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Evaluation of Phytochemical Composition, Antioxidant Properties, and Antibacterial Potential of *Coptis teeta* Walls

Himashree Bora[1], Madhu Kamle[1], Sidharth Chopra[2], Pradeep Kumar[1,3]*

[1]Applied Microbiology Laboratory, Department of Forestry, North eastern regional Institute of Science and Technology, Nirjuli-791109, Arunachal Pradesh, India.
[2]Division of Molecular Microbiology and Immunology, CSIR-Central Drug Research Institute, Sector 10, Janakipuram Extension, Sitapur Road, Lucknow 226031, UP, India.
[3]Department of Botany, University of Lucknow, Lucknow 226007, UP, India.

***E-mail** ✉ pkbiotech@gmail.com

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ABSTRACT

In the current study, an evaluation was conducted on the extract of *Coptis teeta* Walls to determine its phytochemical content, phenolic and flavonoid levels, antioxidant capacity, and antibacterial effects against human pathogenic bacteria. The qualitative analysis revealed the presence of various bioactive compounds, including alkaloids, carbohydrates, saponins, phenols, flavonoids, tannins, terpenoids, cardiac glycosides, coumarin, starch, quinones, phlobatannins, and steroids. Among the extracts, acetone showed the highest concentrations of total phenolics (100.24 ± 0.00 mgGAE/g) and total flavonoids (269.13 ± 0.05 mgQE/g). The highest DPPH radical scavenging activity was recorded in the acetone extract (IC50 7.37 µg/ml), while the n-hexane extract showed the lowest activity (IC50 76.11 µg/ml). For the ABTS assay, the water extract had the highest IC50 value at 1.41 µg/ml, followed by the acetone extract at 1.91 µg/ml, both of which were lower than the IC50 of ascorbic acid of 2.73 µg/ml. In the FRAP assay, the methanol extract showed the highest antioxidant activity, measuring 113.93 µM Fe(II)/g, with the acetone extract following closely at 98.81 µM Fe(II)/g. Antibacterial activity was evaluated by disk diffusion, well diffusion, and determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against human pathogenic bacteria. The largest zone of inhibition (ZOI) was observed in water, methanol, and chloroform fractions at a concentration of 1.6 mg/ml, while the lowest was at 400 µg/ml. The MIC and MBC values for all extracts ranged from 0.625 µg/ml to 5 mg/ml and from 1.25 mg/ml to 5 mg/ml, respectively, across all tested bacterial strains.

Keywords: Phytochemistry, Phytochemicals, Human pathogenic bacteria, Antimicrobial

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Introduction

Coptis teeta Walls., a perennial herb belonging to the Ranunculaceae family, thrives in the Himalayan foothills. Known by different names such as *Mishmi teeta* (in Assamese), Mamira or Tiktamuulaa (in Ayurvedic medicine), and Maamisaa or Maamiraa (in Unani medicine), this plant is found across Bhutan, Nepal, Yunnan province of China, and parts of northeastern India, particularly in the Mishmi mountains of Arunachal Pradesh [1]. The rhizome of *C. teeta* is considered valuable in traditional Chinese medicine, where it is called "Yunan goldthread," and is also recognized for its medicinal benefits among the Mishmi people of Arunachal Pradesh, who have long utilized it to treat various ailments, particularly for its antibacterial and anti-inflammatory effects. The plant is known for addressing a range of health issues such as eye inflammation, vision problems, cataracts, skin conditions, digestive disorders, jaundice, fever, malaria, gonorrhea, urinary issues, cancer, and general inflammation, as well as for detoxifying the body and eliminating heat and dampness [1, 2]. Furthermore, it has been used to treat infectious diseases like bacillary dysentery, typhoid, tuberculosis, cerebrospinal meningitis,

empyrosis, and pertussis [1]. *C. teeta* grows at elevations between 1700 to 2800 meters in temperate broadleaf oak-rhododendron forests within the Mishmi Hills, with populations located in areas such as Malenja, Simbi, Kibithoo in Lohit, and Mayodia hills in Dibang Valley, Chnaglagram, Anini, and Hawai. Some populations also thrive in bamboo thickets and broadleaf tree canopies [3]. Cultivation of the plant has been observed in Dibang Valley and nearby regions, but deforestation and overexploitation for medicinal purposes have caused a 60% population decline, resulting in its classification as an endangered species by the IUCN Red List. The plant grows to a height of 50-60 cm, with rhizomes measuring 5-6 cm in length, dark yellow, and featuring dense nodes and rootlets. The rhizomes contain 8-8.5% berberine, the primary active compound, as well as alkaloids like coptin [4]. Despite its extensive use in traditional medicine, limited systematic studies have been conducted on its phytochemical and pharmacological properties [5–7]. The goal of this study was to evaluate the phytochemical composition, total phenolic content, and flavonoid levels in various solvent extracts, as well as to assess the antioxidant activity using DPPH, ABTS, and FRAP assays. In addition, the antibacterial properties of the extracts were tested against five human pathogenic bacterial strains.

