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ORIGINAL ARTICLE

Anti-bacterial, Anti-oxidant and Anti-inflammatory Activity of Leaf Extract of *Christella acuminata*

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ABSTRACT

Natural source being a valuable resource of medicine, *Christella acuminata* (fern), is considered in the present study, where its ethanolic and aqueous leaf extracts were examined for phytochemical screening, antibacterial activity, antioxidant activity, and anti-inflammatory activity. The presence of phytoconstituents was determined by different phytochemical screening methods. Various concentrations (100-1000μg/ml) of the sample extracts were examined for their anti-bacterial activity by Disc Diffusion Method, anti-oxidant activity by Ferric ion Reducing Anti-oxidant Power (FRAP) assay, and anti-inflammatory effect by Egg Albumin denaturation assay. The presence of various phytochemicals like alkaloids, flavonoids, polyphenols, steroids, and saponins was positive. The efficacy of antibacterial activity of the extracts was observed as the zone of inhibition of bacteria around the discs relative to streptomycin and the zone of inhibition was found to be the highest in an ethanolic extract with the recorded values of 2.35$\pm$0.02mm and 2.14$\pm$0.02mm for gram-positive and gram-negative bacteria respectively whereas the ferric reducing antioxidant power was observed highest in an aqueous extract with recorded values of 1.8566 $\pm$ 0.021.The percentage inhibition of egg albumin denaturation showed the highest activity in an ethanolic extract, with recorded values of 89.7 $\pm$ 0.09 %. These results suggest that the ethanolic and aqueous leaf extract of *Christella acuminata* can be a potent source in drug discovery for improving inflammation, and oxidative stress and treating bacterial infections. Since no previous study is reported for this plant extract as per we know, further detailed studies may be carried out for better insights.

Keywords: Ferns; Anti-bacterial; Anti-oxidant; Anti-inflammatory; Glaphylopteriolopsis

INTRODUCTION

The medicinal value of ferns was acknowledged a long time ago in Traditional Chinese Medicine.[1] India has about 1000 species in 191 genera, with most of them found in the Himalayan area, Central India, and the Western Ghats.[2] The leaves of the fern *Christella acuminata*, also known as *Glaphylopteriolopsis erubescens*, Genus: *Christella*, belong to the family Thelypteridaceae. Nepalese locally call it Pirey uneu and is native to the Eastern Himalayan region of Nepal, Bhutan, and India, including Sikkim, Darjeeling, Assam and other parts of North-East India.[3] The leaves and rhizomes of the Glaphylopteriolopsis genus contain various phytochemical constituents that are used to treat many diseases. Traditionally, *C. acuminata* is used to support fermentation in the making of kinema and marcha, which are fermented edibles.[4] Various species of pteridophytes like *Asplenium Nidus* L. and *Pteris vittata* L. possess antibacterial activity, whereas *Drynaria quercifolia, Pyrossia adnascens*, and *Microsorum punctatum* possess antioxidant and anti-inflammatory activity.[5–7] Though very little medicinal information regarding this particular species of the plant is available, other species of ferns do possess phytochemicals like alkaloids, saponins, anthraquinones, tannins, flavonoids, and terpenoids and are effective in treating rheumatism, bacterial infection, boils, inflammation, oxidative stress, dysentery, etc.[8]

The body's natural reaction to shielding tissues from damage, infection, or illness is inflammation. A complicated and dynamic protective reaction, inflammation occurs in vascularized tissues in response to trauma, toxins, or

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infections by microbes. The synthesis and release of chemical agents by the cells within the damaged, wounded, or infected tissue trigger the inflammatory response. These substances result in pain, redness, swelling, heat, and functional loss.[9]

Whereas a biological system's capacity to detoxify reactive products like oxygen reactive species (ROS) is out of balance, which leads to the condition known as oxidative stress. Oxidative stress can accelerate the ageing process of the body, induce several degenerative and chronic diseases, and result in acute pathologies (such as trauma and stroke) if it is not well managed.[10,11]

This present study focuses on *Christella acuminata*, belonging to the family Thelypteridaceae. It has been indicated in this report that secondary metabolites minimise oxidative damage by inactivating harmful free radicals, mainly the reactive oxygen species, thereby playing a protective role in the cells as antioxidants and anti-inflammatory molecules. This initial report on the antioxidant and anti-inflammatory activities of *C. acuminata* fern using FRAP assay and Egg albumin denaturation assay, as well as antibacterial activity against gram-positive and gram-negative bacteria, was intended to bring attention to this species of fern.

