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Evaluation of Phytochemical Components, Antioxidant, and Antibacterial Activities of Coptis teeta Walls

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ABSTRACT

In the present study, Coptis teeta Walls plant extract is subjected to evaluate the presence of phytochemicals, phenol, flavonoid, antioxidant, and antibacterial activities against human pathogenic bacteria. The qualitative analysis of plant extracts showed the presence of alkaloids, carbohydrates, saponin, phenols, flavonoids, tannin, terpenoids, cardiac glycoside, coumarin, starch, quinone, phlobatannins, and steroids. Total phenolics content (TPC) and total flavonoid content (TFC) were found to be highest in acetone extract with 100.24±0.00 mgGAE/g and 269.13±0.05 mgQE/g respectively. The highest DPPH radical scavenging activity was observed in acetone extract and lowest in n-hexane extract IC_{50} at 7.37 µg/ml and $IC_{50\ at}$ 76.11 µg/ml respectively. Whereas in the ABTS assay IC_{50} was highest for both water extract 1.41 µg/ml and acetone extract 1.91 µg/ml which was lower than that of the ascorbic acid with IC_{50} 2.73 µg/ml. In the FRAP assay, the highest antioxidant activities were observed in methanol extract at 113.93 µM Fe(II)/g followed by acetone extract with 98.81 µM Fe(II)/g. The Antibacterial activity was evaluated using disk diffusion, well diffusion, minimum inhibitory concentration (mic), and minimum bactericidal concentration (mbc) against human pathogenic bacteria. The results showed that ZOI for water, methanol and chloroform fraction at disk diffusion and well diffusion was highest at the concentration of 1.6mg/ml. and lowest at 400µg/ml. For all the extracts the MIC and MBC values was ranging from 0.625µg/ml – 5mg/ml and 1.25 mg/ml – 5mg/ml respectively, for all the tested pathogens.

Key words: *Phytochemicals, Antimicrobial, Human pathogenic bacteria, Phytochemistry*

INTRODUCTION

Coptis teeta Walls. of the family Ranunculaceae is a perennial herbaceous plant found in the foothills of the Himalayan region. The plant is commonly known as Mishmi teeta (Assamese) or Mamira or Tiktamuulaa (Ayurvedic medicine) and Maamisaa or Maamiraa (Unani). The plant is distributed in Bhutan, Nepal, and Yunan province of China and the North Eastern region, especially in the five districts of Mishmi mountains in Arunachal Pradesh of India [1]. Mishmi teeta is an important medicinal plant that contains tenuous rhizome, known as "Yunan goldthread" in the traditional Chinese medicine system, and in India it is indigenous to the people of the Mishmi community of Arunachal Pradesh having predated knowledge about its medicinal usage as an antibacterial and anti-inflammatory agent for a long time and known mainly for treating inflammatory eye diseases, decrease vision, cataract, skin-related problems, indigestion, constipation, jaundice, fever especially in malaria, gonorrhea, and urine disorders cancer and inflammation, clearing heat, eliminating, dampness, purging fire and detoxification [1, 2]. It is also used for the treatment of bacillary dysentery, typhoid, tuberculosis,

epidemic cerebrospinal meningitis, empyrosis, pertussis, and other diseases [1]. The plant grows in the lower and upper elevations of 1700mts-2800mts respectively, in low densities and patchy distribution, narrowly restricted to temperate broad -leaf oak- rhododendron forests in the Mishmi Hills, at Malenja, Simbi, in Kibithoo block of Lohit and Mayodia hills in Dibang Valley, Chnaglagram, Anini and Hawai. Some populations also thrive under the dense bamboo thickets and broad-leaf tree canopy [3]. Cultivation of this plant has also been observed at Suplang, Hunli, and Mayodia in Dibang Valley and a few places near Siang and Subansiri of Arunachal Pradesh. But over the last few decades due degradation of forests and overexploitation of rhizomes for medicinal uses 60% of the population has got declined and hence it has been enlisted as an endangered species in the IUCN Red list of Threatened species. The plant is 50-60 cm in height and the rhizome is 5-6 cm long dark yellow containing dense nodes and rootlets. The plant is extracted for its rhizome which contains 8-8.5% berberine which is the active ingredient along with alkaloids like coptin [4]. Despite a long tradition of use for the treatment of various ailments, limited systematic phytochemical and pharmacological work has ever been carried out. The main objective of the study was to determine the presence of phytochemicals, total phenols, and flavonoids in various solvent extracts. Furthermore, antioxidant activity was determined in these extracts using DPPH, ABTS, and FRAP methods [5-7]. Also, carried out the antibacterial activity of these abstracts against five human pathogenic bacteria.

