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Research Article

Calamintha incana Methanolic Extract: Investigation of Phytochemical Composition and Antioxidant and Antibacterial Activities

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Calamintha incana, a medicinal plant traditionally used for its therapeutic properties, has been investigated for its phytochemical constituents and biological activities. Through a specific LC-MS/MS analysis method, the phytochemical constituents of *Calamintha incana* methanolic extract have been identified and quantified. The 2,2-diphenyl-1-picrylhydrazyl method was used to measure the antioxidant properties. The antibacterial properties of this extract have been tested against four harmful bacteria using disc diffusion and the minimum inhibitory concentration methods. A colorimetric assay has evaluated the total phenolic and flavonoid content. The extract contained 34 compounds, with linolenic acid (11.2%) and myristic acid (10.3%) being the most abundant. Despite the low phenolic and flavonoid content, the extract exhibited antioxidant activity with an IC_{50} of 96.8 ± 0.3 μg/mL. Moreover, the extract demonstrated potent antimicrobial properties against *B. cereus* and *S. aureus*, with minimum inhibitory concentrations of 11.8 and 21.5 μg/mL, respectively. These robust results underscore the potential of *C. incana* methanolic extract in the development of effective antibacterial agents.

1. Introduction

Due to the rise of antibiotic-resistant microorganisms and the side effects of some medications, researchers are stepping up their hunt for new antibacterial drugs [1].

Treatment failures, extended diseases, higher healthcare expenses, and death are some of the dire consequences of antibiotic resistance. Because of this, finding novel antibacterial agents that can fight resistant bacteria and slow their spread is an absolute need [2].

Researchers are actively investigating several ways to create new antibacterial agents. These include finding natural antibacterial compounds, designing synthetic compounds based on existing antibiotics, and creating new therapies like phage therapy and immunotherapy [3].

Using herbal extracts to combat microorganisms has been a well-established practice in traditional medicine for centuries [4]. Many plant extracts and essential oils contain bioactive compounds like phenols (like thymol and carvacrol) and flavonoids (like quercetin, kaempferol, and apigenin). These compounds are recognized to have antimicrobial effects. These compounds are well known for their antimicrobial properties [5, 6]. These natural compounds offer several advantages as antimicrobial agents, including their broad-spectrum activity against various microorganisms, their origin from natural sources, and their

lower likelihood of inducing microbial resistance than conventional antibiotics.

A member of the Lamiaceae family, *Calamintha* is a perennial herb. It is known for its aromatic scent, reminiscent of mint, and its leaves and other aerial parts are commonly used. The *Calamintha* genus is renowned for its diversity and widespread distribution across various regions. Its eight distinct species thrive in multiple habitats. Some species are found in Europe and the Mediterranean, while others are in Eastern Mediterranean and North America [7].

Among these species, *C. incana* (Sm.) Helder is native to Jordan and is commonly found in rocky regions [8]. The leaves of this plant are used as a spice and are also utilized to create herbal tea.

In traditional medicine, it is believed that *C. incana* possesses medicinal properties and can aid in treating abdominal pain, weakness, and other ailments. *C. incana* is diaphoretic, expectorant, and antispasmodic [9]. Due to its medicinal properties, it has been used for generations in Jordanian culture as a natural remedy.

Recent research has illuminated the impressive therapeutic potential of *Calamintha incana* and related species. The plant shows significant antioxidant capabilities through compounds like cis-piperitone oxide, benzenamine-4-methyl-3-nitro, and (2S,4R)-p-mentha-6,8-diene 2-hydroperoxide [10, 11]. Its antimicrobial properties are attributed to a broad array of metabolites, including trans-piperitone oxide, piperitenone oxide, piperitenone, thymol, eugenol, 3-methoxy acetophenone, pulegone, isoborneol, menthone, 1,8-cineole, linalool acetate, sabinene, linalool, α-pinene, β-pinene, camphor, and limonene [12–15]. Cytotoxic effects have been linked to benzenamine-4-methyl-3-nitro, (2S,4R)-p-mentha-6,8-diene 2-hydroperoxide, limonene, and thymol [11, 16]. Its antidiabetic potential is also supported by compounds such as p-linolenic acid, myristic acid, and p-cymene [17]. The plant also inhibits key enzymes like tyrosinase (involved in melanin production) and α-glucosidase and α-amylase (essential for carbohydrate digestion and absorption) [12]. This wide range of biological activities, from antioxidant and antimicrobial to cytotoxic and antidiabetic, highlights the exceptional potential of the *Calamintha* genus and leaves the scientific community impressed.

