

RESEARCH ARTICLE

Antioxidant and antibacterial potential of bioactive extraction from *Cadaba glandulosa* leaves

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Abstract

This study investigated the bioactive components and explored the antioxidant and antibacterial properties of the methanolic leaf extracts of *Cadaba glandulosa* (MLCG). The observed activity is related to the diverse chemical composition of the extract as determined by gas chromatography-mass spectrometry (GC-MS) analysis, which tentatively identified 18 distinct compounds. Notable compounds include methyl dodecanoate, methyl tetradecanoate, 9,12-octadecadienoyl chloride, hexadecanoic acid methyl ester, palmitoleic acid, anethole, brefeldin A and oleic acid. The antioxidant tests showed a significant scavenging activity of 88.2% at a concentration of 381.5 µg/mL, which underlines the effectiveness of the extract in neutralizing free radicals. The total phenolic content in MLCG was found to be 79.5%, corresponding to 250.8 mg of gallic acid equivalents (GAE)/ mL. The antibacterial activity of MLCG showed variability between bacterial strains, with the strongest inhibition observed against *Staphylococcus aureus* and *Streptococcus pyogenes*, both Gram-positive bacteria. The extract showed moderate activity against Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*, while the least activity was observed against *Klebsiella pneumoniae*. In this study, the impressive antioxidant and antibacterial properties of MLCG underline the therapeutic potential of *Cadaba glandulosa* as a natural source of antioxidant and antibacterial agents.

Competing interests: NO authors have competing interests.

Introduction

The worldwide increase in antibiotic-resistant pathogenic bacteria has led to an intensive search for new antimicrobial agents, focusing on natural sources [1–3]. Plants are known for producing secondary metabolites with potent antimicrobial properties [3]. *Cadaba glandulosa*, a member of the Capparaceae family, is a hardy, low-growing shrub native to the arid and semi-arid regions of Africa, the Middle East and parts of Asia. This hardy plant thrives on rocky and sandy soils and can withstand extreme drought. This makes it ideally suited to the harsh desert environment of Saudi Arabia, particularly in the southwestern and central regions, where its drought and heat tolerance ensure its survival in the local flora. *Cadaba glandulosa* is known for its dense, glandular-hispid, rounded leaves and small, greenish-yellow flowers pollinated by insects. It is traditionally used to treat liver ailments, including hepatotoxicity and hepatitis B and C infections [4]. Although this plant has long been valued in traditional medicine, the specific bioactive compounds responsible for its therapeutic effects are still largely unexplored. Recent scientific interest has triggered initial research on its bioactive profile, suggesting that it contains compounds with remarkable therapeutic potential. Bioactive components such as alkaloids, sesquiterpene lactones and cadabacin are found in many *Cadaba* species. *Cadaba farinosa* and *Cadaba glandulosa* are traditionally used for their laxative, vermifugal, antisyphilitic, emmenagogue, aperitive, antiscorbutic and anti-inflammatory properties. They are also used in treating liver disorders, cancer, dysentery, fever and body aches, as well as for their hepatoprotective and hypoglycemic effects [4–10]. The present study aims to determine the bioactive components through gas chromatography-mass spectrometry (GC-MS) analysis, antioxidant, and antibacterial potential of the leaves of *Cadaba glandulosa*.

Materials and methods

Materials

All chemicals used in this study were of analytical grade. Methanol (analytical grade, $\geq 99.8\%$ purity) and other chemicals were purchased from Sigma-Aldrich (USA). HPLC-grade methanol used for GC–MS sample preparation and purification was obtained from Merck KGaA (Darmstadt, Germany). Bacteriological media were obtained from Scharlau (Spain). Additional chemicals and culture media were supplied by Afaq Sada Trading, Riyadh, Kingdom of Saudi Arabia, and Somatco, Jeddah, Kingdom of Saudi Arabia.

Plant collection and identification

Jazan City, the capital of Jazan Province, is in the southwest of Saudi Arabia on the coast of the Red Sea. It is located at 42° – 43° east longitude and 16° – 17° north latitude and is thus on the extreme south-western edge of the country. The region borders Aseer to the north and east, the Red Sea to the west and Yemen to the south and south-east. Jazan is not only known for its coastline and rich agriculture, but also for its arid and semi-arid areas where hardy desert plants thrive. These plants

have adapted to extreme heat, low rainfall and saline soil and play an important role in the region's ecosystem, providing valuable medicinal benefits. Permission for plant collection was obtained from the Wildlife and National Vegetation Cover authorities, Saudi Arabia. The plant species is not classified under any special conservation category, ensuring compliance with regulatory guidelines. *Cadaba glandulosa* leaves were collected from the Jazan region, Kingdom of Saudi Arabia, in April 2023. Fresh *Cadaba glandulosa* leaves were carefully washed with Millipore water, gently blotted dry with sterile wipes, and separated from the plant material. The leaves were air-dried in the shade at ambient room temperature (25–30 °C) for 7–10 days until a constant weight was reached (Fig 1). The dried leaves were then pulverized into a coarse powder and stored in airtight containers at room temperature until extraction. The prepared extracts were stored at 2–8 °C until use. The plant was identified and authenticated by Dr. Remesh Mochikkal, Herbarium Curator, Department of Biology, College of Science, Jazan University, Jazan, Saudi Arabia. A voucher specimen was deposited for future reference. The plant was confirmed as *Cadaba glandulosa* and assigned the reference number 1221 (JAZUH).

Sample preparation and extraction

The dried leaves of *Cadaba glandulosa* were ground to a coarse powder with a grinder and stored in an airtight container for later use. The extraction process followed the method described by Sultan et al. (2020) [11]. The bioactive compounds from the leaves were isolated using the hot continuous percolation technique with methanol as solvent. Approximately 200 g of powdered leaves were placed in a Soxhlet extractor and subjected to constant extraction with methanol at 60 °C for 4 hours. After extraction, the mixture was cooled, and the methanolic extract was transferred to a sterile glass vessel. The methanolic extract obtained from the leaves of *Cadaba glandulosa* was designated as MLCG. The solvent was allowed to evaporate under open-air conditions until a dry residue was obtained. The dried extract (MLCG) was collected and combined before being measured for weight before storage for future analysis. For DPPH assay and total phenolic content determination, dried MLCG was dissolved in analytical-grade methanol to obtain a stock solution of 10 mg/mL. For GC–MS analysis, the dried methanolic MLCG was dissolved in HPLC-grade methanol at a concentration of 1% (w/v; 10 mg/mL). The resulting solution was filtered through a 0.2 µm Millex-GV syringe filter composed of polyvinylidene fluoride (PVDF) (Merck KGaA, Darmstadt, Germany) prior to injection and analysis. For antibacterial evaluation, the dried MLCG was dissolved in dimethyl sulfoxide (DMSO) to prepare a 300 µg/mL solution, and 100 µL of this solution was applied per well or disc during the assay.



