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Original Article

Exploring the Phytochemical Properties and Therapeutic Potential of *Cheilanthes swartzii*: A Novel Fern from the Western Ghats

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ABSTRACT

The pharmaceutical industry has laid major emphasis on finding novel sources of compounds with therapeutic and commercial value, and plant phytochemical screening has made significant strides in this quest. Identification of phytochemicals in medicinal plants is one of the initial steps in the process of developing new plant-based drugs. Plant phytochemicals offer quite a number of advantages, from wound healing to antiviral properties. Phytochemical studies have shown that ferns contain a wide variety of useful compounds. However, compared to angiosperms, ferns are still relatively unknown. The present study reports the phytochemical analysis and anti-diabetic and anti-oxidant properties ofan under-explored fern, *Cheilanthes swartzii* Webb & Berthel., obtained from the Western Ghats region of Southern India. The presence of different phytochemicals in the plant's methanol and petroleum extract was tested using standard protocols. Phytochemicals including alkaloids, tannins, phenol, flavonoids, steroids, terpenoids, and cardio glycosides have been identified, according to the qualitative assessment. DPPH and ABTS+ radical scavenging methods determined antioxidant activity. The results obtained in DPPH radical scavenging activity showed higher levels in methanolic extract and least in petroleum ether extract. The $ABTS^{+}$ radical scavenging rate was found highest in methanol extracts andthe lowest potential in petroleum ether extracts. The *in vitro* antidiabetic activity of α-amylase and β-glucosidase enzymes activity revealed that the methanolic extract of the plant moderately inhibited α-amylase enzymes, while significantly inhibited β-glucosidase enzymes activity exhibiting a low IC_{50}value (54.35 µg/ml). The presence of beneficial phytochemicals, along with antioxidant and anti-diabetic properties, suggests that this plant could be further utilized based on the obtained results.

INTRODUCTION

Pteridophytes account for a significant contribution to the earth's plant diversity. It is the second largest group of vascular plants, forming a significant and dominant component of many plant communities. The Pteridophytes which contain ferns and fern allies are well known to man for more than 2000 years and also have been mentioned in ancient literature [1]. Pteridophytes have a long geological history as pioneer plants and colonized the planet millions of years ago. Approximately 12,000 species of pteridophytes occur in the world flora, more than 1,000 species belong to 70 families and 191 genera are likely to occur in India [2]. Among the Indian pteridophyte species, 170 species are useful in food, flavour, dye, medicine, bio-fertilizers, oil, fibre, and biogas production.

Pteridophytes are also known to occupy a significant place in primary healthcare for cultural and economic reasons. Due to the economic crisis in developing countries, pteridophytes are presently utilized for human ailments. Pteridophytes have been successfully used in different systems of medicine such as Ayurvedic, Unani, Homeopathic, and other systems of medicine [3]. Researchers have reported medicinal, pharmaceutical, and phytochemical uses of various pteridophytic species, which have potential applications in medicinal and industrial usages however only a few information about the medicinal properties of pteridophytes is documented. The ferns contain a variety of alkaloids, glycosides, flavonoids, terpenoids, sterols, phenols sesquiterpenes, etc., as potential compounds are reported to have been used in various industries

[4]. Besides ferns possess numerous bioactivities such as anti-microbial, anti-viral, anti-inflammatory, anti-tussive, and anti-tumor [5]. Researchers have been screening medicinal plants to identify bioactive compounds for human ailments for several decades [6]. 90% of the world's population still relies completely on raw herbs and unrefined extracts as medicines [7]. Although pteridophytes have boundless medicinal properties, phytochemical validation has not yet been explored [8]. Biochemically, pteridophytes are less versatile than angiosperms. Still, some medicinal ferns have been documented for their anti-inflammatory activity namely *Pteris multifida* [9], and *Cheilanthes farinose* [10]; wound healing activities namely, *Davallia solida* [11], and *Angiopteris evecta* [12] respectively. The family Pteridaceae commonly called "Maiden hair ferns" encompasses 53 genera and 1,211 species of ferns, 5 having a remarkable presence in tropical and subtropical regions [13]. *Cheilanthes* commonly known as "lip fern" belongs to the fern subfamily Cheilanthoideae of Pteridaceae. The fern fronds of various members of *Cheilanthes* have been used in folk medicines for many applications such as cough, headache, stomach pain, malarial fever, and hepatic problems [14]. The genus is now recognized as a rich source of flavonoids [15]. Since no record of ferns belonging to genus *Cheilanthes* is reported from the state of Tamil Nadu to date, this present study was made as an attempt to analyze qualitative and quantitative properties and to determine their *in vitro* anti-oxidant and anti-diabetic activity of *Cheilanthes swartzii* collected from the Southern Western Ghats.