Only recently, few studies have been conducted on this plant endemic to Jordan. Moreover, few researchers have investigated the antibacterial and antioxidant potential of *C. incana*. By exploring the phytochemical and biological properties of *C. incana* methanolic extract, we aim to discover its potential as a powerful antibiotic. Uncovering this plant's natural benefits can pave the way for a future of health and wellness.

2. Materials and Methods

2.1. Plant Material. In the spring of 2023, *Calamintha incana* leaves, flowers, and stems were meticulously collected from Ajloun County in Jordan. Prof. Sawsan Oran confirmed the plant material's identification and authenticity, and he then placed a voucher specimen (No. CI/2023/23) in the Department of Biological Sciences' Herbarium in Amman, Jordan.

2.2. Preparation of Methanolic Extracts. The leaves and flowers were subjected to air drying until fully dehydrated. Subsequently, the dried materials were finely powdered. Following the method outlined by Althaher and Mastinu, 20 g of the powdered plant material was soaked in 200 mL of methanol for three days. The resulting extract was then filtered through the Whatman No. 1 filter paper. The solvent was evaporated at 50°C using a rotary evaporator, and the filtered extracts were allowed to dry completely. The dried extract was stored at 4°C for further analysis [17].

2.3. LC-MS/MS Analysis. The present study analyzed a sample using a Shimadzu LC instrument manufactured by Corp. Japan. The LC system comprised a control bus module (CBM-20A), column oven (CTO-30A), liquid chromatograph (LC-30AD), autosampler (SIL-30AC), and liquid chromatograph mass spectrometer (LCMS-8030) (Triple Quad MS) with an electrospray (ESI) source connected to it.

Ten microliters of injection volume and 25 g of extracted sample diluted in 1 mL of methanol were utilized for the LC-MS/MS analysis. A chromatographic separation was carried out at a temperature of 30°C and a flow rate of 0.8 mL/min using an ACE 5 C18-column (250×4.6 mm; 5 μm). The gradient elution employed a mobile phase consisting of three components: A (water with 0.1% acetic acid), B (methanol with 0.1% acetic acid), and C (acetonitrile with 0.1% acetic acid) in a ratio of 80 : 12 : 8. The elution program was initiated with 80 : 12 : 8 (A : B : C) at 8 min, and the polarity was gradually increased to 25 : 60 : 15 at 40–45 min, followed by a return to the mobile phase (80 : 12 : 8) for 5 min to recondition the column. Before processing LC-MS injections, the extract samples and solvents for mobile phases were filtered via a 0.22 μm filtration system (Millipore Corp., Billerica, MA). Each sample was examined in triplicate, and the samples' mass spectral and retention indices were compared with data from the NIST 2016 mass spectral library to determine the constituents of the plant extract. The proportion of each component was determined by comparing its average peak area to the total peak areas of the extract.

2.4. Assessment of Total Phenolic and Flavonoid Contents. To measure the total phenolic content in a plant extract sample, mix 500 μL of Folin–Ciocalteu reagent with 100 μL of the extract. The extract should be 1 mg dissolved in 1 mL of methanol. The Folin–Ciocalteu reagent was diluted ten times with distilled water before use. After allowing the mixture to rest for 5 minutes, 0.4 mL of sodium carbonate (7.5% w/v) was added. After combining the mixture, it was allowed to sit for 40 minutes. At a wavelength of 760 nm, the absorbance was measured. A calibration curve was then generated using gallic acid as the standard to determine the overall phenolic content. The phenolic content was measured in milligrams of gallic acid per gram of methanolic extract [18].