Fig 1. *Cadaba glandulosa*. (A) The collected plant is washed and exposed to open air for drying. (B) Dorsal view of the leaves arranged along the stem of the plant. (C) Leaves are plucked, collected, and air-dried in the shade.

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GC-MS analysis

To ascertain phytochemical composition of the resulting filtered MLCG, it was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The GC-MS analysis utilized Thermo Scientific equipment with an AS 3000 autosampler and an ISQ detector. A 2 μ L extract sample was introduced into a TR 5MS capillary column for partial component separation. Helium served as a carrier gas, maintaining a flow rate of 1.2 mL/min. The oven was initially set to 50°C for 10 minutes and the temperature gradually increased to 70°C, 100°C, 150°C, 200°C, 250°C, 270°C and finally 290°C at a rate of 5°C per minute. At each step, the temperature was held for 10 minutes, resulting in a total run time of 113 minutes. The MS detector (ISQ) was configured to identify molecular masses within the range of 40–650 amu at an ionization energy of 70 eV in positive ion mode. A delay period of 3 minutes was applied to prevent the interference of initial solvent peaks in the recorded spectra. The injector port, MS transfer line, and ion source temperatures were set to 250°C, 270°C, and 280°C, respectively. Mass spectrometry was conducted, and spectral data was analyzed via Xcalibur software. The mass spectra were interpreted using the NIST and MAINLIB software libraries to identify and record the bioactive compounds present.

Antioxidant activity by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The antioxidant capacity of the test sample MLCG was evaluated using the DPPH stable free radical assay method [12,13]. A DPPH solution was prepared by dissolving 2.4 mg DPPH in 100 ml methanol. For the assay, 3.995 mL of this DPPH solution was mixed with 5 μ L of the MLCG sample. The mixture was incubated in the dark for 30 minutes, with gallic acid serving as the standard control due to its well-recognized antioxidant capacity and strong free radical scavenging activity. A calibration curve was constructed using concentrations ranging from 100 to 500 μ g/mL. The antioxidant activity of the sample was expressed as micrograms of gallic acid equivalent to per microgram of dry weight. The absorbance of the test sample (A_T) and the standard control (A_C) was measured at 517 nm using a spectrophotometer. All measurements were performed in triplicate for accuracy.

$$\% \text{ inhibition} = \frac{A_C - A_T}{A_C} \times 100$$

Determination of the total phenol content

The total phenolic content (TPC) of the sample was determined using the Folin–Ciocalteu colorimetric method [14]. In brief, 100 μ L of the MLCG was mixed with 500 μ L of a 10% (v/v) Folin–Ciocalteu reagent and incubated for 5 minutes at room temperature. Subsequently, 400 μ L of a 7.5% sodium carbonate (Na_2CO_3) solution was added, and the reaction mixture was incubated for 30 minutes at room temperature in the dark. The absorbance of the resulting, blue-colored complex was measured at 760 nm using a spectrophotometer. Gallic acid was used as a standard reference compound and a calibration curve was constructed with gallic acid concentrations ranging from 50 to 500 μ g/mL. The total phenolic content was calculated from the calibration curve and expressed as micrograms of gallic acid equivalents (GAE) per microgram dry weight of sample. All measurements were performed in triplicate to ensure accuracy.

Evaluation of the antibacterial potential of MLCG

Bacterial strains utilization and culture standardization. The study employed various bacterial strains, including *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Streptococcus pyogenes* ATCC 19615, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC 700603. A 24-hour culture was initiated and adjusted to a standardized concentration by diluting it in a gradient ranging from 10^{-1} to 10^{-7} using nutrient broth [3]. The bacterial culture viability was determined by quantifying the colony-forming units per milliliter (CFU/mL).

Assessment of antibacterial susceptibility. The antibacterial activity was evaluated following the method described by Moni et al. (2018) [3]. Bacterial cultures were prepared by subculturing stock strains onto Mueller–Hinton agar plates and incubating for 24 h prior to testing. The antibacterial efficacy of the MLCG and the standard ciprofloxacin was assessed using the agar well diffusion method. A sterile cotton swab soaked with a culture containing a specific concentration of CFU/mL for each bacterial strain selected in the study was used for inoculation. The cotton swab was swabbed over the Muller-Hinton agar plates and allowed to dry for about 10 minutes before applying the samples. The agar plates were inoculated, and sterile stainless-steel drills were used to create uniform wells for the agar well diffusion assay. This method was employed to evaluate the activity of the test samples alongside the standard ciprofloxacin at a concentration of 50 µg/mL. Antibacterial activity was assessed by measuring the zones of inhibition around the wells following 24 hours of incubation at 37°C. The results, showing the antibacterial range based on the size of these zones, were methodically documented in “Table 1”, demonstrating the relationship between the diameter of inhibitory zones and the antibacterial activity spectrum.

Statistical analysis

Statistical analysis was performed using Prism 9 (GraphPad Instat Software, USA). All data were expressed as mean ± standard deviation (SD) from three independent experiments (n=3). One-way analysis of variance (ANOVA) was applied, followed by Tukey’s post hoc test to determine the level of significance among groups. Differences were considered statistically significant at $p < 0.05$, $*p < 0.01$, and $**p < 0.001$.

Results

In this study, numerous bioactive compounds were identified by GC-MS analysis, as shown by the different peaks in the GC-MS chromatogram in Fig 1. The main bioactive compounds detected in the MLCG sample are summarized in “Table 2” along with the corresponding retention times (RTs). Figs 2–4 illustrates the chemical structures of these bioactive molecules. A total of 18 bioactive compounds were identified tentatively, each with different retention times, molecular properties and percentages. Among these, methyl dodecanoate was the most abundant compound, accounting for 9.44% of the chromatogram area with an RT of 18.53 minutes, which underlines its importance. Similarly, methyl tetradecanoate accounted for 4.39% of the total area with a RT of 22.79 minutes, indicating its notable presence in the sample. Another important

Table 1. Antibacterial Study of MLCG.

