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ARTICLE

Ecological Value of *Ormenis mixta* Essential Oil from Pre-Rif Morocco as a Natural Antifungal Agent for Sustainable Agriculture

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ABSTRACT

Growing concerns regarding the environmental and health impacts of synthetic pesticides have stimulated increasing interest in plant-derived antimicrobial agents as sustainable alternatives. In this study, the essential oil (EO) of *Ormenis mixta* collected from the Pre-Rif region (Taza, Morocco) was extracted by hydrodistillation using a Clevenger-type apparatus and chemically characterized by gas chromatography–mass spectrometry (GC–MS). Antimicrobial activity was evaluated using the broth microdilution method according to CLSI guidelines against Gram-positive bacteria (Staphylococcus aureus, Bacillus subtilis), Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*), and the opportunistic yeast Candida albicans. The EO yield reached 0.55% (w/w). Chemical analysis revealed a composition dominated by aromatic hydrocarbons (51.18%) and polycyclic aromatic compounds (20.89%), whereas oxygenated monoterpenes were detected in lower proportions. The lowest minimum inhibitory concentration (MIC) was recorded against *C. albicans* (6.50 ± 0.76 mg/mL), indicating notable antifungal activity. Gram-positive bacteria exhibited moderate susceptibility, while Gram-negative strains were comparatively less sensitive, likely due to structural differences in their outer membranes. Molecular docking analysis further suggested that major aromatic constituents, particularly hydrogenated anthracene and trimethyl-substituted naphthalene

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derivatives, may contribute to membrane-related antimicrobial interactions. Overall, these findings describe a distinct Pre-Rif chemotype of *O. mixta* and provide experimental evidence supporting its potential as a natural antimicrobial agent. The results offer a scientific foundation for future ecological, toxicological, and field-based evaluations aimed at integrating this essential oil into sustainable agricultural and environmental management strategies.

Keywords: *Ormenis mixta*; Essential Oil; Ecological Sustainability; Antifungal Activity; Biocontrol Agent; Circular Bioeconomy

1. Introduction

The intensive use of synthetic antimicrobials and pesticides in agriculture has caused serious ecological problems, including soil degradation, water pollution, biodiversity loss, and the emergence of resistant microbial strains. These impacts underline the urgency of developing eco-friendly alternatives that can protect crops and ecosystems without leaving harmful residues. Within this ecological framework, the exploration of local biological resources offers an opportunity to discover natural agents that align with the principles of ecological protection and sustainable agriculture. Interest in medicinal and aromatic plants has been growing significantly in recent years, and international trade in these products is expanding at an anticipated annual rate of approximately 8–10% [1].

Medicinal and aromatic plants (MAPs) are rich in secondary metabolites, particularly essential oils, valued for their therapeutic, food, cosmetic, and industrial applications [2,3]. Extraction techniques—traditional and modern—aim to maximize both yield and quality [4]. The global essential oils market, valued at USD 10.3 billion in 2021, is expected to reach USD 16.0 billion by 2026, reflecting their increasing use as natural alternatives to synthetic chemicals [5,6]. However, industry also generates about 20 million tonnes of by-products annually, often discarded despite being potential sources of bioactive compounds that could provide both environmental and economic benefits [7,8].

Morocco hosts over 800 MAP species [9], many producing essential oils with notable biological properties. Moroccan EOs have shown antifungal activity against *Alternaria alternata*, *Botrytis cinerea*, and *Fusarium oxysporum* [10], as well as antibacterial effects—for instance, *Thapsia transtagana* inhibited both Gram-positive (*Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis*) and Gram-negative species (*Pseudomonas aeruginosa*, *Citrobacter freundii*, *Escherichia coli*, *Klebsiella pneumoniae*) [11]. Other species, such as *Dittrichia viscosa*, *Mentha pulegium*, and *Origanum elongatum*, exhibit antioxidant potential [12–14]. Beyond therapeutic uses, MAP-derived EOs are gaining interest in pest biocontrol [15,16], offering eco-friendly alternatives to chemical pesticides that contribute to health risks, resistance, and biodiversity loss. Indeed, their volatility further reduces environmental persistence and pollution [17–20].

Ormenis mixta (syn. *Chamaemelum mixtum* (L.) All., *Anthemis mixta* L., *Cladanthus mixtus* (L.) Chevall. (Also known as "Moroccan chamomile" or "simple-leaved chamomile")), is an annual species of the Asteraceae family found extensively in western and central Morocco, especially in the plain areas of the region as well as in sandy or stony pastures and along the Atlantic coastline [21,22]. This plant is spontaneous in nature, grows 10–40 cm, and has a distinctive fragrant white and yellow flower; it is collected by the local populations between the months of March and June. *Ormenis mixta* represents an important source for obtaining an essential oil commonly used in the perfume, cosmetic, and pharmaceutical industries, with Morocco being the principal global supplier [23].

The essential oil from *O. mixta* is mainly composed of D-germacrene and 1,8-cineole, and is marketed in aromatherapy for its general tonic, neurotonic, and aphrodisiac activity, besides its anti-infectious, anti-bacterial, parasiticidal, and wound healing properties [22–24]. The plant is also used in traditional medicine to a lesser extent than for industrial purposes, particularly in the Rabat region and Maamora region of Morocco, where it is used to apply the flower heads onto small wounds to promote the healing process [25]. In addition, a few studies have discussed the antioxidant, anti-inflammatory, antidiabetic, hypocholesterolemic, and antimicrobial activity attributed to this species in the literature, signaling the increasing significance of this species for pharmaceutical valorization and as a natural preservative in food-related and medical-related applications [26].

Earlier work on *O. mixta* essential oils from several Moroccan localities has, for the most part, identified chemotypes dominated by oxygenated monoterpenes or chemotypes rich in camphor [27]. These observations illustrate that geographical origin has a huge effect on the production of volatile compounds [28]. The present study shows that the Pre-Rif population of *O. mixta* had a very different chemical composition, containing mainly aromatic and polycyclic hydrocarbons, providing convincing evidence that it contained a novel chemotype which had not been documented, thus far, in Moroccan populations of *O. mixta*. Furthermore, the chemical variability observed may also be associated with functional differences, as the Pre-Rif oil demonstrated a particularly strong antifungal activity toward Candida albicans than toward Gram-negative bacteria. These regional differences highlight the need to formalize documenting intra-specific diversity, which is important for the valorization and sustainable use of Moroccan chamomile essential oils [29,30].

Despite the growing interest in Moroccan medicinal and aromatic plants, research on *O. mixta* essential oil remains limited and geographically fragmented. Most available studies have focused on central and coastal regions, leaving northern areas such as the Pre-Rif largely unexplored. Furthermore, although the antimicrobial properties of *O. mixta* have been previously mentioned, few investigations have systematically linked chemical variability with biological activity. This knowledge gap is particularly relevant because intra-specific diversity in chemotypes can strongly influence bioactivity and, consequently, potential industrial and environmental applications. Addressing this gap is essential to establish the full valorization potential of *O. mixta* and to guide sustainable exploitation strategies.

In this context, the objective of the present study was to determine the chemical composition of *Ormenis mixta* essential oil harvested from the Pre-Rif region (Taza, Morocco) and assess its antibacterial and antifungal potentials on two Gram-positive and two Gram-negative bacteria and on Candida albicans. The study sought to relate the composition and chemical classes of volatile compounds to the observed antimicrobial activity and determine possible pharmaceutical and ecological significance from the native species.

2. Materials and Methods

2.1. Plant Material and Essential Oil Extraction

The specimens of *Ormenis mixta* analyzed in this study were systematically collected in the Taza region (**Figure 1**) at 34°43′15″ N, 4°02′23″ W (altitude of 1,324 m), an area recognized as a center of excellence for traditional Moroccan phytotherapy. This strategic region forms a unique biogeographical corridor between Mediterranean and Atlas influences, creating a mosaic of ecosystems conducive to diversified medicinal flora [31]. The Taza region presents remarkable plant diversity, with more than 800 species recorded, of which nearly 15% are endemic [32,33].

The plant materials used in this research were taxonomically validated by Professor Zidane, a botanist at the Faculty of Sciences, Ibn Tofail University, Kenitra/Morocco. A voucher was deposited in the Herbarium of the Department of Biology, Faculty of Sciences-Kenitra, under number 83/2025 for *O. mixta*.

Figure 1. Taza province on the map, its districts and harvest area (with modifications) [34].

The plant material was air-dried at ambient temperature and hydrodistilled for 3 h using a Clevenger-type device. The essential oil yield was calculated based on the dry weight (% w/w).

2.2. Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The chemical composition of the essential oil was analyzed using GC–MS equipped with an HP-5MS capillary column. Helium was used as a carrier gas at a constant flow of 1.0 mL/min. The injector temperature was set at 250 °C. The oven temperature program was: 50 °C for 5 min, rising at 4 °C/min to 280 °C, and held for 10 min. Mass spectra were acquired in EI mode at 70 eV. Compounds were identified by comparing mass spectra with NIST library data and by retention indices calculated relative to a homologous series of n-alkanes.

2.3. Microorganisms and Culture Conditions

Six microbial strains were used in this study: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Bacillus subtilis*, and *Candida albicans*. All strains were obtained from the microbial collection of the National Institute of Aromatic and Medicinal Plants (Morocco) and are recognized for their clinical and ecological relevance in antimicrobial resistance. The bacteria were maintained on Mueller–Hinton agar and the yeast on Sabouraud dextrose agar under standard culture conditions.

2.4. Antimicrobial Assay

The antimicrobial activity of *O. mixta* essential oil was assessed using the broth microdilution method in sterile 96-well microplates following CLSI guidelines. Mueller–Hinton broth was used for bacteria, and Sabouraud broth for fungi. A stock solution of the essential oil (100 mg/mL) was prepared in 0.2% agar and serially diluted (1/2 to 1/64) to yield a final range of 0.195–50 mg/mL. Each well contained 100 µL of broth, 100 µL of EO dilution, and 10 µL of microbial suspension (10^6 CFU/mL for bacteria and 10^4 CFU/mL for fungi). Plates were incubated at 37 °C for 24 h (bacteria) or 26–28 °C for 24–48 h (fungi).

After incubation, 10 µL of resazurin solution (5 mg/mL) was added, and color change was observed following an additional 3 h incubation. The minimum inhibitory concentration (MIC) was defined as the lowest EO concentration with no color change (no microbial growth). Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined by subculturing 10 µL from wells without growth onto agar plates, with MBC/MFC defined as the lowest concentration reducing viable counts by ≥ 99.99%. The MBC/MIC or MFC/MIC ratio classified the oil as bactericidal/fungicidal (<4) or bacteriostatic/fungistatic (>4) [35]. Each experiment was conducted on three separate occasions to ensure biological reproducibility (independent assays performed on different days). In the agar diffusion assay, every strain–concentration combination was evaluated using three discs per plate (technical replicates), resulting in a total of nine measurements (n = 9) for each condition and microorganism. For MIC and MBC determinations, duplicate wells were prepared within each independent experiment, providing six observations per tested condition (n = 6). Appropriate controls were systematically included. Gentamicin served as the antibacterial reference (10 µg/disc for diffusion assays and 0.5–8 µg/mL for MIC determination), while amphotericin B (0.25–4 µg/mL) was used as the antifungal standard. Vehicle controls containing ≤1% DMSO and 0.1% Tween-80 were incorporated, along with sterility controls to verify media integrity. Microbial suspensions were adjusted to a turbidity equivalent to 0.5 McFarland, corresponding approximately to 1 × 10^6 CFU/mL for bacterial strains and 5 × 10^5 CFU/mL for fungal strains. All procedures were carried out in compliance with BSL-2 laboratory safety guidelines within the institutional microbiology facility. The study did not involve human participants or vertebrate animals, and all biological residues were sterilized by autoclaving prior to disposal.