Materials and Methods

Collection of plant samples

C. teeta specimens were obtained from multiple locations in Anjaw and Anini, situated in the Dibang Valley of Arunachal Pradesh, in April 2021. The plant was identified and its voucher specimen was preserved at the Herbarium of the Botanical Survey of India in Shillong, Meghalaya.

Extraction process

The collected roots were thoroughly washed, air-dried, and ground into a fine powder. The powdered roots were subjected to solvent extraction using Water, Methanol, Chloroform, Acetone, and n-hexane. For each solvent, 5 g of the dried powder was macerated with 50 ml of the solvent for 48 hours, with periodic shaking. After extraction, the mixture was filtered through Whatman No. 1 filter paper, and the solvent was evaporated under reduced pressure using a rotary evaporator set to 40 °C, yielding a dry extract. These extracts were then stored in a freezer at 4 °C for future analyses.

Phytochemical screening

Phytochemical screening was conducted to identify the presence of various compounds in each extract, including alkaloids, carbohydrates, saponins, phenols, flavonoids, tannins, terpenoids, cardiac glycosides, proteins, coumarins, starch, quinones, phlobatannins, and steroids, based on standardized methods [8].

Quantification of total phenolic content

The total phenolic content in the extracts was determined using the Folin-Ciocalteu method with slight modifications [9, 10]. A 250 µl aliquot of the crude extract (one mg/ml) was diluted with distilled water to a total volume of 3 ml, then mixed with one ml of Folin-Ciocalteu reagent. After a 3-minute reaction, 2 ml of 20% sodium carbonate was added, and the solution was incubated in the dark for 60 minutes at room temperature. Absorbance was measured at 735 nm, and the phenolic content was quantified by constructing a calibration curve using gallic acid as the standard, with results expressed as gallic acid equivalents (GAE) per gram of dry weight.

Quantification of total flavonoid content

The total flavonoid content was determined by the aluminum chloride (AlCl3) colorimetric method [11], using quercetin as the standard. A 500 µl sample of the crude extract was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, and 0.1 ml of 1M sodium acetate, followed by 2.8 ml of distilled water. After standing for 30 minutes, the absorbance was measured at 428 nm using a UV-Vis spectrophotometer. Flavonoid content was determined by creating a calibration curve using quercetin, and the results were expressed as quercetin equivalents per gram of dry weight.

Antioxidant activity

DPPH radical scavenging assay

The free radical scavenging activity of the extracts was assessed using the DPPH assay [12]. A 0.1 mM DPPH solution was prepared in methanol, and one ml of this solution was combined with 3 ml of the sample or standard solution (50–500 µg/ml). The mixture was shaken and allowed to stand in the dark for thirty minutes at room temperature. The absorbance was recorded at 517 nm, and ascorbic acid was used as the reference. Radical scavenging activity was calculated using the formula:

$$\% \text{ RSA} = [(\text{Abscontrol} - \text{Abssample})/\text{Abscontrol}] \times 100, \quad (1)$$

Where Abscontrol is the absorbance of the DPPH solution without the sample, and Abssample is the absorbance of the sample extract. The IC50 value was calculated based on the graph of % RSA versus concentration.

ABTS radical scavenging assay

For ABTS radical scavenging, ABTS+ was produced by mixing 7 mM ABTS with 2.4 mM potassium persulfate, and the solution was left in the dark for 14 hours at room temperature [13]. The working solution was adjusted to achieve an absorbance of 0.70 ± 0.01 at 734 nm. Various concentrations of the sample or standard (50–500 µg/ml) were added to 4 ml of the ABTS solution, and incubated for 30 minutes at room temperature, and the absorbance was measured at 734 nm. The ABTS scavenging activity was calculated using the following equation:

$$\text{ABTS RSA (\%)} = [(\text{Abscontrol} - \text{Abssample})/\text{Abscontrol}] \times 100 \quad (2)$$

Where Abscontrol is the absorbance of the ABTS+ solution without the sample, and Abssample is the absorbance of the sample extract mixed with ABTS+.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed following the method outlined by Benzie and Strain [14] and Deepa *et al*. [15], with minor adjustments. An acetate buffer was prepared by mixing 300 mM sodium acetate and glacial acetic acid to achieve a pH of 3.6 (adjustable to pH > 3.6 with drops of glacial acetic acid). A 10 mM solution of TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl and a 20 mM ferric chloride solution were also prepared. The working solution was made by combining sodium acetate, TPTZ, and ferric chloride in a 10:1:1 ratio, followed by heating to 37 °C. 150 µl of the extract was diluted in 3 ml methanol, mixed with 1 ml of the FRAP solution, and incubated in the dark at room temperature for 30 minutes. The optical density was then measured at 593 nm. A standard curve was plotted using ferrous sulfate (FeSO4) at various concentrations, and the results were expressed in µMFe(II)/g dry mass, with ascorbic acid as the reference.

