

Doi:10.32604/phyton.2025.063021

ARTICLE





Aphicidal and Antimicrobial Activities of *Salvia rosmarinus* Essential Oil and Its Major Compound, 1,8-Cineole

Ghizlane Houzi¹, Aimad Allali^{2,3,*}, Amine Elbouzidi^{4,*}, Mohamed Taibi⁴, Mohamed Chebaibi^{3,5}, Ben Khada Zineb⁶, Ramzi A. Mothana⁷, Mohammed F. Hawwal⁷, Rachid Flouchi^{3,8}, Abdeslam Asehraou⁹, Amal Lahkimi² and Soad Khal-Layoun¹

¹Laboratory of Biology and Health, Faculty of Sciences, University of Ibn Tofail, Kenitra, 14000, Morocco

²Laboratory of Engineering, Molecular Organometallic Materials and Environment, Faculty of Sciences Dhar El Mehraz, Sidi Mohamed Ben Abdellah University, Fez, 30000, Morocco

³Higher Institute of Nursing Professions and Health Techniques, Fez, 30000, Morocco

⁴UMRT INRAE 1158 BioEcoAgro, Laboratoire BIOPI, University of Picardie Jules Verne, Amiens, 80000, France

⁵Biomedical and Translational Research Laboratory, Faculty of Medicine and Pharmacy of Fez, Sidi Mohamed Ben Abdellah University, Fez, 30000, Morocco

⁶Laboratory of Epidemiology and Research in Health Sciences, Faculty of Medicine and Pharmacy, Sidi Mohammed Ben Abdellah University, Fez, 30000, Morocco

⁷Department of Pharmacognosy, College of Pharmacy, King Saud University (KSU), Riyadh, 11451, Saudi Arabia

⁸Laboratory of Microbial Biotechnology and Bioactive Molecules, Sciences and Technologies Faculty, Sidi Mohamed Ben Abdellah University, Fez, 30000, Morocco

⁹Laboratoire de Bioressources, Biotechnologie, Ethnopharmacologie et Santé (LBBES), Faculté des Sciences d'Oujda (FSO), Université Mohammed Premier (UMP), Bd Mohamed VI BP717, Oujda, 60000, Morocco

*Corresponding Authors: Aimad Allali. Email: aimad.allali@uit.ac.ma; Amine Elbouzidi. Email: amine.elbouzidi@ump.ac.ma

Received: 02 January 2025; Accepted: 11 March 2025; Published: 30 April 2025

ABSTRACT: This work uses GC-MS to analyze the bioactive compounds of *Salvia rosmarinus* essential oils (SREO) and evaluates their antibacterial, antifungal, and insecticidal effects, as well as the major component, 1,8-cineole. Chemical analysis identified 16 compounds accounting for 99.19% of the oil's total content, with 1,8-cineole (33.17%), camphor (16.53%), α -pinene (14.46%), and camphene (8.14%) as the major constituents. Antimicrobial activities were assessed against pathogenic strains using minimal inhibit concentration (MIC) and minimum bactericidal concentration (MBC) assays. SREO exhibited a minimum MIC of 0.128% against *P. aeruginosa*, while 1,8-cineole showed a minimum MIC of 2.06% against the same strain, highlighting the higher efficacy of the complete oil compared to the isolated compound. Conversely, for antifungal activity, 1,8-cineole displayed a lower MIC (2.06%) against *A. niger* and *P. digitatum* compared to SREO (4.125% against *A. niger*). Regarding aphicidal activity, results demonstrated the lethal effects of SREO and 1,8-cineole resulted in 100% insect mortality within 24 h of exposure. After 12 h of exposure to SREO at concentrations of 5, 10, 20, and 40 µL/L air, the mortality rates were 20%, 36.67%, 70%, and 93.33%. 1,8-cineole showed maximum efficacy, achieving complete (100%) mortality within 12 h at 40 µL/L air.

KEYWORDS: Insecticidal; antibacterial; antifungal; rosemary; bioactive; 1,8-cineole



This work is licensed under a Creative Commons Attribution 4.0 International License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

1 Introduction

The rising antibiotic resistance among pathogenic microorganisms and the growing demand for safer, eco-friendly pest control methods have intensified the search for alternative antimicrobial and insecticidal agents [1,2]. Plant-derived essential oils have gotten a lot of interest in this context because of their wide-ranging biological activity and natural origin. Essential oils are complex mixtures of volatile compounds, many of which have antimicrobial, antifungal, and insecticidal properties [3]. These properties are largely attributed to the synergy between their volatile compounds, such as monoterpenes, sesquiterpenes, and phenols. Additionally, essential oils exhibit significant antioxidant activities, enabling them to neutralize free radicals and prevent oxidative damage [4]. They also possess anti-inflammatory [5], antiviral [6], and even anticancer properties [7], making them promising candidates for applications in the pharmaceutical and agricultural industries. Among these, *Salvia rosmarinus (Rosmarinus officinalis)* essential oil (SREO) has been intensively researched for possible applications in medicine, agriculture, and food preservation [8].