METHODOLOGY

Collection, Identification, and Preparation of Plant Material

The leaves of the fern *C. acuminata*, Genus: *Christella*; Family: Thelypteridaceae, were collected from the local surroundings of Majhitar, East Sikkim, India. The plant was identified and authenticated (authentication no. 2706220009379) by the Botanical Survey of India, Sikkim Himalayan Regional Centre, Gangtok. The leaves were then washed to remove fine impurities. The leaves were shade-dried for 4-5 days to remove moisture content and to preserve the maximum bioactive constituents. The dried leaves were further cut down into small pieces and then ground using a grinder to get the powder form. Then the powder was preserved in a well-closed container.

Method of extraction

The aqueous and ethanolic extracts were prepared by maceration for 5-7 days. 160g of coarsely powdered *C. acuminata* was placed in the conical flasks at 1:20 for aqueous extract and at 1:10 for ethanolic extract, respectively. It was then kept for extraction with consecutive shaking for 5-7 days, followed by filtration using a Whatman filter paper. The filtrates were concentrated by evaporating the solvents. The two different concentrated filtrates as a residue were used for anti-inflammatory, antioxidant, and antibacterial activity.

Qualitative phytochemical analysis of fern extracts

Preliminary phytochemical screening for the presence of alkaloids, saponins, glycosides, polyphenols and tannins, steroids, terpenoids, and flavonoids was carried out as per standard methods.[12,13]

Determination of antibacterial activity

The antibacterial test was conducted in a laminar airflow cabinet using the disc diffusion method. The tests were done in triplicate with sterile petri plates, which were prepared with nutrient agar media. 1 ml of diluted culture was uniformly poured on each Petriplate and kept for 30 minutes at room temperature. Nanoparticles (discs) were placed on the plates, and further, the antibacterial compounds were deposited on each disc. Then the Petri plates were incubated for 24 hours. The efficacy of antibacterial compounds was observed as a zone of inhibition of bacteria around the discs.[14,15]

Determination of antioxidant activity using F.R.A.P. assay

Serial dilutions from 100μg/ml to 1000μg/ml were prepared for *C. acuminata* leaf extracts and reference drug (L-ascorbic acid). 2.5 ml of phosphate buffer (0.2M, pH 6.6) was added to all test tubes. Now add 2.5 ml of 1% potassium ferricyanide to each test tube. Then the sample is mixed properly using a vortexed shaker. Achieve incubation for 20 minutes at an optimum temperature of 50°C. After incubation is completed, add 2.5 mL of 10% trichloroacetic acid to all the test tubes. Centrifuge the sample at 3000rpm for 10 min. After centrifugation is completed, take 2.5 ml of supernatant liquid from all the samples and place it in a test tube with proper labelling. Finally, 2.5 mL of distilled water & 0.5 mL of 0.1% ferric chloride were added to all the test tubes, and absorbance was measured at 700nm using a UV-spectrophotometer and recorded.[16]

Determination of anti-inflammatory activity using albumin denaturation assay

The anti-inflammatory activity of unknown crude extracts can be determined in vitro for the inhibition of egg albumin (protein denaturation). Serial dilution from 100μg/ml to 1000μg/ml was performed for *C. acuminata* leaf extracts and reference drug (diclofenac sodium). All samples contained 5.0 ml of total volume. The reaction mixture was prepared using 2.8 ml of phosphate-buffered saline (pH 6.4) and 0.2 ml of 1-2% egg albumin. Then, 2.5 ml of *C. acuminata* leaf extract from each concentration was mixed gently with the reaction mixtures. A similar procedure was used for the reference drug (diclofenac sodium), and they were used as a positive control for this study. The reaction mixture was incubated in a water bath at 37°C $\pm$ 2°C

for 15-20 min, and later it was heated to 70°C, at which the reaction mixture was maintained for 5 min. Then, the reaction mixture was allowed to cool down at room temperature for 15 min. The absorbance of the reaction mixture before and after denaturation was measured for each concentration (100μg/ml, 200μg/ml, 400μg/ml, 600μg/ml, 800μg/ml, 1000μg/ml) at 680nm using UV spectrophotometry. Each test was repeated thrice, and the mean absorbance was recorded. The percentage inhibition of protein was determined on a percentage basis compared to the control using the following formula:[7,17]

$$\text{Percentage inhibition (\%)} = \frac{\text{Absorbance of Control} - \text{Absorbance of test}}{\text{Absorbance of Control}} \times 100$$

RESULTS

Qualitative phytochemical estimation of leaves of Christella acuminata

The qualitative phytochemical estimation shows the presence of alkaloids, saponins, polyphenols, tannins, steroids, and flavonoids (Table 1).