Antibacterial activity

The antibacterial activity of the plant extracts was tested against 5 human pathogenic bacteria: two gram-positive bacteria, Streptococcus mutans (MTCC 890) and *Streptococcus pyogenes* (MTCC 1926), and three gram-negative bacteria, *Vibrio cholerae* (MTCC 3906), *Shigella flexneri* (MTCC 1457), and *Salmonella typhi* (MTCC 3224).

Agar well diffusion assay

The antibacterial activity was evaluated using the agar well diffusion method described by Allen *et al*. [16] and the Kirby-Bauer technique. Mueller Hinton agar plates were prepared according to the manufacturer's guidelines, and 100 µl of bacterial culture, prepared to a 0.5 McFarland standard in Mueller Hinton broth, was spread onto the agar surface. A 6-mm well was aseptically punched in the agar using a sterile borer. Stock solutions of the extracts (water, methanol, acetone, chloroform, and n-hexane) were prepared in DMSO, and 100 µl of each extract at concentrations of 400, 800, and 1.6 mg/ml was dispensed into the wells. DMSO (5%) was used as the negative control, and streptomycin served as the positive control. The plates were incubated at 37 °C for 12 hours, and the zones of inhibition (ZOI) were measured in centimeters.

Disc diffusion assay

The disc diffusion method was carried out using Mueller Hinton agar plates, with bacterial cultures at a 0.5 McFarland standard evenly distributed over the surface. Sterilized 6-mm discs made from Whatman No. 3 filter paper were impregnated with extracts of the plant (at concentrations of 400, 800, and 1.6 mg/ml) and placed onto the agar surface. A streptomycin antibiotic disc (10 µg) was used as a positive control, and 5% DMSO as a negative control. The plates were incubated at 37 °C for 12 hours, and the ZOI was measured in centimeters. All experiments were conducted in triplicate (n = 3) [17].

Determination of MIC and MBC

The minimum inhibitory concentration (MIC) of the plant extracts was determined using the broth dilution method to assess the antimicrobial susceptibility of the extracts by evaluating visible bacterial growth in the agar broth [18]. A 0.1 ml bacterial suspension (0.5 McFarland standard, ~10^8 CFU/ml) was added to tubes containing varying concentrations of the plant extracts, prepared in two-fold serial dilutions ranging from 5 mg/ml to 0.156 mg/ml, in Mueller Hinton Broth. The tubes were incubated for 8 hours at 37 °C, and the visible growth was noted.

The MIC was determined as the lowest concentration of extract showing no visible growth. For the MBC determination, a 50 µl aliquot of the broth with no visible growth was spread onto freshly prepared Mueller Hinton agar plates, which were incubated at 37 °C for 24 hours. The minimum bactericidal concentration (MBC) was recorded as the lowest concentration of extract at which 99.9% of the bacterial population was killed [19, 20].

Statistical analysis

The data were presented as mean ± standard deviation (SD) based on triplicate determinations (n = 3). Statistical analysis was performed using the chi-square test and one-way ANOVA. Differences were considered statistically significant at $P < 0.05$.

Results and Discussion

The herbarium specimen of the plant was verified by the Botanical Survey of India (BSI), Shillong (Meghalaya), and assigned the accession number 98158. Following this verification, the plant samples were used for further investigations.

Phytochemical screening

Table 1 summarizes the results of the phytochemical screening for various solvent extracts of *C. teeta*. The analysis revealed the presence of several key secondary metabolites, which are known for their medicinal properties. Many modern pharmaceuticals and herbal medicines rely on these compounds for their therapeutic effects. The preliminary qualitative screening detected the presence of alkaloids, carbohydrates, saponins, phenols, flavonoids, tannins, terpenoids, cardiac glycosides, coumarins, starch, quinones, phlobatannins, and steroids. These secondary metabolites play a vital role in plant defense mechanisms against biotic stresses such as insect attacks, microbial infections, and viral diseases [21]. The production of specific secondary metabolites is linked to the plant's bioactivity, particularly in the treatment of various health conditions and chronic diseases, due to their therapeutic potential [22].