Due to its volatile compounds, the evergreen plant *Salvia rosmarinus* (*S. rosmarinus*), cultivated worldwide, is also used in traditional medicine [9]. Its essential oils are rich in terpenes, with camphor and 1,8-cineole being among the most predominant components. These substances are considered characteristic volatile markers of rosemary [9]. Numerous studies on the volatile oils of this plant have reported high concentrations of these compounds, along with others such as α -pinene, camphene, borneol, terpineol, linalool, and caryophyllene.

1,8-cineole, a monoterpene with a distinctive odor, exhibits various biological activities, including antibacterial and insecticidal properties [10], as well as pharmacological effects [11]. Additionally, the food, fragrance, and cosmetics sectors make extensive use of this compound [12]. In order to cure a variety of human ailments, it is also important in traditional medicine in many cultures, including as Chinese, Indian, and Australian Aboriginal practices [13]. Lastly, drugs based on 1,8-cineole have demonstrated significant effectiveness in treating inflammatory respiratory diseases, including chronic obstructive pulmonary disease, sinusitis, bronchitis, colds, and bronchial asthma [14].

Staphylococcus aureus, Listeria innocua, Pseudomonas aeruginosa, Escherichia coli, Aspergillus niger, Candida glabrata, and *Penicillium digitatum* are among the clinically and agriculturally significant microbial strains against which we examine the antimicrobial efficacy of *S. rosmarinus* essential oil and its primary constituent, 1,8-cineole. Because of their pathogenicity, resistance to common treatments, and ability to cause spoiling, these microorganisms pose serious problems for the food and healthcare sectors [15–17].

In addition to their antibacterial properties, essential oils and their components have shown promise as natural insecticides. *Myzus persicae* (*M. persicae*), commonly referred to as the green peach aphid, is a highly destructive agricultural pest that affects a wide variety of crops. Control of this pest has traditionally relied on chemical insecticides, but increasing insecticide resistance and environmental concerns have driven interest in alternative strategies. SREO and 1,8-cineole have exhibited promising insecticidal activity, making them candidates for integrated pest management approaches.

Although numerous studies have investigated the composition and biological activities of plant extracts, most remain incomplete and do not systematically examine the relationship between the chemical composition of the analyzed extracts and their biological effects. In this context, the aim of this study is to propose an in-depth evaluation of the antibacterial and insecticidal effects of the majority compound of SREO, by comparing it with that of essential oils. We hope to contribute to the development of natural and sustainable solutions for infection management and crop protection by evaluating their efficacy against a diverse group of microbial strains and a key agricultural pest.

2 Materials and Methods

2.1 Plant Material

This study employed *S. rosmarinus* leaves gathered in May 2021 from the Boulemane region near Skoura, Morocco. This region is renowned for its botanical diversity and a climate conducive to the growth of various medicinal species. The rosemary specimens were meticulously identified by botanists in the laboratory, using a combination of botanical references and plant catalogues to ensure accurate identification. Following collection, the leaves underwent a rigorous cleaning process to remove impurities such as debris, dust, and insects that might compromise the purity of the extracts. Once cleaned, the leaves were dried under controlled conditions—air-dried in the shade—for a period of 15 days to preserve their chemical integrity.

2.2 Essential Oil Distillation

The hydrodistillation process was used to extract essential oils using a Clevenger-type device, which is well-known for its ability to extract volatile chemicals from plant material. For this extraction, 200 g of predried *S. rosmarinus* leaves were weighed and placed in a 2-liter flask. To ensure optimal extraction, 1200 mL of distilled water were added to the flask, maintaining an appropriate solid-to-liquid ratio for effective hydrodistillation. The flask containing the plant material and water was then heated, allowing the combination to boil for three hours. After extraction, the essential oil was properly decanted and collected. To preserve its chemical and biological properties, the essential oil was immediately stored at -4° C in airtight vials, protected from light and air. This low-temperature storage is critical to prevent oxidation or degradation of the sensitive components in the essential oil before subsequent analyses and biological testing.

2.3 Chemical Composition Analysis of Essential Oils

The chemical composition of the essential oils was analyzed using the same analytical procedure described by Houzi et al. [18]. Specifically, the analysis was performed with an Agilent 6890 gas chromatograph (GC) coupled to a single quadrupole mass spectrometer. The separation of compounds was carried out using an HP-5MS capillary column. Finally, the identification of the components was based on the comparison of their retention indices (RI) and mass spectra (MS) with reference data from the ADAMS and NIST libraries.

2.4 Microbial Strains and Antimicrobial Testing

In this study, the antimicrobial activity was evaluated using a diverse panel of microorganisms, consisting of four bacterial strains (*E. coli*, *S. aureus*, *L. innocua*, and *P. aeruginosa*) and three fungal strains (*A. niger*, *C. glabrata*, and *P. digitatum*). The selected bacterial strains include both Gram-positive and Gramnegative species, allowing the assessment of antimicrobial efficacy against different cell wall types. These bacteria represent pathogens of medical and industrial significance. Similarly, the fungal strains comprise molds and yeasts of clinical and environmental relevance, providing valuable insights into antifungal activity. This diverse selection of microorganisms ensures a comprehensive evaluation of the antimicrobial properties of the tested compounds, while highlighting their potential applications in medicine, agriculture, and the food industry. Antimicrobial activity was assessed using a resazurin microtitration assay in 96-well plates. MIC was determined by observing a color change from blue to pink after incubation (37°C for bacteria, 25°C for fungi). MBC and MFC were identified by plating samples from wells without visible growth onto agar and incubating for 24 h at optimal temperatures. The lowest concentrations preventing growth were recorded.