Organisms	Concentration of 24h culture CFU /mL	Zone of Inhibition in millimeter		
		MLCG (300 µg/mL)	Ciprofloxacin (50 µg/mL)	DMSO
<i>Bacillus subtilis</i> ATCC 6633	2×10^5	14 ± 2.6	27.2 ± 1.2	-
<i>Staphylococcus aureus</i> ATCC 25923	3×10^4	20.5 ± 1.5	26.6 ± 1.3	-
<i>Streptococcus pyogenes</i> ATCC 19615	3×10^4	18.7 ± 1.5	24.16 ± 0.4	-
<i>Escherichia coli</i> ATCC 25922	2×10^5	15.5 ± 2.5	28 ± 2.1	
<i>Pseudomonas aeruginosa</i> ATCC 27853	4×10^4	16.5 ± 2.0	26.6 ± 0.6	-
<i>Klebsiella pneumoniae</i> ATCC 700603	2×10^4	14.5 ± 0.5	28.8 ± 1.3	-

All values represent the mean of three independent batches (n=3) with standard deviation. Statistical analysis was performed using Dunnett’s multiple comparison test (Sample vs. Standard) as a post hoc test, with significance set at $p < 0.05$. The effects observed in the samples were significantly lower than those of the standard ciprofloxacin. Statistical analysis showed that *Staphylococcus aureus* displayed **highly significant differences ($p < 0.001$) compared with *Bacillus subtilis*, *Escherichia coli*, and *Klebsiella pneumoniae*, *Moderately significant differences ($p < 0.01$) were observed when compared with *Streptococcus pyogenes* and *Pseudomonas aeruginosa*, reflecting intermediate sensitivity. *MLCG: Methanolic leaf extracts of *Cadaba glandulosa* (-) No activity.

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Table 2. GC-MS detection of possible bioactive compounds of MLCG.

S.no	Compound name	Molecular formula	Molecular weight	Retention time (Min)	Probability Index	Percent area of curve
1	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	214	18.53	61.40	9.44
2	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	298	40.41	20.19	4.52
3	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	22.79	37.96	4.39
4	Fenretinide	$C_{26}H_{33}NO_2$	391	23.37	7.81	0.17
5	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	32.40	26.48	1.97
6	Anethole	$C_{10}H_{12}O$	148	15.94	23.04	1.59
7	Palmitoleic acid	$C_{16}H_{30}O_2$	254	19.21	26.41	1.04
8	17-Octadecynoic acid ethanol	$C_{18}H_{32}O_2$	280	26.85	16	1
9	Brefeldin A	$C_{16}H_{24}O_4$	280	42.13	19.38	1
10	1-Heptatriacotanol	$C_{37}H_{76}O$	536	32.10	10.02	0.82
11	cis-11-Eicosenoic acid	$C_{20}H_{38}O_2$	310	42.79	18.93	0.77
12	Dasycarpidan-1-methanol, acetate	$C_{20}H_{36}N_2O_2$	326	58.70	16.61	0.75
13	2-Hexadecanol	$C_{16}H_{34}O$	242	25.03	17.45	0.74
14	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{40}O_4$	356	53.25	14.66	0.70
15	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	62.34	15.17	0.56
16	2-Bromotetradecanoic acid	$C_{14}H_{27}BrO_2$	306	50.41	12.90	0.50
17	8-Octadecenal	$C_{18}H_{34}O$	266	25.25	13.68	0.39
18	Oleic Acid	$C_{18}H_{34}O_2$	282	24.81	17.70	0.22

* **MLCG**: Methanolic leaf extracts of *Cadaba glandulosa*.

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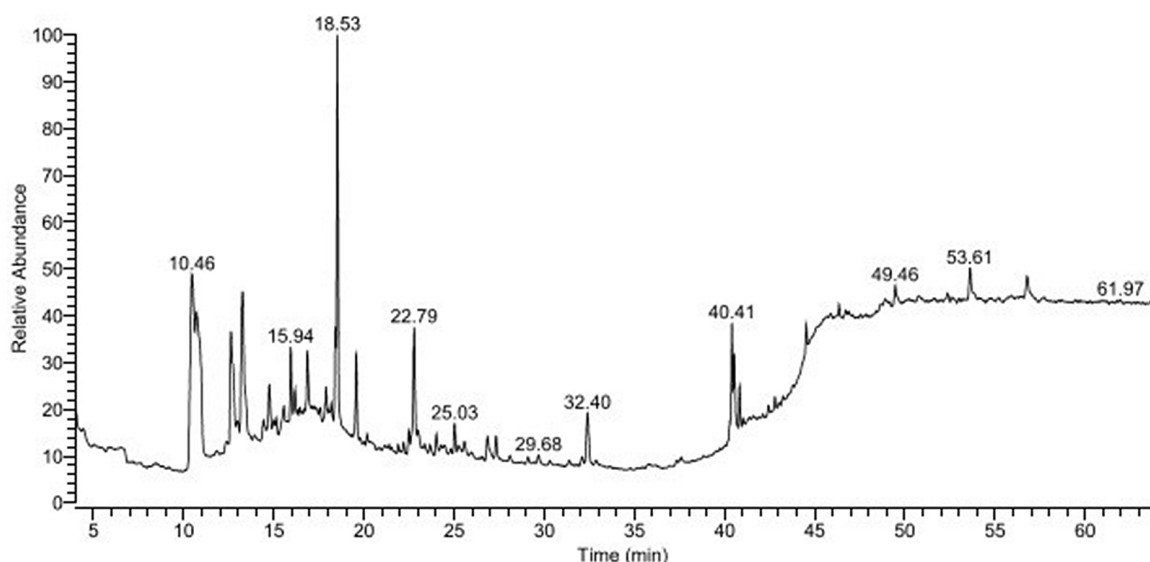


Fig 2. GC-MS chromatogram of MLCG.