Table 1. Results of preliminary phytochemical screening

Chemical Groups	Various solvent extracts				
	Water	**Methanol**	**Acetone**	**Chloroform**	**n-Hexane**
Alkaloid	-	+	-	-	-
Carbohydrates	+	+	+	+	-
Saponin	+	-	+	+	-
Phenols	+	+	+	-	-
Flavonoids	+	+	+	+	+
Tannin	+	+	+	-	-
Terpenoids	+	+	+	+	+
Cardiac glycoside	+	+	+	+	+
Protein	-	-	-	-	-
Coumarin	+	+	+	+	+
Starch	+	+	-	+	+
Quinone	+	+	+	+	+
Phlobatannin	-	-	-	-	-
Steroids	+	+	+	+	+

Total phenolic content

The total phenolic content was quantified as gallic acid equivalents (GAE), with the standard curve equation being $y = 0.0038x + 0.0442$ ($R^2 = 0.9944$), as shown in **Figure 1**. Among the various extracts, acetone exhibited the highest phenolic content, measuring 100.24 ± 0.00 mgGAE/g. This was followed by the water extract, which contained 92.34 ± 0.00 mgGAE/g, while the methanol extract had 45.57 ± 0.01 mgGAE/g. The chloroform extract showed 13.15 ± 0.00 mgGAE/g, and the n-hexane extract had the lowest value at 0.005 ± 0.00 mgGAE/g, as summarized in **Table 2**.

Total flavonoid content

The total flavonoid content was determined as quercetin equivalents (QE) using the standard curve equation $Y = 0.0064x + 0.0062$ ($R^2 = 0.9915$), as presented in **Figure 2**. Acetone again recorded the highest flavonoid

concentration at 269.13 ± 0.05 mgQE/g, followed by the methanol extract at 64.01 ± 0.03 mgQE/g and the water extract at 49.22 ± 0.04 mgQE/g. The chloroform extract contained 8.61 ± 0.00 mgQE/g, while the n-hexane extract had the least amount at 3.87 ± 0.02 mgQE/g, as detailed in **Table 2**.

Table 2. Estimation of total phenol and *flavonoid* contents in various solvents of *C. teeta*

Extractives	Total phenolic content (mgGAE/g)	Total flavonoid content (mgQE/g)
Water	92.34 ± 0.00	49.22 ± 0.04
Methanol	45.57 ± 0.01	64.01 ± 0.03
Acetone	100.24 ± 0.00	269.13 ± 0.05
Chloroform	13.15 ± 0.08	8.61 ± 0.00
n-Hexane	0.005 ± 0.00	3.87 ± 0.02

*Values are the means of three triplicates with Standard deviations (mean ± S.D; n = 3), $P < 0.05$

Antioxidant activity of C. teeta extracts

DPPH test

The radical scavenging potential of *C. teeta* extracts was assessed using the DPPH assay. Among the extracts, acetone exhibited the highest scavenging ability at 350 µg/ml, reaching 56.1 ± 0.006%, followed by water extract (33.7%), methanol (23.64%), chloroform (12.65%), and n-hexane (5.25%). Ascorbic acid, used as a reference, displayed a radical scavenging activity of 83.72%. The IC50 values, representing the concentration at which 50% of the DPPH radicals were inhibited, were 12.3 µg/ml for water, 16.011 µg/ml for methanol, 7.739 µg/ml for acetone, 30.14 µg/ml for chloroform, and 76.11 µg/ml for n-hexane. For ascorbic acid, the IC50 was 3.530 µg/ml. The ranking of the radical scavenging efficiency was as follows: ascorbic acid > acetone > water > methanol > chloroform > n-hexane.

ABTS assay

In the ABTS assay, the methanol extract showed the strongest antioxidant activity at 400 µg/ml, with an inhibition of 99.93%, followed by acetone (98.04%), water (95.93%), chloroform (63.34%), and n-hexane (23.25%). Ascorbic acid had an inhibition of 97.88%. The IC50 values were calculated for each extract: water (1.413 µg/ml), acetone (1.915 µg/ml), methanol (2.36 µg/ml), chloroform (7.609 µg/ml), and n-hexane (16.8 µg/ml). For ascorbic acid, the IC50 was 2.730 µg/ml. The scavenging effectiveness was ranked as follows: water > acetone > methanol > ascorbic acid > chloroform > n-hexane.

FRAP assay

The total antioxidant capacity was measured by the FRAP assay, where the standard curve for FeSO4 was established ($y = 0.0141x + 0.7424$, $R2 = 0.9742$). The methanol extract exhibited the highest antioxidant activity with 113.92 ± 0.03 µM Fe(II)/g, followed by acetone (98.81 ± 0.02 µM Fe(II)/g), and water (37.22 ± 0.03 µM Fe(II)/g). The lowest values were observed in chloroform (0.853 ± 0.01 µM Fe(II)/g) and n-hexane (0.288 ± 0.05 µM Fe(II)/g). Ascorbic acid showed 90.25 ± 0.01 µM Fe(II)/g. Notably, methanol demonstrated a significantly higher antioxidant activity than ascorbic acid.