On the other hand, a 1 mL volume of methanolic extract, with a concentration of 1 mg/mL in methanol, was combined with 1 mL of $AlCl_3$ (2% w/v) and kept at room

temperature for 40 minutes. The resulting mixture was analyzed for its absorbance at a wavelength of 430 nm. A calibration curve was developed using quercetin as a standard to determine the flavonoid content. The flavonoid quantity in the extract was calculated as mg quercetin per gram of methanolic extract [19].

2.5. Antioxidant Activity (Antiradical Activity Using DPPH). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, a gold standard in antioxidant research, is a dependable method for determining the scavenging capacity of antioxidants against the stable DPPH radical. This reliable method, which you are all familiar with, ensures the accuracy of our scientific research. In this assay, 20 μL of extract with varying concentrations of 20–120 μg/mL is mixed with 100 μL of 0.1 mM DPPH solution in methanol. The mixture is then incubated at room temperature without light for 30 minutes. Likewise, a positive control using ascorbic acid is prepared using the same procedure. After the incubation period, the absorbance of the samples and standards is measured at 517 nm to calculate the scavenging ability of DPPH radicals [20], according to the following equation.

$$\text{Scavenging activity } (\%) = \left(\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}\right) \times 100. \quad (1)$$

To determine IC_{50}, the concentration of extract that results in 50% inhibition is plotted against the percentage of inhibition. This value is then compared to the IC_{50} of ascorbic acid, which is used as the positive control.

2.6. Antibacterial Activity

2.6.1. Disc Diffusion Method. The disc diffusion method evaluated the antibacterial efficacy of plant methanolic extract against four distinct bacterial species. The tested bacterial strains comprised two Gram-positive bacteria, *Bacillus cereus* ATCC 23857 and *Staphylococcus aureus* ATCC 65381, and two Gram-negative bacteria, *Escherichia coli* ATCC 25922 and *Proteus penneri* ATCC 35198, which were obtained from the faculty of medicine, University of Jordan (Amman, Jordan). The bacterial dilutions were prepared using the 0.5 McFarland turbidity standard (1×10^5 CFU/mL). Subsequently, 1 mL of each bacterial culture was pipetted into the sterile Petri dish, and Müller–Hinton agar (Oxoid, UK) was poured into the dish containing the inoculum. After being dissolved in DMSO, the extract of *C. incana* was put into the sterile filter paper discs (8 mm in diameter) at different doses (25, 50, 100, and 200 μg/mL) and incubated for one hour at 37°C. The dried discs containing the inocula were then placed on the agar surface. The zone of inhibition (measured in millimeters) that developed following the incubation period was used to determine the antibacterial efficacy of the samples. A negative control was used, DMSO (did not exceed 1% concentration). A positive control comprising of antibiotic discs including ceftazidime (CAZ) 30 mg (for *P. penneri*, *S. aureus*, and *E. coli*) and amikacin (Ak) 30 mg (for *B. cereus*) was also used separately. All test plates underwent a 24 hour incubation period at 37°C. Each experiment was accomplished in triplicate [21].

2.6.2. Minimum Inhibition Concentration (MIC). To determine the tested methanolic extract's minimal inhibitory concentration, a technique outlined by Althaher et al. was employed. To do the test, 96-well microplates were used, and 100 μL of MHB (0.5 McFarland turbidity standard) from Oxoid, UK, was put into each well. Subsequently, 100 μL of plant extract (1.56–200 μg/mL dissolved in DMSO) was added to each microplate well. The plates were then incubated for 18 hours in a 37°C incubator. The experiment had two carefully planned controls to ensure the accuracy of the results: the growth control (GC) used a bacterial suspension without the plant extract, and the serial control (SC) used DMSO at a level that did not go above 1% v/v. Positive controls, ceftazidime (CAZ) 30 mg and amikacin (Ak) 30 mg, were also utilized. Following incubation, the turbidity of the samples was determined using a spectrophotometer (Bio-Tek Synergy HTX Multimode Reader, USA) at 560 nm [21].