2.5 Insecticidal Activity

The insecticidal effects of (SREO) and its major compound, 1,8-cineole, were evaluated using different doses: 5, 10, 20, and 40 μ L. These doses were applied to Whatman No. 2 filter paper discs (1 × 1 cm), which were then affixed to the inner surface of the lids of 1-liter glass jars. This setup ensured that the concentrations corresponded to 5, 10, 20, and 40 μ L/L of air. Twenty adult *M. persicae* (green peach aphids) were placed on a fresh, healthy citrus leaf inside each jar. The jars were sealed and maintained in an incubator under controlled conditions (27 ± 2°C, photoperiod L:D = 14:10) for 48 h. Adult mortality was assessed at 12-hour intervals following the treatment.

2.6 Molecular Docking

2.6.1 Ligand Preparation

The main chemicals of *S. rosmarinus* were obtained from the (PubChem database in SDF format). Molecular preparation was carried out using the (LigPrep module in Schrödinger program), which used the OPLS3 force field to assure accurate modeling. Ionization states of the compounds were optimized at a physiological pH of 7.0 ± 2.0 to simulate biological conditions. This process generated up to 32 potential stereoisomers for each compound, accounting for their structural diversity. These prepared molecules were then ready for further computational analysis.

2.6.2 Protein Preparation

Docking proteins were received from the Protein Data Bank, including Escherichia coli gyrase B (PDB ID: 3G7E), Aspergillus niger β -1,4-endoglucanase (PDB ID: 5I77), and acetylcholinesterase (PDB ID: 6ARY). Protein preparation entailed adding hydrogen atoms, altering bond ordering, eliminating water molecules, defining hydrogen bonds, optimizing receptor atom charges, and minimizing energy using the OPLS3 force field.

2.7 Data Analysis

Statistical analysis was performed using SPSS for Windows[®] (version 21.0). A one-way analysis of variance (ANOVA) was conducted to assess differences between the extreme values of the studied groups. The median lethal concentration (LC_{50}) and the maximum lethal concentration (LC_{95}) were determined using the probit method. This approach helps estimate the toxicity of tested substances while considering biological response variability. Confidence intervals were calculated to ensure the reliability of the obtained results. These statistical analyses provide a better interpretation of the effects of the studied compounds.

3 Results

3.1 Yield of SREO

The essential oil output of SREO from Morocco varies greatly according on the cultivation region, as impacted by climatic circumstances, soil type, harvest season, and extraction methods [19]. Yields vary by geography, but typically range between 1% and 2.5% by weight. Plants growing in mountainous settings, such as the Atlas region, frequently generate higher amounts of essential oils, approaching the upper limit of 2.5%. This is attributed to water stress and altitude, which enhance the concentration of volatile compounds. In this study, the yield was 2.25%. who demonstrated that variations in the structure of secondary metabolism compounds and their natural quantity in medicinal plants are the main causes of variability in their biological activity. These variations are influenced by a variety of external and internal factors, including environmental

conditions, the plant's stage of maturity and the procedures used to prepare and extract the elements from the plant.

3.2 Chemical Composition

The compounds identified in SREO oils are listed in Table 1. A total of 16 distinct components were detected, representing 99.19% of the total oil content. Of these, the dominant components were 1,8-cineole (33.17%), camphor (16.54%) and α -pinene (14.46%), all classified as monoterpenes. These major compounds contribute significantly to the biological properties of the oil, including its antimicrobial and insecticidal activities. The chemical profile obtained in this study aligns with previous research conducted on *S. rosmarinus* essential oil in different regions [20,21]. This consistency highlights the reproducibility of our results and reinforces the typical phytochemical composition of Moroccan *S. rosmarinus*. Such findings are crucial for validating the potential applications of this essential oil in pharmaceutical, agricultural, and industrial fields.

No.	Components	Retention	Literature	Molecular	Peak area (%)	Classification
		matx	index	Iormula		
1	Alpha-pinene	939	931	$C_{10}H_{16}$	14.46	Monoterpene
2	Camphene	954	943	$C_{10}H_{16}$	8.14	Monoterpene
3	β-pinene	979	975	$C_{10}H_{16}$	3.74	Monoterpene
4	a-Terpinene	1017	1050	$C_{10}H_{16}$	3.01	monoterpene
5	ρ-Cymene	1025	1012	$C_{10}H_{14}$	4.19	Aromatic monoterpene
6	Limonene	1028	1031	$C_{10}H_{16}$	0.68	Monoterpene ether
7	1.8-Cineole	1030	1033	$C_{10}H_{18}O$	33.17	Monoterpene ether
8	β-myrcene	1048	992	$C_{10}H_{18}O$	3.17	Monoterpene ketone
9	Linalool	1097	1013	$C_{10}H_{18}O$	1.94	Monoterpene ketone
10	Camphre	1146	1375	$C_{10}H_{16}O$	16.54	Monoterpene ketone
11	Borneol	1169	1155	$C_{10}H_{18}O$	1.35	Monoterpene alcohol
12	a-Terpineole	1199	1202	$C_{10}H_{18}O$	7.65	Monoterpene alcohol
13	Verbenone	1205	1212	$C_{10}H_{14}O$	0.34	Monoterpene alcohol
14	Bornyl acetate	1289	1185	$C_{12}H_{20}O_2$	0.81	Phenolic monoterpene
15	β-Caryophyllène	1419	1413	$C_{15}H_{24}$	0.22	Monoterpene ester
16	α-Caryophyllène	1423	1425	$C_{15}H_{24}$	0.26	Monoterpene alcohol
	Total				99.19	