Table 1: The result of the phytochemical estimation of leaves of *Christella acuminata*

Qualitative tests	Leaf extract
Alkaloid (Mayer's test)	+
Saponins (Foam test)	+
Glycosides	-
Polyphenols and Tannins	+
Steroids	+
Terpenoids (Salkowski's test)	-
Flavonoids (Shinoda test)	+

Determination of antibacterial activity

To test the antibacterial capacity of the selected ferns, four bacteria, *Escherichia coli* (Gram negative), *Salmonella typhi* (Gram negative), *Bacillus subtilis* (Gram positive) and *Staphylococcus aureus* (Gram positive) were assessed.

According to the present study, the most potent extract against the above-mentioned bacteria was the ethanol extract compared to the aqueous extract. The antibacterial activity showed that the ethanol extract produced the maximum zone of inhibition compared to the aqueous extract. The inhibition of bacterial (gram +ve and gram –ve) growth by both the extracts of the leaves of *C. acuminata* (Houtt.) indicated the antibacterial activity of the sample (Table 2).

Table 2: Zone of inhibition of aqueous extract (AE), Ethanolic extract (EE)

Samples	Gram positive	Gram –ve
STD	3.11±0.044mm	3.15±0.02mm
EE	2.35±0.02mm	2.14±0.02mm
AE	2.01±0.04mm	1.85±0.03mm

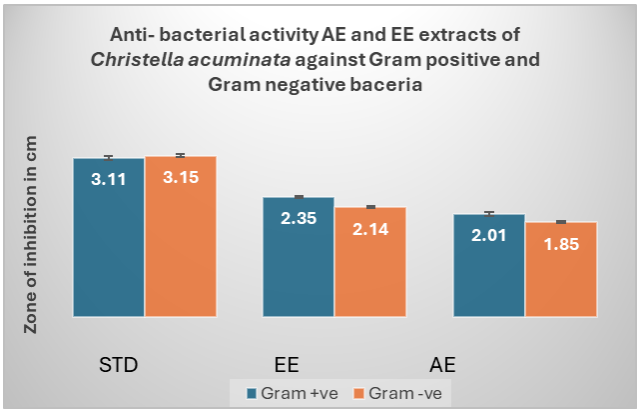

Fig. 1: Zone of inhibition of aqueous extract (AE), Ethanolic extract (EE) [All the values are Mean ±SEM, n=3 *** P<0.001 when compared with the control group. STD: standard drug (Streptomycin), EE: ethanol extract, AE: Aqueous extract of *Christella acuminata*.]

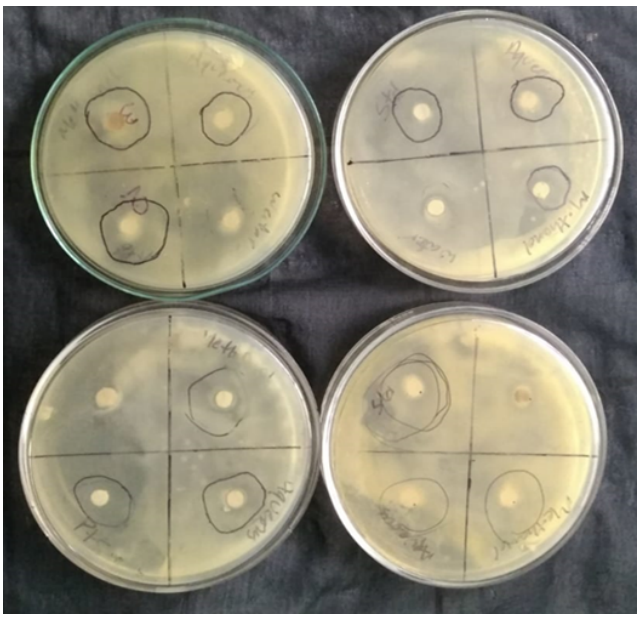

Fig. 2: Zone of inhibition

Determination of antioxidant activity using F.R.A.P. assay

The antioxidant activity of the leaves of ethanolic and aqueous extracts of *C. acuminata* was evaluated for their ferric-reducing antioxidant power by the F.R.A.P assay. Results show that all leaves ethanolic and aqueous extracts of the *C. acuminata* showed the ferric-reducing antioxidant power ranging from 0.188 ± 0.007 to 1.31 ± 0.116 and 0.22 ± 0.005 to 1.8566 ± 0.021 respectively at 700nm by U.V. spectrophotometer. Compared to the different concentrations of standard L-ascorbic acid the aqueous extract exhibits higher ferric-reducing antioxidant power than the ethanolic extract. These observations were carried out in triplicate, and the mean value of absorbance implies that both the ethanolic and aqueous extracts of the fern samples may contain antioxidant molecules that quench the oxidative radicals (Table 3).

