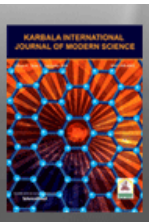

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Humera Khan

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Abstract

1,8-Cineole is a naturally occurring chemical molecule predominantly found in fragrant plants, particularly Myrtle. Its aroma is distinctive and has been the subject of numerous investigations due to its various biological actions. This study examines the characterization of 1,8-Cineole derived from Myrtle and investigates its antibacterial and antioxidant properties. This study seeks to compare 1,8-Cineole with antibiotics like Amoxicillin and Tetracycline, and moreover, to investigate its antioxidant capabilities against diverse bacterial strains (both Gram-positive and Gram-negative). 1,8-Cineole exhibits the most effective antibacterial properties, demonstrating an inhibition zone of 12.00 mm against *Staphylococcus aureus* and 10.00 mm against *Pseudomonas aeruginosa*. In comparison to Gram-negative bacteria, Gram-positive bacteria serve as superior antibacterial agents. Moreover, Amoxicillin showed greater potency than Tetracycline in the comparative analysis of the efficacy of two prevalent antibiotics. This indicates that 1,8-Cineole may exhibit greater efficacy when used in conjunction with other therapies, rather than as a standalone treatment. The antioxidant efficacy of 1,8-Cineole was evaluated using seven antioxidant assays, revealing significant results: ferric reducing antioxidant power (85.32±1.43), reducing power (95.38±2.36), $DPPH^{\cdot}$ scavenging (48.68±0.85), superoxide radical scavenging (65.42±1.92), and metal chelation (95.17±2.04). In conclusion, 1,8-Cineole is a prominent component of the essential oil derived from Myrtus communis. The compound has shown significant antibacterial efficacy against multiple bacterial strains and showed elevated antioxidant activity levels. The results suggest that 1,8-Cineole may be regarded as a viable natural agent for the development of antibacterial and antioxidant medicines.

Keywords

: 1, 8-Cineole; Antibacterial; Antioxidant; Eucalyptol; Plants; Oil.

RESEARCH PAPER

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Abstract

1,8-Cineole is a naturally occurring chemical molecule predominantly found in fragrant plants, particularly Myrtle. Its aroma is distinctive and has been the subject of numerous investigations due to its various biological actions. This study examines the characterization of 1,8-Cineole derived from Myrtle and investigates its antibacterial and antioxidant properties. This study seeks to compare 1,8-Cineole with antibiotics like Amoxicillin and Tetracycline, and moreover, to investigate its antioxidant capabilities against diverse bacterial strains (both Gram-positive and Gram-negative). 1,8-Cineole exhibits the most effective antibacterial properties, demonstrating an inhibition zone of 12.00 mm against *Staphylococcus aureus* and 10.00 mm against *Pseudomonas aeruginosa*. In comparison to Gram-negative bacteria, Gram-positive bacteria serve as superior antibacterial agents. Moreover, Amoxicillin showed greater potency than Tetracycline in the comparative analysis of the efficacy of two prevalent antibiotics. This indicates that 1,8-Cineole may exhibit greater efficacy when used in conjunction with other therapies, rather than as a standalone treatment. The antioxidant efficacy of 1,8-Cineole was evaluated using seven antioxidant assays, revealing significant results: ferric reducing antioxidant power (85.32 ± 1.43), reducing power (95.38 ± 2.36), DPPH scavenging (48.68 ± 0.85), superoxide radical scavenging (65.42 ± 1.92), and metal chelation (95.17 ± 2.04). In conclusion, 1,8-Cineole is a prominent component of the essential oil derived from *Myrtus communis*. The compound has shown significant antibacterial efficacy against multiple bacterial strains and showed elevated antioxidant activity levels. The results suggest that 1,8-Cineole may be regarded as a viable natural agent for the development of antibacterial and antioxidant medicines.