MATERIALS AND METHODS

Collection of plant sample

Coptis teeta were a collection from various sites of Anjaw, Anini (Lat 28°C17’38.593’’N and Log 95°54’55.655’’E), Dibang Valley of Arunachal Pradesh in April 2021. The plant was identified and deposited as the Voucher specimen at the Herbarium of Botanical Survey of India, Shillong (Meghalaya).

Preparation of plant extract

Freshly collected plant roots are washed, air-dried at room temperature, and finely powdered using a grinder. The powdered root was subjected to extraction with Water, Methanol, Acetone, Chloroform, and n-Hexane. All the extracts were obtained by macerating 5g of dry matter powder with 50ml of each solvent for 48hr with intermittent shaking. The extracts were filtered using Whatman filter paper No.1 and concentrated under reduced pressure and temperature using a rotary evaporator at 40°C to total dryness. The dried crude extracts were stored in the freezer at 4° C for future use.

Phytochemical screening

The preliminary phytochemical screening for each extract was tested for the presence of alkaloids, carbohydrates, saponin, phenols, flavonoids, tannin, terpenoids, cardiac glycosides, protein, coumarin, starch, quinone, phlobatannins, and steroids by standard methods [8].

Determination of total phenolic contents

To determine total phenolic content the Folin Ciocalteu method with minor modifications was used [9, 10]. 250µl of crude extract (1mg/ml) was made up to 3ml with distilled water and was mixed with 1ml of Folin-Ciocalteu reagent and allowed to stand for 3 min. After that 2ml of 20% (w/v) Sodium Carbonate was added and incubated in the dark for 60 min at room temperature. The absorbance was measured at 735nm. Gallic acid was used as standard. The Phenolic content was determined by constructing a calibration curve against the standard and results were expressed as gallic acid (GAE) equivalent per gram of dry weight.

Determination of total flavonoid contents

The aluminum chloride ($AlCl_3$**)** colorimetric assay method [11] was used to determine total flavonoid contents, using quercetin as a standard. 500 µl of crude extract was mixed with 1.5ml methanol, 0.1ml of 10% aluminum chloride, and 0.1ml of 1 M Sodium acetate and added to 2.8 ml of distilled water. The solution was allowed to stand for 30 min and absorbance was recorded at 428 nm using a UV-Vis spectrophotometer. flavonoid content was determined by constructing a calibration curve against the standard different concentrations and was expressed as quercetin equivalent per gram of dry weight.

Antioxidant activity

DPPH radical scavenging assay

The free radical scavenging ability of the extracts was tested by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay as per standard procedure [12] with little modification. 0.1mM of DPPH was prepared in methanol and 1ml of this solution was mixed with 3ml of various concentrations of the sample and standard solution (50- 500µg/ml) and shaken vigorously, then it was allowed to stand in the dark for 30 min at room temperature and absorbance was taken at 517 nm. Ascorbic acid was taken as a reference. The optical density was recorded and % inhibition was calculated by the formula %RSA= [($Abs_{control}$- Abs_{sample})/$Abs_{control}$] x100, where Abscontrol is the absorbance of DPPH solution without any sample and Abssample, is the absorbance of sample extract. The IC_{50} extract concentration providing 50% of radical scavenging was calculated from the percent RSA graph against extract concentration.