MATERIALS AND METHODS

The Study Area

The Western Ghats of Nilgiris Biosphere Reserve (NBR) constitutes an important biogeographic region, as one of the nine floristic zones in India and spreads along the states of Tamil Nadu, Kerala, Karnataka and Maharashtra. The present study area Barliyar is confined to a major range of Nilgiri biosphere reserve, Southern Western Ghats of Tamilnadu. It is located in the Udhagamandalam district of the Southwest of Tamil Nadu and lies between 11º.3436° N latitude and 76º.840º E longitude. The elevation of the hills ranges from 1033m / 3389 feet above Mean Sea Level (MSL) and temperature varies between 10 and 22 °C. Vegetation is predominantly deciduous forest types and the average annual rainfall is 1600 mm.

Collection of Plant Material

Fern species of the *Cheilanthes swartzii* Webb & Berthel. (= Syn. *Cheilanthes mysurensis* Wall. ex Hook.) were collected from the Nilgiris, Tamil Nadu, India. The collected plant materials were washed with tap water followed by distilled water to remove unwanted debris and shade dried for 20 days. The completely shade-dried materials were powdered separately and stored in polythene bags for further phytochemical analysis.

Extraction of *Cheilanthes swartzii* whole Plant

50g of fine powder of *C. swartzii* was packed with No.1 Whatman filter paper and placed in a soxhlet apparatus using petroleum ether followed by methanol. The residues were collected and dried at room temperature of 30 °C after which the yield was weighed and then activity was performed.

Phytochemical Studies

Qualitative Analysis

The extracts were screened for the presence of alkaloids, tannins, saponins, phenol, flavonoids, steroids, terpenoids, and cardiac glycosides [16-19].

Quantitative Analysis

Estimation of Total Phenols

Total polyphenol content was measured using the Folin–Ciocalteu method described by Gao *et al* [20]. Plant extracts (100 µl) were mixed with 0.2 ml of Folin–Ciocalteu reagent, 2 ml of H_2O, and 1 ml of 15% Na_2CO_3, and incubated at room temperature for 2 hours. The absorbance was measured in a UV spectrophotometer at 765 nm. Gallic acid served as the standard and results were expressed as Gallic acid equivalents (GAE), mg per 100 g of dry weight.

Estimation of Flavonoid Content

Flavonoid contents were determined by following the method of Zhishen [21]. An aliquot (250µl) of each extract was mixed with 1.25 ml of distilled water and 75 µl of 5% $NaNO_2$ solution. After 6 min, 150 µl of 10% $AlCl_3.H_2O$ solution was added. After 5 min, 0.5 ml of 1 M NaOH solution was added. The absorbance against blank was determined at 510 nm. Rutin was utilized for constructing the standard curve. The results were expressed as mg/g of the extracts (DW).

In vitro Antioxidant Activity

DPPH Radical Scavenging Activity

The 2, 2-diphenylpicryl-1-picryl-hydrazyl (DPPH) radical scavenging activity of entire plant extracts was measured according to the Blois method [22]. IC_{50} values of the extract were calculated.

Metal Chelating Activity

The metal chelating effect on ferrous ions was determined according to the method of Dinis [23]. The different concentration of plant extracts was mixed with 0.05 ml of 2 solution followed by the addition of 0.2 ml of mM ferrozine, which was left to react at room temperature for 10 min and the absorbance of the mixture was measured at 562 nm. The metal chelating activity of the extracts was evaluated and expressed as mg EDTA equivalent/g extracts.

Total Antioxidant Activity by the ABTS+ Assay

ABTS radical cation (ABTS·+) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. Before assay, the solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 30 ºC to give an absorbance at 734 nm of 0.70 ± 0.02 in a 1^{-cm} cuvette [24]. After the addition of 1 ml of diluted ABTS+ solution to 10µl of entire extracts or Trolox standards (Final concentration 0-15 µM) in ethanol, optical density (OD) was taken at 30ºC exactly 30 minutes after the initial mixing. The unit of total antioxidant activity (TAA) is defined as the concentration of Trolox having equivalent antioxidant activity expressed as µmol/g sample extracts on dry matter.