Keywords: 1,8-Cineole, Antibacterial, Antioxidant, Eucalyptol, Plants, Oil

1. Introduction

Antioxidant chemicals function through diverse chemical pathways that inhibit or avert the oxidation of substrates, so aiding in the determination of their biological significance and potential applications [1]. Antioxidants are defined as substances that inhibit the activity of free radicals. Free radicals are very unstable atomic clusters or individual atoms that have unpaired electrons. Such radicals may arise via the interaction of oxygen molecules with certain pharmaceuticals, environmental contaminants, and mitochondrial activities. Free radicals accumulate when antioxidant mechanisms are disrupted, leading to an increase in many diseases [2]. Free radicals induce harm to DNA, proteins, cell membranes, and other essential biological constituents. It causes harm linked to numerous health disorders such as rheumatoid arthritis, atherosclerosis, diabetes, asthma, anemia, and myocardial infarction [3,4]. These free radicals contribute to the progression of diabetes mellitus, impairing physiological functions and exacerbating issues associated with the heart, kidneys, liver, and eyes [5]. Intracellular antioxidants inhibit free radical production and play a role in regulating protein expression and activity in aerobic cells [6]

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Antimicrobial agents, whether typically determined or artificially created, are compounds planned to prevent, eliminate, treat, or inhibit the development of destructive microorganisms and infections, which include fungi, bacteria, and viruses [7,8]. They are used for therapeutic, prophylactic, and empiric purposes to treat infections [9]. However, novel anti-microbial, created through nanotechnology and computational strategies, have moved forward antimicrobial activities and are selective with target destinations, advertising better approaches to treat infectious diseases [10]. This includes antiseptic, disinfection, antibiotic, etc. Antibiotics are medications that fight bacterial diseases in individuals and animals by killing the bacteria or making it difficult for the bacteria to develop and increase [11].

Recent studies emphasize that plants with antimicrobial properties have become progressively critical with the rise of anti-microbial resistance. The misuse and overuse of antibiotics have led to the proliferation of resistant infections, making the exploration of bioactive chemicals from plants a fundamental area of research. These common compounds are being examined as potential options for conventional antibiotics to combat the developing danger of drug-resistant infections. This underscores the significance of vital plants in creating unused antimicrobial treatments in reaction to the worldwide challenge of antibiotic resistance [12,13].

Myrtle is a member of the Myrtaceae family, commonly known as *Myrtus communis*. This expansive and critical family of blooming plants that incorporates over 5000 species and 150 genera. This family is known for its fragrant plants, numerous of which have therapeutic, culinary, and decorative uses. The Myrtaceae family incorporates well-known plants such as eucalyptus, clove, and guava, widely valued for their essential oils and therapeutic/medicinal properties [14].

Investigate shows that Myrtle, like other individuals of the Myrtaceae family, contains an assortment of bioactive compounds, counting fundamental oils known for their potential therapeutic impacts, including antimicrobial, antioxidant, antiseptic, and anti-inflammatory properties [15]. Myrtle contains 15–35 % of 1,8-Cineole (depending upon the region), commonly known as Eucalyptol, a colorless liquid found in different medicinal plants that has potential gastroprotective, anti-bacterial, anti-inflammatory, antioxidant, and hepatoprotective properties [15–17]. Therefore, this study was conducted to study the antibacterial and antioxidant activities of 1,8-Cineole isolated from myrtle grown on the outskirts of Erbil, Kurdistan Region. It will compare 1,8-Cineole with different antibiotics such as Amoxicillin and Tetracycline, and it will also study its antioxidant activities on Gram (positive and negative) bacteria.

2. Related work

1,8-Cineole is a significant component in the essential oils of *M. communis* (myrtle) and contributes to its various medicinal properties [18]. It is chemically known as 1,3,3-trimethyl-2-oxabicyclic [2,2,2] octane with a chemical structural formula depicted in Fig. 1. This characteristic camphor-like odor of the monoterpenoid is one of the principal constituents in the essential oils of different species of Myrtle, accounting for their pharmacological actions. Its presence confirms the potential usage of these species for traditional and modern medicine associated principally with ointment, antibacterial, antimicrobial, anti-inflammatory, and antioxidant activities [19]. Indeed, studies realized that these essential oils, rich in 1,8-Cineole, coming from Myrtle species, showed a high potency of biological activities and were advantageous in developing new natural remedies and pharmaceutical applications [19–21].

2.1. Reviews on antibacterial activities of 1,8-Cineole

1,8-Cineole is the most abundant component found in sage, myrtle, and Laurel (belonging to Lamiaceae, Myrtaceae, and Lauraceae), and it has bactericidal properties and antibiotic activity [22].

