ORIGINAL ARTICLE e-ISSN: 2349-0659 p-ISSN; 2350-0964

Antibacterial Activity of *Ceratopteris thalictroides*: An Unexplored Wild Food

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Abstract

Ceratopteris thalictroides is an important pteridophyte having food and medicinal values. It is easily available near the coastal wetland and in lowland paddy fields. The local communities consume them as a leafy vegetable and it is also a food of herbivores. Therefore, an attempt has been made to gather the information and collect the plant from the coastal areas of Konark, Odisha, India. The survey was made in January 2021 and through Passport Data Form, the information was collected. Results revealed that plant species is used as food and to cure many diseases. The phytochemical screening indicated its medicinal potential. It was observed that antibacterial activity against *Streptococcus mutans* and *Shigella flexneri* was sound. The present study highlights an unexplored fern for future food and formulation of new drugs from it to fight against antimicrobial resistance.

Keywords: Antimicrobial resistance, Food values, Nutraceutical, Unexplored plant

Asian Pac. J. Health Sci., (2022); DOI: 10.21276/apjhs.2022.9.3.48

Introduction

Ceratopteris thalictroides is commonly known as swamp fern and it belongs to the family Pteridaceae. The plant is aquatic or semi-aquatic fern, growing up to 1 m in height tall, either floating in water or rooted in soil. It grows very well in swampy areas, forests, marshes, and natural and manmade ponds, usually in stagnant water bodies. It is widely distributed in the tropics where it prefers a temperature of 20–22°C and a pH of 5–5.6. This plant has a short life cycle of usually <30 days.[1] This fern is mostly found in Africa, Asia-Temperate, Asia-Tropical, Australasia, North America, Pacific, and South America. It is also found in many localities in India from Chamba and Kangra (Himachal Pradesh), Dehra Dun (Garhwal), Nainital, Almora and Pithoragarh (Kumaun), Meghalaya, Nagaland, Kohima, Madhya Pradesh, Rajasthan, Karnataka, Tamil Nadu, and Andaman Nicobar Islands.[2] It is commonly known as "Water Sprite," "Water fern," "Oriental Water fern," "Water Stag-horn fern," and "Water Hornfern." In the Philippines, it is called "*pakung-sungay*," in Japanese Romaji – mizu-warabi, in Spanish (Peru) – helecho de Sumatra, and in Swedish – small vattenbraken.[2] This plant has anti-inflammatory activity, antioxidant activity, and antidiabetic activity. The chloroform and acetone extracts of the plant have been reported to show antibacterial activity against some human pathogens.[1] A novel anti-human immunodeficiency virus protein had earlier been identified from this plant and an investigation on the phytochemical composition of the plant indicated the occurrence of some secondary metabolites.[1] The present study was carried out to determine the phytochemical analysis, antibacterial activity, and thin-layer chromatography (TLC) analysis of the leaves of *C. thalictroides*.

Botanical Description

Aquatic or semi-aquatic plants; erect or sub-erect, thick, fibrous or plump, long roots, apex covered by scales; soft scales. Uniformly light brown, about 2 × 4 mm, ovate, acute, entire, small, curved cell all over. Fronds arranged in rosette; stipes up to 20 × 1.5 cm, terete, fleshy, light green, densely ridge all over with few scattered scales. Lamina dimorphous; sterile lamina bipinnate or tripinnate, ovate, about 32 × 20 cm, acute, cuneate; primary pinnae about five pairs, slightly ascending, alternate, up to 8 cm apart, distinctly stalked, up to 10 × 6 cm, secondary pinnae about four pairs, alternate, shortly stalked, broadly deltoid or ovate, about 4 × 4 cm, pinnatified about 2–3 mm to the costa, ultimate lobes linear, oblong or variously shaped, about 1.5 × 0.7 cm, apex acute, margin entire; veins slightly distinct above and below, copiously anastomosing to form rectangular to hexagonal, elongated areoles of up to 0.5 × 2 mm. Lamina glabrous above and below, light green; texture non-woody. Fertile lamina ovate, up to 40 × 23 cm, tripinnate, ultimate segment needle like, up to 6 × 0.2 cm, acute, margin reflexed, and completely covering the lower surface on which two rows of larger sporangia are borne; spores trilete, 120 µm in diameter, light green, exine with thick dense, convexed ridges [Figure 1].[3]