Discussion

The antioxidant capabilities of *C. teeta* extracts were thoroughly investigated using DPPH, ABTS, and FRAP assays. Reactive oxygen species (ROS), generated during oxidation processes, are involved in inflammatory and angiogenic pathways associated with tumor growth, cellular damage, and food spoilage [23]. Excessive ROS production accelerates lipid oxidation, contributing to various health issues. All extracts from *C. teeta* demonstrated robust antioxidant activities, likely due to their electron-donating properties that reduce Mo(VI), ABTS*, and Fe(III) in the assays [24]. Polyphenolic compounds in plants, which include a diverse range of secondary metabolites, are involved in plant defense mechanisms, serving as hydrogen atom donors, reducing agents, and scavenging singlet oxygen [25]. The correlation between high phenolic and flavonoid content and significant antioxidant activity, especially in water, methanol, and acetone extracts, underscores the role of these metabolites in combating oxidative stress [26].

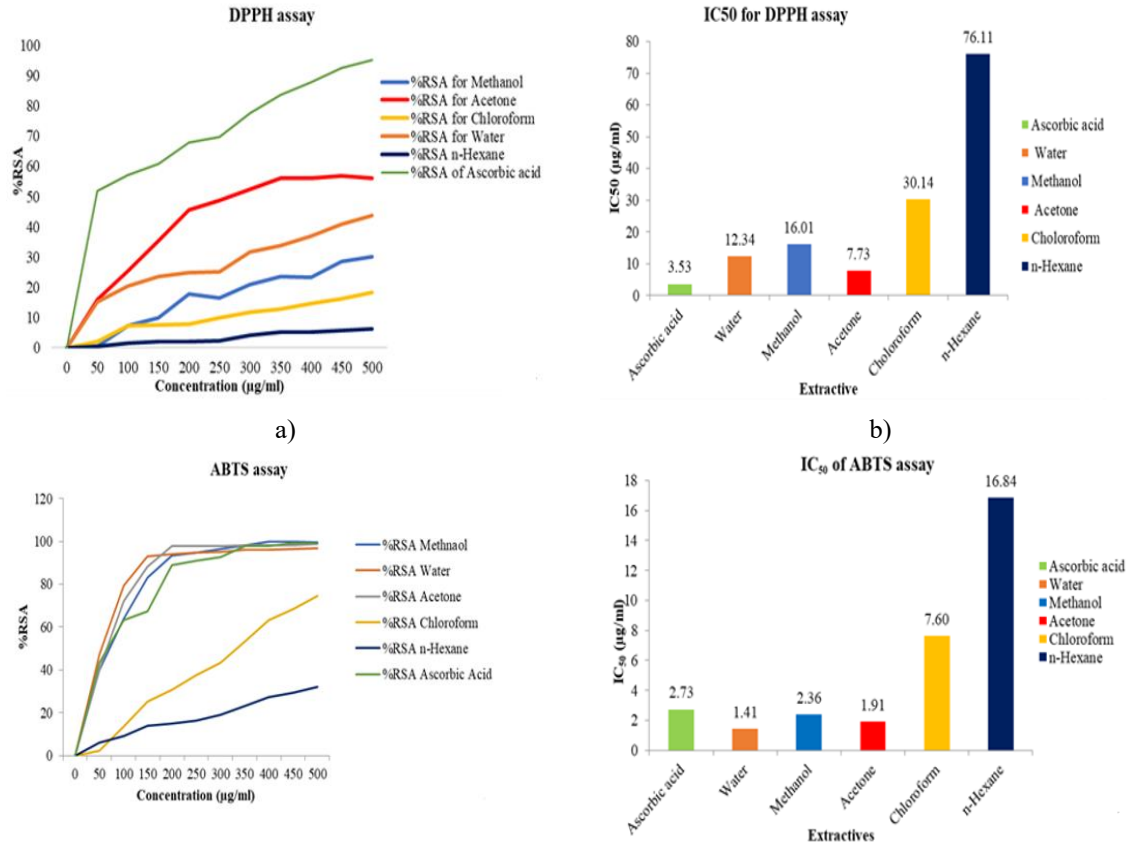

Figure 1. Represents DPPH assay; a) % radical scavenging activity, b) IC50 of DPPH, c) ABTS assay for % radical scavenging activity, and d) IC50 of ABTS