ABTS radical scavenging assay

For the ABTS assay, the procedure was followed according to Cano *et al*. [13] with some modifications. Stock solution $ABTS^+$ radical cation was produced using 7mM of ABTS and 2.4mM of potassium persulphate solution. The working solution was prepared by mixing both the stock solutions equally in (a 1:1 ratio) and kept for 14 hr in the dark at room temperature. The solution was then diluted to obtain the absorbance of 0.70± 0.01 unit at 734 nm using a spectrophotometer before beginning the assay. Various concentrations of sample/ standard were taken (50-500 µg/ml) to the 4 ml of ABTS solution and mixed. The sample was incubated at room temperature for 30 min and optical density was noted at 734 nm. The ABTS scavenging capacity was compared with Ascorbic acid and calculated as ABTS RSA (%) = [($Abs_{control}$- Abs_{sample})/$Abs_{control}$] x100 where $Abs_{control}$ is the absorbance of $ABTS^+$ solution without any sample; Abs_{sample} is the absorbance of sample extract mixed with $ABTS^+$.

Ferric reducing antioxidant power (FRAP) assay

FRAP assay was carried out according to Benzie and Strain [14] and Babu *et al.* [15] with slight modifications. Acetate buffer of 300mM containing Sodium acetate and Glacial acetic acid, pH 3.6 was prepared (the pH is above 3.6 it can be adjusted using drops of glacial acetic acid), 10mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40mM HCl and 20mM of Ferric chloride solution. The working solution was prepared by mixing all Sodium acetate, TPTZ, and FeCl3. 6H2O in (10:1:1) ratio and the temperature was raised to 37°C before use. 150µl of the extract was adjusted to the volume of 3 ml of methanol and was allowed to react with 1ml of FRAP solution and was incubated for 30min in dark at room temperature. The optical density was taken at 593nm. was taken was standard curve Ferrous sulphate (FeSO4) with different concentrations and results are expressed in µMFe(II)/g dry mass and compared with ascorbic acid.

Antibacterial activity

Antibacterial activity was tested against five human pathogenic bacteria, two-gram positive *Streptococcus mutans* (MTCC 890), *Streptococcus pyogenes* (MTCC 1926), and three-gram negative bacteria *Vibrio choleraee* (MTCC 3906), *Shigella flexneri* (MTCC 1457) and *Salmonella typhi* (MTCC 3224).

Agar well diffusion assay

Agar well diffusion method was performed as per Allen *et al.* [16] and Kirby- Bauer technique to determine the antibacterial activity of the plant extracts. Mueller Hinton Agar plates were prepared as per the manufacturer's instruction and 100µl of bacterial cultures prepared in Mueller Hinton Broth (0.5 McFarland Standard) were spread over it and rest it to get dry. A circular 6mm diameter well was punched on the plate aseptically with a sterile borer. The stock solutions of the extracts (Water, Methanol, Acetone, Chloroform, and n-Hexane) prepared in DMSO, 100µl of each extract were dispensed on the well at concentrations of 400µg/ml, 800 µg/ml, and 1.6mg/ml. 5% DMSO was taken as negative control and reference antibiotic (Streptomycin) as positive control and incubated for 12hr at 37°C. the readings were calculated for the zone of inhibition (ZOI) in cm.

Disc diffusion assay

Disc diffusion assay was performed according to. Mueller Hinton Agar plates were prepared and the bacterial cultures (0.5 McFarland standard) were seeded over the plate evenly. A 6mm diameter disc was prepared from Whatman No.3 filter paper and sterilized. The discs were dispensed with the plant extracts of different concentrations of 400µg/ml, 800 µg/ml, and 1.6mg/ml and placed on the surface of the agar plate. Antibiotic disc (Streptomycin 10µg) was taken as a reference and 5% DMSO as a negative control. The plates were incubated

for 12 hrs at 37°C and ZOI was recorded in centimeters around the discs in each plate [17]. All determination was carried out in triplicates (n=3).