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How to cite this article: Behera T, Kaur A, Kumar A, Maggirwar R, Kumar S. Antibacterial Activity of *Ceratopteris thalictroides*: An Unexplored Wild Food. Asian Pac. J. Health Sci., 2022;9(3):235-241.
Source of support: Nil
Conflicts of interest: None.
Received: 01/12/2021 **Revised:** 12/01/2022 **Accepted:** 03/02/2022

Methodology

Identification, Collection of Plant, and Ethnobotanical Survey

C. thalictroides was collected from the coastal areas of Konark, Odisha. The plant was identified by Dr. Sanjeet Kumar, APRF,

Odisha, with the help of available literature[4,5] [Figure 1]. The ethnobotanical information was collected through Passport Data Form from local communities and Adivasi Mela.

Preparation of Plant Extracts

The collected plant parts were collected and dried. The dried parts were grinded to make powder. The powder was use to perform the extraction through Soxhlet apparatus using four solvents (n-hexane, methanol, acetone, and aqueous)

Phytochemical Screening

Phytochemical analysis was carried out on different extracts of different plant parts using standard procedure to identify the bioactive compounds.[6,7]

Tests for Primary Metabolites

Test for protein

A 1 ml of plant extract was mixed with 2–5 drops of Millon's reagent and kept it in water bath for 30 min. The pink coloration indicates the presence of protein.[8]

Test for carbohydrate

A 1 ml of plant extract was mixed with 2–5 drops of Fehling's solution A and B and kept in water bath for 30 min. A reducing sugar reacts with Fehling's reagent in alkaline medium to form an orange to red precipitate. Appearance of this orange or red precipitate indicates the presence of carbohydrate.[9]

Test for starch

To 1 ml of plant extract, 2–3 drops of concentrated HCL were added and heated for 1–2 min. NaOH followed to Benedict's reagent was added to this solution and kept in water bath. The change in coloration from bluish-green to red-orange indicates the presence of starch.[10]

Test for sucrose

A 1 ml of plant extract was mixed with 2–3 drops of iodine. Appearance of blue-black color indicates the presence of sucrose.[11]

Test for lipid

A 1 ml of plant extract was mixed with 2–5 drops of Sudan-III. Appearance of pink droplet indicates the presence of lipid.[12]

Test for amino acid

A 1 ml of plant extract mixed with five drops of Ninhydrin reagent and kept it in water bath for 30 min, Ninhydrin is a powerful oxidizing agent to give a purple-colored product (diketohydrin) termed Ruhemann's purple. Appearance of purple or bluish color indicates the presence of purple of bluish color indicates the presence of amino acid.[13]

Tests for Secondary Metabolites

Test for tannin

A 5 ml of plant extract was added with five drops of 10% lead acetate. Formation of a light yellow precipitate indicates the presence of tannin.

Test for saponin

A 5 ml of plant extract was mixed with 2 ml of normal distilled water and shaken vigorously. The stable persistent froth indicated the presence of saponin.

Test for flavonoids

To 1 ml of the extract, few drops of dilute sodium hydroxide were added. The presence of flavonoids is indicated on production of an intense yellow color in the plant extract which become colorless on addition of 2–3 drops of 50% dilute acid.

Test for terpenoid

A 1 ml of plant extract was mixed with 2 ml of chloroform and equal volume of concentrated sulfuric acid was added. Terpenoids presence is confirmed by a reddish-brown coloration of interface.

Test for phenolic compounds

A 2 ml of plant extract was added with five drops of 1% ferric chloride and 1 ml of potassium ferrocyanide, a bluish-green solution showed the presence of phenolic compound.

Test for reducing sugar

A 2 ml of plant extract was boiled with two drops of Fehling's solution A and B for 5 min. An orange-red precipitate was obtained indicated the presence of reducing sugar.

Test for alkaloids

A 5 ml of plant extract was mixed with 3 ml of aqueous HCL on water bath and then filtered. A 1 ml of Dragendorff's was added in the filtrate. The occurrence of orange-red precipitate indicates the presence of alkaloids in the sample extract.

Test for steroid

A 2 ml of plant extract was dissolved in 5 ml chloroform and then 5 ml of concentrated sulfuric acid was added. Formation of two phases (upper red and lower yellow with green fluorescence) indicates the presence of steroid.