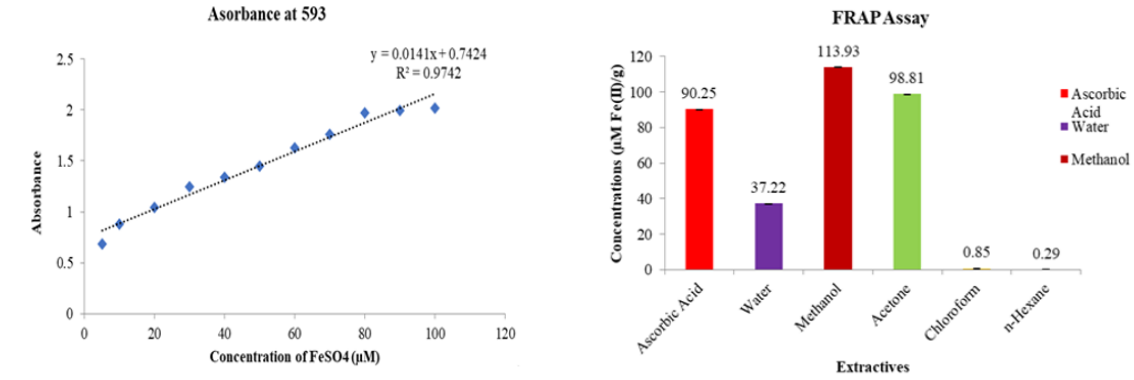

Figure 2. FRAP assay; a) standard curve for $FeSO_4$, and b) concentrations for the total antioxidant present in different solvent

Antibacterial activity

The antimicrobial properties of various *C. teeta* solvent extracts were assessed against two gram-positive bacteria, *Streptococcus mutans* and *Streptococcus pyogenes*, along with three gram-negative strains, *Vibrio cholerae*, *Shigella flexneri*, and *Salmonella typhi*. Methods such as agar disc diffusion, well diffusion, MIC, and MBC were employed to evaluate the inhibitory effects. The extracts showed varying degrees of inhibition across different concentrations, with their effectiveness compared to a reference antibacterial agent. Antibacterial efficacy was significantly influenced by the concentration of the extracts, with marked differences observed ($P < 0.05$) between extracts in both diffusion assays.

The highest inhibition zones (ZOI) were observed at a concentration of 1.6 mg/ml for both methods **(Table 3)**. In disc diffusion, the water extract demonstrated relatively consistent inhibition across all bacterial strains, with ZOIs

ranging from 8 ± 0.00 mm to 8.83 ± 0.28 mm. For the methanol extract, *V. cholerae* exhibited the largest ZOI at 13 ± 1.00 mm, while S. pyogenes had the smallest (8 ± 0.00 mm), and *S. typhi* showed no inhibition. Acetone extract provided significant inhibition against *S. mutans* (12 ± 1.7 mm), while *S. typhi* was least affected (8.33 ± 1.15 mm). The chloroform extract showed its highest ZOI against *S. mutans* (9.66 ± 0.57 mm), with *S. flexneri* exhibiting the smallest (6.66 ± 0.57 mm), and *S. pyogenes* showed no inhibition. In the case of n-hexane, *S. mutans* displayed the highest ZOI (11 ± 1.73 mm), while *V. cholerae* showed the lowest (6.33 ± 0.57 mm).

Table 3. Antimicrobial activities of *C. teeta* extractives against bacterial strain tested by disk diffusion, ZOI in (mm) at various concentrations

Extractives/ Controls	400 µg/ml					800 µg/ml					1.6 mg/ml				
	SM	SP	SF	VC	ST	SM	SP	SF	VC	ST	SM	SP	SF	VC	ST
Water	7 .0± 0.0	7.0 ± 0.0	7 .0± 0.0	7.3 ± 0.57	7.67 ± .57	7.0 ±0.00	7.33 ± .70	7 .0± 0.00	7.0 ± 0.00	7.0 ± 0.00	8.66±0.57	8.0 ± 0.00	8.00 ± 0.00	8.83±0.28	8.0 ± 0.00
Methanol	7.16 ± .28	0	8.00 ± .00	8.66 ± .57	0	8.0 ± 0.00	7.0 ± 0.00	9.00 ± 0.00	13.3 ± 0.57	0	12.66 ± 0.57	8.0 ± 0.00	9.00 ± 0.00	13.0 ± .00	0
Acetone	6.33 ± .57	6.33 ± 0.57	8 ± 0.00	6.00 ± .00	8.33 ± .15	7.0 ± 0.00	7.0 ± 0.00	8.0 ± 0.00	7.0 ± 0.00	8.0 ± 0.00	12.0 ± 1.73	7.33 ± 0.57	9.00 ± 0.00	8.66 ± 0.57	8.33 ± 1.15
Chloroform	0	0	0	0	0	8.00 ± .00	0	6.66 ± 0.57	0	6.66 ± 0.57	9.66 ± 0.57	0	6.66 ± 0.57	7.0 ± 0.00	7.0 ± 0.00
n-Hexane	0	0	0	0	0	7.3 ± 0.57	0	0	0	8 ± 0.00	11.0 ± 1.73	10.66 ± 2.08	7.00 ± 0.00	6.33 ± 0.57	9.330 ± 0.57
Streptomycin (+)	13.6 ± .15	15 ± 0.00	20 ± 0.00	8.3 ± 0.28	14 ± 0.00	13.0 ± 0.00	14.0 ± 0.00	15.0 ± 0.00	9.0 ± 0.00	8.66 ± 0.57	14.33 ± 0.57	14.00 ± 0.0	14.66 ± 0.57	8.33 ± .28	10.0 ± 0.00
DMSO (-)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