2.7. Data Analysis. The mean zone of inhibition of the extract for each bacterium was compared statistically using a one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparison test at a significance level of $p < 0.05$. The experimental data were expressed as mean ± standard deviation (SD) and analyzed using Statistical Package for Social Sciences (SPSS) software (version 20.0).

3. Results and Discussion

3.1. Phytochemical Analysis. In a comprehensive analysis, each compound present in the methanolic extract of *C. incana* was identified and quantified, with its elution order from the chromatographic column, percentage composition, and retention time (min) recorded (Table 1). The LC-MS/MS chromatogram displayed the corresponding peaks of each compound (Figure 1).

Upon doing the chemical analysis, it was discovered that there were 34 components present, accounting for 98.9% of the total makeup. This extract included a significant amount of linolenic acid (11.2%), myristic acid (10.3%), and p-cymene (9.5%), which were the primary components. On the other hand, beta-damascenone was the component found in the extract with the lowest concentration (0.1%). The compounds discovered were arranged into distinct

Table 1: Chemical composition of *C. incana* methanolic extract.

Number	Compound	Percentage (%)	Retention time (min)
1	Azulene	0.3	1.9
2	3-Octanol	4.3	3.8
3	p-Cymene	9.5	5.5
4	Limonene	2.1	7.2
5	3-Nonanone	0.2	7.5
6	Unknown	1.1	9.2
7	Thymol	1.2	9.5
8	Camphor	3.5	11
9	Piperitone	2.6	12.6
10	1,8-Cineole	0.2	14.2
11	Menthol	2.7	15.7
12	p-Coumaric acid	0.6	17.4
13	Jasmone	2.8	18.3
14	Piperitone oxide	4.3	18.7
15	Chysin	2.3	20
16	Gallic acid	1.1	21
17	Caffeic acid	0.8	21.9
18	Beta-damascenone	0.1	22.5
19	Calamenene	0.5	22.8
20	Caryophyllene	2.2	24
21	Germacrene D	0.6	24.9
22	δ-Cadinene	5.1	25.5
23	Spathulenol	6.4	26.6
24	α-Bisabolol	3.7	27.3
25	Myristic acid	10.3	28.8
26	Palmitic acid	6.2	30.6
27	Linolenic acid	11.2	32.5
28	Oleic acid	1.5	33.4
29	Stearic acid	0.1	34.6
30	Catechin	0.4	36.5
31	Quercetin	2.5	37.7
32	Arachidic acid	0.9	39.1
33	Behenic acid	3.2	40.5
34	Chlorogenic acid	5.5	41.6
Total identified compounds			98.9 (%)
Monoterpenes			25
Sesquiterpenes			21.3
Fatty acids			33.4
Phenols			9.2
Alcohol			4.3
Flavonoids			2.9
Alkaloid			2.3
Non-benzenoid aromatic hydrocarbon			0.3
Ketone			0.2

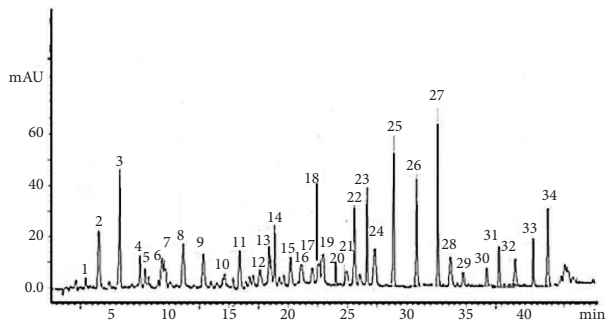


Figure 1: The chromatogram of the methanolic extract of *C. incana*.

groups based on their chemical composition. Monoterpenes (25.0%) and sesquiterpenes (21.3%) were found to be the next most prevalent class, after fatty acids (33.4%), which were determined to be the most abundant class. Ketones, which comprised just 0.2 percent of the extract, were the class with the lowest proportion.