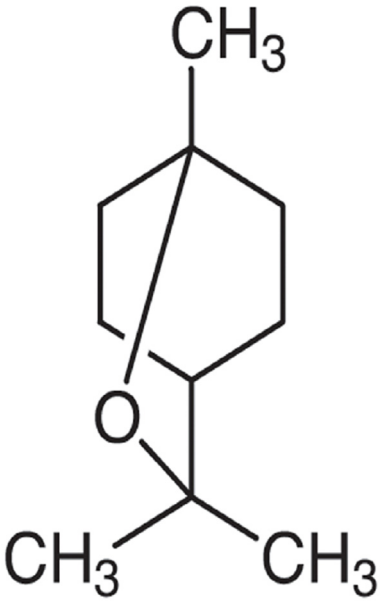

Fig. 1. Chemical structural formula of 1,8-Cineole.

Combining 1,8-Cineole with nisin against *Staphylococcus aureus* shows promising antibacterial activity, reducing each antibacterial compound dose [23]. 1,8-Cineole has been reported for high antibacterial activity, particularly against the Gram-positive bacteria *S. aureus* and the opportunistic pathogen *Streptococcus pneumoniae*. Its mechanism involves disrupting bacterial cell membranes, causing cell lysis and, hence, the death of these organisms. Therefore, this highly names it a prospective natural antimicrobial agent [14]. There have been reports of high antibacterial activities of 1,8-Cineole against Gram-positive and Gram-negative bacteria. It disrupts the membranes of the bacterial cell, which leads to leakage of cell constituents out of the cell, ultimately leading to the cell's death. As a compound occurring naturally in essential oils, this has recently been studied for possible applications in treating resistant infections [24].

2.2. Reviews on antioxidant activities of 1,8-Cineole

To date, less research has been conducted on the metabolism of 1,8-Cineole in humans. Research indicates that 1,8-Cineole significantly diminishes the production of cytokines and intracellular TLR-4 triggered by *Dermatophagoides pteronyssinus* in human bronchial epithelial cells [25].

Other studies further confirm the inhibitory effectiveness of 1,8-Cineole in producing pro-inflammatory cytokines in LPS-induced lung inflammation, human mononuclear cells, and lung macrophages [26]. 1,8-Cineole displayed anti-allergic potential by inhibiting IL-4 production and reduced cytokine-induced airway mucus hypersecretion, acting as an anti-inflammatory, anti-oxidant rather than a secretolytic. These effects give it potential as a steroid alternative in the management of respiratory disorders [27]. 1,8-Cineole isolated from the essential oils of plants having pharmacological properties that are anti-inflammatory and antioxidant, mainly via the regulation of NF-κB and Nrf2. Also, it has been traditionally used for the treatment of respiratory diseases and cardiovascular diseases [28].

1,8-Cineole derived from plants can enhance the performance of broiler chicks. It enhances chicken growth performance, immunity, antioxidant capacity, and intestinal health [29]. Cineole also enhances the antioxidant impact in rats on a daily basis [30].

3. Method

This section delineates the experimental methodologies employed to acquire, segregate, extract, fractionate, and isolate Myrtle for the investigation of the antibacterial and antioxidant properties of 1,8-Cineole.

3.1. Plant material

Myrtle (*M. communis*) was cultivated in the temperate area of Erbil City of Kurdistan region, Iraq, (shown in Fig. 2), particularly in gardens (nursery/greenhouse). The plant was cultivated during the autumn of 2021. The harvest was made at the end of the summer season when its leaves and berries were fully mature. Myrtle harvesting was performed meticulously by villagers and more professional farmers to collect the harvest, followed by drying processes in well-managed facilities to preserve the essential aromatic properties and medicinal values. As such, the cultivation and use reflect the significance of myrtle as a plant used as an ornament and for therapy.

The fully grown-up plant Myrtle (Myrtus Communis) is shown in Fig. 2. It shows mature leaves. The importance of plants' ornamental and therapeutic significance is stressed.

3.2. Methods for extraction, fractionation, and isolation (EFI)

100 g of the plant material *M. communis* were ground into fine powder and then extracted with a 1:1 v/v methanol: chloroform solvent solution for three successive cycles to ensure exhaustion of the

Fig. 2. Harvested myrtle plant.

bioactive compounds. The mixture was filtered, and the solvent was removed using a rotary evaporator at a controlled temperature that would not degrade target compounds.

