



**PRELIMINARY PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT
PROPERTY OF THE FERN, *CHRISTELLA DENTATA* (FORSSK.)**

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ABSTRACT

Aqueous and ethanolic extracts of leaves, petiole and rhizome of *Christella dentata* were subjected to qualitative screening for phytochemicals. Results revealed the presence of various metabolites like ketose, carbohydrate, protein, cardiac glycosides, steroids,

flavonoids, phenols, saponin, terpenoids, alkaloids, tannin, coumarin, acids and quinone in different plant parts. Occurrence of different secondary metabolites and their concentration was much lesser in the leaves. Rhizome revealed the presence of most of the metabolites analysed and at higher concentrations. DPPH radical scavenging test was used for the evaluation of antioxidant property and significantly higher levels of free radical scavenging activity with IC 50 value of 26µg/ml was shown by the alcoholic extract of rhizome which was higher than that of ascorbic acid. Antioxidant activity exhibited by the leaf extract was highly negligible.

KEYWORDS: *Christella dentata*, phytochemicals, antioxidant property.

INTRODUCTION

Pteridophytes, the second largest group of plants next to angiosperms forms a much neglected plant group as far as their economic value is concerned. The use of these plants as folk medicine has been known to man for more than 2,000 years and ample literature is available regarding the medicinal importance of various fern species (Kirtikar and Basu, 1935; Nayar, 1957; Chopra et al., 1958; Kumar and Roy, 1972; Watt, 1972). Pteridophytes have been successfully used medicinally in Ayurvedic, Unani, Homeopathic and other systems of

medicines. Phytochemicals are the chemicals derived from plants and constitute large number of secondary metabolites in plants that provide protection against insect attacks and diseases and provide a number of protective functions for human consumers. Scientific knowledge on the phytochemical and antioxidant property of large group of fern members is still inadequate. The present paper focuses on the qualitative phytochemical screening and antioxidant property of different parts of an unexplored fern *Christella dentata*.

MATERIALS AND METHODS

Collection and preparation of plant extracts

The study material *Christella dentata* was collected from Akamala forest station, Vadakkanchery, Thrissur, Kerala. The collected materials were separated into leaves, petiole and rhizome. These were reduced in size by chopping them into smaller pieces using a blade and then dried using hot air at 50^oc. The dried materials were grinded to fine powder by a domestic grinder and stored in moisture free containers till further use. The extracts of different plant parts were prepared using distilled water and ethanol.

Phytochemical screening

The ethanolic and aqueous extracts were subjected to preliminary phytochemical analysis for screening the presence of various primary and secondary metabolites. Primary metabolites analyzed were carbohydrate (Molisch Test), sugar (Benedict's Test), ketose (Seliwanoff's Test), starch (Iodine Test), proteins (Lowry's Test), aminoacids (Ninhydrin Test), and fats (Filter Paper Test). Secondary metabolites tested were quinone (H₂SO₄ Test), cardiac glycosides (Keller-Killani Test), steroids (Salkowski Test), flavonoids (Shinoda Test), alkaloids (Mayer's Test), phenols (Folin Test), saponin (Foam Test), tannin (Lead Acetate Test), coumarin, acids and terpenoids (Salkowski Test). Standard methods given by Harborne (1973) and Sofowora (1982) were adopted for the phytochemical analysis.

DPPH based free radical scavenging activity

The antioxidant activity of the plant extracts was estimated using DPPH radical scavenging protocol and compared against the activity of ascorbic acid which was taken as standard. DPPH solution (0.004% w/v) was prepared in 95% ethanol. Stock solution of ethanolic extract was prepared in the concentration of 10mg/100ml (10 μ g/ml). From stock solution 2ml, 4ml, 6ml, 8ml and 10ml were taken in five test tubes respectively. With same solvent made the final volume of each test tube up to 10ml whose concentration was then 20 μ g/ml, 40 μ g/ml, 60 μ g/ml, 80 μ g/ml and 100 μ g/ml respectively. 2ml of freshly prepared DPPH

solution (0.004% w/v) was added in each of these test tubes. The reaction mixture was incubated in the dark for 15 minutes and thereafter the optical density was recorded at 523nm against the blank. For the control, 2ml of DPPH solution in ethanol was mixed with 10ml of ethanol and the optical density of the solution was recorded after 30minutes. The assay was carried out in triplicate. The decrease in optical density of DPPH solution on addition of test samples in relation to the control was used to calculate the antioxidant activity, as percentage inhibition of DPPH radical. The capability of scavenging DPPH radical was calculated using the following equation:

$$\text{DPPH Scavenged (\%)} = \frac{(\text{A control} - \text{A test})}{(\text{A control})} \times 100$$

Where “A control” is the absorbance of the control reaction and “A test” is the absorbance of the sample containing plant extracts.

RESULTS AND DISCUSSION

The results of qualitative phytochemical analysis of leaves, petiole and rhizome of *Christella dentata* revealed the presence of various metabolites like ketose, carbohydrate, protein, cardiac glycosides, steroids, flavonoids, phenols, saponin, terpenoids, alkaloids, tannin, coumarin, acids and quinone. Occurrence of different secondary metabolites and their concentration was much lesser in the leaves. Rhizome revealed the presence of most of the metabolites analysed and at higher concentrations as indicated by the development of high color intensity in the various test solutions. Results of phytochemical analysis are depicted in table 1.