In the well diffusion assay, the water extract displayed its most significant zone of inhibition (ZOI) against *Shigella flexneri* with a measurement of 14 ± 1.73 mm, while the least inhibition was observed for *Streptococcus pyogenes* at 8.66 ± 1.15 mm. The methanol extract exhibited its strongest activity against *Vibrio cholerae*, yielding a ZOI of 17.66 ± 0.57 mm, and the weakest against *S. pyogenes* at 7.83 ± 0.28 mm. In acetone extract, *Shigella flexneri* showed the largest ZOI at 13.66 ± 0.57 mm, whereas *Salmonella typhi* exhibited the smallest at 10.66 ± 2.08 mm. Chloroform extract displayed the highest ZOI against *V. cholerae* (12.33 ± 0.57 mm), with the smallest inhibition observed for S. mutans at 9 ± 0.00 mm; notably, no inhibition was seen against *S. pyogenes* and *S. typhi*. The n-hexane extract only showed activity against *S. flexneri* with a ZOI of 6.66 ± 0.57 mm, while no inhibition zones were formed for other bacteria. The detailed results are presented in **Table 4**.

Table 4. Antimicrobial activities of *C. teeta* extractives against bacterial strain tested by well diffusion, ZOI in (mm) at various concentrations

Extractives/ Controls	400 µg/ml					800 µg/ml					1.6 mg/ml				
	SM	SP	SF	VC	ST	SM	SP	SF	VC	ST	SM	SP	SF	VC	ST
Water	2 ± 3.46	0	6.66 ± 0.57	9.33 ± 0.57	0	7.66 ± 0.57	0	9.66 ± 0.57	12.00 ± 0.00	7.00 ± 0.00	9.00 ± 0.00	8.66 ± 1.15	14.00 ± 1.73	13.66 ± 0.57	11.33 ± 1.15

Methanol	9.66 ± 0.57	0	8.66 ± 0.57	10 ± 0.00	0	10.66 ± 1.15	6.00 ± 0.00	13.66 ± 0.57	15 ± 0.00	7.33 ± 0.57	14.00 ± 0.00	7.83 ± 0.28	16.00 ± 1.73	17.66 ± 0.57	8.66 ± 1.154
Acetone	7.33 ± 0.57	0	6.33 ± 0.57	0	0	8.66 ± 0.57	6.83 ± 0.57	10 ± 0.00	10.66 ± 2.30	7.33 ± 0.57	12.66 ± 0.57	13.33	13.66 ± 0.57	12.66 ± 0.57	10.66 ± 2.08
Chloroform	7.33 ± 0.57	0	0	7.33 ± 0.57	0	7.66 ± 0.57	0	9.66 ± 0.57	10.00 ± 0.00	0	9.00 ± 0.00	0	11.00 ± 1.73	12.33 ± 0.57	0
n-Hexane	0	0	0	0	0	0	0	0	0	0	0	0	6.66 ± 0.57	0	0
Streptomycin (+)	21.66 ± 2.88	17.66 ± 1.15	18 ± 3.46	9 ± 0.00	16.33 ± 2.30	25.33 ± 0.57	18.33 ± 0.57	20.66 ± 1.15	9.33 ± 0.57	18.33 ± 1.15	20.00 ± 0.00	16.66 ± 2.88	20.33 ± 0.57	10.66 ±1.15	19.00 ± 0.00
DMSO (-)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of *C. teeta* extracts from five solvents were assessed against five bacterial species, as summarized in **Table 3**. For the water extract, MIC values were recorded at 1.25 mg/ml for *S. flexneri*, *V. cholerae*, and *S. mutans*, and at 2.5 mg/ml for *S. pyogenes* and *S. typhi*. The MBC values for *S. flexneri* and *V. cholerae* were 2.5 mg/ml and 1.25 mg/ml, respectively, while *S. mutans*, *S. pyogenes*, and *S. typhi* had MBC values at 5 mg/ml. The methanol extract showed MIC values of 0.625 mg/ml for *S. flexneri* and *V. cholerae*, 0.312 mg/ml for *S. mutans*, and 2.5 mg/ml for *S. pyogenes* and *S. typhi*. The MBC for *V. cholerae* and *S. mutans* was 0.625 mg/ml, while *S. flexneri* exhibited an MBC at 2.5 mg/ml, and *S. pyogenes* and *S. typhi* at 5 mg/ml. The acetone extract presented MIC values of 1.25 mg/ml for *S. flexneri*, *V. cholerae*, and *S. mutans*, and 2.5 mg/ml for *S. pyogenes* and *S. typhi*. The MBC values for *S. flexneri*, *S. pyogenes*, and *S. typhi* were 5 mg/ml, for *V. cholerae* was 2.5 mg/ml, and for *S. mutans* was 1.25 mg/ml. For the chloroform extract*, S. flexneri* had a MIC of 1.25 mg/ml with an MBC of 2.5 mg/ml, while *V. cholerae* and *S. typhi* had both MIC and MBC values of 5 mg/ml. *S. mutans* and *S. pyogenes* exhibited MIC values of 0.625 mg/ml and 5 mg/ml, respectively, with MBC values greater than 5 mg/ml. In the case of n-hexane extract, *S. flexneri* showed a MIC at 0.625 mg/ml and an MBC at 5 mg/ml, while *V. cholerae* and *S. mutans* showed MIC and MBC values of 1.25 mg/ml and 2.5 mg/ml, respectively. *S. pyogenes* and *S. typhi* had MIC values of 2.5 mg/ml and MBC values of 5 mg/ml **(Table 5)**.