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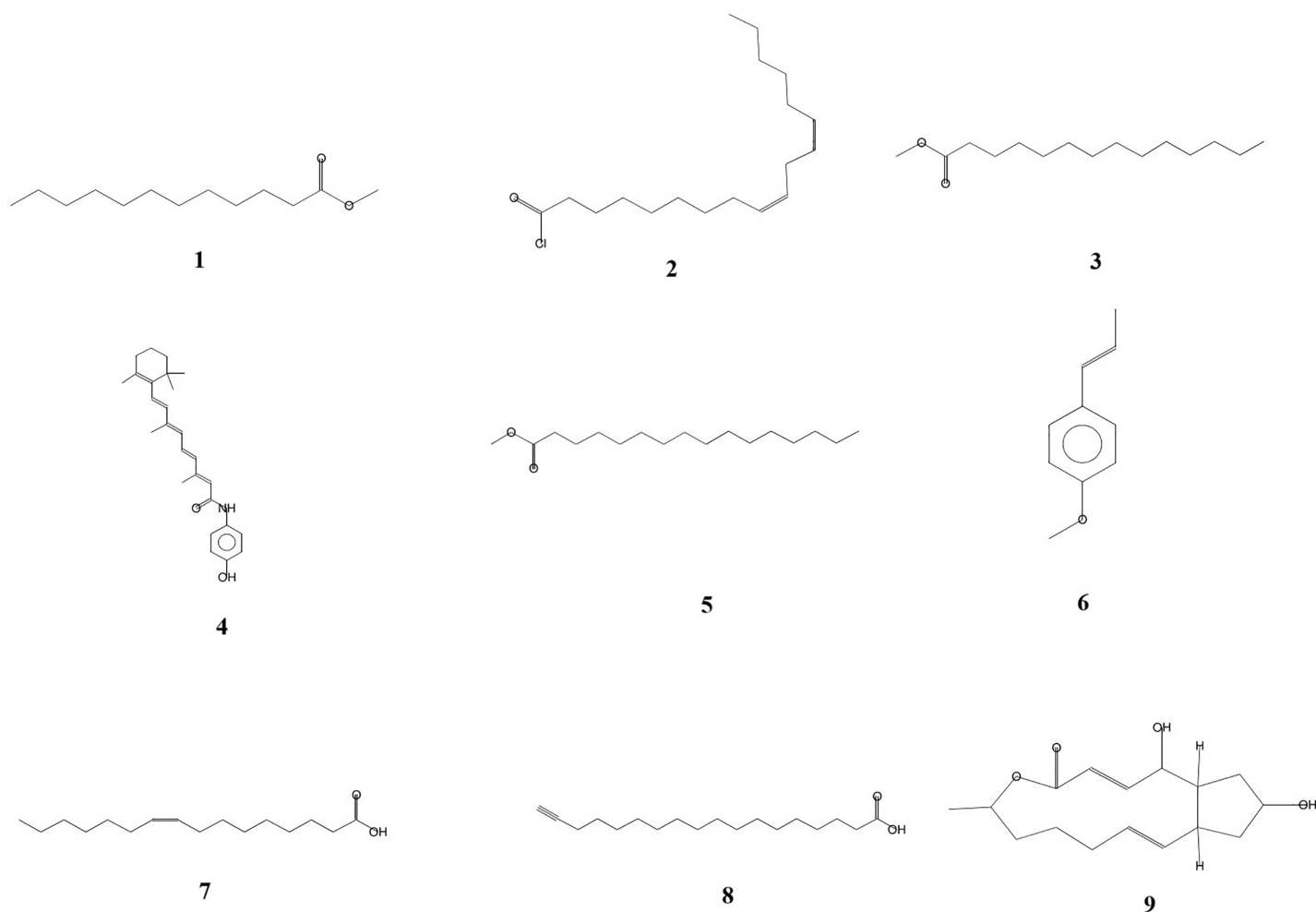


Fig 3. The structure of bioactive compounds of the MLCG. (1) Dodecanoic acid, methyl ester (2) 9,12-Octadecadienoyl chloride, (Z,Z)- (3) Methyl tetradecanoate (4) Fenretinide (5) Hexadecanoic acid, methyl ester (6) Anethole (7) Palmitoleic acid (8) 17-Octadecynoic acid (9) Brefeldin A.

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compound, 9,12-octadecadienoyl chloride, accounted for 4.52% of the area and had a longer RT of 40.41 minutes. Fatty acid derivatives such as hexadecanoic acid, methyl ester (1.97%) and palmitoleic acid (1.04%) emphasize the diversity of fatty acids in MLCG.

In addition, unique compounds with different bioactivities were identified. Anethole, with a RT of 15.94 minutes and an area of 1.59%, and brefeldin A, which occupies 1% of the area at a RT of 42.13 minutes, stand out due to their characteristic chemical structures. Compounds present at lower concentrations also contributed to the molecular diversity of the sample. For example, 1-heptatriacotanol and cis-11-eicosenoic acid were detected at 0.82% and 0.77% at RTs of 32.10 and 42.79 minutes, respectively. Similarly, dasycarpidan-1-methanol acetate and 2-hexadecanol contributed 0.75% and 0.74%, while 9-octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester accounted for 0.70%. It is noteworthy that rare bioactive molecules such as ethyl isoalcoholate (0.56%, RT 62.34 min), 2-bromotetradecanoic acid (0.50%, RT 50.41 min) and 8-octadecenal (RT 25.25 min) were also identified. Oleic acid, a known bioactive fatty acid, was present at 0.22% and a RT of 24.81 minutes. These results demonstrate the wide range of chemical compounds in MLCG, which include fatty acids, esters and rare bioactive molecules. This diversity indicates the potential for a wide range of biological