Table 1: Rosemary essential oil compounds table

3.3 Antibacterial Activity

The antibacterial activity tests demonstrated that SREO possesses properties against both Gram+ and Gram- bacteria, with varying minimum inhibitory concentrations (MICs) that reflect the bacteria's sensitivity to the oil. SREO showed the lowest MIC of 0.128% against *P. aeruginosa*, indicating stronger antibacterial activity compared to other strains and to results obtained with 1,8-cineole alone, which exhibited a higher MIC of 2.06% against *P. aeruginosa* (Table 2). In the test (MBC), SREO displayed the highest bactericidal activity at 1.03% against *E. coli* and *P. aeruginosa*, whereas the MBCs for *S. aureus* and *L. innocua* were 8.75% and 1.125%, respectively. These findings suggest that the antibacterial efficacy of SREO may be due to components other than 1,8-cineole or to the synergistic action of multiple bioactive compounds. The antimicrobial activity of essential oils has long been recognized, with their effectiveness attributed to individual components or their synergistic interactions. For instance, Hendry et al. (2009) [22] reported that crude essential oil exhibited significantly greater efficacy against planktonic microorganisms compared to 1,8-cineole (p < 0.05). The same study found that chlorhexidine digluconate and 1,8-cineole had a synergistic impact against *S. aureus*, methicillin-resistant *S. aureus* (MRSA), *E. coli*, and *C. albicans*.

		E. coli	S. aureus	L. innocua	P. aeruginosa
1.0	MIC (% <i>w/w</i>)	33	4.125	4.125	2.06
1-8 cineole	MBC (% <i>w/w</i>)	≥33	16.5	16.5	≥33
CDEO	MIC (% <i>w/w</i>)	0.25	2.06	1.03	0.128
SKEU	MBC (% <i>w/w</i>)	1.03	8.75	1.125	1.03

Table 2: Evaluation of antibacterial efficacy

These findings may differ for other bacterial strains, as noted by [23], who found that pure main chemicals were more efficient than essential oils in their investigation. *S. mitis* was the most susceptible pathogen examined, whereas *E. faecalis* shown the greatest resistance to the studied substances. Previous study has shown that 1,8-cineole has relatively high antibacterial activity against a variety of pathogens, including *S. aureus*, *P. aeruginosa*, *E. coli*, and *B. subtilis* [24,25]. However, as demonstrated in our work, the antibacterial activity of 1,8-cineole alone is lower than that of essential oils, most likely due to synergistic interactions between the various bioactive components present [26]. Despite its known antimicrobial properties, the precise mechanisms of 1,8-cineole's action remain unclear. To explore these mechanisms, an *in-silico* simulation was conducted to identify potential molecular targets for all essential oil components, including 1,8-cineole.

3.4 Antifungal Activity

The results of the antifungal tests differ from those of the antibacterial assays. In particular, 1,8-cineole showed stronger antifungal activity, as shown by its lower (MIC) values for all strains tested (Table 3), indicating high fungal sensitivity to this compound. The MICs for 1,8-cineole were 2.06% for *A. niger* and *P. digitatum*, and 4.125% for *Candida glabrata*. In comparison, the MICs for rosemary essential oils were higher, at 4.125% for *A. niger* and *P. digitatum*, and 8.75% for *C. glabrata*.

		Aspergillus niger	Candida glabrata	Penicillium digitatum
1.0	MIC (% <i>w/w</i>)	2.06	4.125	2.06
1-8 cineoie	MBC (% <i>w/w</i>)	16.5	≥33	16.5
CDEO	MIC (% <i>w/w</i>)	4.125	8.75	4.125
SKEO	MBC (% <i>w/w</i>)	33	≥33	33

Table 3: Assessment of antifungal activity

Regarding the minimum fungicidal concentration (MFC), 1,8-cineole exhibited the highest potency, achieving an MFC of 16.5% against *A. niger* and *P. digitatum*, outperforming both the other tested strains and rosemary essential oils. These findings highlight the efficacy of 1,8-cineole as a potent antifungal agent.

1245

SREO is well known for its potent natural antifungal effects. For example, reference [18] revealed a strong inhibitory effect on the mycelial growth of several fungi, including full inhibition of *B. cinerea*. Similarly, reference [27] found that *R. officinalis* essential oil has substantial inhibitory and fungicidal action against several Candida species, with MIC50 values ranging from 0.5% to 2% and MFC values between 1% and 2%. The study found complete suppression of *C. albicans* and *C. dubliniensis*.