2.5. Molecular Docking

2.5.1. Preparation of Ligands

In order to assess the interactions of the compounds discovered by GC-MS based on the primary molecules, molecular docking experiments were carried out. The identified biomolecules were acquired in SDF 3D format from the PubChem database. PyMOL was used to convert them into PDB files [36]. Following their unique input into AutoDock, the produced ligands (**Figure 2**) were stored in pdbqt format.

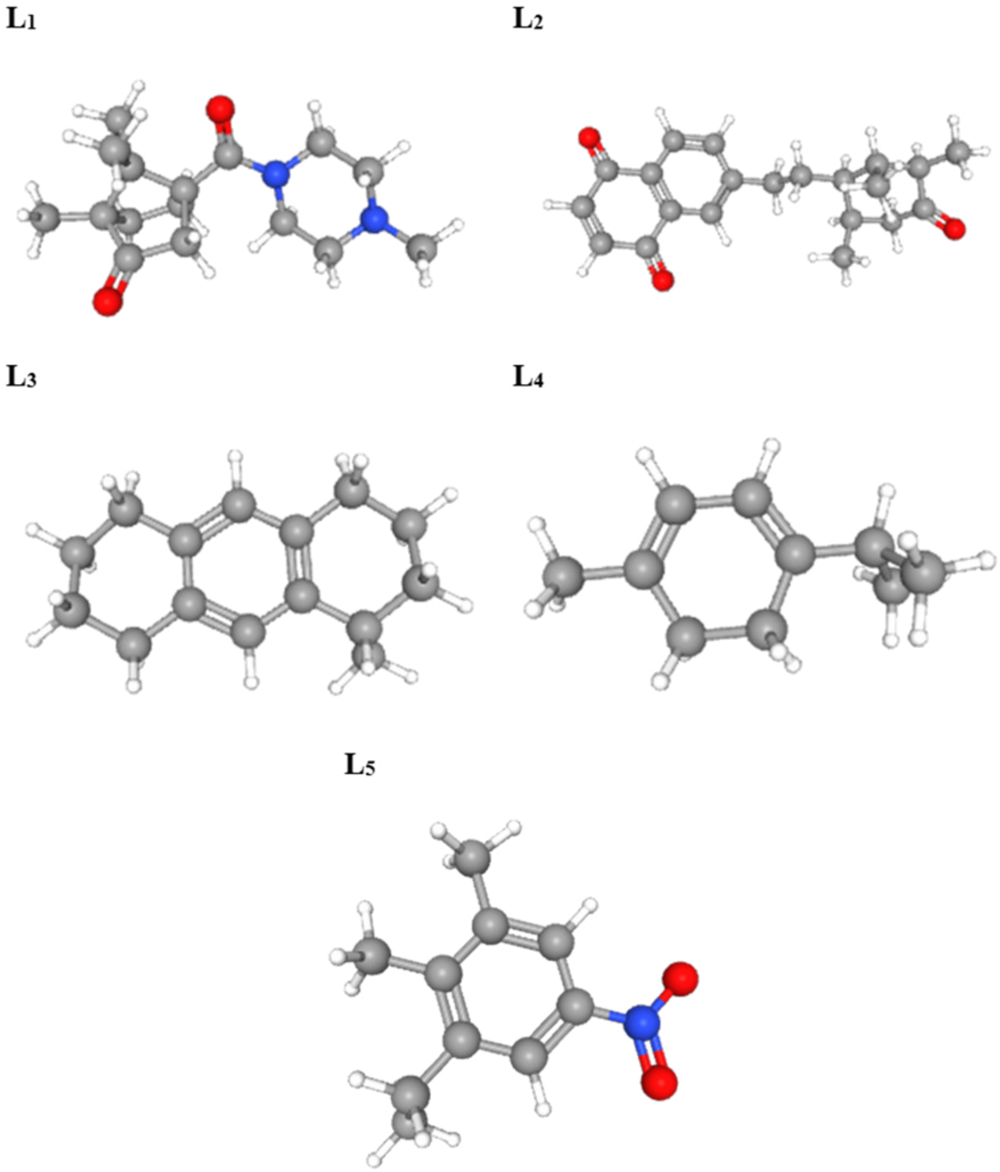
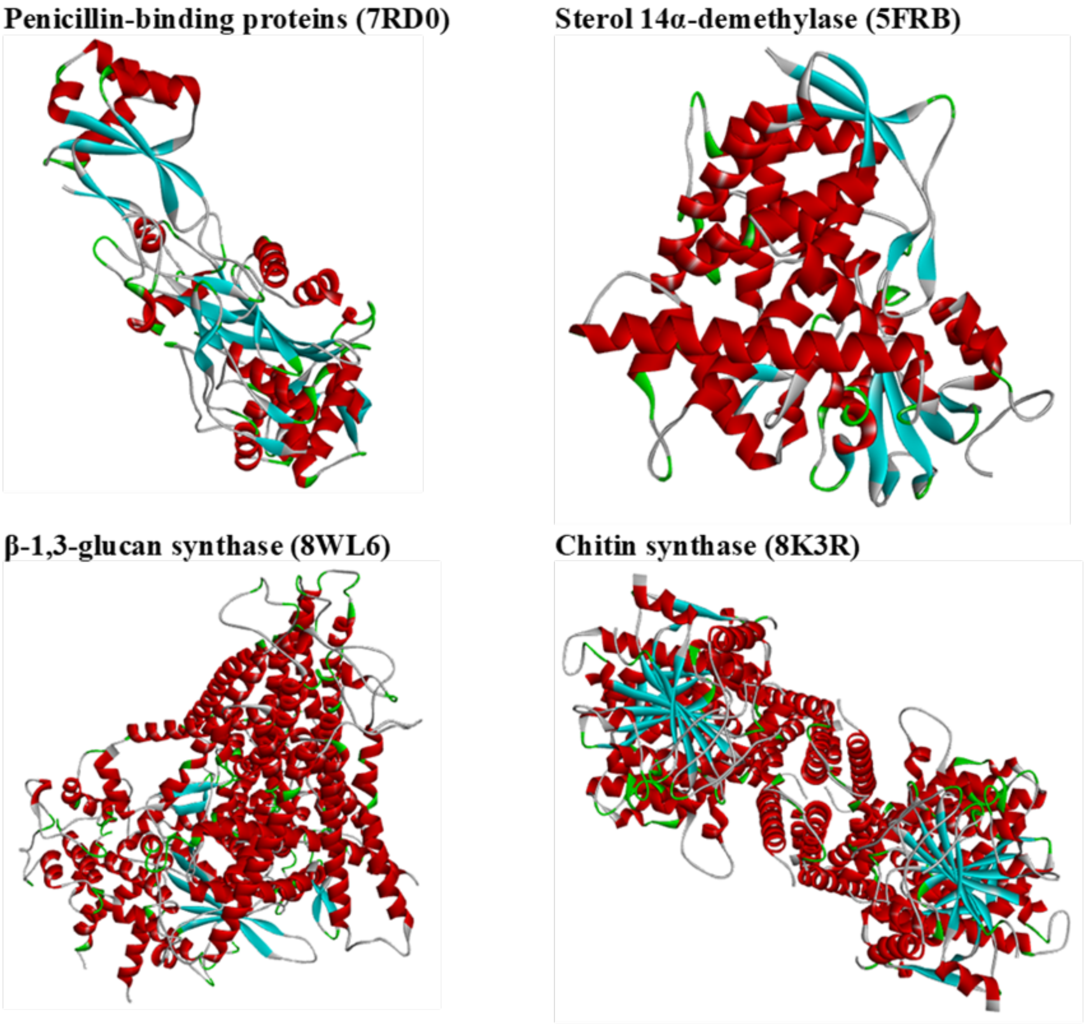


Figure 2. 3D structure of the ligands.

Note: L$_1$: Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S); L$_2$: Naphthalene, 2,3,6-trimethyl; L$_3$: Anthracene, 1,2,3,4,5,6,7,8-octahydro; L$_4$: 1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl); L$_5$: Benzene, 1-ethyl-2,3-dimethyl.

2.5.2. Preparation of Proteins

Before being included into AutoDock, the crystallographic structures of sterol 14α-demethylase (PDB ID: 5FRB), β-1,3-glucan synthase (PDB ID: 8WL6), chitin synthase (PDB ID: 8K3R), and penicillin-binding proteins (PDB ID: 7RD0) were retrieved from the Protein Data Bank. As part of their preparation, they used the Biovia Discovery Studio 2024 software (Accelrys, San Diego, USA) to remove the water molecules and co-crystallized ligands from the active site. The missing hydrogen atoms were then filled in, the Kollman-type partial charges were assigned, and the protein structures (**Figure 3**) were converted to the pdbqt format using the AutoDockTools program (v.1.5.7, 4.2.6).


Penicillin-binding proteins (7RD0)

Sterol 14α-demethylase (5FRB)

β-1,3-glucan synthase (8WL6)

Chitin synthase (8K3R)

Figure 3. 3D structure of the proteins.

2.5.3. Molecular Docking Analysis

Using AutoDock Vina and an automated script, the molecular interactions of the bioactive compounds were analyzed. The configuration file.txt contains the docking grid's parameters. The genetic algorithm combined with local search (LGA) served as the foundation for the simulations [36]. Using AutoGrid, the active areas of each enzyme were defined by centering the grid on the co-crystallized ligands with a cubic mesh of 60 Å per side and a spacing of 0.500 Å, and the (x, y, z) coordinates of the centered grid box were specified for each protein studied. The box of the sterol 14α-demethylase was centered at the coordinates (290.960, 16.750, 6.010), β-1,3-glucan synthase at (131.832, 130.674, 113.486), chitin synthase at (116.598, 116.604, 129.727) and penicillin-binding proteins at (0.551, −18.521, 14.179). Every binding affinity value was taken into account throughout the dynamic simulations. Biovia Discovery Studio 2024 was used to identify the amino acids implicated in the interactions and view the complex conformations.

2.6. Statistical Analysis

Results are reported as mean ± SD. MIC and MBC/MFC values were summarized using descriptive statistics, and the killing index was expressed using the ratio (MBC/MIC). For the GC–MS dataset, each identified compound was reported as a relative percentage of the total chromatographic area and then ordered from highest to lowest abundance. To illustrate how much of the oil composition was accounted for by the most abundant constituents, we plotted a cumulative contribution curve based on the ranked percentages. This representation highlights dominant compounds and shows whether a small set of volatiles explains most of the profile. Associations between oil yield, the abundance of major constituents, and antimicrobial indices were examined using Pearson's correlation (r), and the correlation matrix was displayed as a heatmap to facilitate interpretation. Analyses and figures were produced in Python 3.11 using pandas, numpy, seaborn, and matplotlib, with statistical significance defined as $p < 0.05$.

3. Results

3.1. Extraction Efficiency

Following hydrodistillation, the yield of essential oil extracted from the aerial parts of *O. mixta* was 0.55% (w/w) based on the dry weight of the plant material. The oil appeared as a pale yellow liquid with a characteristic aromatic odor. This essential oil yield is in the low to moderate range as previously reported for *O. mixta* in Morocco, indicating that variation can occur depending on geographical origin, climate, period of harvesting, and drying processes. For example, previous research conducted in various Moroccan regions has found the essential oil yield for *O. mixta* to be between 0.30% and 0.80%, demonstrating the effect ecological and edaphic factors can have on essential oil yield. This kind of variability is not unusual for species within the Asteraceae family; rather, it reflects the plant's ability to adapt to changing environmental conditions. Thus, the current results classify the Pre-Rif population within the expected yield range, confirming its potential as a local viable source of essential oil for pharmaceutical and environmental uses.

