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Study of Antioxidant Properties of Genus *Cheilanthes* Sw

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Abstract

The genus Cheilanthes Sw. belonging to family Pteridaceae commonly known as silver fern. It is growing in several habitats such as moist soils, along the edges of the hills or rocky regions, frequently growing in small crevices. Maximum species are used by tribal people (like Bheel, Meena, Garasia and Saharia) to remedy on cold, cough, peptic ulcer, asthma, bronchitis and diabetes. The antioxidant properties of Cheilanthes extracts were tested for their total antioxidant measurements by using DPPH, DMPD radical scavenging assay as well as FRAP assay in vitro. The four ethnomedicinal Cheilanthes species are obtained from different localities of Northern Westerns Ghats of India.

INTRODUCTION

The *Cheilanthes* species whole plant with rhizome powder possesses good antioxidant properties as well as their potential antioxidant capacity against DPPH radical, ferric reducing power and DMPD radical. *Cheilanthes* species whole plant with rhizome is a rich source of phytochemicals including total antioxidant and phenolic compounds and it offers to the development of value-added products. So enhance today's opportunities in nutraceuticals and food applications for Human Health. The four ethnomedicinal *Cheilanthes* species are obtained from different localities of Northern Western Ghats of India. Antioxidant properties have been investigated from number of medicinal plants and are the naturally form of raw extracts, also their chemical ingredients and they are appropriate effective to inhibit the damaging processes affected by oxidative stress. Antioxidants stabilize otherwise deactivate free radicals as well as they frequently attack targets in biological cells (Nunes *et al*., 2012) Essential antioxidative mechanism has an Human body and mostly the biological functions such as the anti-carcinogenic, antimutagenic, and anti-aging reactions initiate from this property (Gocer and Gulcin,2011; Gulcin, 2012; Naima *et al*., 2012). Antioxidants are considerably used in pharmaceutical products, food and cosmetic for the reason they possess multifacetedness in their gathering and amount of activity and provide huge scope in correcting imbalance (Djeridane *et al*., 2006). The antioxidant properties of *Cheilanthes* species whole plant with rhizome powder extracts were tested for their total antioxidants capacity with 3-different methods. Highest total antioxidant activity was recorded by Total Phenolic Content (TPC) in methanol solvents as well as Total Flavonoids Content

(TFC) in ethanol and acetone solvents extract of genus *Cheilanthes* species. The antioxidant properties of *Cheilanthes* extracts were tested for their total antioxidants measurements by using DPPH, DMPD radical scavenging assay as well as FRAP assay in vitro.

MATERIAL AND METHODS

DPPH Radical Scavenging Activity

Potential of Antioxidant activity of the *Cheilanthes* species extracts was evaluated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay (Lee *et al*., 2003) with some modifications. In short, the prepared by stock reagent solution was dissolving 24 mg of DPPH in 100 ml methanol as well as kept stored at −20°C until use. The obtained by working solution was mixing of stock solution 10 ml with 45 ml methanol and using a spectrophotometer obtain an absorbance value of 1.1±0.02 at 517 nm. Allowed to react with 3 ml of DPPH solution with the different concentrations of *Cheilanthes* extracts. The vigorously shaken was mixture simultaneously kept at room temperature for 30 min in the dark. The spectrophotometrically measured mixture was at 517 nm. Extract was also analyzed without added control sample and the grades were produced as radical scavenging activity (% RSA).

$$\% \text{ RSA} = (\text{A control} - \text{A sample}) \times 100 / \text{A control}$$

Here, A = absorbance at 517 nm.

By plotting the percentage (%) of free radical scavenging activity of ascorbic acid against its concentration then arranged a standard curve. The expressed closing results of mg ascorbic acid as equal antioxidant capacity in 1 g of sample (mg AEAC g−1).