Compared with studies conducted on related species, our research has provided invaluable insights into the chemical profiles of different varieties within the *Calamintha* genus. These findings underscore the significant variability in chemical composition even among closely related species, emphasizing the crucial role of thorough characterization for each plant species or variety.

The study conducted in Jordan in 2021 provided valuable insights into the main component of *C. incana* and the essential oil extracted from its aerial parts, constituting a significant portion of the plant (78.76%). Within the essential oil, non-terpenoid aromatic compounds were identified as substantial constituents. The most important ones were benzenamine-4-methyl-3-nitro (34.11%) and (2S,4R)-p-mentha-6,8-diene 2-hydroperoxide, constituting 31.48% of the essential oil (31.48%) [11].

The phytochemical profile of the *C. incana* (Sm.) Boiss essential oil from Turkey indicated a prevalence of oxygenated monoterpenes, such as trans-piperitone oxide (41.37%), the most abundant constituent, comprising 41.37% of the essential oil, piperitenone oxide (34.47%), piperitenone (6.67%), and the monoterpene phenol thymol, which was found in a notable percentage, representing 3.37% of the essential oil [12].

About 34 distinct components were in the essential oil extracted from the *C. officinalis* Moench species. Notable components included β-bisabolene, which made up about 9.9% of the essential oil, followed by piperitenone, which made up about 5.3%, β-bourbonene, which made up 7.4%, and germacrene D, which made up 7.6% of the essential oil (5.3%) [22].

On the other hand, it was found that the primary constituents of *C. nepeta* oil collected from different parts of Italy were pulegone (64.4–39.9%), piperitenone oxide (2.5–19.1%), and piperitenone (6.4–7.7%) [7]. Conversely, isomenthone constituted the majority, accounting for 35.8% to 51.3% of the essential oil extracted from Portuguese *C. nepeta*. 1,8-Cineole, or eucalyptol, is another vital ingredient that makes up 21.1% to 21.4% of essential oil. 7.8% to 6.0% of the essential oil was composed of trans-isopulegone [23].

Our extract's major components, including linolenic acid (11.2%), myristic acid (10.3%), and p-cymene (9.5%), are consistent with those reported in other studies, though they differ in concentration. While compounds such as trans-piperitone oxide and piperitenone oxide are present in various *Calamintha* species [11, 12, 22, 23], their concentrations in our extract vary. Additionally, metabolites like benzenamine-4-methyl-3-nitro and (2S,4R)-p-mentha-6,8-diene 2-hydroperoxide, which are significantly represented in *Calamintha incana* essential oil, are less frequently reported in other *Calamintha* species. This suggests that these compounds may be distinctive to *Calamintha incana*,

underscoring the significance of our research in uncovering the unique chemical composition of this plant species.

3.2. Assessment of Total Phenolic and Flavonoid Contents. This study tested the methanolic extract for total phenolic and flavonoid contents within a 1 mg/mL concentration range. Figure 2(a) shows the results plotted against the gallic acid standard calibration curve (10–100 μg/mL) ($y = 0.1025x \times 0.0216$, $R^2 = 0.9994$), and Figure 2(b) shows the quercetin standard calibration curve (10–100 μg/mL) ($y = 0.0211x + 0.0286$, $R^2 = 0.9993$), respectively. The units of representation for the phenolic content test findings were milligrams of gallic acid equivalent (GAE) per gram of extract. As an alternative, the flavonoid concentration was stated as milligrams of quercetin equivalent per gram. The findings, which are presented as the average of three replicates ±SD, revealed that the methanolic extract contained more phenols (27.31 ± 0.12 mg GAE/g of extract) than flavonoids (3.76 ± 0.15 mg QE/g of extract).

Plants are rich sources of essential compounds known as phenolic and flavonoid compounds. These compounds are essential for preserving cellular health and have strong antioxidant qualities that help prevent oxidative stress. Flavonoids, a type of polyphenolic compound, are found in abundance in various plant parts. Their unique ability to neutralize oxygen radicals empowers us to make healthier choices and significantly enhances our body's ability to combat damage and promote good health [24, 25].