In vitro anti-diabetic Activity

In vitro α-amylase Enzyme Inhibitory Effect

A modified procedure by McCue and Shetty [25] was followed. A total of 250 µl of extract (1.25–10 mg/ml) was placed in a tube and 250 µl of 0.02M sodium phosphate buffer (pH 6.9) containing α -amylase solution (0.5mg/ml) was added. This solution was pre-incubated at 25 °C for 10 min, after which 250 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at various time intervals and then they were further incubated at 25 °C for 10 min. The reaction was terminated by adding 500 µl of dinitrosalicylic acid (DNS) reagent. The tubes were then incubated in boiling water for 5 minutes and cooled to room temperature. The reaction mixture was diluted with 5 ml of distilled water and the absorbance was measured at 540 nm using a spectrophotometer. A control was prepared using the same procedure replacing the extract with distilled water. The α -α-amylase inhibitory activity was calculated as the percentage inhibition:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of Control- Absorbance of sample}}{\text{Absorbance of Control}}$$

In vitro β -glucosidase Enzyme Inhibitory Activity

The β-glucosidase inhibitory activity was determined using the standard method described by Kim *et al* [26]. The enzyme solution was prepared by dissolving 0.5 mg α-glucosidase in 10 ml phosphate buffer (pH 7.0) containing 20 mg of bovine serum albumin. It was diluted further to 1:10 with phosphate buffer just before use. Sample solutions were prepared by dissolving 4 mg of sample extract with 400 µl DMSO. Five concentrations: 50, 100, 150, 200, and 250 µg/ml were prepared and 5 µl each of the sample solutions or DMSO (sample blank) was then added to 250 µl of 20 mM p-nitrophenyl-α-D –glucopyranoside and 495 µl of 100 mM phosphate buffer (pH 7.0). It was pre-incubated at 37 °C for 5 min and the reaction was initiated by the addition of 250 µl of the enzyme solution, after which it was incubated at 37 °C for exactly 15 min. 250 µl of phosphate buffer was added instead of enzyme for blank. The reaction was then stopped by the addition of 1000 µl of 200 mM Na_2 CO_3 solution and the amount of p-nitrophenol released was measured against a sample blank (containing DMSO with no sample) at 400 nm using UV-visible.

RESULTS

Identification and Botanical Description of Plant Material

The collected ferns were identified by using 'The Manual of Pteridophyte Flora of the Western Ghats, South India [27]. The voucher specimens of *Cheilanthes swartzii* Webb & Berthel. were deposited in the Botany Department Herbarium, PSG College of Arts & Science, Coimbatore.

Phytochemical Studies

Qualitative Analysis

In the present study, the preliminary phytochemical analysis of petroleum ether and methanolic extracts of whole plant parts of *C.swartzii* are presented in (Table 1). The preliminary phytochemical screening of both petroleum and methanolic extracts of the plant revealed the presence of Alkaloids, Tannins, Phenols, Flavonoids, Steroids, Terpenoids, and Cardiac glycosides. In petroleum ether extract, the saponins were not observed but, surprisingly it was present in methanol extract.

Quantitative Analysis

Estimation of Total Phenol

The present study revealed that the methanolic extracts of *C. swartzii* presented the highest amount of phenolic content (16.01 ± 3.60) than that of petroleum ether extract (8.79 ± 1.23).

Estimation of Total Flavonoids

The total flavonoid content of *C. swartzii* methanol and petroleum ether extracts is presented in (Table 2). Comparably, the methanolic extract exhibited the highest value (7.20 ± 0.30) of total flavonoid contents followed by petroleum ether extracts (4.31 ± 0.21).

In- vitro Antioxidant Activity

DPPH (1, 1-diphenyl-2-picrylhydrazyl) Radical Scavenging Activity

The results on DPPH radical scavenging activity of methanol and petroleum ether solvent extracts are presented in (Table- 3). The stable DPPH free radicals can be used to evaluate the antioxidative activities in a relatively short time. The absorbance decreased as a result of the color change from purple to yellow as the radical was scavenged by antioxidants through the donation of hydrogen to form the stable DPPH molecule. Therefore, lower IC_{50} indicated a higher antioxidant activity. Methanolic extract of (38.01µg/ml) plant extracts showed higher levels of free radical scavenging activity compared to the petroleum ether extract and standard drug (27.9 µg/ml) Butylated Hydroxy Toluene (BHT). The DPPH radical scavenging activity was found to be the least in petroleum ether extracts (55.33 µg/ml).