3.2. Chemical Composition

The GC–MS chromatogram of *O. mixta* EO revealed a chemically diverse profile with major peaks detected between 10 and 35 min (**Figure 4**), indicating the predominance of aromatic and polycyclic hydrocarbons. Key constituents included Benzene, 1-ethyl-2,3-dimethyl (RT 11.92 min), bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl (RT 16.32 min), anthracene derivatives (RT 28.51 min), and Naphthalene, 2,3,6-trimethyl (RT 34.68 min). Early eluting compounds corresponded mainly to monoterpenes (RT 9–12 min), whereas heavier compounds were detected at longer retention times.

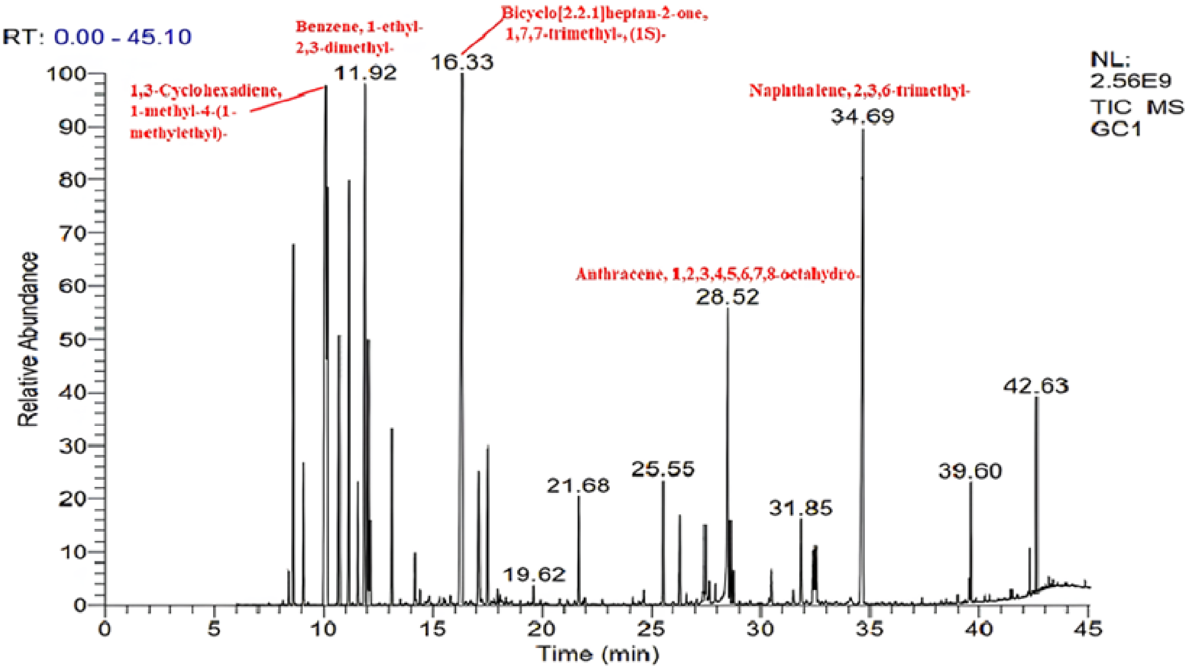


Figure 4. Chromatogram of *O. mixta* EO.

Notes: The x-axis represents the retention time (RT, in minutes), while the y-axis indicates the relative abundance of detected ions. Each peak corresponds to a compound separated by gas chromatography and identified by mass spectrometry. Major identified compounds are labelled in red, including 1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- (RT = 10.09 min), Benzene, 1-ethyl-2,3-dimethyl- (RT = 11.92 min), Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)- (RT = 16.32 min), Anthracene, 1,2,3,4,5,6,7,8-octahydro- (RT = 28.51 min), and Naphthalene, 2,3,6-trimethyl- (RT = 34.68 min). The notation NL (2.56E9) indicates the normalization level, i.e., the maximum signal intensity used as a reference for relative abundance scaling.

The abundance of peaks for aromatic and polycyclic compounds reflects the quantitative findings from the compositional analysis, where aromatic hydrocarbons accounted for around 50% of the oil. The properties of these molecules explain their hydrophobic nature, leading them to interact with microbial membranes and thus perhaps account for some of the microbial suppression we observed. The very small number of oxygenated monoterpenes, such as eucalyptol, and then a few minor ketones, will contribute a very small amount to the bioactivity of the oil (if indeed there is bioactivity present) but may act in combination with and support the sub-structure of the major hydrocarbons, as a component of bioactivity. Therefore, the chromatographic profile confirms that *O. mixta* EO does in fact contain a dual structure: a small number of very dominant aromatic peaks, and many minor peaks that provide chemical diversity and potential functional-site synergism.

Table 1 shows the chemical composition of *O. mixta* EO using GC–MS. A total of 30 constituents were found, which collectively accounted for nearly all of the oil fraction (96.13%). The chromatographic data revealed prominent amounts of aromatic hydrocarbons and polycyclic aromatic compounds; moderate amounts of monoterpene hydrocarbons; and trace amounts of oxygenated monoterpenes. The major compounds were 1,3-cyclohexadiene, 1-methyl-4-(1-methylethyl)- (15.71%); bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl- (13.99%); and naphthalene, 2,3,6-trimethyl- (10.95%), these major compounds comprised over 40% of the EO. Other notable constituents were benzene, 1-ethyl-2,3-dimethyl- (8.85%) and anthracene derivatives (5.59%); along with α-phellandrene (5.80%), confirming this study reflects the dominance of terpenoid and aromatic classes generally accepted for this family, Asteraceae.

Table 1. GC–MS profile of *O. mixta* EO: Retention time and relative abundance of identified constituents (%).

RT (min)	Compounds Name	Contents (%)
8.60	Tricyclo[2.2.1.0(2,6)]heptane, 1,3,3-trimethyl-	3.87
9.09	Camphene	1.54
10.09	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	15.71
10.16	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	4.79
10.72	a-Myrcene	3.32
11.15	a-Phellandrene	5.80
11.57	Cyclohexene, 1-methyl-4-(1 methylethylidene)-	1.33
11.92	Benzene, 1-ethyl-2,3-dimethyl-	8.85
12.05	D-Limonene	3.21
12.11	Eucalyptol	0.78
16.32	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	13.99
17.11	endo-Borneol	2.05
17.49	Isopulegol	2.08
19.62	Benzaldehyde, 4-(1-methylethyl)-	0.24
21.67	Phenol, m-tert-butyl-	1.30
25.55	Tricyclo[3.3.1.1(3,7)]decane-1-carbonitrile	1.68
26.27	Bicyclo[3.1.1]hept-2-ene-2-ethano l, 6,6-dimethyl-	1.16
27.46	1-Phenylcyclohexanol	1.27
27.62	Camphene	0.42
27.92	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	0.28
28.51	Anthracene, 1,2,3,4,5,6,7,8-octahydro-	5.59
28.62	Naphthalene, 1,4-dimethyl-	0.86
28.73	Carveol	0.41
31.85	Phenanthrene, 1,2,3,4,5,6,7,8-octahydro-	1.29
32.43	Phenol,4-(1,1-dimethylethyl)-2-methyl-	0.76
34.07	1,4,5,8,9,10-Hexahydroanthracene	0.25
34.68	Naphthalene, 2,3,6-trimethyl-	10.95
39.60	Hexane, 1-chloro-5-methyl-	1.00
-	Identified	94.78
-	Not identified	5.22

Conversely, while specific oxygenated components, eucalyptol, endo-borneol, and isopulegol were less abundant in oil yield (0.78%, 2.05%, and 2.08% respectively), they may serve a critical biological role in conjunction with hydrocarbon fractions. Less significant constituents (1.30%) phenolic derivatives and aldehydes (0.24%) contribute some wealth. The dominant constituents being excluded by oxygenated monoterpenes or sesquiterpenes is evidence of the aromatic and polycyclic nature of a chemotype. Overall, the Pre-Rif samples chemotype was contrasted with the other *O. mixta* chemotypes recognized within Morocco, which were characterized by oxygenated constituents as dominant. This range of chemical constituents must be recognized and relates to the impacts of environmental and ecological parameters (rainfall, temperature, and soil types) on EO biosynthesis, a characteristic feature of the species. On a functional level, hydrocarbons likely interact with microbial membranes through a lipophilic pathway contributing to antimicrobial capacity, and although limited, the role of oxygenated derivatives alongside increased oil potency may be attributed to disrupting microbial enzymatic and metabolic pathways. Both the dominant and non-dominant biological importance of the role of *O. mixta* EO is explored in this study's chemical characterization.

The cumulative contribution curve (**Figure 5**) illustrates that a very small number of important compounds make up a majority of the chemical composition of *O. mixta* EO extract. The first 5 compounds already account for >55% of the oil, while the 10 greatest contributing compounds accounted for >75%. After ~20 compounds, the curve stabilizes and contributes successively less to the overall total. This is a common occurrence in EOs, with a few most common dominant volatiles determining the main bioactivity and aroma of the oil, as well as many minor and trace compounds providing primarily chemical model diversity and potential synergies.

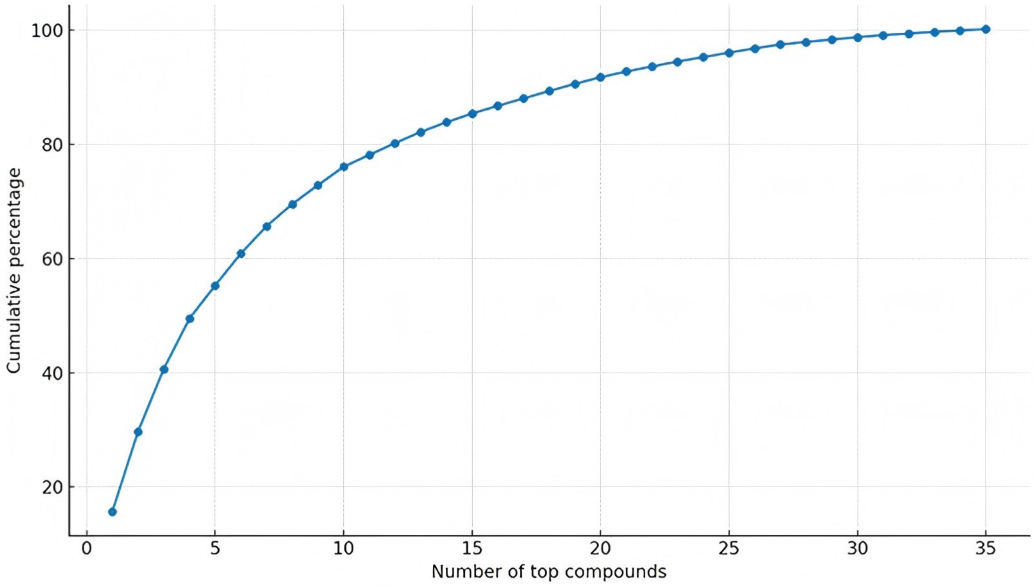


Figure 5. Cumulative contribution curve.