Antibacterial Activity

The extracts of experimental plant parts were screened for antibacterial activity against Gram-positive bacteria *Streptococcus mutans* (microbial type culture collection [MTCC] 497) and Gram-negative bacteria *Shigella flexneri* (MTCC 1457). All used MTCC bacterial strains were collected from Institute of Microbial Technology, Chandigarh. Antibacterial activity was done using slightly modification of standard method of agar well diffusion assay,[12] disk diffusion method,[14-16] and broth dilution assay.[17]

Agar well diffusion assay

Agar well diffusion method[18] was followed to test the antibacterial activity of extracts of experimental plant part against the five bacterial strains. Nutrient agar plates were prepared as per manufacturer's instructions. A 100 µL of nutrient broth cultures of the test microbes prepared a day before were poured over the plates uniformly and a lawn culture was prepared using a sterile spreader in a laminar hood. Wells (6 mm) were made using sterile borer. Stock solutions of samples were prepared in 100% DMSO (Sigma) and 2-fold serial dilutions were made in amount of 100 µL per well ranged from 0.5 to 2.0 mg/mL. A 100 µL of samples were added by sterile syringes into the wells in three above-mentioned concentrations and allowed to diffuse at room temperature for 2 h. Plates were incubated at 35 ± 2°C for 18–24 h. Kanamycin and ampicillin served as standard antibiotic control. Triplicate was maintained and the experiment was repeated thrice. For each replicate, the readings (diameter of zone of inhibition of cm) were taken and the mean ± SD values (diameter of zone of inhibition) were recorded.

Disk diffusion assay

Antibacterial activity using disk diffusion assay was done using the 6 mm of disc prepared from Whatman filter paper.[15] Each extract was dissolved in dimethyl sulfoxide. The sets of three dilutions (0.5, 1.0, and 2.0 mg/mL) of crude extracts and standard drugs were prepared. A 6 mm of discs were kept in the drugs for 12 h before placing to the agar plates. The zones of growth inhibition around the disks were measured after 18–24 h of incubation at 37°C for bacteria. The sensitivities of the microbial species to the plant extracts were determined by measuring the sizes of inhibitory zone (including the diameter of disc) on the agar surface around the disks, and value <8 mm was considered as not active against microorganism.

Media used

Nutrient broth was used to maintain broth cultures. The constituents of the nutrient broth included 0.5 g NaCl,[19] 0.5 g peptone, and 0.3 g beef per 100 mL. An additional 1.5 g of agar was added to the nutrient agar medium.

MIC using broth dilution assay

All the extracts of experimental plant parts were screened for their antibacterial activity.[17] Antibacterial activity was assessed by minimum inhibitory concentration (MIC) by serial dilution method. Selected colonies of aforesaid bacteria were picked off to a fresh isolation plate and inoculated in corresponding tubes containing 5 mL of trypticase soya broth. The broth was incubated for 8 ± 1 h at 35 ± 2°C until there was visible growth. McFarland No.5 standard and phosphate buffer saline were used to adjust the turbidity to get 10^5 CFU/mL.

Data interpretation

After the incubation, the tubes showing no visible growth after 8 h till 12 h were considered to be inhibition of bacteria which represent MIC values of a respective concentration. Inoculums control showed visible growth due to no antimicrobial agents, whereas the broth control showed no growth due to the absence of bacteria. Triplicates were maintained and the experiment was repeated thrice, for each triplicates. The readings were taken as foresaid.

TLC

Chromatographic analysis was done using standard methods to evaluate the secondary metabolites.[20]

Preparative TLC

Glass plate was cleaned with ethyl acetate then left for 15 min. A 3 g of silica gel was taken in a beaker and 15 ml water was added. Then, slurry was poured over the glass plate and was allowed to dry, then heated by hot plate for 10 min for the activation of TLC plate.

Mobile Phase

The mobile phase was taken as the solvent system of methanol: chloroform (9:1).

Rf values

The behavior of an individual compound in TLC is characterized by *Rf* and is expressed as a decimal fraction. The *Rf* is calculated by dividing the distance the compound traveled from the original position by the distance the solvent traveled from the original position (the solvent front).

Results

Food and Medicinal Values

C. thalictroides is used as leafy vegetable by many tribal communities. The aerial part of this fern is also edible by many herbivores. It is also used as an ornamental plant in fish aquarium. The fern having many medicinal values also like fresh juice of leaves is used to stop bleeding immediately and the paste of leaves used for skin infections.

