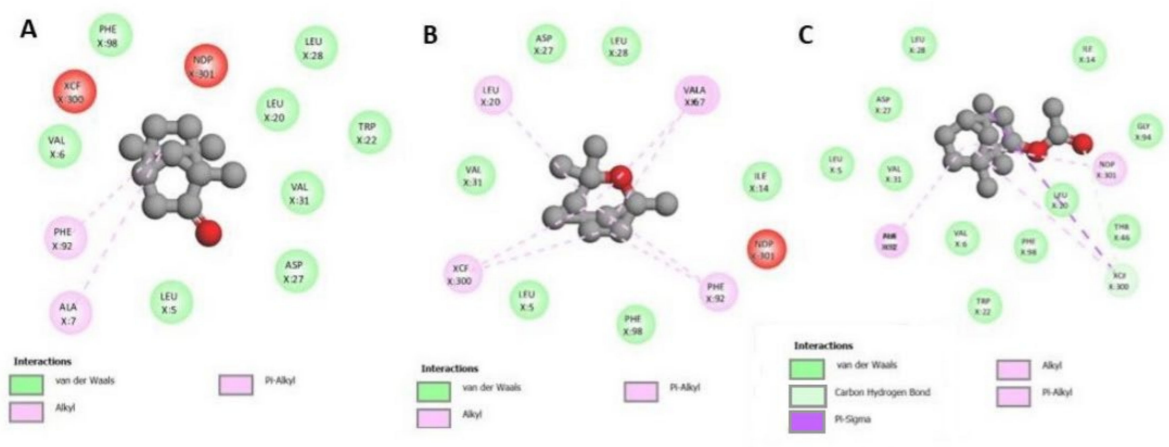

Figure 8. The interaction between dihydrofolate reductase (DHFR) and tested compounds. (**A**): Beta-terpineol; (**B**): Terpinen-4-ol (**C**): 3-epimoretenol.

4. Conclusions

The aim of the present study was to determine the chemical compounds and antibacterial activity of *Origanum vulgare* essential oil. The GC-MS results in the present work showed that terpinen-4-ol, beta-terpineol and 3-epimoretenol are the main compounds of *Origanum vulgare* essential oil. The results of the antibacterial activity have indicated that *Origanum vulgare* essential oil can be considered a potent against the antibiotic-resistant bacteria that were used in the present study. In addition, the results of molecular docking showed that among the three main compounds, 3-epimoretenol had the best interaction with the tyrosyl-tRNA synthetase (TyrRS), DNA gyrase, and dihydrofolate reductase (DHFR) receptors followed by beta-terpineol, and this confirms the antibacterial activity of this essential oil. According to the findings, the present study indicates that *Origanum vulgare* essential oil can be used as a nutraceutical to treat infectious diseases.

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Abbreviation

GC-MS	Gas chromatography–mass spectrometry
TyrRS	Tyrosyl-tRNA synthetase
DHFR	Dihydrofolate reductase
EO	Essential oil
DMSO	Dimethyl sulfoxide

CFU	Colony forming unit
PDB	Protein data bank
RT	Retention time
mm	Millimeter

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