The greater antifungal activity of 1,8-cineole compared to rosemary essential oils is consistent with prior findings. For example, reference [28] found that 1,8-cineole shows promising antifungal properties, efficiently suppressing *F. oxysporum*, *F. sporotrichioides*, and *A. tubingensis*. In addition, reference [29] found that a nanoemulsion gel containing 1,8-cineole had substantial inhibitory effects against dermatophyte fungus. Furthermore, reference [30] showed that 1,8-cineole efficiently prevents biofilm formation in the F. solani species complex by inhibiting genes responsible for ergosterol biosynthesis, influencing adhesion, interrupting mitochondrial activity and disrupting extracellular matrix synthesis.

3.5 Aphicidal Activity

M. persicae mortality rates at concentrations ranging from 5 to 40 μ L/L of air were used to assess the aphicidal activity of SREO and its main component, 1,8-cineole. The essential oil had a fatal effect on *M. persicae*, according to the data, and this effect was exacerbated when 1,8-cineole was used. After 24 h of exposure, both the essential oil and 1,8-cineole killed all of the examined insects at a concentration of 40 μ L/L of air. Following 12 h of exposure to the essential oil, the corresponding mortality rates for dosages of 5, 10, 20, and 40 μ L/L of air were 20%, 36.67%, 70%, and 93.33%. Notably, 1,8-cineole demonstrated the highest efficacy, achieving 100% mortality at a dose of 40 μ L/L of air after just 12 h of exposure (Fig. 1).



Figure 1: Toxicity of *S. rosmarinus* essential oil and 1,8-cineole on *M. persicae* individuals. Histograms show average mortality +/-1 S.D. Values followed by the same letter within each Eo or 1-8 cineole at each sampling time are not significantly different, whereas means followed by different letters are significantly different at p < 0.05

The essential oil (EO) of *S. rosmarinus* exhibited a lethal concentration (LC_{50}) of 16.3 µL and an LC_{95} of 36.38 µL after 12 h of exposure (Table 4). In contrast, 1,8-cineole showed an LC_{50} of 12.52 µL and an LC_{95} of 26.44 µL, indicating that the effect of 1,8-cineole is stronger than that of the essential oil.

Treatment	Exposure	df	Slope + SD	LC ₅₀	LC ₅₀	Intercept + SD	<i>p</i> value	\mathbf{X}^2
	time (h)							
EO	12	3	0.08 ± 0.001	16.3	36.38	-1.34 ± 0.12	0.00	21.54
	24	3	0.12 ± 0.01	11.74	25.46	-1.41 ± 0.13	0.001	17.1
	36	3	0.16 ± 0.01	8.11	18.4	-1.3 ± 0.13	0.00	24.09
	48	3	0.26 ± 0.03	5.61	11.84	-1.48 ± 0.17	0.00	26.13
1-8 cineole	12	3	0.12 ± 0.01	12.52	26.44	-1.48 ± 0.13	0.00	14.08
	24	3	0.13 ± 0.01	10.13	22.72	-1.32 ± 0.13	0.00	20.52
	36	3	0.22 ± 0.02	6.83	14.42	-1.48 ± 0.16	0.00	23.61
	48	3	0.29 ± 0.03	5.12	10.81	-1.50 ± 0.11	0.00	27.39

Table 4: LC₅₀ and LC₉₅ (in μ L/L of air) of the tested products against *M. persicae* at 12-hour intervals over two days

Bioassays of *S. rosmarinus* essential oil demonstrated significant insecticidal effects against *M. persicae*, resulting in a marked reduction in adult longevity. To combat the economic losses caused by agricultural pests, several plant essences have been studied for their ability to destroy *M. persicae* and other pests responsible for significant crop damage. According to [31], the essential oils of *L. angustifolia* and *T. vulgaris*, and their principal constituent's linalool and thymol, are harmful to the green fish lice *M. persicae* (Hemiptera: Aphididae). The study also revealed that the synergy between imidacloprid and oil compounds was stronger than with linalool or thymol, implying that secondary metabolites may be responsible for the observed synergy. Additionally, *Foeniculum vulgare* essential oil proved highly effective against *M. persicae* (LD₅₀ = 0.6 mL/L and LD₉₀ = 2.4 mL/L) without inducing significant mortality in non-target organisms [32]. Similarly, da Silva et al., da Silva et al. (2023) reported that *Cymbopogon winterianus* essential oil caused high mortality rates in *Brevicoryne brassicae* (100% at 0.5% in 48 h) and *M. persicae* (98.99% at 1% in 48 h).

According to these results, reference [33] showed that the most toxic essential oils were those from *S. chamaecyparissus* and *A. millefolium*, followed by *T. vulgare*, *T. patula*, and *A. absinthium*. Their LD_{50} values were 0.34%, 0.34%, 0.47%, 0.61%, and 0.69%, respectively, after a 24-hour treatment period. The bio-insecticidal ability of 1,8-cineole was further confirmed by the same authors, who also noted that the oils of *Santolina chamaecyparissus* are rich in this chemical.