Qualitative Tests for Primary Metabolites

The result of the primary metabolites of aqueous extract of *C. thalictroides* showed the presence of Fehling's solution and starch. Acetone extract showed the presence of Fehling's solution and sucrose. Methanol extract showed only the presence of Fehling's solution. Moreover, n-hexane extract showed the presence of starch and lipid [Table 1].

Table 1: Phytochemical screening of the primary metabolites of *Ceratopteris thalictroides*

Primary metabolites	*Solvent*			
	Aqueous	*Acetone*	*Methanol*	*n-hexane*
Phytochemicals				
Protein	−ve	−ve	−ve	−ve
Carbohydrate	+ve	+ve	+ve	−ve
Starch	+ve	−ve	−ve	+ve
Sucrose	−ve	+ve	−ve	−ve
Lipid	−ve	−ve	−ve	+ve
Amino acid	−ve	−ve	−ve	−ve

Tests for Secondary Metabolites

The result of phytochemical screening of the aqueous extract of *C. thalictroides* showed the presence of tannin, saponin, phenolic compounds, and reducing sugar. Similarly, acetone extract showed the presence of phenolic compounds, reducing sugar, and steroids. Methanol extract showed the presence of tannin, flavonoids, phenolic compound, and reducing sugar. Extract of n-hexane showed only the presence of phenolic compounds [Table 2].

Antibacterial Analysis

Disk diffusion assay for aqueous extract of C. thalictroides

The Gram-positive bacteria *S. mutans* showed the different zone of inhibition in different concentrations [Table 3 and Figure 2]. The concentration of 100 mg/ml the zone of inhibition is 1.0 cm, 50 mg/ml the zone of inhibition is 0.8 cm, and 25 mg/ml the zone of inhibition is 0.8 cm. Similarly, the Gram-negative *S. flexneri* showed the different inhibition zone in different concentration.

Figure 1: Collection of plant and traditional uses, (1 and 2) collection of *Ceratopteris thalictroides* from Konark, (3 and 4) data collection from local communities, (5 and 6) data collection from Adivasi Mela

Table 2: Phytochemical screening of the secondary metabolites of *Ceratopteris thalictroides*

Secondary metabolites	*Solvent*			
	Aqueous	*Acetone*	*Methanol*	*n-hexane*
Phytochemicals				
Tannin	+ve	−ve	+ve	−ve
Saponin	+ve	−ve	−ve	−ve
Flavonoids	−ve	−ve	+ve	−ve
Terpenoid	−ve	−ve	−ve	−ve
Phenolic compounds	+ve	+ve	+ve	+ve
Reducing sugar	+ve	+ve	+ve	−ve
Alkaloids	−ve	−ve	−ve	−ve
Steroids	−ve	+ve	−ve	−ve

The concentration of 100 mg/ml the zone of inhibition is 1.2 cm, 50 mg/ml the zone of inhibition 1.0 cm, and 25 mg/ml the zone of inhibition is 0.8 cm. We used penicillin for +ve control and its zone of inhibition is 4.4 cm (*S. mutans*) and 3.8 cm (*S. flexneri*).

Here, we used *S. mutans* and its zone of inhibitions is 1.7 cm in 100 mg/ml concentration, 1.5 cm in 50 mg/ml concentration, and 1.3 cm in 25 mg/ml concentration And then used Gram-negative bacteria *S. flexneri* and its zone of inhibitions [Figure 2 and Table 4] is 2.0 cm in 100 mg/ml concentration, 1.5 cm in 50 mg/ml concentration, and 1.2 cm in 25 mg/ml concentration. Ampicillin was used for +ve control, the zones of inhibitions are 6.4 cm (*S. mutans*) and 6.5 cm (*S. flexneri*).

MIC using broth dilution assay for aqueous extract of C. thalictroides

The MIC of *S. mutans* is 100 mg/ml and the MIC of *S. flexneri* is 50 mg/ml. Broth work as control and inoculums is the bacterial broth [Table 5 and Figure 2].