The residue, after removal of the solvent, was further fractionated. In series, it yielded four fractions by solvent elution: hexane, dichloromethane (DCM), ethyl acetate, and methanol. Each fraction was dried in a lyophiliser to remove residual solvents. The methanol fraction demonstrated considerable antibacterial and antioxidant activities, so it was further fractionated by Sephadex column chromatography for thorough fractionation. Fractions were analyzed by thin-layer chromatography to pool the fractions of similar compositions, which led to nine sub-fractions.

Preparative high-performance liquid chromatography was employed for higher sensitivity and purity. Chromatographic separation was carried out on the Thermo Scientific™ Dionex™ UltiMate™ 3000 with a preparative column of Thermo Scientific™ Hypersil™ Prep BDS C18. The gradient elution is composed of methanol and pure water. A volume of 100 mg of each fraction was melted in a 1:1 (w:v) methanol and water solution, filtered using a 0.22 μm membrane, and then put into 5 mL vials for subsequent HPLC analysis. UV detection was followed up to monitor the separation process, and the separation method was optimized according to the appropriate UV wavelength [31].

The 1,8-Cineole was dissolved in deuterated methanol to elucidate its structural structure. High-resolution NMR spectroscopy was recorded on the Agilent-Premium Compact 14.1 T 600 MHz NMR system for its structural identification. Two-dimensional COSY, HMBC, HSQC, and ^{1}H and ^{13}C NMR were recorded.

Mass Spectra (MS) was conducted on the Agilent 6460 Triple Quad and HPLC for further characterization. The MS was connected to HPLC without an analytical column to obtain accurate mass data. Acetonitrile was used as the eluent, containing 5 MM of ammonium format with 0.1% formic acid. The volume injected was 4 μL with a flow of 0.400 mL/min. MS spectrum was acquired within the 50–1200 *m/z* range for 90 s [32].

3.3. Schematic for the extraction, fractionation, and isolation method

Figure 3 presents a comprehensive schematic illustration of the extraction, fractionation, and isolation methodology utilized in this research. It delineates the sequential procedure, emphasizing the principal techniques employed for the isolation of the target compound.

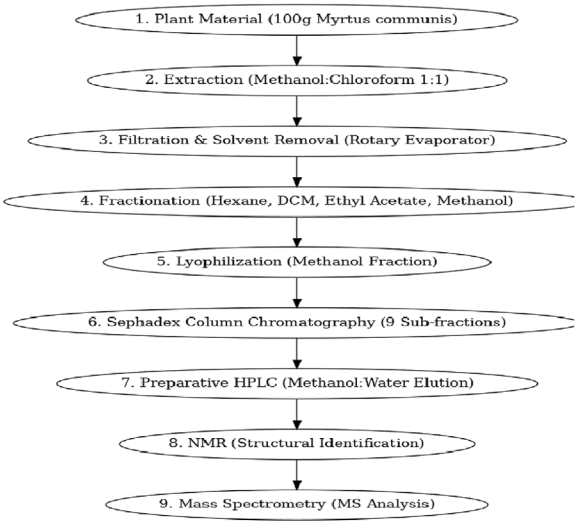

Fig. 3. Flowchart for EFI

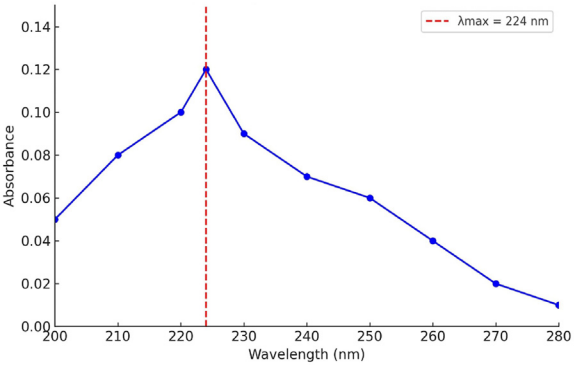

Fig. 4. UV–Vis absorption spectrum of isolated 1,8-Cineole.

3.4. Antibacterial activities

The activity of the principal constituent of *M. communis*, 1,8-Cineole, was screened against six bacterial strains, three of which were Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 15442, and *Klebsiella pneumoniae* ATCC 10031). Three were Gram-

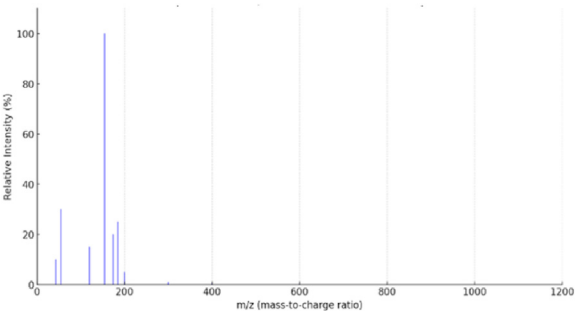

Fig. 5. Mass spectrum of isolated 1,8-Cineole.

positive bacteria (*Enterococcus faecalis* ATCC 29212, *Bacillus cereus* CCM 99, and *S. aureus* ATCC 25213).