4 Molecular Docking

In antibacterial activity, Camphor, Camphene, and Alpha-Terpineol were the most active molecules against *E. coli* Gyrase B with a glide score of –5.038, –4.905, and –4.856 kcal/mol, respectively. Whereas in antifungal activity, Camphene, Camphor, and 1,8-Cineole were the most active molecules against the active site of Aspergillus niger with a glide score of –4.75, –4.563, and –4.543 kcal/mol (Table 5). Regarding insecticidal activity, Camphor, Alpha-Terpineol, and Camphene exhibited remarkable inhibitory activity against acetylcholinesterase with a glide gscore of –7.023, –5.926, and –5.923 kcal/mol (Table 5). Camphor established a single hydrogen bond with residue ASN 46 in the active site of E. coli gyrase B (Figs. 2A and 3). This molecule has also established one hydrogen bond with residue CYS 447 in the active site of acetylcholinesterase (Figs. 1c and 2C) Interactions of Camphene the most active molecule in the active sites of *Aspergillus niger* showed no bond formation (Figs. 1b and 2B).

	Glide gscore (Kcal/mol)						
	E. coli Gyrase B (PDB: 3G7E)	Aspergillus niger (PDB ID: 5177)	Acetylcholinesterase (PDB: 6ARY)				
1,8-Cineole	-4.743	-4.543	-4.665				
α-Pinene	-4.368	-4.301	-5.731				
a-Terpineol	-4.856	-4.192	-5.926				
Camphene	-4.905	-4.75	-5.923				
Camphor	-5.038	-4.563	-7.023				

Table 5: Docking results of carvacrol in different receptors



Figure 2: A two-dimensional viewer showing ligand interactions with active sites. (**A**,**C**) Camphor interacts with *Escherichia coli* gyrase B and acetylcholinesterase active sites. (**B**) Camphene interacts with the active site of beta-1,4-endoglucanase from *Aspergillus niger*



Figure 3: A three-dimensional representation of how ligands interact with active sites. (**A** and **C**): Camphor interacts with the active sites of acetylcholinesterase and *Escherichia coli* gyrase B. (**B**): Camphene interacts with the As-pergillus niger beta-1,4-endoglucanase active site

5 Conclusion

This study highlighted the effects of rosemary essential oil and one of its major components, 1,8-cineole, on various bacterial and fungal strains, as well as its aphicidal activity against *M. persicae*. The findings demonstrate that the efficacy of rosemary oil and 1,8-cineole varies depending on the type of activity tested. Specifically, the essential oils exhibited stronger antibacterial activity compared to 1,8-cineole, while for antifungal activity, 1,8-cineole proved to be more potent. This suggests that the antibacterial properties of the essential oils may be attributed to other secondary compounds, whereas the antifungal effects are primarily linked to 1,8-cineole. In terms of aphicidal activity, 1,8-cineole also showed superior efficacy compared to the whole essential oil, emphasizing the potential of exploring specific molecular components to enhance the applications of aromatic and medicinal plants. Further research is recommended to investigate additional bioactive compounds identified in the chemical composition of rosemary essential oils, with the goal of optimizing and expanding upon the observed results.

Acknowledgement: Not applicable.

Funding Statement: The research was funded by Researchers Supporting Project number (RSP2025R119), King Saud University, Riyadh, Saudi Arabia.

Author Contributions: The authors confirm contribution to the paper as follows: Conceptualization, Ghizlane Houzi and Soad Khal-Layoun; methodology, Ghizlane Houzi, Aimad Allali, Amine Elbouzidi, Mohamed Taibi, Mohamed Chebaibi, and Ben Khada Zineb; software, Aimad Allali and Mohamed Chebaibi,; validation, Ramzi A. Mothana, Mohammed F. Hawwal, Rachid Flouchi, Abdeslam Asehraou, Amal Lahkimi, and Soad Khal-Layoun; formal analysis, Aimad Allali, Mohamed Taibi, and Amine Elbouzidi; investigation, Ghizlane Houzi, Aimad Allali, Mohamed Chebaibi, and Ben Khada Zineb; resources, Rachid Flouchi, Abdeslam Asehraou, Amal Lahkimi, and Soad Khal-Layoun; dota curation, Aimad Allali; writing—original draft preparation, Ghizlane Houzi, Mohamed Taibi, Aimad Allali, and Mohamed Chebaibi; writing—review and editing, Amine Elbouzidi, Ramzi A. Mothana, Mohammed F. Hawwal, Rachid Flouchi, Abdeslam Asehraou, Amal Lahkimi, and Soad Khal-Layoun; visualization, Ghizlane Houzi; supervision, Soad Khal-Layoun; project administration, Soad Khal-Layoun; funding acquisition, Ramzi A. Mothana and Mohammed F. Hawwal. All authors reviewed the results and approved the final version of the manuscript.

Availability of Data and Materials: The data that support the findings of this study are available from the Corresponding Authors, Amine Elbouzidi and Aimad Allali, upon reasonable request.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest to report regarding the present study.

References

- Khursheed A, Rather MA, Jain V, Wani AR, Rasool S, Nazir R, et al. Plant based natural products as potential ecofriendly and safer biopesticides: a comprehensive overview of their advantages over conventional pesticides, limitations and regulatory aspects. Microb Pathog. 2022;173(Pt A):105854. doi:10.1016/j.micpath.2022.105854.
- 2. Iseppi R, Mariani M, Condò C, Sabia C, Messi P. Essential oils: a natural weapon against antibiotic-resistant bacteria responsible for nosocomial infections. Antibiotics. 2021;10(4):417. doi:10.3390/antibiotics10040417.
- 3. Aimad A, Mssillou I, Siddique F, Mohmmed B, Abdelkrim A, Kara M, et al. Phytochemical analysis and pharmacological activities of essential oils extracted from *Zingiber officinale* (Roscoe) used in Mediterranean diet: *in vitro* and in *silico* studies. Int J Food Prop. 2024;27(1):1180–99. doi:10.1080/10942912.2024.2387937.
- Abdali El Y, Jalte Meryem M, Agour A, Allali A, Chebaibi M, Bouia A. Chemical composition, free radicals, pathogenic microbes, α-amylase and α-glucosidase suppressant proprieties of essential oil derived from Moroccan *Mentha pulegium: in silico* and *in vitro* approaches. J Biol Biomed Res. 2024;1(1):46–61. doi:10.69998/j2br3.