Determination of MIC and MBC

To determine the efficacy of the extracts, the MIC by broth dilution method was used to study the antimicrobial susceptibility test by evaluating the visible growth of the bacteria in the agar broth [18]. 0.1 ml of the bacterial suspension adjusted to 0.5 McFarland standard (10^8 CFU/ml) was inoculated into the tubes containing different concentrations of two-fold serial dilution extracts ranging from 5mg/ml to 0.156mg/ml in Mueller Hinton Broth were incubated for 8hrs at 37°C until there was visible growth. The visual turbidity of the broth was noted down to confirm the MIC.

After MIC determination of the different extracts of the plant, an aliquot of 50µl of the broth from MIC which showed no visible bacterial growth was spread over fresh prepared Mueller Hinton Agar plates and incubated for 24 hrs at 37 °C and the MBC was read and recorded for 99.9% of the bacterial population are killed at the lowest concentration of the extract [19, 20].

Statistical analysis

The data were expressed as triplicated (n=3) with mean ±standard deviation (SD) and analyzed using the Chi-square test and using one-way analysis of variance (ANOVA) $P<0.05$ was statistically significant.

RESULTS AND DISCUSSION

The collected herbarium of the specimen plant was confirmed by BSI, Shillong (Meghalaya), and received the accession number 98158. After confirmation plant samples were further utilized for further analysis.

Phytochemical screening

The quantitative screening of phytochemicals from *C. teeta* for various solvent extracts was summarized in **Table 1**. The photochemical analysis conducted on the various solvents of *C. teeta* plant extracts revealed the presence of the major constituent which is known to exhibit medicinal properties. Medicinal herbs and many modern medicines rely on secondary metabolites for their action. The preliminary qualitative analysis revealed the presence of secondary metabolites such as alkaloids, carbohydrates, saponin, phenols, flavonoids, tannins, terpenoids, cardiac glycoside, coumarin, starch, quinone, phlobataninn, and steroids. The secondary metabolites act as a defense mechanism in plants against various biotic stress such as insect predation, microbial infestation, viral diseases, etc. [21]. The synthesis of secondary metabolites with specific compounds are correlated to the bioactivity of plant for treating health issues, and chronic diseases because of its therapeutic properties [22].

Table 1. Results of Preliminary of 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

Total phenolic content in terms of (GAE) gallic acid equivalent (The standard curve equation y=0.0038x+0.0442, R^2= 0.9944) **Figure 1**, the acetone extracts constitute the highest amount of phenols about 100. 24 ±0.00 mgGAE/g followed by water extract 92.34 ± 0.00 mgGAE/g, methanol extract 45.57 ± 0.01 mgGAE/g, Chloroform extract 13.15±0.00 mgGAE/g and. The least was found in n-Hexane 0.005 ± 0.00 mgGAE/g **(Table 2)**.

Total flavanoid content

Total flavonoid (Standard curve equation: Y=0.0064x+0.0062, R^2=0.9915) in terms of quecetin (QE) **Figure 2**, the acetone extract again recorded the highest concentration for flavonoid 269.13 ± 0.05 mgQE/g, then methanol extract 64.01± 0.03 mgQE/g and water 49.22 ±0.04 mgQE/g. The low content of flavonoids was found in Chloroform 8.61±0.00 mgQE/g and n-Hexane 3.87±0.02 mgQE/g **(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

DPPH test

The radical scavenging activity of *C. teeta* extracts of acetone possessed the highest activity at 350µg/ml with 56.1±0.006 % followed by water at 33.7%, Methanol at 23.64 %, Chloroform at 12.65 %, and n-Hexane 5.25 % whereas for the standard ascorbic acid it was 83.72 % **(Figure 1a)**. The IC_{50} value was defined as the concentration of the sample that inhibited 50% of the DPPH. IC_{50} for Water, Methanol, Acetone, Chloroform, and n-Hexane was 12.3 µg/ml, 16.011 µg/ml, 7.739 µg/ml, 30.14 µg/ml, 76.11 µg/ml respectively and for ascorbic acid 3.530 µg/ml **(Figure 1b)**. the free radical scavenging effect of different extracts and ascorbic acid were in the order ascorbic acid> acetone> water> methanol> chloroform and>n-hexane.