In Kirby–Bauer antibiotic testing (established in 1940), 100 μL of each bacterial suspension, calibrated to the 0.5 McFarland standard, was uniformly distributed on Mueller Hinton Agar plates. Sterile 6 mm discs were saturated with 20 μL of the 1,8-Cineole solution at a concentration of 1024 μg/mL. The discs were placed on the agar plates and incubated for 16–18 h at 37 °C [33]. The antibacterial activity was quantified in millimeters by measuring the diameter of the clear zone of inhibition (refereeing Figure 6) surrounding the discs. Tetracycline and Amoxicillin served as standard antibiotics in the comparative research.

A cation-adjusted Mueller Hinton Broth medium was made for the determination of Minimum Inhibitory Concentration (MIC), which included Mueller Hinton Broth, 400 μL of $MgCl_2$, and 1 mL of $CaCl_2$ at a concentration of 2 mg/mL. This medium was supplemented to each well of a sterile 96-well microplate [34]. The 1,8-Cineole solution was added to the wells at an initial concentration of 1024 μg/mL and serially diluted. A quantity of 100 μL of the sample or antibiotic was added into each well after cooling and mixing well. Subsequently, 5 μL of the bacterial suspension was prepared with the inoculum to a turbidity of 0.5 McFarland in the cationic Mueller Hinton Broth medium and added to every well.

Microplate incubation was carried out for 2 h at +4 °C and continued at 37 °C for 16–18 h inside the incubator. The MIC value is expressed in μg/mL, the lowest concentration without apparent bacterial growth. Tetracycline and Amoxicillin were taken as reference standards for comparison in the assay.

3.5. Antioxidant activities

The Ferric Reducing Antioxidant Power (FRAP) assay was carried out to assess the antioxidant activity of 1,8-Cineole. The method involves the reduction of Fe^{3+} to Fe^{2+}. The blue-colored complex that forms Fe^{2+}-TPTZ was measured spectrophotometrically at 593 nm [35]. The reducing power of 1,8-Cineole was also evaluated by lessening the Fe^{3+}/ferricyanide complex to its ferrous form, with the green shade formation of Fe^{2+} measured at 700 nm [36]. The method is based on measuring the principle of orange complex formation by the ferrous ion, Fe^{2+}, with 1,10-phenanthroline, determined at 510 nm. Furthermore, at 517 nm, the obtained result was about the DPPH scavenging activity of 1,8-Cineole. The antioxidant capacity was also read at 734 nm with the results of 2, 2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ammonium salt oxidation by peroxy-disulfate [37]. The superoxide radical scavenging activity was assayed in the present study at 560 nm, reorienting the conversion of yellow NBT^{2+} to formazan blue due to its reaction with the superoxide radical generated by the PMS-NADH system. The ferrous ion chelating activity was finally ascertained at 562 nm due to the Fe^{2+}-ferrozine complex formation [38]. Antioxidant activity in vivo is precisely measured using the LPIC assay; a robust association between LPIC (Inco) and LPIC (Mixed) values is seen, indicating