3.3. Antimicrobial Activity Evaluation

Antimicrobial activity showed clear differences between microbial species (**Table 2**). Strains of Candida albicans (group a) exhibited the lowest MIC values (≈6–7 mg/mL), with moderate variability and MBC values equal to MIC, generating a ratio close to 1, suggesting a strong fungicidal action. Bacillus subtilis (group b) exhibited low MIC values (≈12 mg/mL), with MBC values very close to their corresponding MIC values, generating ratios close to 1, confirming bactericidal effects. The intra-assay variability was low (CV ~ 6–7%). Staphylococcus aureus (group b) showed slightly higher MIC values (≈14–16 mg/mL), with MBC values of the same order of magnitude, confirming a ratio also close to 1, supporting a bactericidal profile. Some additional fluctuation (one replicate had MBC < MIC) is acceptable and most likely due to experimental noise when reading resazurin.

Table 2. Minimum inhibitory concentration (MIC), minimum bactericidal/fungicidal concentration (MBC/MFC), and killing effect of *Ormenis mixta* EO against the tested microorganisms (mean ± SD).

Species	MIC (mg/mL)	MBC or MCF (mg/mL)	Ratio (MIC/MBC)	Effect	Tukey Grouping
Escherichia coli	25.67 ± 0.80	51.40 ± 3.99	0.50 ± 0.04	Bactericide	c
Staphylococcus aureus	14.56 ± 1.32	12.00 ± 2.00	1.13 ± 0.13	Bactericide	b
Pseudomonas aeruginosa	49.33 ± 1.52	97.33 ± 3.51	0.51 ± 0.01	Bactericide	d
Klebsiella pneumoniae	24.67 ± 1.53	49.67 ± 2.52	0.50 ± 0.03	Bactericide	c
Bacillus subtilis	12.17 ± 0.76	12.33 ± 1.15	0.99 ± 0.09	Bactericide	b
Candida albicans	6.50 ± 0.76	6.67 ± 0.58	0.98 ± 0.08	Fungicide	a

Note: MIC = Minimum Inhibitory Concentration; MBC = Minimum Bactericidal Concentration; MCF = Minimum Fungicidal Concentration.

Intermediate values were shown for *Escherichia coli* and *Klebsiella pneumoniae* (group c) with MIC values of ~25–26 mg/mL, and the MBCs were nearly double the MICs, so the ratios were ~0.5. These results still indicate a bactericidal effect as MBC/MIC ≤ 4 using the midpoint method. The variability was observed to be low (CV ~ 3–6%). Finally, *Pseudomonas aeruginosa* (group d) had the highest MICs (~49–51 mg/mL) and MBCs, which were almost double the MICs, consistent with its elevated level of resistance. This strain was statistically significantly distinct from the other strains. In synthesis, the hierarchy of sensitivity is the following: *C. albicans* < *B. subtilis* ≈ *S. aureus* < *E. coli* ≈ *K. pneumoniae* < *P. aeruginosa*. The ratios were close to 1 for the fungus and Gram-positive bacteria, and ~0.5 for Gram-negative bacteria, showing a mostly bactericidal or fungicidal profile.

3.4. Correlation

The correlation analysis (**Figure 6**) revealed a negative correlation between EO yield, and the antimicrobial ratios (i.e., the highest value from the four different antibiotic ratios) of *Bacillus subtilis* (r = −0.40) and *Candida albicans* (r = −0.52); suggesting that higher yield, as a percentage of the overall composition, contributed to a greater effect, as measured *B. subtilis* was more sensitive, and therefore overall *C. albicans* and Gram-positive bacterial sensitivity was more reliant upon yield. Of the major constituents, cyclohexadiene (15.71%) exhibited a strong positive correlation with *Klebsiella pneumoniae* (r = 0.72), bicycloheptanone (13.99%) exhibited a moderate correlation with Staphylococcus aureus (r = 0.47) Naphthalene, 2,3,6-trimethyl- (10.95%) exhibited a moderate positive correlation via an effect on *Escherichia coli* (r = 0.51), and contributing complementary mode of actions. Taken together, we can establish that both the yield (% composition) and specific dominant compounds are contributing to the antimicrobial activity profile, with corresponding higher yield and balanced profile resulting in more sensitivity, particularly to exposure to *C. albicans* and Gram-positive bacteria.

3.5. Molecular Docking Interpretation

The molecular docking is a crucial information tool for the discovery of new drugs. It enables the precise selection of potentially active components and the analysis of the establishment of contacts at the level of the liaison site in the protein-ligand complex. The affinity between a ligand and its receptor is largely determined by the liaison energy; the lower this energy, the higher the affinity [36].

In this regard, five bioactive compounds were tested against proteins and their corresponding interaction energies were compared. The obtained values, expressed in kcal/mol, together with the inhibition constants and amino acid residues involved in the interactions, were recorded at the end of the docking procedure (**Table 3**).

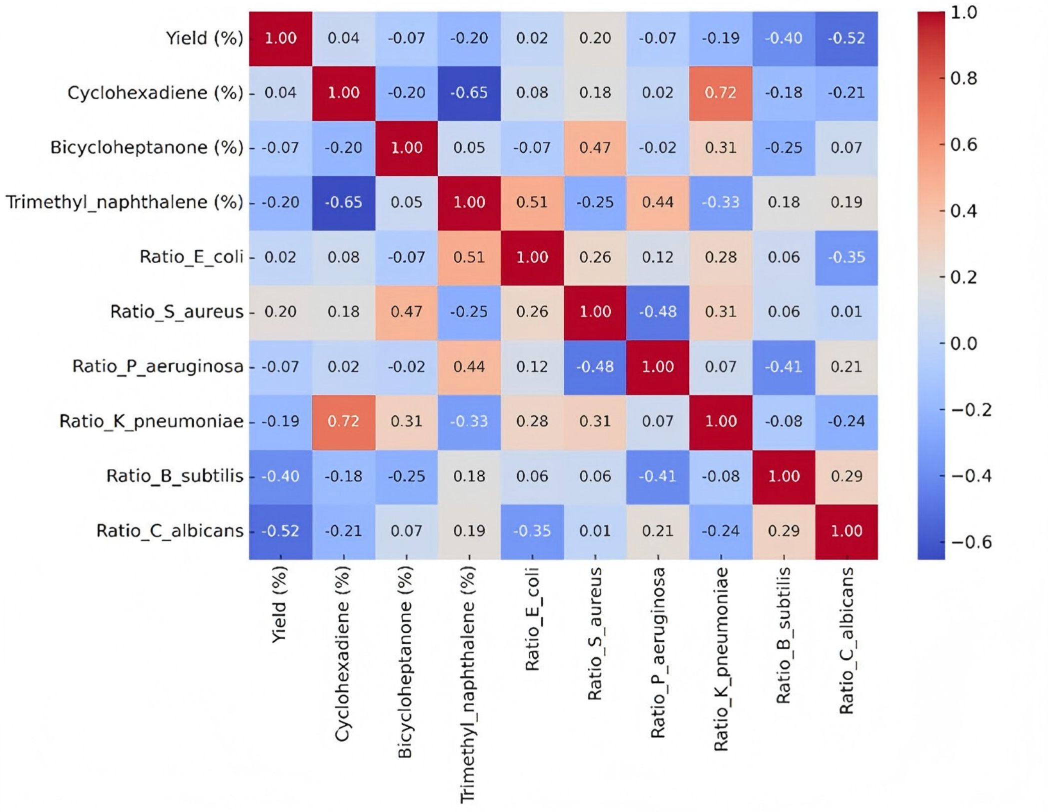


Figure 6. Correlation heatmap between EO yield, major compounds, and antimicrobial ratios (MBC/MIC) of *Ormenis mixta* EO.

Table 3. Interaction scores between bioactive compounds and proteins.

	7RD0		**5FRB**		**8WL6**		**8K3R**	
	Be	**Ic**	**Be**	**Ic**	**Be**	**Ic**	**Be**	**Ic**
L_1	−5.53	88.52 µM	−6.90	8.82 µM	−6.24	26.70 µM	−5.02	209.38 µM
L_2	−6.87	9.18 µM	−9.55	99.88 nM	−8.84	333.83 nM	−7.57	2.84 µM
L_3	−5.67	69.65 µM	−7.61	2.66 µM	−6.89	8.88 µM	−6.05	36.71 µM
L_4	−5.20	153.53 µM	−6.46	18.42 µM	−5.43	104.22 µM	−4.63	400.83 µM
L_5	−6.21	28.05 µM	−6.79	10.58 µM	−5.78	58.41 µM	−5.72	64.24 µM

Note: Be: Binding Energy (kcal/mol); Ic: Inhibition Constant.

Naphthalene, 2,3,6-trimethyl, had the strongest affinity for the protein Penicillin-binding proteins (7RD0) among the five ligands, with a constant of inhibition of 9.18 µM and a liaison energy of −6.87 kcal/mol. The other ligands, such as 1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl) (153.53 µM) and Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S) (88.52 µM), exhibit significantly weaker interactions.

The analysis of molecular interactions with Penicillin-binding proteins (**Figure 7**) shows that each bioactive compound has a series of unique interactions with the residues of the site of liaison, conditioning its affinity and potential inhibitor: Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S) forms a hydrogen bond with Tyr A 440 and three hydrogen bonding contacts with Leu A 390, Glu A 393, and Ser A 180. Naphthalene, 2,3,6-trimethyl, interacts by hydrogen bonds with Tyr A 438, Gly A 389, Lys A 385, Leu A 390, and Ile A 388, a Pi-Alkyle bond with Tyr A 440, and two Pi-anion bonds with Asp A 391 and Asp A 387. Anthracene, 1,2,3,4,5,6,7,8-octahydro, forms three Pi-alkyle relationships with Pro A 248, Pro A 268, and Arg A 267. The 1,3-cyclohexadiene, 1-methyl-4-(1-methylethyl), has hydrogen bonds with Ile A 163, Ala A 154, Tyr A 232, and Leu A 158. Finally, 1-ethyl-2,3-dimethylbenzene forms classical hydrogen bonds with His A 151, Phe A 270, Phe 286, and Tyr A 165 (**Figure 7**).