Table 3: Antibacterial activity using DD assay

Organism used	*Concentration*	*Zone of inhibition*
Streptococcus mutans	100 mg/ml	1.0 cm
	50 mg/ml	0.8 cm
	25 mg/ml	0.8 cm
Shigella flexneri	100 mg/ml	1.2 cm
	50 mg/ml	1.0 cm
	25 mg/ml	0.8 cm
+ve control (SM) penicillin	-	4.4 cm
+ve control (SF) penicillin	-	3.8 cm
−ve control (SM) D/W	-	0 cm
−ve control (SF) D/W	-	0 cm

SM: *Streptococcus mutans*, SF: *Shigella flexneri*, DD: Disk diffusion

Table 4: Antibacterial activity using AWD assay

Organism used	*Concentration*	*Zone of inhibition*
Streptococcus mutans	100 mg/ml	1.7 cm
	50 mg/ml	1.5 cm
	25 mg/ml	1.3 cm
Shigella flexneri	100 mg/ml	2.0 cm
	50 mg/ml	1.5 cm
	25 mg/ml	1.2 cm
+ve control (SM) ampicillin	-	6.4 cm
+ve control (SF) ampicillin	-	6.5 cm
−ve control (SM) D/W	-	0.0 cm
−ve control (SF) D/W	-	0.0 cm

AWD: Agar well diffusion, SM: *Streptococcus mutans*, SF: *Shigella flexneri*

Table 5: MIC analysis of aqueous extracts of *Ceratopteris thalictroides*

Concentration of drugs	*Observation*		*MIC*	
	SM	*SF*	*SM*	*SF*
100 mg/ml	−ve	−ve	Observed MIC	Observed
50 mg/ml	+ve	−ve	NIL	Observed MIC
25 mg/ml	+ve	+ve	NIL	NIL
12.5 mg/ml	+ve	+ve	NIL	NIL
6.25 mg/ml	+ve	+ve	NIL	NIL
3.125 mg/ml	+ve	+ve	NIL	NIL
+ve control inoculum	+ve	+ve	NIL	NIL
−ve control broth	−ve	−ve	NIL	NIL

MIC: Minimum inhibitory concentration, SM: *Streptococcus mutans*, SF: *Shigella flexneri*

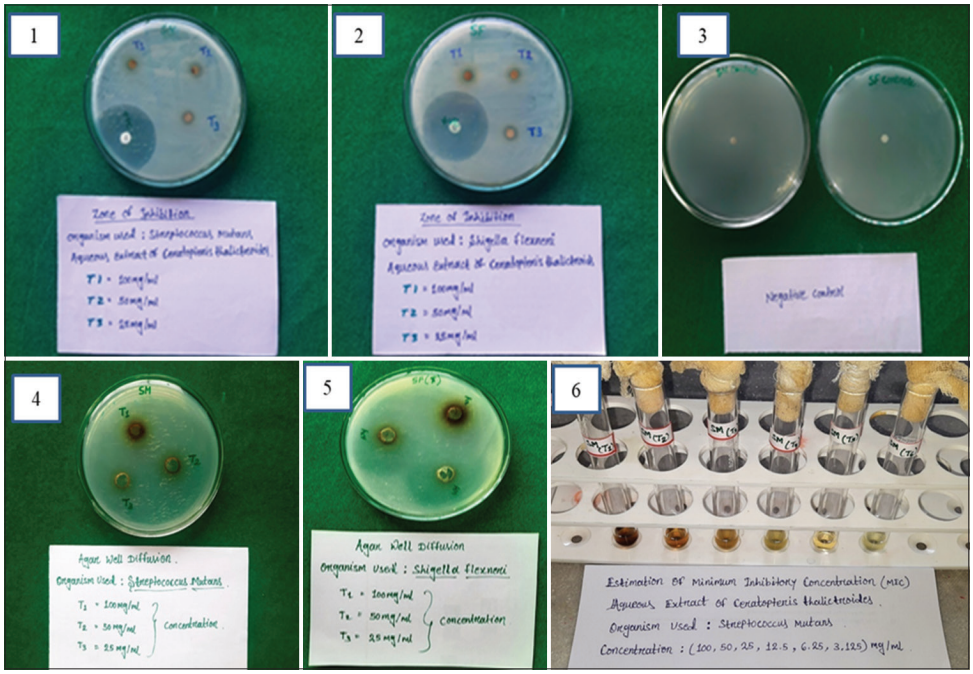

Figure 2: Antimicrobial activities, (1) disk diffusion of aqueous extract against *Streptococcus mutans,* (2) disk diffusion of aqueous extract against *Shigella flexneri,* (3) negative control used in disk diffusion, (4) agar well diffusion of aqueous extract against *Streptococcus mutans,* (5) *Shigella flexneri,* (6) broth dilution assay of aqueous extract against *Streptococcus mutans*