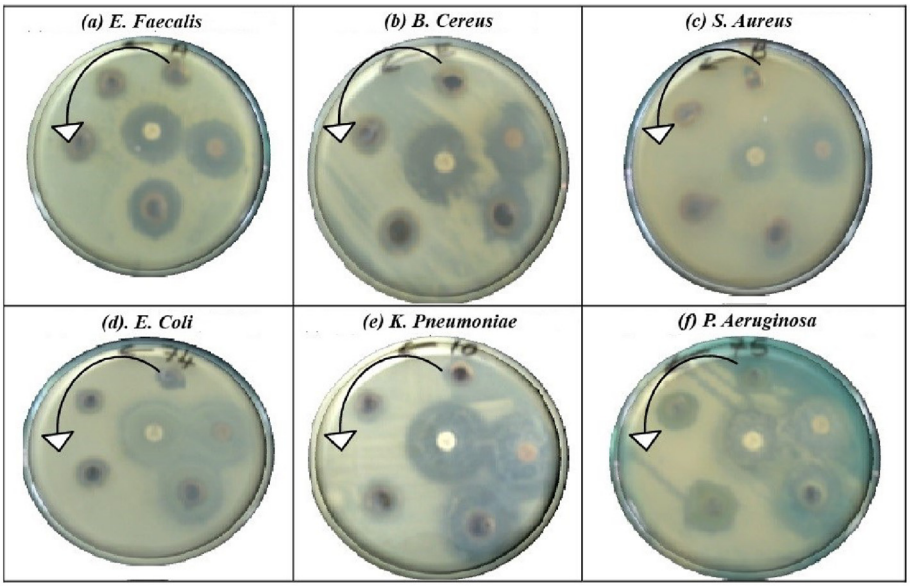

Fig. 6. Antibacterial Test: 1, 8-Cineole showing Inhibition.

the interplay between lipophilic and hydrophilic antioxidants [39].

4. Data analysis

The plant specimen was gathered in May 2024. The calculation of standard deviations for each parameter and the findings from triple replication in vitro organic action tests were employed. The analysis was conducted utilizing the statistical software SPSS (version 24). ANOVA was conducted to examine the interaction of many independent factors and their effect on the dependent variable. The movement was compared to the significance level of the values, and the result was shown using $p < 0.05$.

5. Results and discussion

The following section presents, some key findings, emanating from the characterization of 1,8-Cineole extracted from *M. communis*. The data concerning antibacterial and antioxidant activities are analyzed and discussed, accompanied by tables and figures showing the trends and correlations.

5.1. Characterization of isolated 1,8-Cineole by spectroscopic and analytical techniques

The compound 1,8-Cineole was isolated from *M. communis*, and several spectroscopic analyses and other tools confirmed its structural identity. To test its possible antibacterial and antioxidant properties, it was essential to characterize the compound and fully determine its degree of purity. Some techniques such as UV-V spectroscopy, ^{1}H NMR, ^{13}C NMR, and mass spectrometry (MS) worked, which made it possible to obtain complete information about the molecular structure of 1,8-Cineole.

The UV–Vis absorption data related to the isolated 1,8-Cineole percentages are given in Table 1. (See Tables 2–4) The absorption maxima at 224 nm are typical of the compound and are based on values reported in the literature, confirming the expected presence of chromospheres. While lacking solid chromospheres, 1,8-Cineole does have this UV absorption peak (this peak is shown in Figure 4), ensuring the purity of the compound and its identity as being within the literature.

In the graph, the absorbance values are plotted against a wavelength range with a discernible 224 nm peak, which is said to represent the λ_{max} of the compound-overview of the UV–Vis absorption spectrum of isolated 1,8-Cineole.

Table 1. UV–Vis absorption data of isolated 1,8-Cineole.

Peak	λ_{max} (nm)	λ_{max} (nm)	λ_{max} (nm)
1	224	0.12	224

Table 2. Computed & experimental ^{13}C NMR and ^{1}H NMR chemical shift (δ) of 1,8-Cineole by DFT Calculations.

Atom Position	^{13}C NMR		^{1}H NMR	
	δ (ppm) B3LYP	δ (ppm) M06–2X	δ (ppm) Experimental	δ (ppm) B3LYP
C1	76.735	72.618	73.62	–
C2	35.937	35.662	31.57	1.555
C3	28.711	28.052	22.90	2.066
C4	38.407	34.916	33.00	1.302
C5	28.714	28.037	22.90	2.066
C6	35.937	35.685	31.57	1.555
C7	81.141	76.903	69.77	–
C8	30.544	33.203	28.92	0.944
C9	30.544	33.226	28.92	0.944
C10	30.167	33.575	27.61	0.859
H2(α)	–	–	–	1.555
H2(β)	–	–	–	1.605
H3(α)	–	–	–	1.493
H3(β)	–	–	–	2.066
H4	–	–	–	1.302
H5(α)	–	–	–	1.492
H5(β)	–	–	–	2.066
H6(α)	–	–	–	1.555
H6(β)	–	–	–	1.605
H8(α)	–	–	–	0.944
H8(β)	–	–	–	1.505
H8(γ)	–	–	–	1.154
H9(α)	–	–	–	0.944
H9(β)	–	–	–	1.506
H9(γ)	–	–	–	1.154
H10(α)	–	–	–	0.859