Antimicrobial agents present in plants are composed of various chemical compounds that inhibit microbial growth or induce cell death [20]. Compounds such as alkaloids, polyphenols, and terpenoids have evolved to defend plants against harmful organisms and are known to play a key role in treating infections [20]. Given the rise in antibiotic resistance and the unregulated use of synthetic antibiotics, plant-based antimicrobial agents have become increasingly important [27]. The antibacterial activity of the plant extracts was assessed using diffusion and dilution methods against both gram-positive (*S. mutans*, *S. pyogenes*) and gram-negative (*S. flexneri*, *V. cholerae*, *S. typhi*) bacteria. It was observed that polar solvents like water, methanol, and acetone demonstrated activity against all five bacterial strains, consistent with previous reports suggesting that polar solvents tend to be more effective in antimicrobial assays [20]. During the study, it became apparent that the observed antimicrobial activity varied due to the additional membrane layers found in gram-negative bacteria, which can impact the effectiveness of plant extracts [28]. Numerous studies have explored the antimicrobial potential of plant extracts and their compounds, with researchers noting that compounds such as terpenoids, alkaloids, and phenolics interact with microbial cell membranes and enzymes, disrupting their functions and inducing cell death [19, 20, 28-30]. Additionally, the hydrophobic properties of certain plant extracts allow them to interact with and alter the structure and permeability of microbial cell membranes and mitochondria [19, 20, 29-32].

Table 5. MIC and MBC of *C. teeta* extractives against bacterial strain tested using (+ for no growth/inhibition, – for growth/ no inhibition)

Minimum inhibitory concentration (MIC)																										
Conc. (mg/ml)/ Control	Water					Methanol					Acetone					Chloroform					n-Hexane				
	SM	SP	SF	VC	ST	SM	SP	SF	VC	ST	SM	SP	SF	VC	ST	SM	SP	SF	VC	ST	SM	SP	SF	VC	ST
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	+	+	+	+
1.25	+	-	+	+	-	+	-	+	+	-	+	-	+	+	-	+	-	+	-	-	+	-	+	+	-
0.625	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-
0.312	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.156	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(+)C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
(-) C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Minimum bactericidal concentration (MBC)																										
Conc. (mg/ml)/ Control	Water					Methanol					Acetone					Chloroform					n-Hexane				
	SM	SP	SF	VC	ST	SM	SP	SF	VC	ST	SM	SP	SF	VC	ST	SM	SP	SF	VC	ST	SM	SP	SF	VC	ST
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	>5	>5	+	+	+	+	+	+	+	+
2.5	-	-	+	+	-	+	-	+	+	+	+	-	-	+	-			+	-	-	+	-	-	+	-
1.25	-	-	-	+	-	+	-	-	+	-	+	-	-	-	-			-	-	-	-	-	-	-	-
0.625	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-			-	-	-	-	-	-	-	-
0.312	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-
0.156	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-
(+) C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
(-) C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Conclusion

C. teeta, a plant traditionally used by the tribes of Arunachal Pradesh for treating various ailments, has been found to contain significant phytochemicals. This study lays the foundation for further exploration into the identification of novel bioactive compounds, offering a potential solution in the fight against antimicrobial resistance caused by diverse pathogens. The research provides additional scientific validation to support tribal claims regarding the plant's medicinal properties. With its promising antibacterial effects on certain pathogens, *C. teeta* holds potential for pharmaceutical applications as an alternative antimicrobial agent. Further studies are required to isolate and investigate the specific bioactive compounds present in this plant.

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