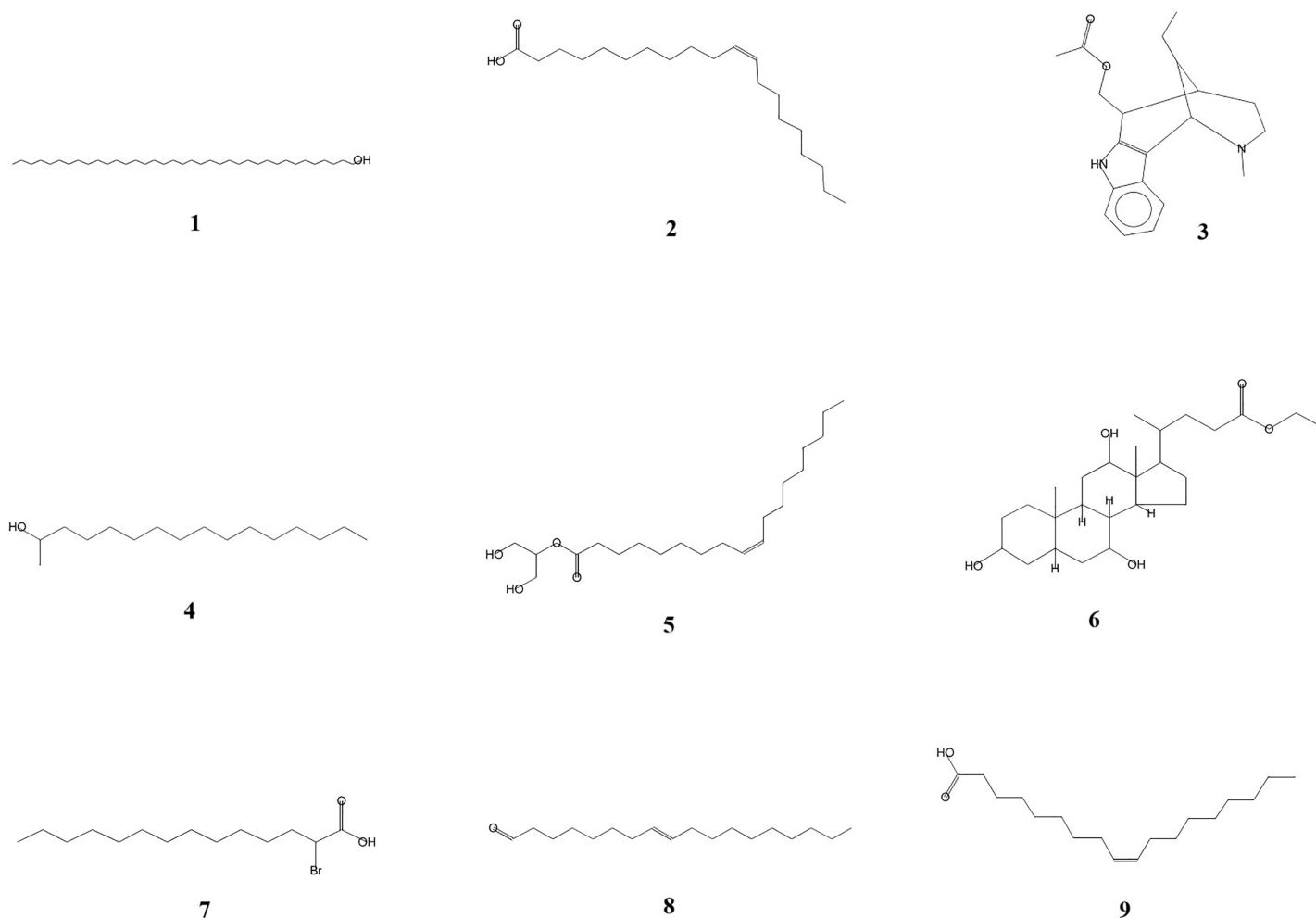


Fig 4. The structure of bioactive compounds of the MLCG. (A) 1-Heptatriacotanol (2) cis-11-Eicosenoic acid (3) Dasycarpidan-1-methanol, acetate (4) 2-Hexadecanol (5) 9-Octadecenoic acid (Z), 2-hydroxy-1-(hydroxymethyl)ethyl ester (6) Ethyl iso-allocholate (7) 2-Bromotetradecanoic acid (8) 8-Octadecenal (9) Oleic Acid.

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activities and applications. These findings confirm that the high phenolic content of MLCG contributes significantly to its strong free radical scavenging potential. Fig 5 illustrates the DPPH radical scavenging activity of gallic acid, MLCG and gallic acid standard curve for total phenolic content (TPC). As shown in Fig 5A, gallic acid exhibited a strong and linear increase in DPPH radical scavenging activity with increasing concentration, confirming the validity and reliability of the assay. Likewise, Fig 5B demonstrates that MLCG displayed a pronounced dose-dependent enhancement in DPPH inhibition, indicating significant antioxidant potential. MLCG achieved a high level of antioxidant activity, with 88.2% DPPH inhibition at 381.5 $\mu\text{g/mL}$, whereas the gallic acid standard exhibited a stronger scavenging effect, reaching approximately 94.5% inhibition at 472 $\mu\text{g/mL}$. Furthermore, the total phenolic content of MLCG, calculated using the gallic acid calibration curve (Fig 5C), was determined to be 79.5%, corresponding to 250.8 mg gallic acid equivalents (GAE)/mL, suggesting a close association between phenolic content and antioxidant activity.

The antibacterial activity of MLCG (300 $\mu\text{g/mL}$) was investigated against a range of human pathogenic bacteria, and its efficacy was compared with ciprofloxacin (50 $\mu\text{g/mL}$) as a standard antibiotic ("Table 1"). MLCG showed varying degrees

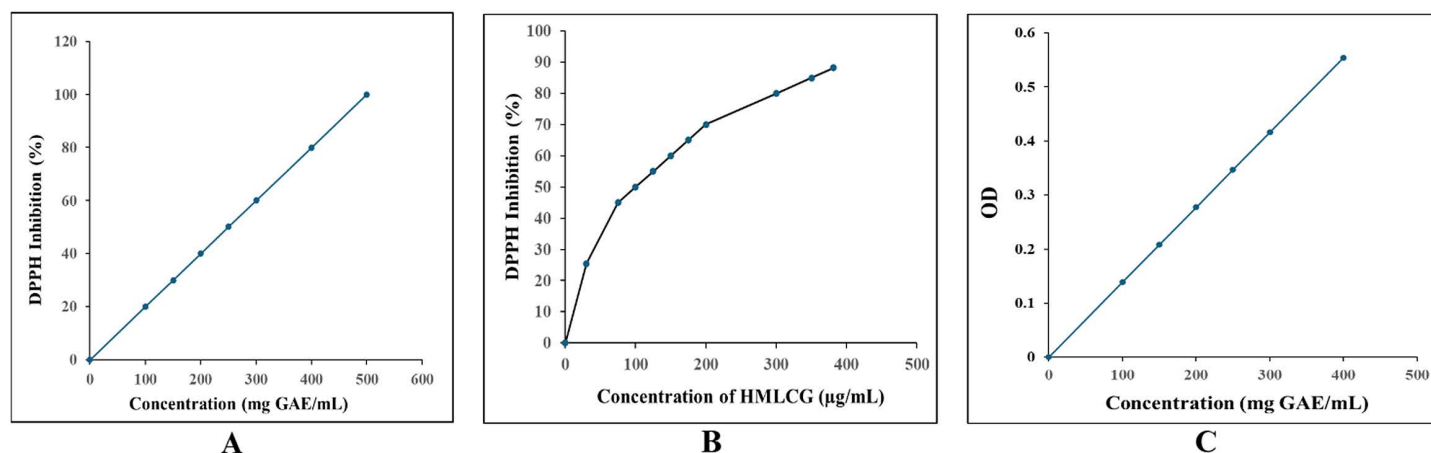


Fig 5. Evaluation of antioxidant activity and phenolic content of *Cadaba glandulosa* leaf extract. (A) DPPH radical scavenging activity of gallic acid (standard). (B) Percentage DPPH radical inhibition by the methanolic leaf extract of *Cadaba glandulosa* (MLCG) at different concentrations. (C) Standard calibration curve of gallic acid for total phenolic content (TPC) determination.

