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Chief Editors

Dr.S.Chitra
Dr.S.Devimeenakshi

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DEPARTMENT OF CHEMISTRY & BIO - CHEMISTY

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PRELIMINARY PHYTOCHEMICAL ANALYSIS OF THE FERN *CHEILOSORIAMYSURENSIS*(WALL. EX HOOK.) CHING & SHING AND ITS ANTIOXIDANT ACTIVITY

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Abstract

Cheilosoriamysurensis is a medicinal fern used to treat a variety of ailments, with few researches done on its phytochemical makeup and potential bioactivity. The main aim of the study was to support that traditional herbal medicine with active phytochemicals would be a good substitute for allopathic medicine. The methanol extract was tested for preliminary phytochemical analysis and its antioxidant activity. *Cheilosoriamysurensis* exhibited significant reducing power and total antioxidant activity, and its absorbance increased with an increase in concentration of sample. Overall, the findings suggest that this fern could be useful for medical purposes as a source of phytochemicals.

Keywords: *Cheilosoriamysurensis*; antioxidant; medicinal fern; Reducing power: Total antioxidant assay:

Introduction

focuses on locally available plant species and plant-based products, as well as ancient knowledge, traditional medicine can be found all over the world (AwasandDemissew, 2009). Human society faces numerous obstacles, and coping with health problems is a serious societal concern, mainly in growing international locations with restricted resources (Mangambu, 2013). The development in biology and medicine, many people in growing countries do not have sufficient fitness care (Singh and Singh,2012). The world population, especially the rural population in developing countriesare still largely dependent on herbal treatments (Islam, 2014).

A number of diseases have become increasingly important in recent times, of which infectious diseases caused by bacteria and cancer are two areas that need immediate attention. Plant sources with diverse natural bioactive ingredients play an important role in curing dreaded diseases (Atanasov, *et al*., 2015). Many chemical compounds show impressive in vitro activity against pathogenic microorganisms resistant to recent allergenic drugs. The identification and characterization of these lead molecules in terms of their biological activity on the spectrum, potency, toxicity and safety is most needed.

Pharmacological researchand traditional medicine havebrought many drugs into the international pharmacopoeia. Pteridophytes are immune to many microbial diseases, itis possible that this is one of the reasons for their evolutionary success and the fact that they have been around for over 350 million years. Screening plant extracts for antibacterial, antioxidant, and anti-cancer effects could be beneficial to both humans and plants, given the enormous diversity of Indian medicinal plants and pteridophytes.In recent years, antimicrobial resistance has become a global issue. Because medicinal ferns are biodegradable, harmless and have fewer side effects, it is necessary to screen them for novel bioactive chemicals (Prusti, 2008).

Recently, biological research has concentrated on the medicinal, pharmaceutical, and phytochemical aspects of pteridophytes, which have significant value for medical and industrial uses. Many pteridophyte species are still being studied for possible uses and for the extraction of new active components. Hence, in this work is carried out thepreliminary phytochemical analysis and its antioxidant activity of the fern, *Cheilosoriamysurensis,* found in the Western Ghats, Tamilnadu.

Materials and Methods

Plant material

Cheilosoriamysurensis(wall. ex hook.) ching& K.Hshing were collected from the Gopalaswamy hills, Western Ghats of Tamilnadu, South India.The fern species was identified and authenticated by BSI,Southern circle, Coimbatore,Tamilnadu. A voucher specimenwas reposited as herbarium at the Department of Botany, NallamuthuGounderMahalingam College, Pollachi.

Sample preparation

The methanolic extract of the fern leaves (Dried) were prepared according to the method described by Harbone, 1998. The sample was extracted using a soxhlet apparatus. The collected extract was concentrated under reduced pressure at 40°C. The extractwas dried and stored at 4°C in storage vials for experimental use.

Qualitative phytochemical Analysis

Test for alkaloids

About 2 g of plant materials were crushed then added 1 mL of ammonia. Furthermore, 10 mL of chloroform was added, then crushed and filtered. The filtrate was added 10 mL of sulfuric acid 2N, shaken vigorously, left for a minute until the sulfuric acid solution and chloroform separated. The sulfuric acid layer is taken into a test tubes and tested by Meyer reagents to determine the presence of alkaloids. The addition of Meyer reagent established white precipitate indicate the presence of alkaloids (Miles, 1985).

Test for Terpenoid, Steroid, and Saponin:

The methanol extract wasconcentrated and partitioned with hexane. The soluble extract in hexane was tested with the Liberman-Bourchard reagent. The blue or green color exhibits the presence of steroids and red color for terpenoids. The insoluble residue in hexane is added water and shaken vigorously. The presence of the stable foam for 30 min indicates the existence of saponins, if positive for saponins, the solution was hydrolyzed with HCl and tested with the Liberman-Bourchard reagent. The green or blue color indicates the presence of steroidal saponins and the purple or red color shows the existence of terpenoidsaponins (Lajis,1994).