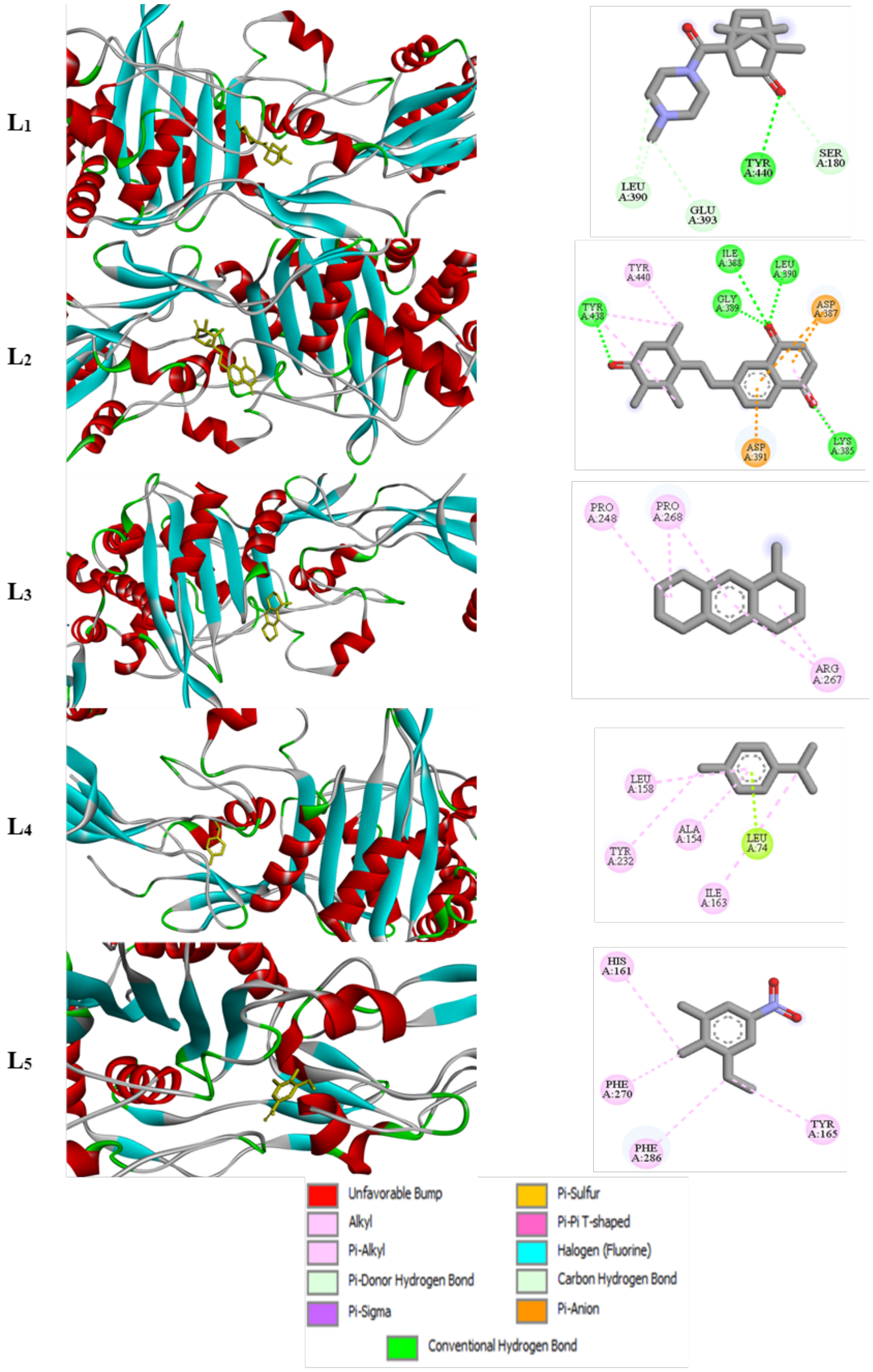


Figure 7. 2D and 3D interaction of ligands with active site of Penicillin-binding proteins.

Anthracene, 1,2,3,4,5,6,7,8-octahydro is distinguished by a strong inhibitory activity on sterol 14α-demethylase (Ic = 2.66 nM) coupled with a favorable binding energy (Be = −7.61 kcal/mol). This duality indicates a high affinity and a potential pharmacological efficacy (**Table 3**).

The analysis of molecular interactions with Sterol 14α-demethylase (**Figure 8**) shows that each bioactive compound has a series of specific interactions with the residues of the site of liaison, conditioning its affinity and potential inhibitor: Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S) forms four Pi-Alkyle interactions with His A 374, Ile A 373, Phe A 229, and Phe A 504, as well as a Pi-hydrogen donor liaison with Leu A 503. Naphthalene, 2,3,6-trimethyl, interacts with Tyr A 136, Ser A 375, and His A 374 through hydrogen bonds; four Pi-Alkyle bonds with Ile A 337, Ile A 377, Leu A 503, Ala A 307, and Phe A 234; and a Pi-pi T-shaped bond with Tyr A 122. Anthracene, 1,2,3,4,5,6,7,8-octahydro, forms five Pi-alkyle relationships with Leu A 471, Ala A 430, Leu A 356, Ala A 360, and Leu A 353, as well as eight halogen relationships with Leu A 159, Gln A 467, Gly A 474, Thr 475, His A 359, Ile A 363, Gln A 354, and Tyr A 470. Ile A 376, Ile A 373, Pro A 403, Ala A 371, and Pro A 455 are the Pi-Alkyle relationships for 1,3-cyclohexadiene, 1-methyl-4-(1-methylethyl). Finally, Benzene, 1-ethyl-2,3-dimethyl, forms a hydrogen bond with His A 461 and six Pi-Alkyle bonds with Val A 135, Leu A 143, Lys A 147, Ile A 464, Leu A 307, and Cys A 463 (**Figure 8**).

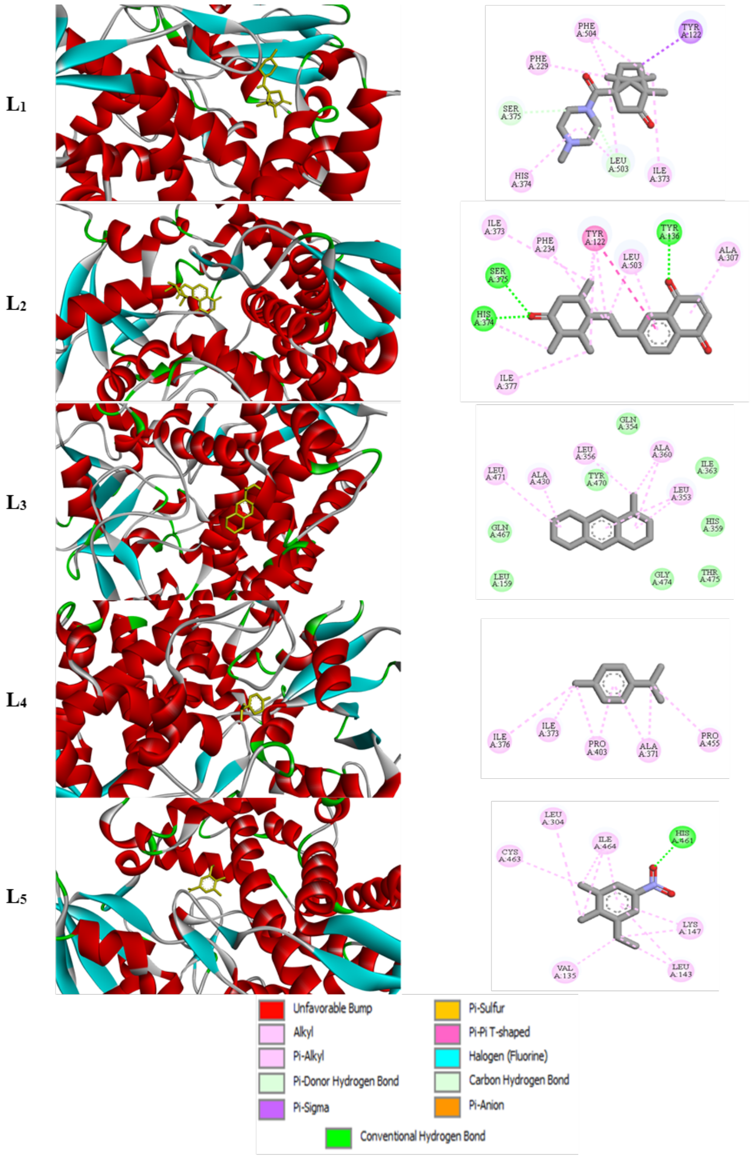


Figure 8. 2D and 3D interaction of ligands with active site of sterol 14α-demethylase.

For the protein β-1,3-glucan synthase, naphthalene, 2,3,6-trimethyl, shows a consistent inhibition (333.83 nM) and a liaison energy of −8.84 kcal/mol. Anthracene, 1,2,3,4,5,6,7,8-octahydro, has an intermediate activity (Ic = 8.88 µM), whereas the other ligands remain less effective (**Table 3**).

The analysis of molecular interactions with the protein β-1,3-glucan synthase (**Figure 9**) shows that each bioactive compound has a series of specific interactions with the residues of the site of liaison, conditioning its affinity and potential inhibitor: Bicyclo[2.2.1]heptan-2-one, 1,7,7-triméthyl-, (1s) forms six Pi-Alkyle contacts with His A 1298, Pro A 1295, Leu A 1299, Met A 1270, and Ala A 1264, as well as two Pi-Donor hydrogen interactions with Glu A 1268 and Gly A 1267. Naphtalé, 2,3,6-trimethyle interacts with Asn A 451 by a hydrogen bond, five Pi-Alkyle bonds with Pro A 1295, His A 1298, Leu A 1271, Leu A 1302, and Leu A 1299, as well as a Pi-Sigma bond with Leu A 448. Anthracène, 1,2,3,4,5,6,7,8-octahydro, forms five Pi-alkyle relationships with Leu A 1299, Leu A 1271, Leu A 448, Pro A 1295, and His A 1298. His A 1298, Pro A 1295, Met A 1270, Leu A 1271, and Leu A 448 are the Pi-Alkyle relations for the 1,3-cyclohexadiène, the 1-methyl-4- (1-methyléthyle).

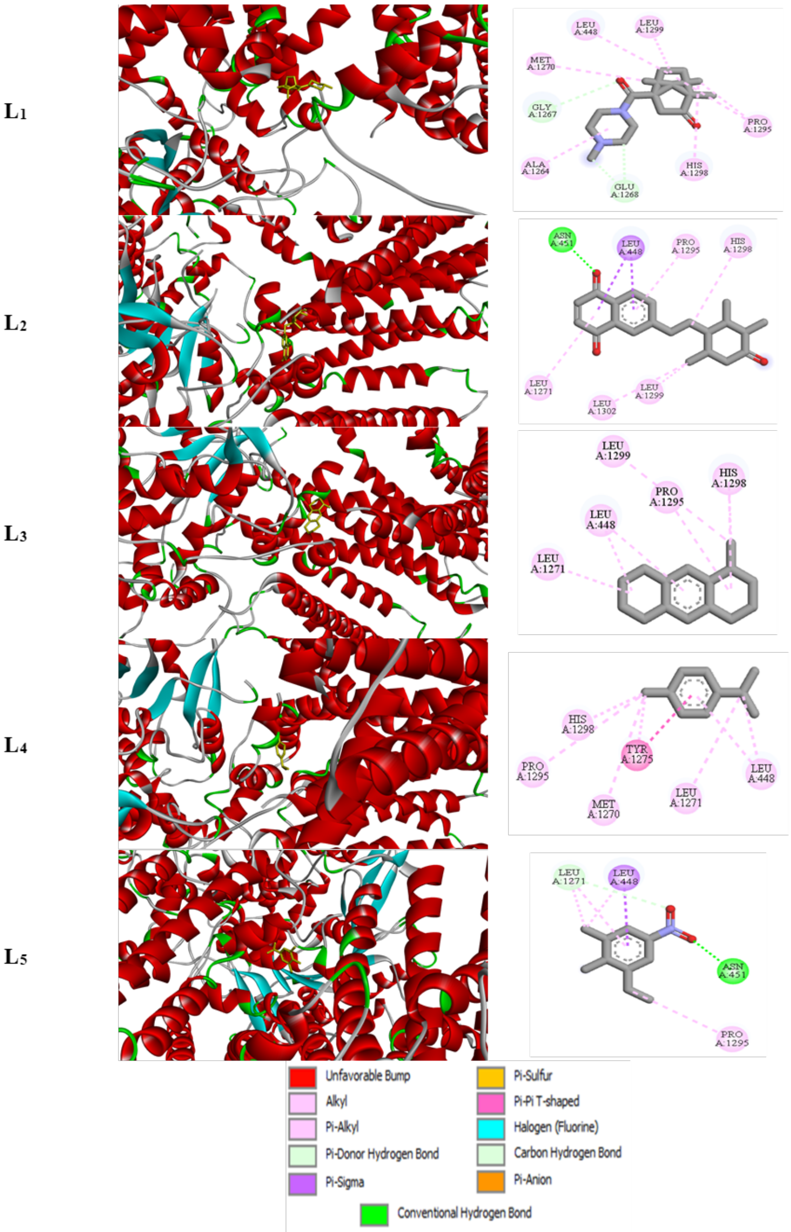


Figure 9. 2D and 3D interaction of ligands with active site of β-1,3-glucan synthase.