MIC of *S. mutans* = 100 mg/ml
MIC of *S. flexneri* = 50 mg/ml

Disk diffusion assay for methanol extract of C. thalictroides

Here, organism used *S. mutans*, the zones of inhibitions are 1.1 cm in 100 mg/ml concentration, 0.9 cm in 50 mg/ml concentration, and 0.7 cm in 25 mg/ml concentration. The 2nd organism we used *S. flexneri*, the zones of inhibitions are 1.1 cm in 100 mg/ml concentration, 0.8 cm in 50 mg/ml concentration, and 0.7 cm in 25 mg/ml. Penicillin is used for +ve control, the zone of inhibitions is 3.8 cm in both organisms [Table 6].

Agar well diffusion assay for methanol extract of C. thalictroides

We used *S. mutans* and the zones of inhibitions [Table 7] are 1.3 cm in 100 mg/ml concentration, 1.1 cm in 50 mg/ml concentration, and 0.7 cm in 25 mg/ml concentration and then used Gram-negative bacteria *S. flexneri* and its zone of inhibitions are 1.4 cm in 100 mg/ml concentration, 1.0 cm in 50 mg/ml concentration, and 0.7 cm in 25 mg/ml concentration. Ampicillin was used for +ve controls, the zones of inhibitions are 6.0 cm (*S. mutans*) and 6.3 cm (*S. flexneri*).

MIC using broth dilution assay for methanol extract of C. thalictroides

The MIC of *S. mutans* is 100 mg/ml and the MIC of *S. flexneri* is 50 mg/ml. Broth work as control and inoculums is the bacterial broth [Table 8].

MIC of *S. mutans* = 100 mg/ml
MIC of *S. flexneri* = 50 mg/ml

Table 6: Disk diffusion assay for methanol extract of *Ceratopteris thalictroides*

Organism used	*Concentration*	*Zone of inhibition*
Streptococcus mutans	100 mg/ml	1.1 cm
	50 mg/ml	0.9 cm
	25 mg/ml	0.7 cm
Shigella flexneri	100 mg/ml	1.1 cm
	50 mg/ml	0.8 cm
	25 mg/ml	0.7 cm
+ve control penicillin (SM)	5 mg/ml	3.8 cm
+ve control penicillin (SF)	5 mg/ml	3.8 cm
−ve control (SM) D/W	-	0 cm
−ve control (SF) D/W	-	0 cm

SM: *Streptococcus mutans*, SF: *Shigella flexneri*

Table 7: Agar well diffusion assay for methanol extract of *Ceratopteris thalictroides*

Organism used	*Concentration*	*Zone of inhibition*
Streptococcus mutans	100 mg/ml	1.3 cm
	50 mg/ml	1.1 cm
	25 mg/ml	0.7 cm
Shigella flexneri	100 mg/ml	1.4 cm
	50 mg/ml	1.0 cm
	25 mg/ml	0.7 cm
+ve control (SM) ampicillin	5 mg/ml	6.0 cm
+ve control (SF) ampicillin	5 mg/ml	6.3 cm
−ve control (SM) D/W	-	0.0 cm
−ve control (SF) D/W	-	0.0 cm

SM: *Streptococcus mutans*, SF: *Shigella flexneri*

TLC Analysis [Table 9 and Figure 3]

Component – Aqueous and Methanol
Solvent – Methanol: chloroform (9:1).