Test for Flavonoid:

The methanol extract was concentrated and partitioned with hexane. The residue was extracted with 10 mL of 80% ethanol, subsequently added 0.5 mg of magnesium and HCl 0.5 M. The pink or purple color shows the presence of flavonoids (Halimatussakdiah and Amna, 2016)

Test for Phenol:

Methanol extract tested by Ferric Chloride. Add 3 – 4 drops of FeCl3 solution into extract, the formation of bluish black color exhibits the phenol compound (Tiwari *et al*., 2011).

Test for Tannin:

About 0.5 g of methanol extract was boiled in 10 ml of water in the test tube and then filtered. Add a few drops of FeCl3 0.1%. Forming of a brownish green or bluish black color indicates tannins (Ayoola*et al*., 2008)

Test for Glycosides (Keller-Killani Test):

Glacial acetic acid was added into 2 ml. extract and one drop 5% FeCl3 and conc. H2SO4. Reddish brown color appears at the junction of the two liquid layers and the upper layer of bluish green indicates the presence of glycosides (Kokate*et al*., 2001).

Antioxidant activity

Sample preparation

The methanol leaf extract of *Cheilosoriamysurensis* 1.0 mg was dissolved in 1 ml of DMSO. From the above 125, 250, 500 and 1000µg/ml concentration of sample was prepared and used for different assays. Ascorbic acid was used as standard for comparing the activities.

Reducing power assay

The reducing power of methanolic leaf extracts of *C.mysurensis*was determined according to the method previously described by Oyaizu (1986). Different concentrations of methanol extracts of *C.*

*mysurensis*was mixed with phosphate buffer (2.5ml, 0.2M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%) separately. The mixtures were incubated at 50°C for 20 min. Then, 2.5 ml of trichloroacetic acid (10%) was added to the mixtures and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5ml) and $FeCl_3$ (0.5 ml. 0.1%) and the absorbance was measured at 700 nm. Ascorbic acid was used as the standard. Phosphate buffer (pH 6.6) was used as blank.

Total antioxidant capacity by phosphomolybednum assay

The spectrophotometric measurement of Total antioxidant capacity (TAC) is based on the reduction of Mo (VI) to Mo (V) by antioxidant compound and the formation of green phosphate / Mo (v) complex at acidic pH (Prieto *et al,.* 1999). Different concentrations (125- 1000µg/ml) of methanolic leaf extracts were combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution was incubated at 95˚C for 90 min. Then the absorbance of the solution was measured at 695 nm. Methanol (0.3 ml) in the place of extract was used as the blank. Ascorbic acid was used as a reference standard.

Results and Discussion

Phytochemical analysis:

The preliminary phytochemical analysis results of *Cheilosoriamysurensis*methanol extract was recorded (Table-1). *C. mysurensis* contains Alkaloids, steroids, saponins, phenols tannins and coumarins.

Table-1Phytochemical analysis of methanol extract of *Cheilosoriamysurensis*

Secondary Metabolites	Methanol Extract of *Cheilosoriamysurensis*
Alkaloid	+
Terpenoid	-
Steroid	+
Saponin	+
Flavonoid	-
Phenol	+
Tannin	+
Glycosides	+

Pradnya*et al*. (2015) reported phytochemical analysis of four *Cheilanthes* species from northern Western Ghats of India. The methanolic extract of these ferns contains steroids, triterpenoids, reducing sugar, sugars, alkaloids, phenolic compounds, flavonoids, catechins and saponins. In our present investigation methanol extract of *Cheilosoriamysurensis* expressed the positive results for various phytochemical results. Pandey *et al*. (2014) investigated phytochemical analysis of methanolic extract

of *Adiantum* and *Pteris* leaf and stem contains tannins, saponins, flavonoids, and terpenoids steroid terpenoid. Smiliar results were obtained in the present study.

Damaynati*et al*. (2019) reported secondary metabolites like alkaloids, flavonoids, carbohydrates, glycosides, phenol, saponin, steroid, tannins were present in the methanol extract of *Aspleniumindicum, Lepisorusnudum* and *Microsorummembranecium* . In the present study methanol extract of *Cheilosoriamysurensis* expressed the positive results for various phytochemical results.Bharti (2018) reported that methanolic extract of *Ampelopterisprolifera* and *Lygodiumflexosum*shows phytochemical compounds of carbohydrates, protein, aminoacids, alkaloids, glycosides, saponins,flavonoids, phenolics and tannins. In the present study methanol extract of *Cheilosoriamysurensis* expressed the positive results for various phytochemical results.

Herin*et al*. (2013) reported the methanolic extract of *Pterisargyreae, P.confuse, P.vittata , P. biaurita*and *P. multiauriita* shows phytochemical compounds of steroids, alkaloids, saponins , flavonoids, triterpenoids, phenolic compounds and tannins. In present study methanol extract of *Cheilosoriamysurensis* results were directly coincided with the above results.