ABTS

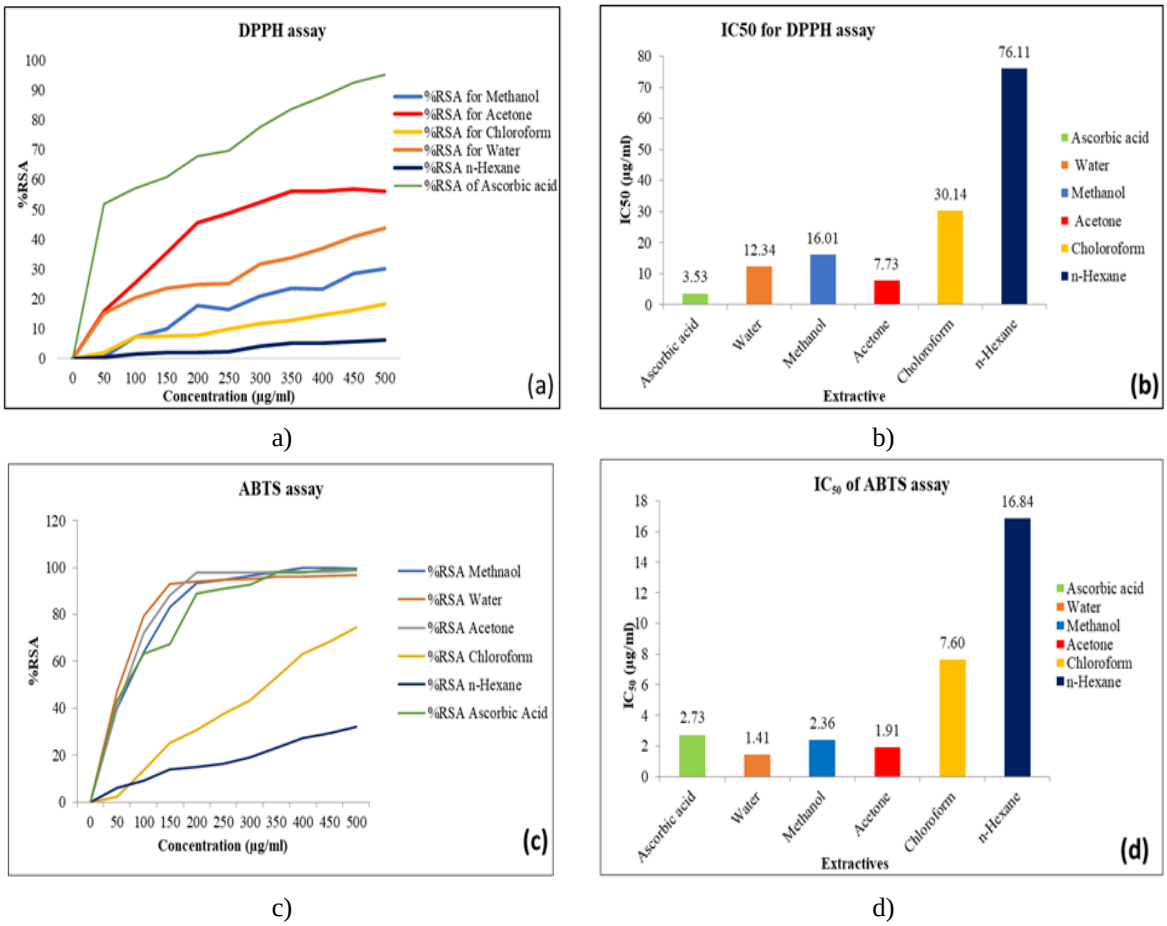
For ABTS Methanol extract contains the highest activity at 400 µg/ml with 99.93% inhibition followed by acetone 98.04 %, water 95.93%, Chloroform 63.34%, and n-hexane (23.25%) with the standard ascorbic acid 97.88% **(Figure 1c)**. The IC_{50} was found to be highest in Water and Acetone extract,1.413 µg/ml and 1.915 µg/ml respectively followed by methanol 2.36 µg/ml, Chloroform 7.609 µg/ml, n-hexane16.8 µg/ml and the standard ascorbic acid 2.730 µg/ml. The order of scavenging effect for ABTS shown in **(Figure 1d)** are as follows: Water>Acetone>Methanol> Ascorbic acid>Chloroform > n-Hexane.