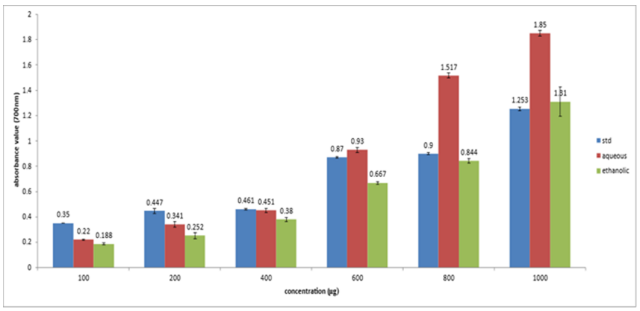

Fig. 3: Reducing power of the std (ascorbic acid), aqueous and ethanolic extract of *C. acuminata* evaluated by FRAP assay [All the values are Mean ± SEM, n=3. STD= Standard drug (ascorbic acid), EE (Ethanolic extract), AE (Aqueous extract) of *Christella acuminata*.]

Determination of anti-inflammatory activity using egg albumin denaturation assay

When we heat the egg albumin, it generally undergoes denaturation, and antigens are released, which are associated with hypersensitivity reactions related to diseases such as glomerulonephritis, serum sickness, rheumatoid arthritis, and systemic lupus erythematosus.

The results show that both ethanolic and aqueous extracts of the *C. acuminata* showed the inhibition of the denaturation of albumin in vitro, ranging from 24.77 ± 0.110 to 89.7 ± 0.09 and 18.67 ± 0.420 to 83.973 ± 0.072, respectively. After comparing the results with the standard (Diclofenac sodium) it was found that the ethanolic extract showed better anti-inflammatory activity by percentage inhibition to albumin denaturation (Table 4).

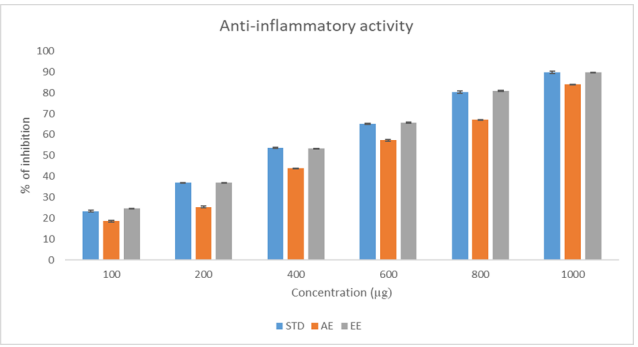

Fig. 4: Percentage inhibition of albumin denaturation of the std (Diclofenac sodium), aqueous and ethanolic extract of *C. acuminata* [All the values are Mean ± SEM, n=3. STD= Standard drug (Diclofenac sodium), EE: Ethanolic extract, AE: Aqueous extract of *Christella acuminata*.]

DISCUSSION

We conducted an investigational study on anti-bacterial, anti-inflammatory and antioxidant activity of fern *C. acuminata* belonging to the family Thelypteridaceae. The plant was collected from the local area of Majhitar, Rangpo, East Sikkim. The plants were washed and dried in the shade for one week. The process was carried out by the maceration extraction process. Approximately 160g of powdered crude drug of *C. acuminata* leaves were exhaustively soaked in ethanol and water at 1:10 and 1:20, respectively, for 3-5 days. Occasionally, stirring was done during the soaking by the maceration process. Samples were then filtered using Whatman no.1 filter paper, and the extract was concentrated to a definite volume using a water bath and heating mantle. The test for phytochemicals like Alkaloids, Saponins, Glycosides, Polyphenols & Tannins, Steroids, Terpenoids, and Flavonoids was performed.