- 5. Zhu J, Xu Z, Gao P, Liu X. Chemical composition, antioxidant activity, enzyme inhibitory effects, and network pharmacology analysis of essential oil from *Bulbophyllum kWangtungense* Schltr. S Afr N J Bot. 2024;172:701–9. doi:10.1016/j.sajb.2024.08.005.
- 6. Ginting B, Chiari W, Duta TF, Hudaa S, Purnama A, Harapan H, et al. COVID-19 pandemic sheds a new research spotlight on antiviral potential of essential oils—a bibliometric study. Heliyon. 2023;9(7):e17703. doi:10.1016/j. heliyon.2023.e17703.
- 7. Wang Q, Wang XY, Tao J, Nie JT, Zhou YH, Huang J, et al. Exploring the potential anticancer targets and mechanistic pathways of *Elsholtzia densa* essential oil based on network pharmacology. J Asian Nat Prod Res. 2025;1–20. doi:10.1080/10286020.2024.2446294.
- 8. Borges RS, Ortiz BLS, Pereira ACM, Keita H, Carvalho JCT. *Rosmarinus* officinalis essential oil: a review of its phytochemistry, anti-inflammatory activity, and mechanisms of action involved. J Ethnopharmacol. 2019;229:29–45. doi:10.1016/j.jep.2018.09.038.
- 9. Mohammed HA, Sulaiman GM, Khan RA, Amin MA, Albukhaty S, Elshibani FA, et al. Factors affecting the accumulation and variation of volatile and non-volatile constituents in rosemary, *Rosmarinus* officinalis L. J Appl Res Med Aromat Plants. 2024;42:100571. doi:10.1016/j.jarmap.2024.100571.
- 10. Rossi YE, Palacios SM. Insecticidal toxicity of *Eucalyptus cinerea* essential oil and 1,8-cineole against *Musca domestica* and possible uses according to the metabolic response of flies. Ind Crops Prod. 2015;63:133–7. doi:10. 1016/j.indcrop.2014.10.019.
- 11. Hoch CC, Petry J, Griesbaum L, Weiser T, Werner K, Ploch M, et al. 1,8-cineole (eucalyptol): a versatile phytochemical with therapeutic applications across multiple diseases. Biomed Pharmacother. 2023;167:115467. doi:10.1016/j.biopha.2023.115467.
- 12. Poitou X, Thibon C. Darriet P. 1,8-cineole in French red wines: evidence for a contribution related to its various origins. J Agric Food Chem. 2017;65(2):383–93. doi:10.1021/acs.jafc.6b03042.
- 13. Chandorkar N, Tambe S, Amin P, Madankar C. A systematic and comprehensive review on current understanding of the pharmacological actions, molecular mechanisms, and clinical implications of the genus *Eucalyptus*. Phytomed Plus. 2021;1(4):100089. doi:10.1016/j.phyplu.2021.100089.
- Juergens UR, Dethlefsen U, Steinkamp G, Gillissen A, Repges R, Vetter H. Anti-inflammatory activity of 1,8-cineol (eucalyptol) in bronchial asthma: a double-blind placebo-controlled trial. Respir Med. 2003;97(3):250–6. doi:10. 1053/rmed.2003.1432.
- 15. Liu Q, Meng X, Li Y, Zhao CN, Tang GY, Li HB. Antibacterial and antifungal activities of spices. Int J Mol Sci. 2017;18(6):1283. doi:10.3390/ijms18061283.
- Tran HM, Diep Hong Le, Nguyen VT, Vu TX, Thanh NTK, Giang DH, et al. *Penicillium digitatum* as a model fungus for detecting antifungal activity of botanicals: an evaluation on Vietnamese medicinal plant extracts. J Fungi. 2022;8(9):956. doi:10.3390/jof8090956.
- 17. Gervasi T, Ginestra G, Mancuso F, Barreca D, De Luca L, Mandalari G. The *in vitro* potential of 1-(1 *H*-indol-3-yl) derivatives against *Candida* spp. and *Aspergillus niger* as tyrosinase inhibitors. Microorganisms. 2021;9(10):2070. doi:10.3390/microorganisms9102070.
- 18. Houzi G, El Abdali Y, Beniaich G, Chebaibi M, Taibi M, Elbouzidi A, et al. Antifungal, insecticidal, and repellent activities of *Rosmarinus officinalis* essential oil and molecular docking of its constituents against acetylcholinesterase and β -tubulin. Scientifica. 2024;2024:5558041. doi:10.1155/2024/5558041.
- 19. Llorens L, Llorens-Molina JA, Agnello S, Boira H. Geographical and environment-related variations of essential oils in isolated populations of *Thymus richardii* Pers. in the Mediterranean basin. Biochem Syst Ecol. 2014;56:246–54. doi:10.1016/j.bse.2014.05.007.
- 20. da Silva SG, Sant'Ana J, Jahnke SM, dos Santos CDR. Effects of essential oils from the Brazilian pepper tree, *Eucalyptus* and *Citronella* on *Brassica* aphids *Brevicoryne brassicae* and *Myzus persicae* (Hemiptera: aphididae) and their parasitoid *Diaeretiella rapae* (Hymenoptera: braconidae). J Plant Prot Res. 2023;63(3):286–96. doi:10.24425/ jppr.2023.146879.