The ^{13}C and ^{1}H NMR chemical shifts of 1,8-Cineole computed with the B3LYP and M06–2X functionals agree with the experimental data. The critical carbon shifts like C1 and C7 have differences of less than 1 ppm, which are very close to the experimental values of 73.62 ppm and 69.77 ppm, respectively. Moreover, the H2(α) and H3(β) proton shifts show minimal deviation from the experimental values. Some discrepancies, such as for C3 and C5 (around 4–5 ppm), are likely due to computational limitations. Nevertheless, the DFT calculations largely support the experimental identification of 1,8-Cineole.

5.2. Mass spectrum of 1,8-Cineole and other compound

The mass spectrum of 1,8-Cineole (clearly shown in Figure 5) has a clear and powerful molecular ion peak at *m/z* 154, exactly corresponding to the molecular weight of 1,8-Cineole ($C_{10}H_{18}O$). This is the compound's molecular identity. Moreover, a few small peaks are found, which are fragment ions probably generated from the decomposition of the

Table 3. Antibacterial potential of 1,8-Cineole.

Bacteria	Bacterial Strain Type	Inhibition Zone (mm)			Minimum Inhibition Concentration (μg/mL)		
		1,8-Cineole	Amoxil	Tetracycline	1,8-Cineole	Amoxil	Tetracycline
Enterococcus faecalis ATCC 29212	Gram-positive bacteria	–	–	32.00 ± 0.00	10	1024	10
Bacillus cereus CCM 99		–	27.00 ± 0.00	36.00 ± 0.00	255	<1	<0.5
Klebsiella pneumoniae ATCC 10031	Gram-negative bacteria	–	–	31.00 ± 0.00	>514	1024	10
Staphylococcus aureus		12.00 ± 0.05	21.00 ± 5.67	32.00 ± 0.00	255	1024	10
Escherichia coli ATCC 25922		9.50 ± 0.10		31.00 ± 0.00	>514	1024	<2
Pseudomonas aeruginosa		10.00 ± 0.00	8.00 ± 0.00	31.00 ± 0.00	>514	1024	10

Table 4. Antioxidant potential of 1,8-Cineole.

Antioxidant Assay	Activity	1,8-Cineole	α-Tocopherol	Ascorbic Acid	Ethylenediaminetetraacetic acid
Ferric reducing antioxidant power	A0.5 (μg/mL)	85.32 ± 1.43[a]	45.89 ± 0.77	60.22 ± 1.19	–
Reducing power		95.38 ± 2.36[a]	54.31 ± 1.52	74.11 ± 2.08	–
H2O2 scavenging		70.35 ± 1.6 [b]	52.92 ± 1.42	63.42 ± 1.77	–
DPPH scavenging	EC_{50} (μg/mL)	48.68 ± 0.85[a]	21.45 ± 0.38	31.34 ± 0.47	–
Superoxide radical scavenging		65.42 ± 1.92[a]	30.59 ± 0.93	40.73 ± 1.12	–
Metal chelate		95.17 ± 2.04[a]	–	–	32.15 ± 0.89
Lipid peroxidation inhibition assay		73.88 ± 1.27[b]	35.48 ± 0.82	54.13 ± 1.32	–

molecule. These ions are essentially the result of bond cleavage from various locations within the 1,8-Cineole molecule. The peak height indicates that the molecular ions are the most frequently present species.

In contrast, the ones with lower intensities show the typical fragment loss that occurs in the demotion of terpenoids of 1,8-Cineole. This formation is in line with all the previous mass spectra of 1,8-Cineole. Thus, it completely supports that the compound isolated is indeed 1,8-Cineole.

5.3. Antibacterial potential of 1,8-Cineole

The antibacterial activity of *M. communis* essential oil major compound, 1,8-Cineole, was assessed on Gram-negative and Gram-positive bacterial strains (Table 3). These Gram-positive bacteria contain a barrier around the cells, facilitating the cells to be easily permeated by hydrophobic compounds into the cell wall and cytoplasm, the reason for the extreme antibacterial effects. In contrast, some authors reported that the cell barrier structure of Gram-negative bacteria was more complex: it differed by the existence of a lipopolysaccharide layer made up of lipid A, core polysaccharide, and O antigen, which offers stronger resistance to be more hydrophobic and penetrate the cell [40].