Naphthalene, 2,3,6-trimethyl, clearly distinguishes itself from the chitin synthase by combining a favorable interaction energy (−7.57 kcal/mol) with the lowest consistent inhibition (Ic = 2.84 µM). The results of the other ligands are generally less favorable, especially 1,3-cyclohexadiene and 1-methyl-4-(1-methylethyl), which exhibit a consistently high level of inhibition (400.83 µM) (**Table 3**).

The analysis of the molecular interactions with chitin synthase shows that each bioactive compound has a series of specific interactions with the residues of the binding site (**Figure 10**), conditioning its affinity and its potential inhibitor: Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1s) forms three Pi-Alkyl interactions with Pro A 1026, Trp A 969, and Lys A 1031. Naphthalene, 2,3,6-trimethyl, interacts with Arg A 1040 and Trp A 969 by hydrogen bonds; a Pi-Alkyl bond with Trp B 969, and a Pi-hydrogen donor bond with Tyr A 122. Trp B 969 and Leu B 967 are the Pi-Alkyl relationships for Anthracene, 1,2,3,4,5,6,7,8-octahydro. 1,3-cyclohexadiene, 1-methyl-4-(1-methylethyl) forms two Pi-Alkyl interactions with Phe A 864, Leu A 852, and two Pi-Sigma bonds with Leu A 852 and Phe A 864. Finally, benzene, 1-ethyl-2,3-dimethyl, forms two hydrogen bonds with Arg A 1040 and Asn B 963, two Pi-Alkyl interactions with Leu B 967, and Trp B 969, as well as a pi-anion bond with Lys B 857.

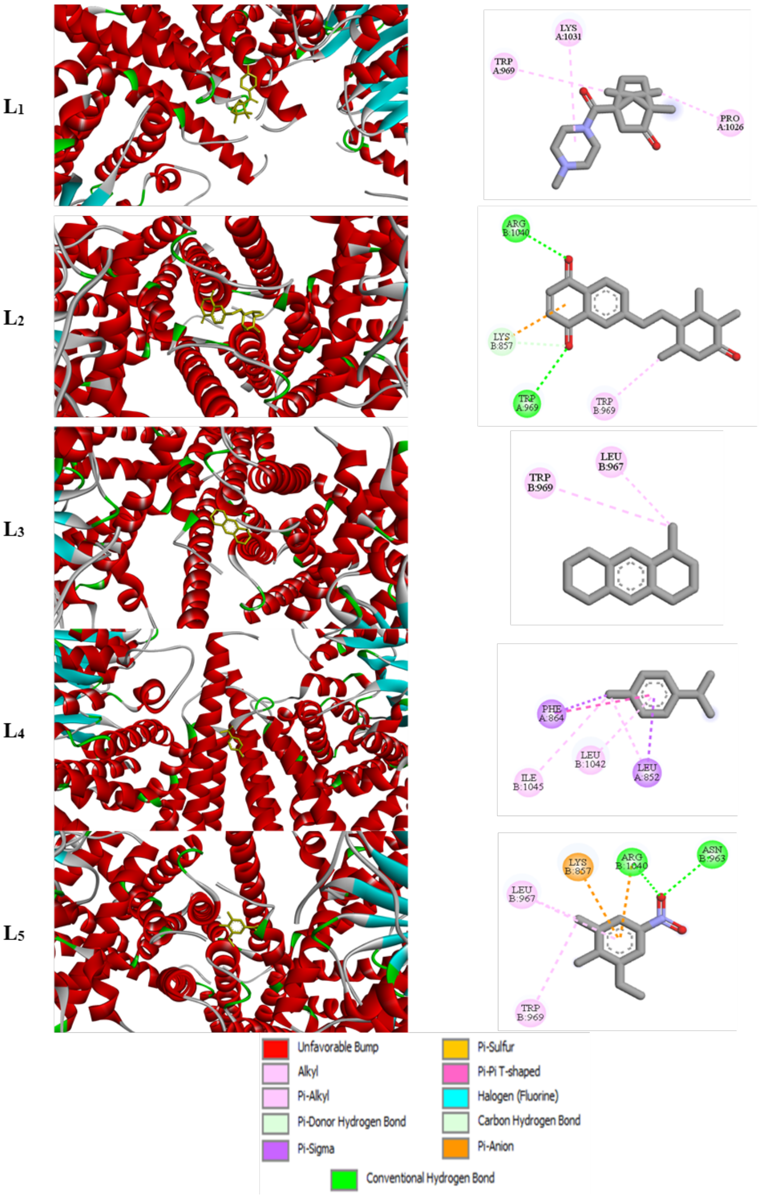


Figure 10. 2D and 3D interaction of ligands with active site of chitin synthase.

4. Discussion

The present study identifies a distinct Pre-Rif chemotype of *O. mixta*, characterized by a predominance of aromatic and polycyclic hydrocarbons rather than the oxygenated monoterpenes commonly reported in other Moroccan populations. This regional chemical divergence likely reflects local ecological conditions, including rainfall regime, soil composition, and temperature patterns.

4.1. Extraction Yield and Variability

EO yield obtained for *O. mixta* in the present study (0.55%) is consistent with values previously reported for Moroccan populations. Elouaddari et al. recorded an extraction yield of 0.47% [37], while Zrira et al. found a slightly lower yield of 0.40% [25]. Comparable yields were also recorded for the Oujda region (0.40%) [34], whereas a higher value was reported from Bouznika (0.80%) [38]. Across studies, reported yields span a broad range (0.1–1.5%), indicating that yield is highly dependent on the collection site [39]. In addition, Elouaddari et al. linked this variability to rainfall patterns: in areas receiving 229–350 mm of precipitation, yields typically ranged from 0.3% to 0.8%, while more humid regions (550–950 mm rainfall) showed markedly lower yields (0.1–0.2%) [28]. One plausible explanation is that excessive moisture can limit oil biosynthesis, as plants under high water availability may develop fewer branches and exhibit reduced leaf integrity. Similar relationships between precipitation and EO yield have been reported for other aromatic species [40]. Taken together, these observations underscore the influence of ecological factors—particularly rainfall—on *O. mixta* oil yield and support the need for region-specific characterization.

4.2. Chemical Composition Analysis

The chemical diversity of *O. mixta* essential oils has been widely documented and varies with geographic provenance and environmental factors [39]. For example, Elouaddari et al. reported camphor as the main component of oils from Kenitra, Settat, and Benguerir, while Bouznika and Oujda populations yielded chemotypes dominated by 2-methyl-2-trans-butenyl methacrylate/ar-curcumene and trans-β-farnesene, respectively [29]. Other studies identified Santolina alcohol, α-pinene, limonene, 1,8-cineole, and germacrene-D as major constituents [25,37,41]. This chemical variability results from harvest time, soil type, altitude, and climatic factors, leading to distinct chemotypes. Such variability necessitates standardization for consistency in quality and use, but also enhances pharmacological potential through diverse bioactivities [42–44].

In terms of antimicrobial activity, *O. mixta* essential oils have shown variable effects depending on chemical composition and microbial strains tested. Strong antibacterial activity has been reported against *E. coli*, *B. subtilis*, *S. aureus*, and *Micrococcus luteus*, while antifungal activity was generally weak against *Penicillium parasiticus*, *Aspergillus niger*, and *Trametes pini* [32]. Wanner et al. observed strong inhibition zones (15–30 mm) and a MIC of 90 µg/mL against *S. aureus*, *E. coli*, *Citrobacter freundii*, *Enterococcus faecalis*, *Listeria monocytogenes*, and *K. pneumoniae* [45]. Other studies indicated moderate activity against *P. aeruginosa* (MIC 50–100 mg/mL) [46] and *E. coli* (MIC 25 mg/mL, MBC 50 mg/mL), the latter being more susceptible when combined with other essential oils [30]. In contrast, *S. aureus* and *B. subtilis* were highly sensitive (MIC and MBC 12.5 mg/mL), in line with oils rich in 1,8-cineole and germacrene-D. These differences underline the link between chemical variability and antimicrobial effectiveness [37,47].

Our findings support this relationship, as the Pre-Rif chemotype, earlier studies described chemotypes predominantly rich in oxygenated monoterpenes (such as camphor and 1,8-cineole), germacrene-D, Santolina alcohol, or trans-β-farnesene [22–25]. In contrast, the Pre-Rif population examined in this study is characterized by a marked predominance of aromatic hydrocarbons (51.18%) and polycyclic aromatic compounds (20.89%), a compositional profile not previously documented at the national level. This richness in aromatic and polycyclic hydrocarbons demonstrated strong antifungal activity against *C. albicans* and marked bactericidal effects against Gram-positive bacteria, while Gram-negative strains exhibited higher resistance. However, antimicrobial testing design in this study only tested two Gram-positive, two Gram-negative bacterial strains, and one type of fungus. Although these microorganisms provide an initial evaluation of bioactivity, a more comprehensive microbial testing panel containing the additional breadth of microbial species relevant in clinical scenarios, including multidrug-resistant species, would need to be conducted to fully assess the extent of therapeutic and environmental value of the *O. mixta* essential oil product in this study. Future studies should be conducted to test multidrug-resistant pathogens and even more fungal species with relevance to agriculture and medicine. These studies would significantly add to the translational significance of our study findings to better define the antimicrobial action and potential uses of this essential oil in practice.

4.3. Sustainability Perspectives

The global essential oils market reached nearly USD 18.96 billion by 2023, with strong growth driven by consumer demand for natural products [48]. However, essential oil production also generates large volumes of solid waste and hydrolats—estimated at over 200,000 t annually—that are often improperly disposed of [49,50]. Such practices have negative environmental consequences, including soil and water pollution, VOC emissions, and greenhouse gas release [51,52]. For example, the production of 1 kg of rose oil requires 3,500–4,000 kg of fresh flowers, generating significant wet biomass waste [53]. In China, the use of medicinal plant waste (MPW) is a solid fuel. The MPW was roasted at different temperatures and atmospheres, which improved its flammability and reduced its moisture absorption. The results show that roasted MPW could be used as a solid fuel or as a raw material for granulation [54].

Recent research highlights valorization opportunities, such as using hydrodistillation by-products of *Centaurea nicaeensis* for heavy metal remediation [55], rosemary residues for wastewater treatment and ceramic membrane enhancement [56], and Chinese herbal waste for chromium adsorption [57]. These approaches exemplify how waste streams can be reintegrated into the circular bioeconomy, supporting both environmental protection and value creation. Hydrolats, for instance, are already recognized as natural preservatives in food systems [58] and as cosmetic ingredients in Europe [59], while residues (roots, flowers, seeds, leaves, fruit) represent low-cost, biodegradable resources [60] suitable for renewable energy production or biodiesel generation [61,62].

In this context, *O. mixta* essential oil holds potential applications that align with Sustainable Development Goals. Its fungicidal and bactericidal activity suggests use in biocontrol of crops and wood, in natural food preservation and active packaging systems, and in wastewater sanitation to reduce microbial load and biofilm formation. Such applications would reduce dependence on synthetic chemicals, support safer agricultural and industrial practices, and benefit rural economies through sustainable exploitation of Moroccan aromatic plants. Nevertheless, further studies are needed for industrial translation. Toxicological and phytotoxicity evaluations, stability tests (light, oxidation, pH), and Life Cycle Assessments (LCA) are essential to ensure safety and scalability. Future research should also explore large-scale cultivation and field validation to facilitate integration of *O. mixta* EO into sustainable development and circular bioeconomy frameworks.