Comparing our results with studies on other *Calamintha* species reveals variability in phenolic and flavonoid contents across different varieties. As far as total phenolic content and flavonoid content are concerned, Althaher et al. found that *C. incana* essential oil contained 24.33 ± 0.034 mg GAE/g of oil extract and 3.03 ± 0.002 mg QE/g, respectively [11]. The essential oil of *C. nepeta* has a relatively low phenol concentration of 3.36 ± 0.23 mg GAE/g oil [26]. In addition, Formisano et al. found that the methanolic extract of *C. origanifolia* has total phenols of 199.90 ± 0.03 mg GAE/L and flavonoids of 26.50 ± 0.07 mg QE/L [27]. The methanolic extract of *C. vulgaris* was found to contain a phenolic content of 39.41 ± 0.18 mg GAE/g and a flavonoid content of 12.03 ± 0.23 mg QE/g [28]. These variations underscore the importance of species-specific investigations to understand each plant's chemical composition and therapeutic potential fully.

3.3. Antioxidant Activity (DPPH Assay). The antioxidant capabilities of the extracted plant were meticulously determined using the DPPH radical scavenging assay. The findings were encouraging, with the extract exhibiting good action. The extract's DPPH radical scavenging activity ranged from 20 to 120 μg/mL, inhibiting DPPH by 10.20%–60.30% (Figure 3). The *C. incana* extract inhibited the DPPH radical by 50% at 96.8 ± 0.3 μg/mL. The IC_{50} for ascorbic acid (positive control) was 56.8 ± 0.2 μg/mL for comparison.

In one study, *C. incana* essential oil had an IC_{50} value of 15.38 mg/mL when the DPPH radical scavenging assay was employed [11]. The antioxidant activity findings for essential oils extracted from *C. incana* and *C. origanifolia* were reported. The IC_{50} value for *C. incana* essential oil (from Turkey) was 19.28 ± 0.74 mg Trolox Equivalent (TE)/g oil [7]. In contrast, the IC_{50} value for the oil extract from *C. origanifolia* was 0.05 ± 0.01 mmol TE/L. The reported activity surpassed methanolic and ethanolic extracts and the positive control (BHT) at the same dose [27].

3.4. Antibacterial Activity

3.4.1. Disc Diffusion Method. The research found that the effectiveness of *C. incana* methanolic extracts varied with concentration, as shown by the mean value zone of inhibition (Table 2). The results showed that *B. cereus* had the largest mean zone of inhibition diameter at 14.2 ± 1.0 mm when the highest dose of plant extract (200 μg/mL) was used. *Staphylococcus aureus* had the second largest at 9.3 ± 0.3 mm. In contrast, *P. penneri* exhibited a minor zone of inhibition diameter even when tested with the lowest quantity of plant extract (25 μg/mL). The zone of inhibition diameter for *E. coli* was modest at the same dose. When comparing the measured plant extract concentrations to the positive control, it is worth mentioning that there were substantial variances across all tested microorganisms.

It is fascinating to learn that the *C. incana* plant extract exhibited solid antimicrobial activity, effectively inhibiting the growth of the test microorganisms. This suggests that the extract contains compounds with antimicrobial properties capable of preventing the development of microbial pathogens.

The researcher in this study calculated the MIC of the *C. incana* plant extract. This required a series of dilutions to determine the smallest amount of plant extract that, after an overnight incubation, stopped the observable development of microbes. The extract's efficacy as an antibacterial agent may be inferred from the MIC value derived from this meticulous procedure.

The plant's methanolic extract has shown remarkable results, with a maximum MIC of 65.7 μg/mL against *P. penneri* and a lowest MIC value of 11.8 μg/mL against *B. cereus* (Table 3). Notably, the MIC values of the plant extract were consistent with the inhibition zone diameters observed for all the tested bacteria. This means that a larger zone of inhibition corresponds to a higher degree of inhibition and a lower concentration of the plant extract. Also, the positive control had a MIC range of 0.120–9 μg/mL, while the negative control, which had a DMSO content of less than 1% v/v, did not inhibit any tested strains.