Total antioxidant activity (Ferric Reducing Antioxidant Power FRAP)

In the FRAP assay, the total antioxidant activity of the standard curve equation was Y=0.0141x+0.7424, R^2 = 0.9742 for $FeSO_4$ **(Figure 2a)**. that the highest amount was found in methanol extract with 113.92 ± 0.03 µM Fe(II)/g followed by Acetone 98.81 ± 0.02 µM Fe(II)/g, water extract with 37.22 ± 0.03 µM Fe(II)/g and was lowest in chloroform and n-Hexane 0.853 ± 0.01 µM Fe(II)/g and 0.288 ± 0.05 µM Fe(II)/g respectively. For the reference ascorbic acid it was 90.25 ± 0.01 µM Fe(II)/g **(Figure 2b)**. The FRAP value for Methanol was significantly higher than that of ascorbic acid.

To evaluate the antioxidant effect of the extracts DPPH, ABTS radical scavenging activity, and FRAP assay were determined. The ROS or the free radicals are produced in the oxidation reaction which is responsible for an inflammatory and angiogenic process involving tumor growth, cell damage, and in damaging food [23]. Extreme production of free radicals rapids up the oxidation of lipid molecules. All the extracts of this plant showed strong antioxidant activities marked by reducing Mo(VI), ABTS*, and Fe (III) by gaining an electron from the corresponding assays [24]. Plant polyphenols are a group of large heterogenous, ubiquitous secondary metabolites

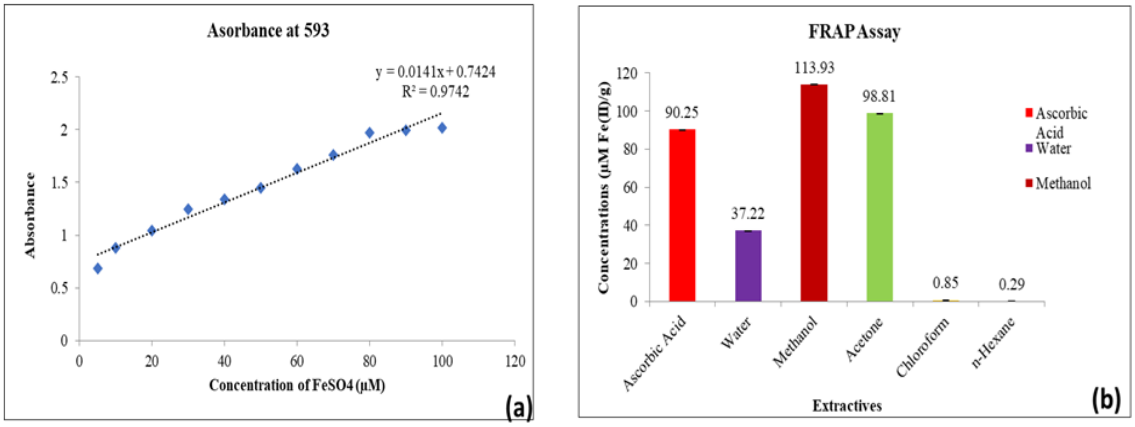
and are generally involved in defense against ultraviolet radiation or invasion of the pathogen, they can perform as hydrogen atom donators, reducing agents, and singlet oxygen scavengers [25]. Thus, the determination of total phenol content and total flavonoid content of *C. teeta* extracts were correlated to the radical scavenging assays (DPPH, ABTS, and FRAP). The high antioxidant activity of water, methanol, and acetone extracts was due to the high phenolic and flavonoid content of the extracts [26].