The antibacterial activities of different extracts (Aqueous extract and ethanolic extract) were to be tested against *Escherichia coli* (Gram-negative), *Salmonella typhi* (Gram-negative), *Bacillus subtilis* (Gram-positive), and *Staphylococcus aureus* (Gram-positive). The results showed that all the plant extracts and their solvents gave better inhibition at high concentrations of the extract. According to the present study, the most potent extract against the above-mentioned bacteria was the ethanol extract then followed by the aqueous extract. As a conclusion, the antibacterial activity showed that the ethanol extract produced the maximum zone of inhibition compared to the aqueous extract. Hence, the inhibition of bacterial (gram +ve and gram –ve) growth by both the extracts of the leaves of *C. acuminata* (Houtt.) shows the antibacterial activity of our sample. During the FRAP test, we prepared different sample concentrations, i.e., 100-1000 μg/ml, using the stock solution. After which, UV Spectroscopy was performed. The absorbance was in increasing order, and the highest absorbance was found for

Table 3: Ferric Reducing Antioxidant Power (F.R.A.P.) at different concentrations of STD (ascorbic acid), Aqueous extract (AE), Ethanolic extract (EE)

Samples	Mean absorbance values ± SEM at different concentrations					
	100	200	400	600	800	1000
STD	0.350 ± 0.00	0.447 ± 0.021	0.561 ± 0.005	0.8703 ± 0.006	0.90 ± 0.009	1.253 ± 0.014
AE	0.22 ± 0.005	0.341 ± 0.023	0.451 ± 0.015	0.9366 ± 0.020	1.517 ± 0.020	1.8566 ± 0.021
EE	0.188 ± 0.007	0.252 ± 0.023	0.38 ± 0.015	0.667 ± 0.011	0.844 ± 0.017	1.31 ± 0.116

Table 4: Percentage inhibition of albumin denaturation at different concentrations of STD (diclofenac sodium), Aqueous extract (AE), Ethanolic extract (EE)

Samples	Percentage inhibition of albumin denaturation ± SEM at different concentrations					
	100	200	400	600	800	1000
STD	23.37 ± 0.461	36.93 ± 0.07	53.60 ± 0.253	65.15± 0.1769	80.393 ± 0.539	89.766 ± 0.4788
AE	18.67 ± 0.420	25.42 ± 0.424	43.88 ± 0.130	57.363 ± 0.435	67.126 ± 0.151	83.973 ± 0.072
EE	24.77 ± 0.110	36.93 ± 0.042	53.306 ± 0.16	65.78 ± 0.220	80.846 ± 0.283	89.7 ± 0.09

1000 μl of both ethanolic and aqueous extract; however, the percentage inhibition of Ferric-Reducing Antioxidant Power was found to be highest in the aqueous extract of *C. acuminata*. During the egg albumin denaturation for anti-inflammatory activity again we prepared different sample concentrations for both ethanolic and aqueous leaf extracts, i.e., from 100-1000 μg/ml using a stock solution, followed by UV spectroscopy. The absorbance was found to be in decreasing order due to the turbidity, and again, the lowest absorbance was found for 1000 μl. The percentage inhibition of denaturation was calculated by comparing the absorbance of the samples and the control, the percentage inhibition was found to be in increasing order with increasing concentration. The highest percentage of inhibition was found in the ethanolic extract of *C. acuminata*.

CONCLUSION

An attempt has been made to evaluate the presence of various phytochemicals and antibacterial, antioxidant, and anti-inflammatory properties of leaves of *C. acuminata*. The identification of the plant material taxonomically and pharmacognostically is important to provide pharmacognostic standards and also to avoid spurious or adulterated drugs. The detailed botanical and pharmacognostical studies help in evolving specific diagnostic characteristics to fulfill this objective.

Although this plant was reputed for its anti-infective properties in traditional medicinal systems, only a limited number of studies have been carried out to evaluate its biological and pharmacological properties. Hence, the present studies have been carried out to evaluate their biological and pharmacological properties. The present study confirmed the occurrence of strong antibacterial, antioxidant, and anti-inflammatory properties in leaf extracts of *C. acuminata*, which may be due to the presence of polyphenolic compounds such as flavonoids. However, the ethanolic leaf extracts were found to be more effective against both gram-positive and gram-negative bacteria as compared to the aqueous extract. The aqueous extract was found to be more effective for anti-oxidant activity, whereas the ethanolic extract showed better results for anti-inflammatory activity. The findings obtained in this study correspond well with the ethnopharmacological use of *C. acuminata* for the treatment of bacterial infection, inflammation, and oxidative stress. This study will further pave the way for the isolation and characterisation of useful compounds from this medicinally significant pteridophytic fern. Further *in vivo* and *in vitro* studies should be performed to establish the anti-bacterial, antioxidant, and anti-inflammatory activity of *C. acuminata* leaf.

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