Metal Chelating Assay

The results of the experiment conducted to assess the metal chelating activity of *Cheilanthes swartzii*, are presented in (Table 4). The methanolic extract displayed an apparent antioxidant activity as they were able to chelate (31.35 µg/g), ferrous metal ions more efficiently than the petroleum ether extracts (52.97 µg/g).

ABTS+ radical Scavenging Assay

The ability of the test sample to scavenge ABTS+ radical cations was equivalent to Trolox solution, having a total antioxidant ability equivalent to 1g dry weight of the extract under the experimental investigation. The highest ABTS radical scavenging rate was found to be in methanol extract 5491.04 µmol/g, while the lowest total scavenging potential was found in petroleum ether extract (3761.36 µmol/g) (Table-5).

In-vitro Anti-Diabetic Activity

α-amylase and β- glucosidase Enzymes Activity Methanolic Extract of *Cheilanthes swartzii*

The methanolic extract and its concentration of 10, 20, 40, 60, 80, and 100 µg/ml exhibited fractions of α-amylase and β-glucosidase inhibitory activity respectively. It showed α-amylase inhibitory activity with an IC_{50}value of (69.52 µg/ml) and β-glucosidase inhibitory activity with an IC_{50} value of (20.20 µg/ml) (Table 6, 7). Evaluation of plot percentage of α-amylase and β-glucosidase inhibition (Fig. 1 & 2) revealed that methanolic extract of *Cheilanthes swartzii* at 100 µg/ml concentration had the highest ὰ -amylase inhibition of (70.40%) followed by concentrations in addition to β -glucosidase inhibition (52%) respectively when compared with acarbose (43.49 and 54.35 µg/ml).

In-vitro Anti-Diabetic Activity

α-amylase and β- glucosidase Enzymes Activity Methanolic Extract of *Cheilanthes Swartzii*

The methanol extract of *Cheilanthes swartzii* and its concentration of10, 20, 40, 60, 80 and 100 µg/ml exhibited fractions of α-amylase and β-glucosidase inhibitory activity respectively. Acarbose was used as a standard reference drug, which showed α-amylase inhibitory activity with an IC_{50} value of (69.52 µg/ml) and β-glucosidase inhibitory activity with an IC_{50} value of (20.20 µg/ml) (Table -6, 7). Evaluating the plot of percentage α-amylase and β-glucosidase inhibition as a function of extract concentrations (Fig- 1& 2) the methanolic extract of *Cheilanthes swartzii* at 100 µg/mL concentration,

had the highest ά -amylase inhibition of (70.40%) followed by concentrations in addition to than β -glucosidase inhibition (52%) respectively when compared with acarbose (43.49 and 54.35µg/ml).

DISCUSSION

Phytochemical screening of medicinal plants is very important in identifying new sources of therapeutically and industrially important compounds [28]. Different phytochemicals have been found to possess a wide range of activities, for example, Phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids, and alkaloids have anti-inflammatory effects [29]. Rupasinghe et al [30] have reported that saponins possess hypocholesterolemic and antidiabetic properties.

Research on drug discovery especially the phyto drug investigation has become one of the frontier areas in phytochemistry and the integrated research in this arena has been used for many valuable drugs and lead compounds [31]. In the present study, the primary phytochemical screening of *Cheilanthes swartzii* plant extract with methanol revealed the presence of secondary metabolites with many therapeutic properties.

Flavonoids

Flavonoids are natural phenolic compounds. In the present findings, Flavonoids, Phenol, Tannin, and Vitamin E content are present in *C.swartzii* extracts. In various studies, the antioxidant activity of the plant extracts was found to be fairly high which are rich in flavonoids [32]. Some flavonoids were reported to exhibit potential for anti–human immunodeficiency virus functions [33]. Methanol is a good solvent for *C. swartzii* and many phenolic compounds.

Compounds like flavonoids are responsible for the free radical scavenging effect in plant crude extracts [34]. The plant-derived constituents are capable of terminating the free radical reaction and preventing our body from oxidative damage [35]. This finding is very similar to that of the antioxidant effect of *Mellitus officinalis* of DPPH free radicals done by Pourmorad [36].