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of inhibitory activity against both Gram-positive and Gram-negative organisms (Fig 6). Among the Gram-positive bacteria tested, *Staphylococcus aureus* (ATCC 25923) showed the highest sensitivity to MLCG, with a zone of inhibition of 20.5 ± 1.52 mm, which was, however, lower than that of ciprofloxacin at 26.6 ± 1.3 mm. Similarly, *Streptococcus pyogenes* (ATCC 19615) showed a considerable zone of inhibition of 18.7 ± 1.52 mm, compared to 24.16 ± 0.4 mm for ciprofloxacin, while *Bacillus subtilis* (ATCC 6633) showed a zone of inhibition of 14 ± 2.64 mm against MLCG, compared to 27.2 ± 1.2 mm for ciprofloxacin. In Gram-negative bacteria, MLCG also showed remarkable antibacterial activity. *Escherichia coli* (ATCC 25922) showed an inhibition zone of 15.5 ± 2.5 mm, compared to 28 ± 2.1 mm for ciprofloxacin. *Pseudomonas aeruginosa* (ATCC 27853) had an inhibition zone of 16.5 ± 2 mm, while ciprofloxacin reached 26.6 ± 0.6 mm. *Klebsiella pneumoniae* (ATCC 700603) was inhibited with a zone of 14.5 ± 0.5 mm, while ciprofloxacin reached 28.8 ± 1.3 mm. Overall, statistical analysis confirmed that the antibacterial activity of the methanolic leaf extract of MLCG varied significantly among the tested bacterial strains when compared with the standard antibiotic, ciprofloxacin. One-way ANOVA followed by Tukey's post hoc test demonstrated that all inhibition zone values for MLCG were significantly lower than those of ciprofloxacin. The comparative analysis among bacterial strains revealed $p < 0.05$ indicating significance, $*p < 0.01$ indicating moderate significance, and $**p < 0.001$ indicating high significance. The highest level of significance ($**p < 0.001$) was observed in most bacterial comparisons, particularly for *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*, when analyzed relative to ciprofloxacin (Figs 6 and 7). In the antibacterial assay, DMSO used as the negative control showed no inhibitory effect against any tested bacterial strains, confirming that the observed antibacterial effects were due to MLCG rather than the solvent. These findings support the reliability and consistency of the experimental data, demonstrating that ciprofloxacin exhibited significantly greater antibacterial potency, while MLCG showed statistically significant but comparatively lower inhibitory activity. Nevertheless, the broad-spectrum antibacterial response of MLCG against both Gram-positive and Gram-negative bacteria highlights its potential as a natural antibacterial agent, warranting further optimization and mechanistic investigation.

Discussion

Cadaba glandulosa is a plant traditionally known for its therapeutic applications and is important in natural medicine. A previous patented study has highlighted the potential of the extract of *C. glandulosa* leaves for therapeutic purposes, demonstrating its hepatoprotective, hypoglycemic and hypolipidemic properties [4]. The current study focuses on the

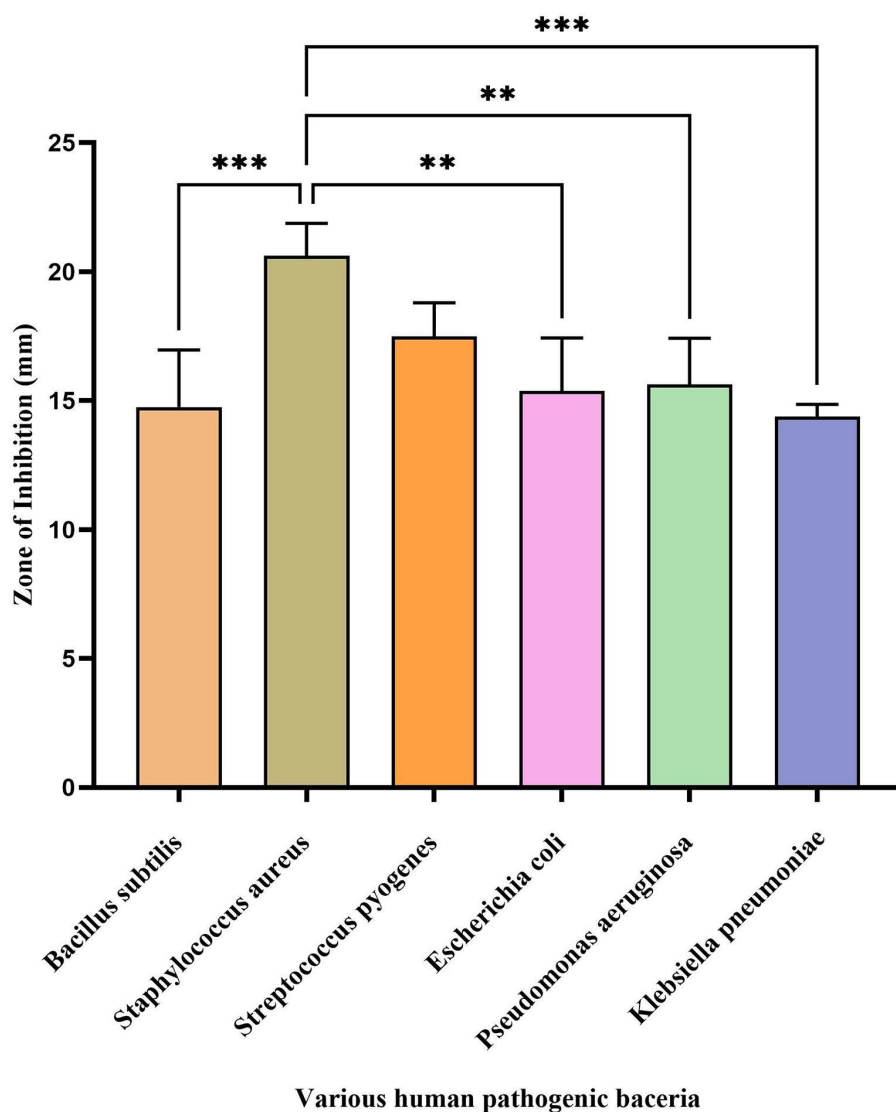


Fig 6. The antibacterial activity of MLCG. **Highly significant differences ($p < 0.001$) were observed when *Staphylococcus aureus* was compared with *Escherichia coli* and *Klebsiella pneumoniae*, indicating a markedly greater sensitivity of the Gram-positive strain. *Moderately significant differences ($p < 0.01$) were noted between *Staphylococcus aureus* and *Bacillus subtilis*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa*.

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investigation of the bioactive potential, antioxidant and antibacterial properties of the leaves of *C. glandulosa* from Jazan, Saudi Arabia. The results of the present study emphasize the excellent chemical diversity and bioactivity of the bioactive compounds identified in MLCG by GC-MS analysis. A total of 18 bioactive compounds were tentatively identified, characterized, showcasing their potential contributions to the observed antioxidant and antibacterial activities. These findings are consistent with existing literature, further underscoring the pharmacological relevance of the identified compounds. The impressive biological activity can be attributed to the presence of key bioactive compounds such as methyl dodecanoate, 9,12-Octadecadienoyl chloride, (Z,Z)-, methyl tetradecanoate. Methyl dodecanoate, the most abundant compound, has been reported to exhibit moderate antioxidant properties by acting as a radical scavenger. It is widely used in finds applications in cosmetics and personal care products as an emollient and antimicrobial agent [15]. Methyl tetradecanoate,