An important ecological consideration is the sustainable management of *O. mixta* populations. Harvesting strategies must be designed to avoid overexploitation and ensure that wild populations maintain their ecological niche and reproductive capacity. Ecological monitoring could help determine harvestable biomass thresholds without compromising species survival. By combining sustainable harvesting, valorization of extraction residues, and integration into the local bioeconomy, *O. mixta* can serve as a model species for the ecological utilization of Moroccan aromatic plants.

Sustainable harvesting of *O. mixta* in rural communities requires the implementation of controlled collection practices to prevent overexploitation of wild populations. This may include rotational harvesting zones, limiting collection to specific phenological stages (e.g., post-flowering), and maintaining a minimum percentage of plants for natural regeneration. Community-based management systems, supported by local cooperatives and agricultural extension services, could ensure traceability and compliance with ecological guidelines. Additionally, integrating small-scale cultivation alongside wild harvesting would reduce pressure on natural populations while stabilizing income sources. Such measures would allow ecological conservation to coexist with economic valorization, reinforcing long-term sustainability.

5. Conclusions

This study demonstrated that the essential oil of *Ormenis mixta* from the Pre-Rif region of Morocco exhibits a distinctive chemical profile dominated by aromatic and polycyclic hydrocarbons, along with a smaller proportion of oxygenated monoterpenes. Biological assays revealed pronounced antifungal activity and strong bactericidal effects, particularly against Gram-positive bacteria, whereas Gram-negative strains were less sensitive. The compositional analyses indicate that a limited set of dominant compounds accounts for most of the oil profile, supporting a functional link between the Pre-Rif chemical signature and the observed antimicrobial performance. Overall, the Pre-Rif chemotype emerges as a promising candidate for development as a natural biocontrol agent, with potential applications in sustainable agriculture and related antimicrobial uses. Future work should prioritize toxicological and phytotoxicity evaluation, stability testing, and field-scale validation to support safe translation and practical deployment. From an ecological perspective, the antifungal and bactericidal activity of *O. mixta* essential oil can be directly applied to sustainable plant protection strategies. Its use in the ecological control of crop diseases could substantially reduce dependence on chemical pesticides in orchards and fields, thereby limiting soil and water contamination and preserving beneficial microbiota. Moreover, the antimicrobial properties of this essential oil hold potential for protecting natural ecosystems, for example, by reducing microbial loads in irrigation water or preventing the disruption of soil microbial communities. Future work should prioritize scale-up strategies for cultivation, alongside toxicological evaluation, stability studies, and field-based validation to enable safe and effective deployment within sustainable development and circular bioeconomy frameworks. Beyond its pharmaceutical and industrial interest, *O. mixta* may also contribute to approaches that couple environmental protection with rural development when integrated into circular-value chains.

Author Contributions

Conceptualization, H.K., S.E.M., M.A., Y.M., and E.M.A.; methodology, H.K. and O.C.; software, M.A., Y.M.; validation, E.M.A.; formal analysis, H.K.; investigation, H.K.; resources, B.E.O.; data curation, H.K.; writing—original draft preparation, H.K.; writing—review and editing, Y.M., M.A., and S.E.M.; visualization, H.K.; supervision, E.M.A.; funding acquisition, E.M.A. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

Not applicable.

Data Availability Statement

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

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Conflicts of Interest

The authors declare no conflict of interest.

References

[1] Chandra, P., Sharma, V., 2019. Marketing information system and strategies for sustainable and competitive medicinal and aromatic plants trade. Information Development. 35(5), 806–818. DOI: https://doi.org/10.1177/0266666918802415

[2] Ait Bouzid, H., Oubannin, S., Ibourki, M., et al., 2023. Comparative evaluation of chemical composi-

tion, antioxidant capacity, and some contaminants in six Moroccan medicinal and aromatic plants. Biocatalysis and Agricultural Biotechnology. 47, 102569. DOI: https://doi.org/10.1016/j.bcab.2022.102569

[3] Durczyńska, Z., Żukowska, G., 2024. Properties and Applications of Essential Oils: A Review. Journal of Ecological Engineering. 25(2), 333–340. DOI: https://doi.org/10.12911/22998993/177404

[4] Souiy, Z., 2024. Essential Oil Extraction Process. In: Jonas (Ed.). Biochemistry. IntechOpen: London, UK. DOI: https://doi.org/10.5772/intechopen.113311

[5] Machado, C.A., Oliveira, F.O., De Andrade, M.A., et al., 2022. Steam Distillation for Essential Oil Extraction: An Evaluation of Technological Advances Based on an Analysis of Patent Documents. Sustainability. 14(12), 7119. DOI: https://doi.org/10.3390/su14127119

[6] Memarzadeh, S.M., Gholami, A., Pirbalouti, A.G., et al., 2020. Bakhtiari savory (*Satureja bachtiarica Bunge.*) essential oil and its chemical profile, antioxidant activities, and leaf micromorphology under green and conventional extraction techniques. Industrial Crops and Products. 154, 112719. DOI: https://doi.org/10.1016/j.indcrop.2020.112719

[7] Khiri, S., Ullah, N., Boubal, Z., et al., 2025. Transforming essential oil extraction Wastes: Sustainable valorization approaches for agricultural, industrial, and cosmetic applications. Biomass and Bioenergy. 201, 108109. DOI: https://doi.org/10.1016/j.biombioe.2025.108109

[8] Saha, A., Basak, B.B., 2020. Scope of value addition and utilization of residual biomass from medicinal and aromatic plants. Industrial Crops and Products. 145, 111979. DOI: https://doi.org/10.1016/j.indcrop.2019.111979

[9] Zrira, S., 2017. Some Important Aromatic and Medicinal Plants of Morocco. In: Neffati, M., Najjaa, H., Máthé, Á. (Eds.). Medicinal and Aromatic Plants of the World—Africa Volume 3, Medicinal and Aromatic Plants of the World. Springer: Dordrecht, Netherlands. pp. 91–125. DOI: https://doi.org/10.1007/978-94-024-1120-1_5

[10] El Abdali, Y., Agour, A., Allali, A., et al., 2022. *Lavandula dentata* L.: Phytochemical Analysis, Antioxidant, Antifungal and Insecticidal Activities of Its Essential Oil. Plants. 11(3), 311. DOI: https://doi.org/10.3390/plants11030311

[11] Alilou, H., Akssira, M., 2021. Chemical composition, antibacterial, antioxidant and insecticidal activities of Moroccan *Thapsia transtagana* essential oil. Saudi Journal of Biological Sciences. 28(12), 6756–6764. DOI: https://doi.org/10.1016/j.sjbs.2021.07.052

[12] Boulamtat, R., Lhaloui, S., Sabraoui, A., et al., 2020. Antifeedant and larvicidal activities of *Mentha pulegium* on chickpea pod borer *Helicoverpa armigera* (Lepidoptera: Noctuidae). International Journal of Tropical Insect Science. 40(1), 151–156. DOI: https://doi.org/10.1007/s42690-019-00064-z

[13] Mssillou, I., Agour, A., Slighoua, M., et al., 2022. Phytochemical characterization, antioxidant activity, and in vitro investigation of antimicrobial potential of *Dittrichia viscosa* L. leaf extracts against nosocomial infections. Acta Ecologica Sinica. 42(6), 661–669. DOI: https://doi.org/10.1016/j.chnaes.2021.09.021

[14] Tagnaout, I., Zerkani, H., Bencheikh, N., et al., 2023. Chemical Composition, Antioxidants, Antibacterial, and Insecticidal Activities of *Origanum elongatum* (Bonnet) Emberger & Maire Aerial Part Essential Oil from Morocco. Antibiotics. 12(1), 174. DOI: https://doi.org/10.3390/antibiotics12010174

[15] Smith, H.H., Idris, O.A., Maboeta, M.S., 2021. Global Trends of Green Pesticide Research from 1994 to 2019: A Bibliometric Analysis. Journal of Toxicology. 2021, 1–11. DOI: https://doi.org/10.1155/2021/6637516

[16] Nboyine, J.A., Kusi, F., Abudulai, M., et al., 2020. A new pest, *Spodoptera frugiperda* (J.E. Smith), in tropical Africa: Its seasonal dynamics and damage in maize fields in northern Ghana. Crop Protection. 127, 104960. DOI: https://doi.org/10.1016/j.cropro.2019.104960

[17] Kaseya, J., Dereje, N., Tajudeen, R., et al., 2024. Climate change and malaria, dengue and cholera outbreaks in Africa: A call for concerted actions. BMJ Global Health. 9(3), e015370. DOI: https://doi.org/10.1136/bmjgh-2024-015370

[18] Seo, S.-H., Choi, S.-D., Batterman, S., et al., 2022. Health risk assessment of exposure to organochlorine pesticides in the general population in Seoul, Korea over 12 years: A cross-sectional epidemiological study. Journal of Hazardous Materials. 424, 127381. DOI: https://doi.org/10.1016/j.jhazmat.2021.127381

[19] Singh, S., Nebapure, S.M., Taria, S., et al., 2023. Current status of phosphine resistance in Indian field populations of *Tribolium castaneum* and its influence on antioxidant enzyme activities. Scientific Reports. 13(1), 16497. DOI: https://doi.org/10.1038/s41598-023-43681-y

[20] Annaz, H., Bouayad, N., Kounnoun, A., et al., 2025. Sustainable pest management with Moroccan essential oils: Insights into insecticidal, behavioral effects, and factors influencing bioactivity. Journal of Natural

Pesticide Research. 13, 100137. DOI: https://doi.org/10.1016/j.napere.2025.100137

[21] da Silva, J.A.T., 2004. Mining the Essential Oils of the Anthemideae. African Journal of Biotechnology. 3(12), 706–720. Available from: https://www.ajol.info/index.php/ajb/article/view/15042

[22] Anass, E., El Amrani, A., Eddine, J.J., 2015. Effect of the Parts of Plant Material (Flowers and Leaves) on Essential Oil Chemical Composition of *Ormenis mixta* from Morocco. Journal of Essential Oil Bearing Plants. 18(2), 398–408. DOI: https://doi.org/10.1080/0972060X.2014.935022

[23] Shukla, S., Patra, D., Sinha, A., et al., 2025. Medicinal and Aromatic Plant Cultivation, Improvement and Conservation for Sustainable Development. In: Kumar, N. (Ed.). Industrial Crops Improvement, Sustainable Landscape Planning and Natural Resources Management. Springer Nature: Cham, Switzerland. pp. 183–204. DOI: https://doi.org/10.1007/978-3-031-75937-6_11

[24] Benkhaira, N., Ech-Chibani, N., Fikri-Benbrahim, K., 2021. Ethnobotanical survey on the medicinal usage of two common medicinal plants in Taounate Region: Artemisia herba-alba Asso and *Ormenis mixta* (L.) Dumort. Ethnobotany Research and Applications. 22. DOI: https://doi.org/10.32859/era.22.48.1-19

[25] Zrira, S., Menut, C., Bessiere, J.M., et al., 2007. Chemical Composition of the Essential Oils of Moroccan *Ormenis mixta* (L.) Dumort. ssp. Multicaulis. Journal of Essential Oil Bearing Plants. 10(5), 378–385. DOI: https://doi.org/10.1080/0972060X.2007.10643570

[26] Jeddi, M., El Hachlafi, N., Fadil, M., et al., 2023. Combination of Chemically-Characterized Essential Oils from *Eucalyptus polybractea, Ormenis mixta,* and *Lavandula burnatii:* Optimization of a New Complete Antibacterial Formulation Using Simplex-Centroid Mixture Design. Advances in Pharmacological and Pharmaceutical Sciences. 2023, 1–21. DOI: https://doi.org/10.1155/2023/5593350