Zone against different bacterial strains.

Results indicated that 1,8-Cineole showed notable antibacterial activity against different strains of bacteria. The MIC of 1,8-Cineole was 12 μg/mL against *S. aureus*, a Gram-positive bacterium, indicating potent antibacterial activity. The compound also produced inhibition zones of 9.50 ± 0.10 and 10.00 ± 0.00 mm against *E. coli* and *P. aeruginosa*, respectively. These results imply that 1,8-Cineole has high potential as an antibacterial agent, especially against Gram-positive bacteria. At the same time, its effect is weak on Gram-negative bacteria compared to the standard antibiotics amoxyl and tetracycline (Table 1).

This compound was as effective as Amoxicillin but not tetracycline. Concretely, Minimum Inhibitory Concentrations (MICs) of 1,8-Cineole against Gram-positive and Gram-negative bacteria such as *S. aureus* and *P. aeruginosa* revealed its prospect as an antimicrobial agent. As a reference antibiotic, Amoxicillin had higher potency than tetracycline, which suggests that 1,8-Cineole may be best suited for adjunctive therapy rather than a stand-alone treatment approach.

5.4. Antioxidant potential of 1,8-Cineole

The antioxidant activities of 1,8-Cineole and α-Tocopherol were determined in the present study using different assays, such as the Ferric Reducing Antioxidant Power, reducing power assay, H_2O_2 Scavenging DPPH scavenging assay, superoxide radical scavenging, metal chelation assay, and Lipid Peroxidation Inhibition Assay revealing its ability to neutralize free radicals and significantly reduce oxidative stress effectively (Table 4). Ascorbic Acid and Ethylenediaminetetraacetic acid were used in the antioxidant test.

For instance, in the reducing power test using the Ferric Reducing Antioxidant Power, 1,8-Cineole exhibited potent reducing activity with an EC50 value relatively close to the values provided by the standard antioxidants, such as ascorbic acid. The reducing power of 1,8-Cineole remarkably effectively reduced Fe^{3+} to Fe^{2+}. The scavenging activity against DPPH· of 1,8-Cineole presented potent free radical scavenging activity, with an EC50 value of about 48.68 μg/mL. Analogously, 1,8-Cineole revealed potent antioxidant activity in the Superoxide radical scavenging. The EC50 is about 65.42 μg/mL. On the other hand, 1,8-Cineole showed a marked metal chelating activity depending on the formation of the Fe^{2+}-ferrozine complex, with an IC50 of about 95.17 μg/mL. These results, therefore, would reflect a vigorous antioxidant activity exerted by 1,8-Cineole, a promising natural compound for investigations into conditions related to oxidative stress.

1,8-Cineole shows moderate to significant antioxidant effects in different tests, suggesting its promise as a natural antioxidant. It demonstrates high efficacy in scavenging DPPH radicals and moderate efficacy in other tests. Even though 1,8-Cineole is not as strong as α-Tocopherol and Ascorbic Acid, it still shows critical antioxidant abilities that may help decrease oxidative stress and shield against damage caused by ROS.

6. Conclusion

The compound 1,8-Cineole demonstrated remarkable antibacterial activity against various strains of bacteria. It was as powerful as Amoxicillin but not tetracycline. Specifically, the Minimum Inhibitory Concentrations (MICs) for 1,8-Cineole against Gram-positive and Gram-negative bacteria like *S. aureus* and *P. aeruginosa* confirmed its potential as an antimicrobial agent.

1,8-Cineole Shows direct to critical antioxidant action over different tests, demonstrating its potential as a characteristic antioxidant. It is a solid action in rummaging DPPH radicals and direct exercises in other tests. Whereas 1,8-Cineole is less potent than α-Tocopherol and Ascorbic Corrosive, it still illustrates essential antioxidant properties, which can help decrease oxidative stretch and secure against ROS-induced harm.

The research indicated that 1,8-Cineole constitutes a key component of the essential oil derived from *M. communis*. The chemical exhibited considerable antibacterial activity against multiple pathogenic strains and displayed robust antioxidant capabilities. The findings indicate that 1,8-Cineole may serve as a promising natural agent for the development of new antibacterial and antioxidant medicines. These findings underscore the significance of 1,8-Cineole as a bioactive agent in combating oxidative stress and bacterial infections, highlighting its potential application in health and wellness products.

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Conflict of interest

The authors report no conflict of interest in this work.

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