Antioxidant activity

Reducing power assay

The reducing power of *C. mysurensis* methanol extract was shown in **Table 2.** The reducing power of methanol extract of absorbance (0.10 ± 0.01 to 0.03 ±0.006) increased with concentration of methanol extracts of 12.5 µg/ml to 100 µg/ml. The *C. mysurensis* extract showed potent ferric reducing power at 100 µg/ml concentration. Ascorbic acid had increase in absorbance value indicate increase in Reducing power capacity.

How yeelai *et al*. (2011) evaluated that antioxidant activities of the methanolic extracts of selected ferns in Malaysia. In this FRP method measured the ability of an antioxidant to donate electron to Fe (III) resuting in the reducing of Fe^{3+} / ferricynaide complex to Fe^{2+} complex, which would be monitored at 700 nm. Results we expressed as Gallic acid equivalent (GAE)/100 g leaves. The higher the FRP value, the greater is the reducing power of tested compound, thus greater the antioxidant activity. In the present study, the results indicate that the reducing ability of the extracts increased with the concentration. The result of *C. mysurensis* was significant. The reducing power of *C. mysurensis* extracts in the descending order. The reducing capacity of ascorbic acid was found to be higher than the extracts at each concentration points.

Gupta*et al*. (2014) reported the In vitro antioxidant activity of methanolic extracts of some ferns from Mawsynram of Meghalaya, India. The reducing ability of Aleuritopterisflava extracts was determined with ascorbic acid equivalent. Higher ascorbic acid equivalent value indicates higher reducing capacity

of sample, thus greater antioxidant potential.In the present study, the results indicate that the reducing ability of the extracts of *C. mysurensis* was increased with the ascorbic acid. Pandey *et al*. (2014) reported that antioxidant potential of methanolic extract of Adiantum and Pteris ferns. Analysis of leaf of Adiantum and Pteris ferns has a more 33 reducing power compared to their stem. There is a significant increase in absorbance of the reaction mixture indicates reducing power. In the present study, the result indicate that the reducing ability of the extracts of*C. mysurensis* was increased with the absorbance.

Table 2: Reducing power assay of *C. mysurensis*

Concentration	12.5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml
Methanol extract (Absorbance)	0.03 ± 0.006	0.07 ± 0.03	0.09 ± 0.01	0.10 ± 0.01
Concentration	12.5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml
Ascorbic acid (Absorbance)	0.101	0.109	0.374	0.762

Total antioxidant capacity assay

Increase in absorbance indicates increase in total antioxidant capacity. The methanolic extract of *C.mysurensis* exhibited significant activity and its absorbance increased with increase in concentration and the values were in comparison with ascorbic acid. The methanol extract exhibited significant absorbance of 2.1±0.14 for 100µg/ml. Ascorbic acid had an absorbance 0.74 at 100 µg/ml. Jose *et al*,(2017) studied the antioxidant effects of hexane, ethyl acetate, and methanolic extract of *Pyrrosiaheterophylla*. The assay is based on reduction of phosphate – molybednum (IV) to phosphate molybednum (v) by various concentrations (50,100,200,400 and 800µg/ml). The result of *C. mysurensis* also show smiliar activity.

Salehaet *al*.(2014) reported the total antioxidant activity of fern Diplaziumesculentum. The total antioxidant potentials of DECH and DEM showed higher antioxidant activity compared to standard ascorbic acid. In the present study, methanolic extract of C. mysurensis also show the same result. Selvaraj*et al*. (2015) revealed the antioxidant effects of methanolic extract of *Azollamicrophylla*. From this analysis, it was evident that antioxidant increases with increase in concentration. The result of *C. mysurensis* exhibited significant activity and also antioxidant increases with increase in concentration. The total antioxidant capacity of *C. mysurensis* was shown in Table 3.

Table 3: Total antioxidant capacity by phosphomolybednum of *C. mysurensis*

Concentration	**12.5 µg/ml**	**25 µg/ml**	**50 µg/ml**	**100 µg/ml**
Methanol extract (Absorbance)	0.4 ± 0.04	0.7 ± 0.05	1.2 ± 0.02	2.1 ± 0.14
Concentration	**12.5 µg/ml**	**25 µg/ml**	**50 µg/ml**	**100 µg/ml**
Ascorbic acid (Absorbance)	0.068	0.158	0.313	0.700

Conclusion

The chemical composition, antioxidant, and cytotoxity activities of the methanol extract of *C. mysurensis* from the Western Ghats of South India are reported for the first time. According to the findings of this study methanol extract of the fern contains contain Alkaloids, steroids, saponins, phenols tannins and coumarins. Furthermore, the findings demonstrated that the *C. mysurensis* methanol extract contains powerful antioxidant activities, as well as being a promising source for developing future antioxidant and anticancer medications.

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