Seraoui et al. conducted a study on the Algerian *C. baborensis* Batt and found that extracts from its leaves, specifically hexanoic and chloroformic extracts, exhibited incredible antibacterial activity against *E. coli*. The inhibition zones reached an impressive 19 mm and 19.2 mm, while the MIC values were recorded at 43 and 43.4 μg/mL, respectively [13]. In contrast, a study found that *Staphylococcus aureus* was sensitive to extracts of *C. sylvatica* subsp. *sylvatica* Bromf obtained from natural and micropropagated seedlings. The MIC values were recorded at 6.25 and 3.33 mg/mL [29].

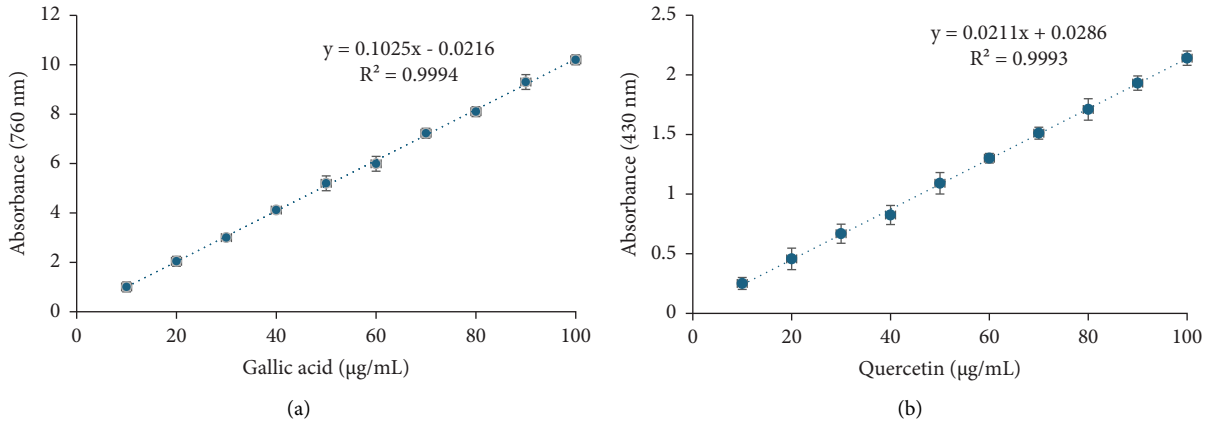


Figure 2: A calibration curve of gallic acid (a) and quercetin (b) was used to determine the total phenolic and flavonoid contents, respectively, in *C. incana* methanolic extract (mean ± SD, $N = 3$). To get the regression equations, plot (10–100 μg/mL of gallic acid) against the absorbance at 760 nm and (10–100 μg/mL of quercetin) against the absorbance at 430 nm.

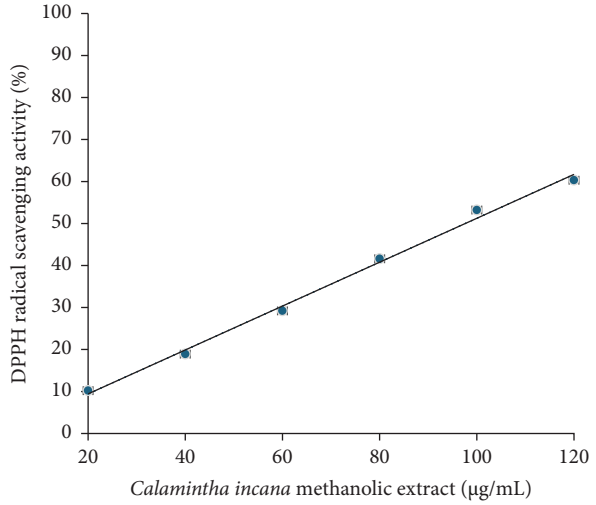


Figure 3: DPPH radical scavenging activity (%) against various concentrations of *C. incana* methanolic extract. Data were exhibited as mean ± SD ($N = 3$).