Natural antioxidants that are present in the herbs and spices either prevent the generation of toxic oxidants, intercept any that are generated and inactivate them and there by block the chain propagation reaction produced by these oxidants. DPPH radical scavenging test is used for evaluation of the antioxidant capacity of pure compounds and extract in a short period of time. The lower IC 50 value indicates the stronger radical scavenging capacity. Analysis of antioxidant property of different parts of *Christella dentata* revealed that leaves possessed no or very little antioxidant activity, while free radical scavenging activity was at significantly higher levels in alcoholic extract of rhizome with IC 50 value of 26µg/ml followed by that of petiole (IC 50-42µg/ml) (table2, fig.1).

Table 1: Qualitative phytochemical analysis of *Christella dentata*.

Primary/ Secondary metabolites	Leaf		Petiole		Rhizome	
	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol
Carbohydrate	+	-	+	++	+++	+++
Sugar	-	-	-	-	-	-
Ketose	-	+++	-	-	+++	+++
Proteins	+++	-	++	++	++	+++
Fats	-	-	-	-	-	-
Quinone	-	-	-	-	+	+++
Cardiac glycosides	+	+++	+	-	+	-
Terpenoids	+	-	++	+	++	+++
Phenols	-	+++	-	-	-	-
Flavonoids	+	-	++	++	++	+
Saponins	++	++	+	+	+	+
Alkaloid	+	+	+++	+++	+++	+++
Tannin	+	-	+	-	++	++
Amino acid	-	-	-	-	-	-
Steroids	+++	++	+	+	+	+++
Coumarin	+++	-	+	-	+	+
Acid	+	-	+	++	-	-

- Absent, +-present, ++-moderately present, +++appreciable amount

Table 2: DPPH scavenging activity of *Christella dentata*.

Concentration (µg/ml)	% inhibition of DPPH			
	Petiole	IC50	Rhizome	IC50
20	36.45±1.31	42µg/ml	40.71±0.41	26µg/ml
40	47.91±0.93		84.43±0.87	
60	72.91±0.60		93.11±0.41	
80	84.37±0.41		94.31±0.34	
100	86.45±1.44		95.80±1.14	

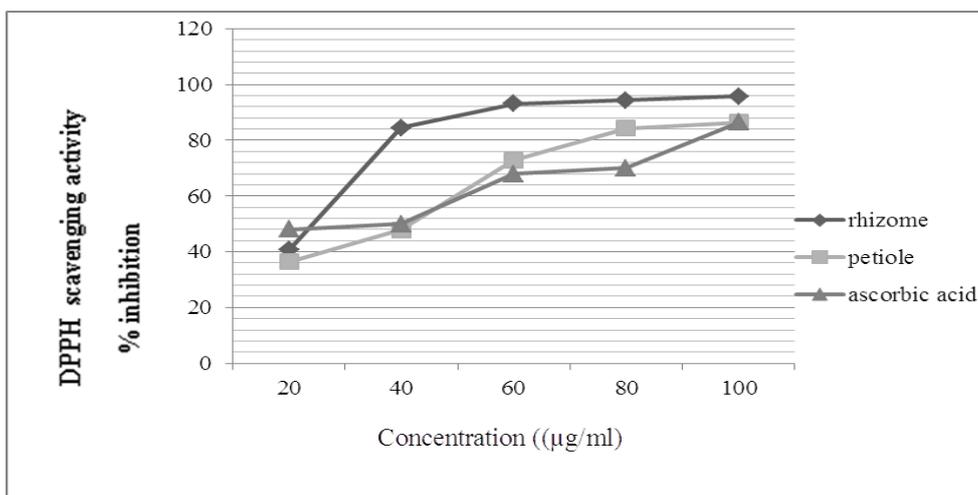


Fig.1: Antioxidant property of *Christella dentata*.

Plant derived natural products such as flavonoids, terpenoids and steroids etc have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity (De Feudis et al., 2003; Takeoka and Dao, 2003). In the present investigation, flavonoids and terpenoids were not detected in the ethanolic extract of leaves. It might be the reason for the absence of antioxidant activity in leaves. While higher levels of above mentioned secondary metabolites in the rhizome followed by petiole might have resulted in the significantly higher levels of antioxidant property in the rhizome and petiole. Hassan et al. (2015) studied antioxidant property of eight fern species from North of Iran and found that rhizome extract were stronger radical scavengers than the aerial part extract in all the ferns which is in agreement with the results of current investigation.

CONCLUSION

Results of the current study revealed that the rhizome of *Christella dentata* is a good source of various secondary metabolites followed by petiole. Rhizome also exhibited excellent antioxidant property higher than that of ascorbic acid. Further work on the profile and nature of chemical constituents of *Christella dentata* will provide more information regarding the use of this plant parts as a possible new source of natural antioxidants.

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