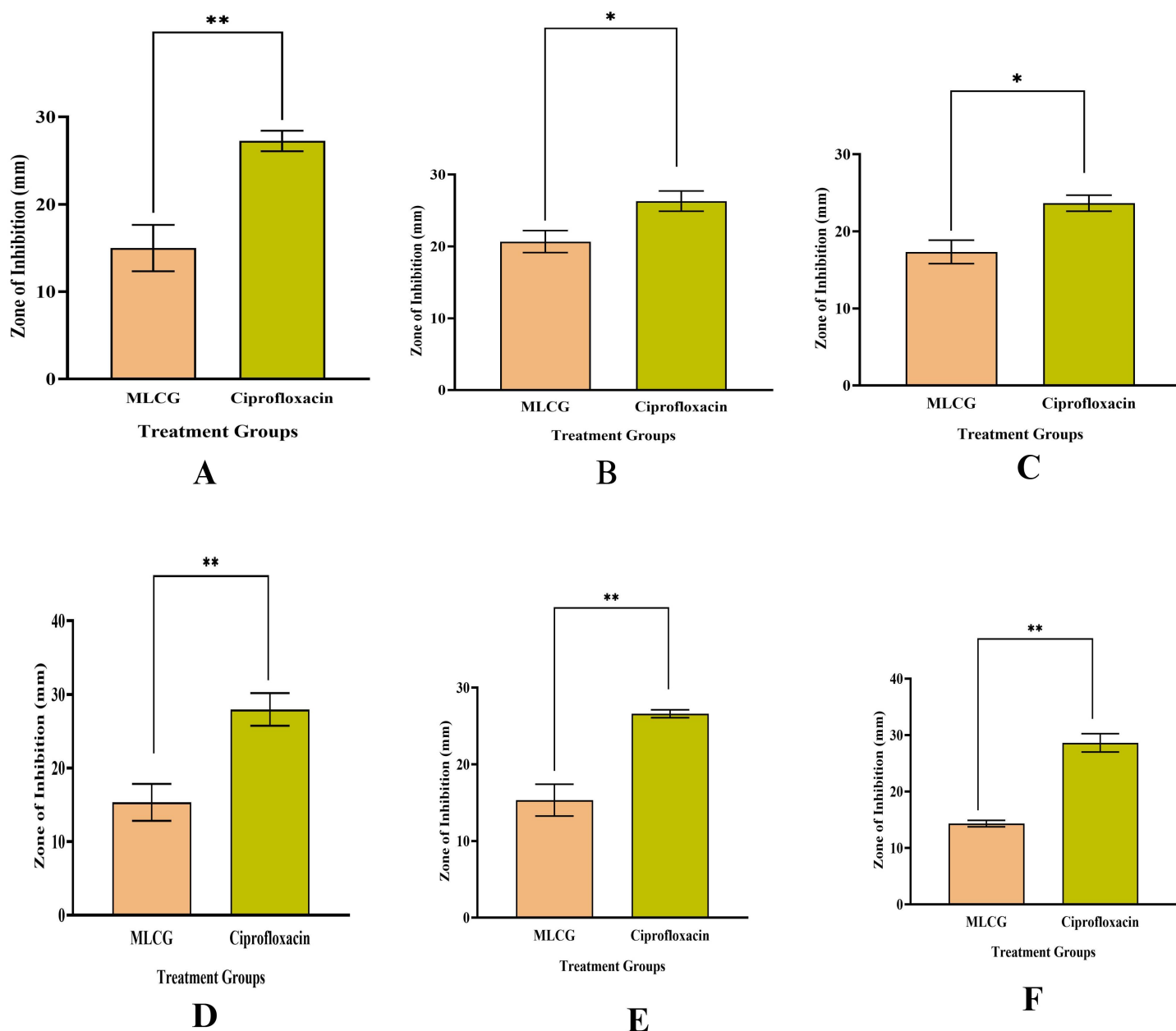


Fig 7. Comparative antibacterial activity of MLCG and ciprofloxacin against selected human pathogenic bacteria. Data are expressed as mean $\pm$ SD (n=3). (A) Effect of MLCG and ciprofloxacin on *Staphylococcus aureus*; (B) Effect of MLCG and ciprofloxacin on *Streptococcus pyogenes*; (C) Effect of MLCG and ciprofloxacin on *Bacillus subtilis*; (D) Effect of MLCG and ciprofloxacin on *Escherichia coli*; (E) Effect of MLCG and ciprofloxacin on *Pseudomonas aeruginosa*; and (F) Effect of MLCG and ciprofloxacin on *Klebsiella pneumoniae*. *Moderately significant difference at $p < 0.01$, and ** Highly significant difference at $p < 0.001$ between MLCG and ciprofloxacin for each bacterial strain.

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commonly known as methyl myristate, is a fatty acid methyl ester with diverse biological and industrial roles. It functions as a plant metabolite and is widely used in various products as a flavoring agent and fragrance [16].

Additionally, methyl tetradecanoate acts as a membrane stabilizer, contributing to cellular integrity, and serves as an energy source by storing and providing energy when required. It also plays a crucial role as a nutrient source for

the protoplasm, supporting essential cellular processes and metabolic functions [17,18]. 9,12-Octadecadienoic acid (Z, Z)-methyl ester otherwise known as linoleic acid was identified in the MLCG. Linoleic acid, the diet's most prevalent omega-6 fatty acid and an important structural component of cell membranes, influences membrane function. It is also a precursor of eicosanoids, hormones that regulate renal and pulmonary function, vascular tone, and inflammation responses [19]. In a previous study, similar bioactive compounds were reported in a cold-macerated methanolic leaf extract of *Cadaba indicia* [20].

Fenretinide, also known as retinoic acid p-hydroxyanilide was determined in MLCG. It is an orally active synthetic phenylretinamide analogue of retinol with promising antineoplastic and chemopreventive properties. By binding to and activating retinoic acid receptors (RARs), fenretinide promotes cell differentiation and induces apoptosis in certain tumor cells, making it a valuable compound in cancer therapy and prevention [21]. Hexadecanoic acid methyl ester, also known as methyl palmitate, is a fatty acid methyl ester found in MLCG, which occurs naturally in various plant and animal fats and is known for its anti-inflammatory, anti-fibrotic and antioxidant properties, making it an important subject of pharmaceutical research [22].

Anethole is a naturally occurring aromatic compound belonging to the class of phenylpropanoids that was determined in MLCG. Anethole is widely used as a flavoring agent in the food and beverage industry and as a fragrance in cosmetics and perfumery. Beyond its sensory applications, anethole exhibits a range of bioactive properties, including anti-inflammatory, antimicrobial, antioxidant, and anticancer effects. It has also been studied for its potential as a natural insecticide and for its ability to modulate metabolic pathways, making it a compound of interest in both traditional medicine and modern pharmaceutical research [23–25]. Palmitoleic acid, a monounsaturated omega-7 fatty acid, is widely known for its health-promoting properties, including anti-inflammatory, antimicrobial and lipid-lowering effects. It has also been shown to positively affect insulin sensitivity, cholesterol metabolism and hemostasis, making it a valuable component in the prevention and treatment of metabolic and cardiovascular diseases [26–28].