FRAP (Ferric Reducing/Antioxidant Power) Assay

This assay was completed by Benzie and Strain as before described (Benzie and Strain, 1996) with certain modifications. For a moment the employed Ferric Reducing/Antioxidant Power reagent produced by mixing 300 mM acetate buffer (pH 3.6) 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) in 40 mM HCL and 20 mM $FeCl3 \cdot 6H2O$ in 10:1:1 ratio earlier to use then heated to 37°C in water bath for 10 min. *Cheilanthes* species extracts were permissible to react with 2.7 ml of the Ferric Reducing/Antioxidant Power reagent of several concentrations (1–5 mg/ml). The reaction mixture was made up to 3 ml of closing volume with distilled water. The kept reaction mixture was left in dark for 30 min. The colored product readings are taken at 593 nm (ferrous tripyridyltriazine complex). The output was expressed as mM Trolox equivalent g−1 Sample of dry weight.

DMPD (N, N-dimethyl-p-phenylendiamine) Assay

This assay is created on the principle of reduction of the purple radical cation DMPD+ (N, N-dimethyl-phenylendiamine) (Schlesier *et al*., 2002). Dissolving 209 mg DMPD in 10 ml distilled water was arranged by A 100 mM DMPD solution. 100 ml 0.1 M acetate buffer (pH 5.25) was added to one ml of this solution. The purple radical actions DMPD+ resulted in adding 0.2 ml of a 0.05 M ferric chloride solution, which was measured at equilibrated to an absorbance of 0.900±0.100 and 505 nm. Up to 12 h The DMPD radical action was stable. 50 µl antioxidant solutions and 1 µl of DMPD+ solution were mixed 10 min at 25°C endlessly. This solution was measuring at 505 nm after mixing of the absorbance (Schlesier *et al*., 2002). The potential of antioxidative was evaluated the four constituents as display for the DPPH assay.

RESULTS AND DISCUSSION

Radical Scavenging Activity- (RSA)

1-diphenyl-2-picrylhydrazyl (DPPH) Radical Assay

The quantification of free radical scavenging activity is used by the DPPH method worldwide, foreign to biological system and in vitro (Lampila *et al*., 2009). Antioxidant activity is unique mechanism to

investigate the scavenging result on proton radicals. In recent study, the total antioxidant capacity investigation was measured as the compounds of cumulative ability existing in the sample to scavenge stable organic free radicals as well as deep violet color, using the DPPH reaction which gave the maximum absorbance within 515– 528 nm range,. The antioxidant occurrence in the sample leads to disappearance of DPPH radical chromo gens, which can be noticed spectrophotometrically at 517 nm. Type of solvents and Light, pH, Oxygen, are methods sensitive to use (Harris and Brannan, 2009). Extracts of CSWPR of all are the radical scavenging effects denoted in Table 1. Entire extracts assessed were able to reduce the stable, purple color DPPH · radical reaching 50% of reduction. It has been observed that flavonoids and phenolic compounds are present in the plants and are responsible for essential antioxidant activity (Zhou and Yu, 2004).The methanol and water Extracts of CSWPR evidenced their capacity as an antioxidant from the above results.

Table 1: Showing DPPH Assay in *Chelianthes* species.

Solvent	*C. farinosa*	*C. anceps*	*C. tenuifolia*	*C. albomarginata*
Water	0.24	0.27	0.22	0.26
Methanol	0.44	0.32	0.42	0.37s
Ethanol	0.22	0.26	0.28	0.24
Acetone	0.24	0.22	0.26	0.24

Values are expressed as mg/ml.