[27] El Orche, A., El Mrabet, A., Said, A.A.H., et al., 2024. Integration of FTIR Spectroscopy, Volatile Compound Profiling, and Chemometric Techniques for Advanced Geographical and Varietal Analysis of Moroccan Eucalyptus Essential Oils. Sensors. 24(22), 7337. DOI: https://doi.org/10.3390/s24227337

[28] Castro-Vázquez, L., Díaz-Maroto, M.C., De Torres, C., et al., 2010. Effect of geographical origin on the chemical and sensory characteristics of chestnut honeys. Food Research International. 43(10), 2335–2340. DOI: https://doi.org/10.1016/j.foodres.2010.07.007

[29] Elouaddari, A., El Amrani, A., Eddine, J.J., et al., 2013. Yield and chemical composition of the essential oil of Moroccan chamomile [*Cladanthus mixtus* (L.) Chevall.] growing wild at different sites in Morocco. Flavour and Fragrance Journal. 28(6), 360–366. DOI: https://doi.org/10.1002/ffj.3146

[30] Chraibi, M., Fadil, M., Farah, A., et al., 2021. Antimicrobial combined action of Mentha pulegium, *Ormenis mixta* and Mentha piperita essential oils against S. aureus, E. coli and C. tropicalis: Application of mixture design methodology. LWT. 145, 111352. DOI: https://doi.org/10.1016/j.lwt.2021.111352

[31] Bencheikh, N., Elbouzidi, A., Baraich, A., et al., 2024. Ethnobotanical survey and scientific validation of liver-healing plants in northeastern Morocco. Frontiers in Pharmacology. 15, 1414190. DOI: https://doi.org/10.3389/fphar.2024.1414190

[32] Chauiyakh, O., El Fahime, E., Aarabi, S., et al., 2023. In vitro biological control of Cedrus atlantica' s lignivorous fungi by the extracted essential oils from the infected wood. International Wood Products Journal. 14(3–4), 146–154. DOI: https://doi.org/10.1080/20426445.2023.2265249

[33] Kodad, H., Chauiyakh, O., Farissi, H.E., et al., 2025. Ethnobotanical Knowledge and Therapeutic Uses of Ormenis mixta and Citrus limon in the Taza Region of Morocco. Sarhad Journal of Agriculture. 41(5). DOI: https://doi.org/10.17582/journal.sja/2025/41.5.195.209

[34] Hakkour, M., Hmamouch, A., El Alem, M.M., et al., 2016. New epidemiological aspects of visceral and cutaneous leishmaniasis in Taza, Morocco. Parasites & Vectors. 9(1), 612. DOI: https://doi.org/10.1186/s13071-016-1910-x

[35] Kodad, H., Chauiyakh, O., Soufiani, A., et al., 2025. Antimicrobial Potential of Citrus limon Peel Essential Oil from Morocco: A Sustainable Approach to Citrus Waste Valorization. Research in Ecology. DOI: https://doi.org/10.30564/re.v7i5.11689

[36] Zirari, M., Aouji, M., Baghdad, W., et al., 2025. In-depth study of the nutritional composition, phytochemicals, antioxidant activity, molecular docking interactions, and toxicological evaluation of Abies marocana Trab. woody biomass. Frontiers in Sustainable Food Systems. 8, 1525572. DOI: https://doi.org/10.3389/fsufs.2024.1525572

[37] Elouaddari, A., Amrani, A.E., Cayuela Sánchez, J.A., et al., 2019. Chemical Composition and Biological Activities of the Cladanthus mixtus Essential Oil: A Review. Analytical Chemistry Letters. 9(5), 649–663. DOI: https://doi.org/10.1080/22297928.2019.168266

5

[38] Elouaddari, A., Amrani, A.E., JamalEddine, J., et al., 2014. Intraspecific Variability of the Essential Oil of Cladanthus mixtus from Morocco. Natural Product Communications. 9(1), 1934578X1400900137. DOI: https://doi.org/10.1177/1934578X1400900137

[39] Touati, S., Acila, S., Boujnah, D., et al., 2022. Geographical location and cultivar-linked changes on chemical properties of olive oils from Algeria. Food Science & Nutrition. 10(6), 1937–1949. DOI: https://doi.org/10.1002/fsn3.2810

[40] Fernández-Sestelo, M., Carrillo, J., 2020. Environmental Effects on Yield and Composition of Essential Oil in Wild Populations of Spike Lavender (Lavandula latifolia Medik.). Agriculture. 10(12), 626. DOI: https://doi.org/10.3390/agriculture10120626

[41] Darriet, F., Bendahou, M., Costa, J., et al., 2012. Chemical Compositions of the Essential Oils of the Aerial Parts of Chamaemelum mixtum (L.) Alloni. Journal of Agricultural and Food Chemistry. 60(6), 1494–1502. DOI: https://doi.org/10.1021/jf203872z

[42] Formisano, C., Rigano, D., Senatore, F., et al., 2012. Essential Oil Composition and Antibacterial Activity of *Anthemis mixta* and *A. Tomentosa* (Asteraceae). Natural Product Communications. 7(10), 1934578X1200701035. DOI: https://doi.org/10.1177/1934578X1200701035

[43] De Sousa, D.P., Damasceno, R.O.S., Amorati, R., et al., 2023. Essential Oils: Chemistry and Pharmacological Activities. Biomolecules. 13(7), 1144. DOI: https://doi.org/10.3390/biom13071144

[44] Etri, K., Pluhár, Z., 2024. Exploring Chemical Variability in the Essential Oils of the Thymus Genus. Plants. 13(10), 1375. DOI: https://doi.org/10.3390/plants13101375

[45] Wanner, J., Schmidt, E., Bail, S., et al., 2010. Chemical Composition, Olfactory Evaluation and Antimicrobial Activity of Selected Essential Oils and Absolutes from Morocco. Natural Product Communications. 5(9), 1934578X1000500903. DOI: https://doi.org/10.1177/1934578X1000500903

[46] Ouedrhiri, W., Balouiri, M., Bouhdid, S., et al., 2018. Antioxidant and antibacterial activities of *Pelargonium asperum* and *Ormenis mixta* essential oils and their synergistic antibacterial effect. Environmental Science and Pollution Research. 25(30), 29860–29867. DOI: https://doi.org/10.1007/s11356-017-9739-1

[47] Abd Rashed, A., Rathi, D.-N.G., Ahmad Nasir, N.A.H., et al., 2021. Antifungal Properties of Essential Oils and Their Compounds for Application in Skin Fungal Infections: Conventional and Nonconventional Approaches. Molecules. 26(4), 1093. DOI: https://doi.org/10.3390/molecules26041093

[48] Abu Bakar, M.S., Ahmed, A., Jeffery, D.M., et al., 2020. Pyrolysis of solid waste residues from Lemon Myrtle essential oils extraction for bio-oil production. Bioresource Technology. 318, 123913. DOI: https://doi.org/10.1016/j.biortech.2020.123913

[49] Yan, M.-R., Wang, C.-H., Jose Cruz Flores, N., et al., 2019. Targeting Open Market with Strategic Business Innovations: A Case Study of Growth Dynamics in Essential Oil and Aromatherapy Industry. Journal of Open Innovation: Technology, Market, and Complexity. 5(1), 7. DOI: https://doi.org/10.3390/joitmc5010007

[50] Gavarić, N., Kladar, N., Mišan, A., et al., 2015. Post-distillation waste material of thyme (*Thymus vulgaris* L., Lamiaceae) as a potential source of biologically active compounds. Industrial Crops and Products. 74, 457–464. DOI: https://doi.org/10.1016/j.indcrop.2015.05.070

[51] Sayed-Ahmad, B., Talou, T., Saad, Z., et al., 2017. The Apiaceae: Ethnomedicinal family as source for industrial uses. Industrial Crops and Products. 109, 661–671. DOI: https://doi.org/10.1016/j.indcrop.2017.09.027

[52] Carrión-Paladines, V., Fries, A., Caballero, R.E., et al., 2019. Biodegradation of Residues from the Palo Santo (Bursera graveolens) Essential Oil Extraction and Their Potential for Enzyme Production Using Native Xylaria Fungi from Southern Ecuador. Fermentation. 5(3), 76. DOI: https://doi.org/10.3390/fermentation5030076

[53] Slavov, A., Vasileva, I., Stefanov, L., et al., 2017. Valorization of wastes from the rose oil industry. Reviews in Environmental Science and Bio/Technology. 16(2), 309–325. DOI: https://doi.org/10.1007/s11157-017-9430-5

[54] Xin, S., Huang, F., Liu, X., et al., 2019. Torrefaction of herbal medicine wastes: Characterization of the physicochemical properties and combustion behaviors. Bioresource Technology. 287, 121408. DOI: https://doi.org/10.1016/j.biortech.2019.121408

[55] Dhouibi, N., Binous, H., Dhaouadi, H., et al., 2020. Hydrodistillation residues of Centaurea nicaeensis plant for copper and zinc ions removal: Novel concept for waste re-use. Journal of Cleaner Production. 261, 121106. DOI: https://doi.org/10.1016/j.jclepro.2020.121106

[56] Rafya, M., Misrar, W., Saâdi, L., et al., 2023. Ceramic membrane support based on kaolin and solid waste

from hydrodistillation of Rosmarinus officinalis L. Materials Chemistry and Physics. 295, 127030. DOI: https://doi.org/10.1016/j.matchemphys.2022.127030

[57] Zhu, G.Y., Xiao, Z.B., Zhou, R.J., et al., 2012. The Utilization of Aromatic Plant Waste Resource. Advanced Materials Research. 518–523, 3561–3565. DOI: https://doi.org/10.4028/www.scientific.net/AMR.518-523.3561

[58] Elhawary, E.A., Nilofar, N., Zengin, G., et al., 2024. Variation of the essential oil components of *Citrus aurantium* leaves upon using different distillation techniques and evaluation of their antioxidant, antidiabetic, and neuroprotective effect against Alzheimer's disease. BMC Complementary Medicine and Therapies. 24(1), 73. DOI: https://doi.org/10.1186/s12906-024-04380-x

[59] Vinciguerra, V., Di Martile, M., Mollica Graziano, M., et al., 2024. LVI and DI-SPME Combined with GC/MS and GC/MS for Volatile Chemical Profile Investigation and Cytotoxic Power Evaluation of Essential Oil and Hydrolate from *Cannabis sativa* L. cv. Carmagnola. Molecules. 29(14), 3299. DOI: https://doi.org/10.3390/molecules29143299

[60] Nguyen, T.L., Ora, A., Häkkinen, S.T., et al., 2024. Innovative extraction technologies of bioactive compounds from plant by-products for textile colorants and antimicrobial agents. Biomass Conversion and Biorefinery. 14(20), 24973–25002. DOI: https://doi.org/10.1007/s13399-023-04726-4

[61] Goel, A., Kulshrestha, S., 2024. An Overview on Floral Waste Management, Conversion to Value-Added Products and its Effects on Environment. Water, Air, & Soil Pollution. 235(9), 605. DOI: https://doi.org/10.1007/s11270-024-07403-y

[62] Zhang, C., Wang, Y., Tan, D., et al., 2024. Efficient lipid production from herbal extraction residue hydrolysate by the oleaginous yeast Cutaneotrichosporon oleaginosum for biodiesel production. Biomass Conversion and Biorefinery. 14(7), 8681–8692. DOI: https://doi.org/10.1007/s13399-022-03148-y