Antioxidant activity

DPPH stable free radical method is easy, rapid, and sensitive to the antioxidant activity of a specific compound or plant extracts. The highest radical scavenging activity was observed by *Melliotus offcinalis* with IC_{50} = 0.018 mg ml-1, which is higher than that of BHT ($P < 0.05$). In the present investigation, *C.swartzii* extracts showed a higher potency than that of BHT (synthetic standard drugs) in scavenging of DPPH free radicals. The present finding exhibited methanolic extracts in ABTS+ assay as the most efficient in free radical scavenging activity 5491.04 µmol/g and 3761.36 µmol/g petroleum ether extract. This result is similar to the findings of Yuan *et al* [37] on the bioactive compound from *C. swartzii*. This implies that the plant extract may be useful for treating radical-related pathological damage, especially at higher concentrations, and may provide protection against chronic diseases, including cancer and neurodegenerative diseases.

a

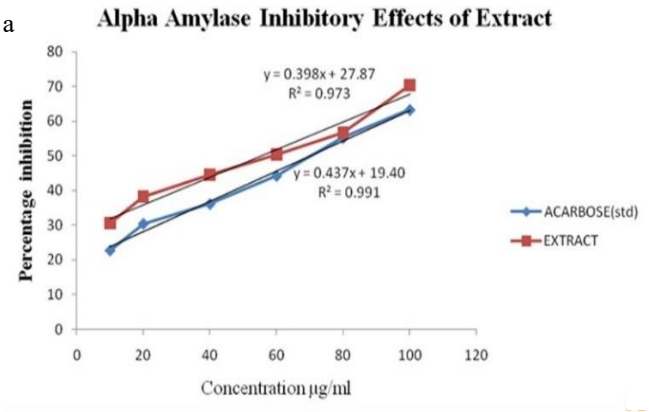

Fig. 1 In-*vitro* Antidiabetic activity of *Cheilanthes swartzii*

b

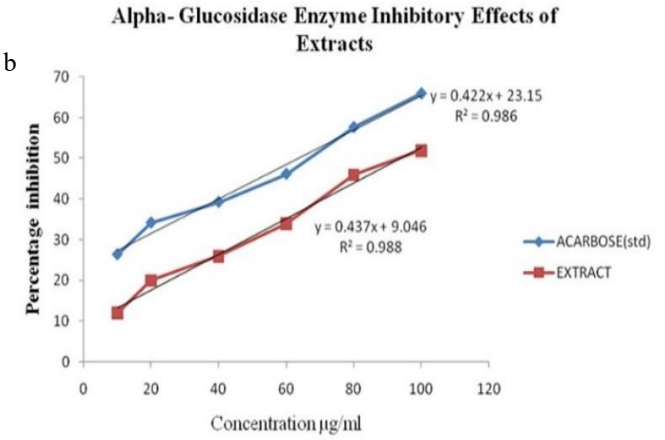

Fig. 2 In-*vitro* Antidiabetic activity of *Cheilanthes swartzii*

Table 1 Preliminary phytochemical constituents of whole plant parts of petroleum ether and methanol extracts of *Cheilanthes swartzii*

Sl. No	Phytochemical constituents	Petroleum ether	Methanol
1	Alkaloids	+	+
2	Tannins	+	+
3	Saponin	-	+
4	Phenol	+	+
5	Flavonoids	+	+
6	Steroids	+	+
7	Terpenoids	+	+
8	Cardiac glycosides	+	+

Present (+); Absent (-)

Table 2 The estimation of total phenol and flavonoid content of whole plants parts of *Cheilanthes swartzii*

S.No.	Solvents	Phenol (mg/g)	Flavonoid.(mg/g)
1.	Petroleum ether	8.79 ± 1.23 b	4.31 ± 0.21 b
2.	Methanol	16.01 ± 3.60 a	7.20 ± 0.30 a

Each value represents the mean ± SD, n=3 mean followed by same letter(s) in each column are not significantly different at p< 0.001 according to Duncan's multiple range tests.