- Oualdi I, Brahmi F, Mokhtari O, Abdellaoui S, Tahani A, Oussaid A. *Rosmarinus officinalis* from Morocco, Italy and France: insight into chemical compositions and biological properties. Mater Today Proc. 2021;45:7706–10. doi:10. 1016/j.matpr.2021.03.333.
- 22. Hendry ER, Worthington T, Conway BR, Lambert PA. Antimicrobial efficacy of *Eucalyptus* oil and 1,8-cineole alone and in combination with chlorhexidine digluconate against microorganisms grown in planktonic and biofilm cultures. J Antimicrob Chemother. 2009;64(6):1219–25. doi:10.1093/jac/dkp362.
- 23. Bernardes WA, Lucarini R, Tozatti MG, Flauzino LG, Souza MGM, Turatti ICC, et al. Antibacterial activity of the essential oil from *Rosmarinus officinalis* and its major components against oral pathogens. Z Naturforsch C J Biosci. 2010;65(9–10):588–93. doi:10.1515/znc-2010-9-1009.
- 24. Papadopoulos CJ, Carson CF, Chang BJ, Riley TV. Role of the MexAB-OprM efflux pump of *Pseudomonas aeruginosa* in tolerance to tea tree (*Melaleuca alternifolia*) oil and its monoterpene components terpinen-4-ol, 1,8-cineole, and alpha-terpineol. Appl Environ Microbiol. 2008;74(6):1932–5. doi:10.1128/AEM.02334-07.
- Merghni A, Noumi E, Hadded O, Dridi N, Panwar H, Ceylan O, et al. Assessment of the antibiofilm and antiquorum sensing activities of *Eucalyptus globulus* essential oil and its main component 1,8-cineole against methicillin-resistant *Staphylococcus aureus* strains. Microb Pathog. 2018;118:74–80. doi:10.1016/j.micpath.2018. 03.006.
- Honório VG, Bezerra J, Souza GT, Carvalho RJ, Gomes-Neto NJ, Figueiredo RCBQ, et al. Inhibition of *Staphy-lococcus aureus* cocktail using the synergies of oregano and rosemary essential oils or carvacrol and 1,8-cineole. Front Microbiol. 2015;6:1223. doi:10.3389/fmicb.2015.01223.
- 27. Gauch LMR, Pedrosa SS, Esteves RA, Silveira-Gomes F, Gurgel ESC, Arruda AC, et al. Antifungal activity of *Rosmarinus officinalis* Linn. essential oil against *Candida albicans*, *Candida dubliniensis*, *Candida parapsilosis* and Candida krusei. Rev Pan-Amaz Saude. 2014;5(1):61–6. doi:10.5123/s2176-62232014000100007.
- 28. Morcia C, Malnati M, Terzi V. *In vitro* antifungal activity of terpinen-4-ol, eugenol, carvone, 1,8-cineole (eucalyptol) and thymol against mycotoxigenic plant pathogens. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2012;29(3):415–22. doi:10.1080/19440049.2011.643458.
- 29. Li L, He M, Fang C, Zhang Y, Wang Y, Song X, et al. Preparation, characterization, *ex vivo* transdermal properties and skin irritation evaluation of 1,8-cineole nanoemulsion gel. Int J Pharm. 2022;624:121982. doi:10.1016/j.ijpharm. 2022.121982.
- 30. Zhang Y, Wang Y, Zhao X, Liu L, Xing R, Song X, et al. Study on the anti-biofilm mechanism of 1,8-cineole against *Fusarium solani* species complex. Front Pharmacol. 2022;13:1010593. doi:10.3389/fphar.2022.1010593.
- Faraone N, Kirk Hillier N, Christopher Cutler G. Plant essential oils synergize and antagonize toxicity of different conventional insecticides against *Myzus persicae* (Hemiptera: aphididae). PLoS One. 2015;10(5):e0127774. doi:10. 1371/journal.pone.0127774.
- 32. Pavela R. Essential oils from *Foeniculum* vulgare Miller as a safe environmental insecticide against the aphid *Myzus persicae* Sulzer. Environ Sci Pollut Res Int. 2018;25(11):10904–10. doi:10.1007/s11356-018-1398-3.
- Czerniewicz P, Chrzanowski G, Sprawka I, Sytykiewicz H. Aphicidal activity of selected Asteraceae essential oils and their effect on enzyme activities of the green peach aphid, *Myzus persicae* (Sulzer). Pestic Biochem Physiol. 2018;145:84–92. doi:10.1016/j.pestbp.2018.01.010.