a) b)

c) d)

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



a) b)

Figure 2. FRAP Assay. a) Standard curve for $FeSO_4$, b) concentrations for the total antioxidant present in different solvent

Antibacterial activity

The antimicrobial activity for various solvents of *C. teeta* against two gram-positive bacteria namely *S. mutans*, *S. pyogenes*, and three-gram negative bacteria namely *V. choleraee*, *S. flexneri*, and *S. typhi.* Used by agar disk

diffusion, well diffusion, MIC, and MBC method. The extracts inhibited the growth of tested microbes at different concentrations are shown by their zone of inhibition in diameter (mm) and the antibacterial activity was compared with the reference antibacterial drug. The antibacterial activities are mostly dependent largely on the concentration of the extracts and It was found that there were significant differences ($P<0.05$) for each concentration among different extracts for both disk and well diffusion.

The highest ZOI for both disk diffusion and well diffusion was at a concentration of 1.6mg/ml **(Table 3)**. In disk diffusion, the ZOI in water was almost equal for all the bacteria ranging from (8± 0.00mm- 8.83 ± 0.28 mm). For methanol extract highest ZOI was for *V. cholerae* (13±1.00 mm), the lowest was for *S. pyogenes* (8±0.00mm) and *S.typhi* did not show any inhibition. In Acetone extract good inhibition was shown by *S. mutans* (12 ± 1.7 mm) and the lowest ZOI was shown by *S. typhi* (8.33 ± 1.15 mm). In Chloroform extract highest ZOI was in *S. mutans* (9.66 ± 0.57 mm), the lowest was for *S. flexneri* (6.66 ±0.57mm), whereas *S. pyogenes* did not show any inhibition. in the n-Hexane extract highest ZOI was for *S. mutans* (11 ± 1.73mm) and the lowest was for *V. choleraee* (6.33 ±0.57mm).