Aqueous extract

Distance traveled by component (mean) = 12.2/6 = 2.03
Distance traveled by solvent (mean) = 40.7/6 = 6.78

$$Rf = \frac{\text{Distance travelled by component}}{\text{Distance travelled by solvent}}$$

Rf = 2.03/6.78 = 0.299

Methanol extract

Distance traveled by component (mean) = 32.2/6 = 5.36
Distance traveled by solvent (mean) = 39.3/6 = 6.55
Rf = 5.36/6.55 = 0.818

Table 8: MIC using broth dilution assay for methanol extract of *Ceratopteris thalictroides*

Concentration of drugs	*Observation*		*MIC*	
	SM	*SF*	*SM*	*SF*
100 mg/ml	−ve	−ve	Observed MIC	Observed MIC
50 mg/ml	+ve	−ve	NIL	Observed MIC
25 mg/ml	+ve	+ve	NIL	NIL
12.5 mg/ml	+ve	+ve	NIL	NIL
6.25 mg/ml	+ve	+ve	NIL	NIL
3.125 mg/ml	+ve	+ve	NIL	NIL
+ve control inoculum	+ve	+ve	NIL	NIL
−ve control broth	−ve	−ve	NIL	NIL

MIC: Minimum inhibitory concentration

Table 9: Detected spots using TLC

Extract	*Distance traveled by component*	*Distance traveled by solvent*	*Rf values*
Aqueous	2.3 cm	6.3 cm	0.36 cm
	2.1 cm	6.2 cm	0.33 cm
	1.7 cm	6.5 cm	0.26 cm
	2.7 cm	7.0 cm	0.38 cm
	1.8 cm	7.3 cm	0.24 cm
	1.6 cm	7.4 cm	0.21cm
Methanol	3.6 cm	5.0 cm	0.72 cm
	5.7 cm	6.2 cm	0.91cm
	5.6 cm	6.5 cm	0.86 cm
	5.5 cm	6.9 cm	0.79 cm
	6.2 cm	7.4 cm	0.83 cm
	5.6 cm	7.3 cm	0.76 cm

TLC: Thin-layer chromatography

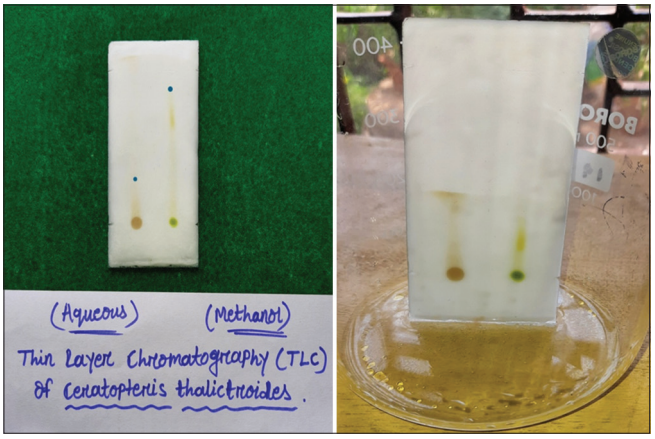

Figure 3: Thin-layer chromatography of both aqueous and methanol extracts in one plate

Discussion

Different experimental methods have been carried out by many scientists based on *C. thalictroides* plants using different methods. An experiment was carried out to screen the biologically active compounds in plant material, *C. thalictroides*. Phytochemical screening proved the presence of alkaloids, steroids, coumarin, tannins, saponins, flavonoids, quinine, anthroquinone, phenol, protein, xanthoprotein, carbohydrate, glycosides, catechin, sugar, and terpenoids in the extracts of whole plant.[21] Another experiment reported a protocol for efficient stable genetic transformation of *Ceratopteris richardii* and *C. thalictroides* using microparticle bombardment. Indeterminate callus was generated and maintained from the sporophytes of both species using cytokinin treatment.[22] Other methods of experiment were conducted to determine the nutritional and anti-nutritional composition of the leaves of *C. thalictroides* using standard procedures. The phytochemical screening and gas chromatography/mass spectrometry analysis of the leaf extracts were also carried out.[1] The present work has been done on the antibacterial effect of the different concentration of extracts of *C. thalictroides* when tested against *S. mutans* and *S. flexneri* and has showing remarkable inhibitory effect. The disk diffusion assay and agar well diffusion assay were carried out to draw more assertive conclusion. The results revealed that all the experimental plants of *C. thalictroides* showed significant inhibitory activity against tested bacterial strains.

Conclusion

Many people believe that the natural products are always safe, good, and have fewer side effects. Therefore, they prefer to use herbal medicines for maintaining and improvement of their health. Keeping in the view, the present study was designed to use *C. thalictroides* against microbial infections. We concluded that the plant possesses diverse bioactive compounds which are responsible for its medicinal properties. Hence, this plant can be of a potential use in therapeutics.