Table 3 The effect of DPPH radical scavenging activity of *Cheilanthes swartzii*

S. No	Sample concentration		% Of inhibition		IC_{50}	
	Petroleum ether	Methanol	Petroleum ether	Methanol	Petroleum ether	Methanol
1	25	10	58.85 ± 0.58 d	34.98 ± 0.88 bc	55.33	38.01
2	50	20	73.11 ± 0.99 b	35.90 ± 0.49 b		
3	75	30	77.11 ± 0.69 a	39.01 ± 0.75 ab		
4	100	40	69.90.98 c	40.23 ± 0.76 a		
5	Butylated Hydroxy Toluene (BHT)				27.9	

Each value represents the mean ± SD, n=3 mean followed by the same letter(s) in each column are not significantly different at p< 0.001 according to Duncan's multiple range tests

Table 4 Metal chelating activity of *Cheilanthes swartzii*

S. No	Sample concentration		% Of inhibition		IC_{50}	
	Petroleum ether	Methanol	Petroleum ether	Methanol	Petroleum ether	Methanol
1	25	10	60.78 ± 1.04 a	57.138 ± 1.11c	52.97	31.35
2	50	20	59.61 ± 0.93 ab	58.180 ± 0.75 bc		
3	75	30	57.65 ± 0.79 b	59.61 ± 0.89 b		
4	100	40	55.96 ± 0.53 c	63.64 ± 0.471 a		
5	EDTA				44.96	

Each value represents the mean ± SD, n=3 mean followed by same letter(s) in each column are not significantly different at p< 0.001 according to Duncan's multiple range tests.

Table 5 Total antioxidant activity by $ABTS^{+}$ of *Cheilanthes swartzii*

S. No	Sample concentration		% Of Total antioxidant activity (TAA) (µmol/g)		IC_{50}	
	Petroleum ether	Methanol	Petroleum ether	Methanol	Petroleum ether	Methanol
1	25	10	5235.17 ± 9.13 a	9719.94 ± 5.79 a	3761.36	5491.04
2	50	20	2795.13 ± 3.13 b	4880.22 ± 6.35 b		
3	75	30	1830.68 ± 5.14 c	3543.72 ± 4.02 c		
4	100	40	1347.86. ± 1.56 cd	2860.24 ± 3.52 d		

Each value represents the mean ± SD, n=3 mean followed by same letter(s) in each column are not significantly different at p< 0.001 according to Duncan's multiple range tests.

Table 6 α- amylase enzymes inhibitory effects of methanolic extracts of whole plant parts of *Cheilanthes swartzii.*

SampleConcentration (mg/ml)	Acarbose (std) % Of Inhibition	*Methanol extract* % Of Inhibition
10	22.72 ± 0.15 a	30.56 ± 0.07 a
20	30.44 ± 1.93 b	38.22 ± 1.87 b
40	36.16 ± 0.05 c	44.56 ± 0.54 c
60	44.28 ± 1.43 c	50.40 ± 0.76 d
80	55.21 ± 1.77 e	56.68 ± 1.18 e
100	63.36 ± 0.54 f	70.40 ± 1.04 f
IC50 Volume µg/ml)	IC50 = 43.89µg/ml	IC50 = 69.52µg/ml

Each value represents the mean ± SD, n=3 mean followed by same letter(s) in each column are not significantly different at p< 0.001 according to Duncan's multiple range tests.

Table 7 β- glucosidase enzymes inhibitory effects of methanolic extracts of whole Plant parts of *Cheilanthes swartzii*

Sampleconcentration (mg/ml)	Acarbose(std) % Of Inhibition	*Methanol extract* % Of Inhibition
10	12.4 ± 0.5 a	26.4 ± 1.11 a
20	20.11 ± 0.65 b	34.2 ± 0.14 b
40	26.24 ± 1.72 c	39.3 ± 0.42 c
60	34.11 ± 0.24 d	46.2 ± 0.56 d
80	46.87 ± 1.02 e	57.7 ± 0.67 e
100	52.41 ± 0.35 f	66.0 ± 1.43 f
IC50 Volume (µg/ml)	IC50 = 20.20 µg/ml	IC50 = 54.35 µg/ml

Each value represents the mean ± SD, n=3 mean followed by same letter(s) in each column are not significantly different at p< 0.001 according to Duncan's multiple range tests.

CONCLUSION

This study provides a pharmacological understanding of the antioxidant and anti-diabetic properties of the less-explored fern, *Cheilanthes swartzii*, marking the first exploration of these attributes. The methanolic extract of the plant when compared to the ethanolic extracts exhibited high anti-oxidant and antidiabetic activities which implies the medicinal properties of the fern. The presence of essential phytochemicals in the plant as per the qualitative analysis reveals the plant's potential to be used in further processes**.**

CONFLICT OF INTERESTS

The authors declare no competing interests.

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