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

Extractives/	400 µg/ml					800 µg/ml					1.6 mg/ml				
Controls	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 Well Diffusion the larger ZOI in water extract was for *S. flexneri* (14±1.73mm) and the lowest was shown by *S. pyogene* (8.66 ± 1.15mm). For Methanol extract highest ZOI was shown by *V. choleraee* (17.66±0.57 mm), and lowest by *S. pyogene* (7.83±0.28mm). In Acetone extract highest was *S. flexneri* (13.66±0.57mm) and the lowest was *S. typhi* (10.66±2.08mm). in Chloroform extract highest ZOI was for *V. choleare* (12.33±0.57mm) and the lowest was for *S. mutans* (9±0.00mm) where *S. pyogenes* and *S. typhi* did not show any inhibition. The n-Hexane inhibition zone was shown only by *S. flexneri* (6.66± 0.57 mm) whereas the others did not form any ZOI. The results obtained are given 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, mg/ml) and Minimum bactericidal concentration (MBC, mg/ml) of *C. teeta* for the five solvents were tested against five organisms are shown in **Table 3** The water extract showed MIC for *S. flexneri, V. cholerae, S. mutans*at 1.25 mg/ml, and for *S. pyogene and Salmonella typhi* at 2.5 mg/ml while *S. flexneri, V. cholerae* showed MBC at 2.5mg/ml and 1.25mg/ml respectively, while at 5 mg/ml *S. mutans*, *S. pyogene* and *S. typhi* exhibited MBC. For methanolic extract at 0.625 mg/ml MIC was shown by *S. flexneri, V. choleraee*, for *S. mutans*it was 0.312mg/ml and for *Streptococcus pyogene and Salmonella typhi* it was 2.5 mg/ml while MBC for the tested organism was at 0.625 mg/ml for *V. cholerae* and *S. mutans* while, *S. flexneri* at 2.5mg/ml exhibited MBC, *S. pyogene* and *S. typhi* exhibited MBC at 5mg/ml. Similarly, Acetone extract at 1.25 mg/ml MIC was exhibited by *S. flexneri, V. cholerae, and S. mutants* and at 2.5 mg/ml *Streptococcus pyogene and Salmonella typhi* exhibited MIC. MBC was also demonstrated for acetone extract, at 5 mg/ml *S. flexneri, Streptococcus pyogene,* and *S.a typhi* exhibited MBC, for *V. choleraee at* 2.5 mg/ml showed MBC and at 1.25 mg/ml MBC was for *S. mutans.* For Chloroform extract MIC for *S. flexneri* was 1.25 mg/ml and MBC was 2.5 mg/ml, while for *V. choleare and S. typhi;* MIC and MBC concentration was the same i,e. 5mg/ml, and for *S. mutans* and *S. pyogene MIC* was 0.625 mg/ml and 5 mg/ml respectively while MBC was > 5mg/ml. Lastly, for n-Hexane extract, *S. flexneri* showed MIC at 0.625 mg/ml and MBC at 5mg/ml while *V. choleare* and *S. mutansat at* 1.25 mg/ml exhibited MIC and MBC at 2.5 mg/ml. For *S. pyogene and S. typhi* MIC was exhibited at 2.5 mg/ml and MBC at 5mg/ml **(Table 5)**. Antimicrobial agents are found to be present in plants as various chemical compounds that retard microbial growth or cause death [20]. Phytochemical compounds like alkaloids, polyphenols, terpenoids, etc have been developed over thousands of years throughout the evolution to defend against organisms and play an important role to treat infectious diseases [20]. It has now become a substitute for synthetic antibiotics because of the increase in unregulated usage and increasing antibiotic resistance in bacteria, hence the evaluation of a novel antimicrobial agent from a natural source is becoming very important [27]. We

examined the antibacterial activity of plant extracts by the diffusion and dilution method against gram-positive (*S. mutans, S. pyogenes)* and gram-negative bacteria (*S. flexneri, V. choleraee, S. typhi*). The polar solvents i.e., water; methanol, and acetone were susceptible to all the five selected bacteria as reports suggested that polar solvents exhibit more antibacterial activity [20]. During the study, it was found that the plant extracts exhibit different effects of antimicrobial activities it is because of the presence of additional membranes surrounding the cell wall of gram-negative bacteria [28]. Researcher around the globe investigated the efficacy of various plant, parts extracts and their effective compounds as antimicrobial agents to control the growth of human pathogenic bacteria. Many researchers suggested that antimicrobial components of the plant extracts (terpenoid, alkaloid and phenolic compounds) interact with enzymes and proteins of the microbial cell membrane causing its disruption to disperse a flux of protons towards cell exterior which induces cell death or may inhibit enzymes necessary for amino acids biosynthesis [19, 20, 28-30]. However, other researchers reported the inhibitory effect of these plant extracts to hydrophobicity characters of these plants extracts which enable them to react with protein of microbial cell membrane and mitochondria disturbing their structures and changing their permeability [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

Coptis teeta commonly used by the tribes of Arunachal Pradesh for curing various human health ailments. Current study reveals the the presence of phytochemicals in the plants. The investigation provides a baseline information to screen the new bioactive compounds and to formulate new drugs to fight against anti-microbial resistance by the board spectrum pathogenic microorganisms. Present study might be useful to supplement scientific information to establish tribal claims for the presence of phytocompounds and medicinal values. The plant

exhibiting significant antibacterial activity against selected human pathogens could be potential efficacy for using pharma industries as an alternative means of antimicrobial. Further research is needed to document the detail bioactive compounds present in the plant.

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