Brefeldin A (BFA) is a bicyclic lactone identified as one of the bioactive compounds in MLCG. Studies suggested that BFA is a metabolite that exhibits a wide range of antibiotic activity. Furthermore, BFA exhibits a range of bioactivities, including antifungal, antiviral, and anticancer properties. Notably, it holds significant potential in cancer treatment due to its cytotoxic effects against various cancer cell lines, such as HeLa, MCF-7, and HL-60. This makes Brefeldin A a promising candidate for therapeutic applications, as supported by studies highlighting its efficacy in targeting these cell lines [29–31]. 1-Heptatriacotanol and cis-11-eicosenoic acid contribute to antioxidant and anti-inflammatory properties, while dasycarpidan-1-methanol, acetate shows promising antimicrobial and anticancer activities [31,32].

Dasycarpidan-1-methanol, acetate is an alkaloidal compound found in MLCG. It has been studied for its anti-inflammatory, antimicrobial, and cytotoxic effects [33]. 2-Hexadecanol and 9-octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester were identified in MLCG. Despite their presence, the pharmacological and biological activities of these compounds have not yet been explored or reported. Further research is required to investigate their potential therapeutic applications and biological properties. In the present study, MLCG demonstrated the presence of oleic acid, which is known for its antioxidant, cardioprotective, antimicrobial and anti-inflammatory properties [34–37].

The antioxidant evaluation of the methanolic leaf extract of MLCG revealed a remarkable scavenging capacity, highlighting its potent free radical neutralizing ability. This strong activity is attributed to a diverse range of bioactive compounds, as identified through GC-MS analysis, including methyl dodecanoate, methyl tetradecanoate, 9,12-octadecadienoic acid (Z,Z)-methyl ester (linoleic acid), and oleic acid. These compounds play a crucial role in stabilizing cellular membranes, supporting metabolic processes, and maintaining cell integrity, thereby contributing significantly to the extract's antioxidant potential [19]. Additionally, the presence of methyl palmitate, palmitoleic acid, and anethole enhances the antioxidant properties of MLCG due to their anti-inflammatory and free radical scavenging effects [19,38]. The DPPH scavenging assay demonstrated a dose-dependent enhancement in the antioxidant activity of MLCG, confirming its effectiveness in counteracting oxidative stress. Compared to gallic acid, the standard antioxidant control, MLCG showed substantial free

radical scavenging potential. This activity is further supported by its high total phenolic content, indicating a rich presence of polyphenolic compounds known for their strong antioxidant capacity. The findings underscore the promising applications of MLCG in mitigating oxidative stress-related diseases and in the development of antioxidant-based therapeutics. In this study, MLCG demonstrated strong antioxidant activity, achieving 88.2% DPPH scavenging at 381.5 µg/mL along with a high total phenolic content of 79.5% (250.8 mg GAE/mL). Interestingly, the GC–MS analysis did not prominently detect phenolic compounds; however, this does not contradict the total phenolic content (TPC) results. Many phenolic constituents are typically present at low concentrations and are often non-volatile, thermolabile, or highly polar, which can limit their detection by GC–MS without prior derivatization. In contrast, the TPC assay is a sensitive spectrophotometric method specifically designed to quantify total phenolics. Thus, the TPC findings confirm the presence of phenolic compounds in MLCG and demonstrate their significant contribution to the observed antioxidant activity.

In contrast, an earlier study on the methanolic extract of *C. glandulosa* reported only 41 ± 0.04% scavenging activity [39]. Similarly, Udhaya Lavinya et al. (2014) found that the methanolic leaf extract of *Cadaba fruticosa* contained a total phenolic content of 39.8 ± 1.92 mg GAE/g (dry weight) and exhibited DPPH scavenging activity markedly lower than the standard quercetin [40]. This study highlights the different antibacterial activity of MLCG against different types of bacteria. The extract showed a broad spectrum of activity, with Gram-positive bacteria being more sensitive than Gram-negative bacteria. Among the strains tested, *Staphylococcus aureus* showed the highest inhibitory effect, followed by *Streptococcus pyogenes*. Although *Bacillus subtilis* was also affected, its inhibition zone was significantly smaller than that of *Staphylococcus aureus* and *Streptococcus pyogenes*, indicating a lower susceptibility of the Gram-positive strains. In contrast, *Escherichia coli* and *Pseudomonas aeruginosa* showed moderate inhibition, while *Klebsiella pneumoniae* showed the weakest response. The significantly larger zone of inhibition for *S. aureus* (***p* < 0.001) suggests a strong antibacterial potential, while *S. pyogenes* (**p* < 0.01) also showed remarkable sensitivity [41]. However, *B. subtilis* showed a significantly lower zone of inhibition, suggesting that not all Gram-positive bacteria are equally sensitive. The Gram-negative strains (*E. coli*, *P. aeruginosa*, *Kl. pneumoniae*) showed smaller zones of inhibition, indicating greater resistance (**p* < 0.01 and ***p* < 0.001), probably due to their outer membrane acting as a barrier to bioactive compounds. Further studies are needed to clarify the underlying mechanisms of action and to improve the antibacterial efficacy of MLCG. Although this work is limited to in vitro antioxidant and antibacterial evaluations, the observed biological activities of MLCG are likely mediated by multiple complementary mechanisms. The antibacterial effect of these compounds could result from their ability to damage bacterial membranes and make them more permeable or by blocking vital proteins which bacteria need for their metabolism. The antibacterial properties of phenolic and flavonoid compounds which exist in methanolic plant extracts become active through their ability to damage cell membranes and their power to break down proteins and block essential biological pathways. The antioxidant properties of MLCG create an indirect effect on bacterial killing through its ability to control microbial oxidative stress and their redox systems. The therapeutic value of MLCG needs verification through enzyme inhibition assays and membrane integrity analyses and in vivo validation models which will serve as future research studies.

Conclusion

In this study, *Cadaba glandulosa* was investigated as a potential source of antioxidant and antimicrobial properties. The analysis revealed a rich composition of bioactive compounds in the plant extract, which showed significant antioxidant activity and different antibacterial effects against different bacterial strains. Although the extract showed encouraging results, its efficacy was lower compared to a standard antibiotic. Future research should focus on the isolation and characterization of the main bioactive compounds responsible for these activities, as well as on the optimization of extraction procedures to increase their yield and efficacy. In addition, in vivo studies are required to evaluate the pharmacokinetics, toxicity and therapeutic potential of the extract. Understanding the mechanisms of action of its antibacterial and antioxidant properties will be crucial for the development of new antimicrobial agents and natural antioxidants. Due to increasing

demand for herbal therapeutics, *Cadaba glandulosa* could be further investigated for its potential applications in pharmaceuticals, dietary supplements and cosmetics. Furthermore, elucidating the mechanisms of action and exploring potential synergistic interactions among the identified compounds are essential steps in advancing their development into effective therapeutic agents.

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