FRAP (Ferric Reducing/Antioxidant Power) Assay

The Ferric Reducing/Antioxidant Power assay is very beneficial for routine analysis and one of the most simple, rapid, inexpensive tests. The direct test of total antioxidant power of a sample is developed for Ferric Reducing/Antioxidant Power assay. The antioxidant activities of CSWPR extracts using FRAP assay are shown in Table 2 and arranged were diverse concentrations (1–5 mg/ml). The CSWPR extracts increased with increasing in concentration (1–5 mg/ml) of ferric reducing power. The CSWPR extract (5 mg/ml) higher ability showed to reduce Fe3+ to Fe2+. The ethanol soluble factor is commonly responsible for reducing potential of the extracts. The essential role in determining the antioxidant properties which played a phenolic phytochemicals exhibited redox properties (Rice-Evans *et al.,* 1995; Rice-Evans and Miller, 1997). Hence the ability of reducing extracts was strongly correlated with the phenolic and flavonoid content.

Table 2: Showing FRAP Assay in *Chelianthes* species.

Solvent	*C. farinosa*	*C. anceps*	*C. tenuifolia*	*C. albomarginata*
Water	0.22	0.24	0.24	0.18
Methanol	0.24	0.22	0.23	0.18
Ethanol	0.26	0.32	0.24	0.22
Acetone	0.18	0.26	0.20	0.24

Values are expressed as mg/ml

DMPD (N, N-dimethyl-p-phenylendiamine) Assay

Several benefits of the DMPD assay have high stability of the end point, cost effective and fewer cumbersome rapid reaction times. DMPD is transformed to stable and cooler DMPD radical cation (DMPD·+, absorption maxima 505 nm) in the occurrence of an oxidant solution (ferric chloride) at acidic pH. The sample capable to transfer a hydrogen atom to DMPD·+ and caused discoloration, which was proportional to their concentration of the present antioxidant compounds (Fogliano *et al*., 1999). Antioxidant activity (% RSA) of the diverse CSWPR extracts the Data in Table 3 showed and all the extracts demonstrated antioxidant activity and clearly indicated that Results in Table 3 and it was quantity dependent methanol > ethanol > water > acetone. The CSWPR extracts of concentrations that cause 50% inhibition (IC50). All extracts of CSWPR exhibited that was experimental lower free radical scavenging activity than the standard ascorbic acid.

Table 3: showing DMPD Assay in *Chelianthes* species.

Solvent	*C. farinosa*	*C. anceps*	*C. tenuifolia*	*C. albomarginata*
Water	0.22	0.28	0.22	0.26
Methanol	0.24	0.32	0.23	0.37
Ethanol	0.26	0.26	0.24	0.24
Acetone	0.18	0.24	0.22	0.24

Values are expressed as mg/ml.

Presence of antioxidant activity in genus *Cheilanthes* extracts prepared in different solvents such as methanol > ethanol > water > acetone indicates that phytochemicals contributing to the antioxidant activity of the genus *Cheilanthes* such as *C. farinosa, C. anceps, C. tenuifolia and C. albomarginata* tested belong to different groups of plant metabolites and respect to their chemical properties. Further research analysis for isolation and identification of phytoconstituents responsible for antioxidant activity is necessary. Such discoveries can contribute to the increasing database of the medicinal plants and may be of significance in varietal enhancement, cosmetics, food preservatives, nutraceuticals, and biopharmaceuticals in a race with the degenerative diseases like cardiovascular diseases, cancer and neurodegenerative diseases. The result signifies that the potentiality of *Cheilanthes* sp. whole plant with rhizome is one of the natural sources of antioxidant compounds as well as their potential antioxidant capacity against DPPH radical, ferric reducing power and DMPD radical. *Cheilanthes* species whole plant with rhizome is a rich source of phytochemicals including total antioxidant and phenolic compounds and it offers to the development of value-added products from *Cheilanthes* species whole plant. So enhance today's opportunities in nutraceuticals and food applications for Human Health.

CONCLUSION

It is observed that the *Cheilanthes* species from whole plant with rhizome powder possess good antioxidant properties as well as their potential antioxidant capacity against DPPH radical, ferric reducing power and DMPD radical. It is a rich source of phytochemicals including total antioxidant and phenolic compounds and it offers to the development of value-added products from *Cheilanthes* species whole plants so enhance today's opportunities in nutraceuticals and food applications for human health.

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