References

1. Adebiyi AO, Oyeyemi SD, Tedela PO, Oja VI. Chemical composition of the leaves of *Ceratopteris thalictroides* (L.) Brongn. Scholars Acad J Biosci 2019;7:377-86.
2. Joshi B, Tewari S, Srivastava A, Mishra RK. Ecology and ethnobotany of *Ceratopteris thalictroides* (L.) Brongn. In: Horticulture and Ecology-Studies and Management. New Delhi: Discovery Publishing House Pvt. Ltd.; 2019. p. 140-6.
3. Shingh SK, Rajkumar SD. Distribution and richness of wetland pteridophytes of Uttar Pradesh, India. Int J Res Anal Rev 2018;5:48-55.
4. Haines HH. The botany of Bihar and Orissa. London: Adlard and Son and West Newman Ltd.; 1925.
5. Saxsena HO, Brahmam M. The Flora of Orissa. Vol. 4. Bhubaneswar: Orissa Forest Development Corporation Ltd and Regional Research Laboratory; 1995.
6. Trease GE, Evans WC. Pharmacognosy. London: WB Scanders Company, Ltd.; 2009. p. 89-300.
7. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. Ibadan: Spectrum Books Limited; 1993.
8. Maurya SK, Asthana A, Maurya SP, Maurya P, Maurya A. Qualitative analysis for protein egg albumin and milk. Indian J Drug 2019;7:30-3.
9. Jahangirpuria HD, Makwana SA, Patel CG. Qualitative test, for identification of Monosacharid, disacharide and polysaecharide

belongs to carbohydrate functional group. Int J Sci Res 2018;3:1-12.
10. Wuttisela K, Shobsngob S, Triampo W, Triampo D. Amylose/Amylo pectine simple determination in acid hydrolyzed tapioc starch. J Chilean Chem Soc 2008;53:1565-7.
11. Fleicher H. The iodine test for reducing sugars a safe, quick and easy alternative to copper (II) and silver (I) based reagents. World J Chem Educ 2019;7:45-52.
12. Marani E, Usunoff KG, Feirabend HK. Lipofuscin and lipofuscinosis. Encyclopedia Neurosci 2009;481-6. DOI: 10.1016/b978-008045046-9.00126-1
13. Friedman M. Application of the Ninhyahin reaction for analysis of amino acids, peptides and proteins to agricultural and biomedical sciences. J Agric Food Chem 2004;52:385-406.
14. Scorzoni L, Benaducci T, Almeida AM, Silva DH, Bolzani VD, Gianinni MG. The use of standard methodology for determination of antifungal activity of natural products against medical yeasts *Candida* sp and *Cryptococcus* sp. Braz J Microbiol 2007;38:391-7.
15. Driscoll AJ, Bhat N, Karron RS, O'Brien KL, Murdoch DR. Disk diffusion bioassays for the detection of antibiotic activity in body fluids: Applications for the pneumonia etiology research for child health project. Clin Infect Dis 2012;54:159-64.
16. Zare f, Rostami FM, Shshsafi M. Prevalence and pattern of antibiotic resistance of gram-negative bacteria isolated from urinary tract infections in patients referring to Neka Laboratories-Iran. Int J Biomed Public Health 2018;1:30-6.
17. Owuama CI. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using a novel dilution method. Afr J Microbiol Res 2017;11:980-21.
18. Allen KL, Molan PC, Reid GM. A survey of the antibacterial activity of some New Zealand honeys. J Pharm Pharmacol 1991;43:817-22.
19. Deaker R, Casteriano A, Gemell LG, Hartley E. Making the Most of High Quality Inoculants: Survival of Rhizobia during Inoculation of Legumes. In: Fremantle W, editor. Proceedings of the 17th International Congress on Nitrogen Fixation. 17 November-1 December. Murdoch: Centre for Rhizobium Studies, Murdoch University; 2011.
20. Raaman N. Phytochemical Techniques. New Delhi: New India Publishing Agency; 2006. p. 2-285.
21. Smitha V, Vadivel V. Phytochemical Screening for active compounds in *Ceratopteris thalictroides* (L.) Brogn. J Pharmacogn Phytochem 2019;8:3556-9.
22. Plackett AR, Huang L, Sanders HL, Langdale JA. High efficiency stable Transformation of the model fern species *Ceratopteris richardii* via microparticle bombardment. Plant Physiol 2014;165:3-14.