Table 2: The zone of inhibition (mm) of *C. incana* methanolic extract for the examined bacteria.

Tested bacteria	Methanolic extract of *C. incana* (mg/mL)				Positive control (antibiotic)	
	25	50	100	200	CAZ 30 mg	Ak 30 mg
Gram-negative bacteria						
Escherichia coli	$1.0 \pm 0.1^{*}$	$1.6 \pm 0.8^{*}$	$5.3 \pm 0.3^{**}$	$7.0 \pm 0.6^{*}$	27.0 ± 0.5	—
Proteus penneri	0	0	$0.4 \pm 0.3^{*}$	$0.8 \pm 0.2^{*}$	32.0 ± 0.2	—
Gram-positive bacteria						
Bacillus cereus	$4.0 \pm 0.3^{***}$	$7.1 \pm 0.3^{*}$	$9.6 \pm 0.2^{**}$	$14.2 \pm 1.0^{**}$	—	19.9 ± 0.4
Staphylococcus aureus	$3.7 \pm 0.9^{*}$	$5.5 \pm 0.7^{**}$	$7.6 \pm 0.6^{**}$	$9.3 \pm 0.3^{**}$	16.8 ± 0.5	—

The essential oil of *C. mentifolia* has been found to possess incredible antibacterial properties. It has shown remarkable efficacy against notorious bacterial strains like *S. albus*, *E. coli*, and *P. fluorescens*. The inhibition zones ranged from 40.2 ± 0.2 to 45.2 ± 0.2 mm in diameter, as reported in a study [30].

Despite their apparent similarities, observing the variability in phytochemical constituents, phenolic and flavonoid contents, and biological activities among *Calamintha* species is remarkable. Such discrepancies can be ascribed to extraction methods and environmental circumstances. Several compounds in the *C. incana* methanolic extract stand out for their distinct roles in the observed antioxidant and antibacterial activities. Linolenic acid (11.2%), a polyunsaturated fatty acid, is well regarded for its antioxidant properties [31], and its high concentration

Table 3: Minimum inhibitory concentration (MIC) for *C. incana* methanolic extracts.

Tested bacteria	Methanolic extract of *C. incana* MIC (μg/mL)	Positive control (antibiotic) MIC (μg/mL)	
		CAZ 30 mg	Ak 30 mg
Escherichia coli	30.5	0.130	—
Proteus penneri	55.6	0.120	—
Bacillus cereus	11.8	—	5
Staphylococcus aureus	21.5	9	—

CAZ 30: ceftazidime; Ak 30: amikacin.

suggests it plays a significant role in scavenging free radicals. Myristic acid (10.3%) also contributes to the antioxidant activity of the extract [31]. Phenolic compounds like gallic acid (1.1%) and chlorogenic acid (5.5%) are known for their potent antioxidant effects [32], and their presence, though in smaller quantities, likely enhances the extract's overall antioxidant capacity. For antibacterial activity, p-cymene (9.5%), a monoterpene, is known for its antimicrobial effects and may be crucial in combating various bacterial strains [33]. Thymol (1.2%), a monoterpene phenol, is noted for its strong antibacterial effects, particularly against Gram-positive bacteria [33]. Chlorogenic acid (5.5%) supports antioxidant activity and antimicrobial properties [34], potentially boosting the extract's antibacterial efficacy. The potential of *C. incana* plant extract as a natural alternative to synthetic antimicrobials *is an* inspiring prospect, motivating the audience with the potential impact of the research in the fight against microbial infections.

4. Conclusions

The study highlights the potential of the *C. incana* methanolic extract as an antioxidant and natural alternative to synthetic antimicrobial agents. The extract demonstrated significant antimicrobial activity against diverse bacterial strains, and further research is needed to identify the specific bioactive compounds responsible for this activity. Investigating the mechanism of action and conducting in vivo studies are necessary to validate the extract's efficacy and safety for therapeutic use as a novel antimicrobial agent.

Data Availability

The findings of this study are supported by data that can be obtained from the corresponding authors upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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