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Research Article

POTENTIAL OF ANTIOXIDANT CAPACITY AND PHENOL CONTENT IN FOUR *CHEILANTHES* SPECIES FROM NORTHERN WESTERN GHATS

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ABSTRACT

Objective: The aim of current work was to assess the total phenolic content (TPC), total flavonoid content (TFC), phenolic compounds and antioxidant properties of different extracts of four *Cheilanthes* sp.: *Cheilanthes farinosa* (Forssk.) Kaulf., *Cheilanthes anceps* Sw., *Cheilanthes tenuifolia* (Burm.f.) Sw., and *Cheilanthes albomarginata* Clarke. which is collected from Northern Western Ghats of India.

Methods: Analysis of the scavenging activity using 1, 1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing power assays and N, N-dimethyl-p-phenylendiamine (DMPD) radical cation decolorization assay.

Results: *Cheilanthes* sp.whole plant with rhizome was analyzed for total flavonoids content (TFC) and phenolic content (TPC). The ethyl alcohol and water are the best solvents for the extracting phenols and flavonoids from the *Cheilanthes* sp.whole plant with rhizome (CSWPR).The antioxidant activities of *Cheilanthes* sp. whole plant with rhizome extracts were a positive correlation between total flavonoids and phenolic content capacity and observed DPPH radical scavenging activity based on these results, the result signifies that the potentiality *Cheilanthes* sp. whole plant with rhizome is one of the natural sources of antioxidant compounds.

Conclusion: We have reported in this study 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. *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 plants so enhance today's opportunities in nutraceuticals and food applications for Human Health.

Keywords: Antioxidant activity; DMPD radical; DPPH radical; Total flavonoids content; Total phenolic content.

INTRODUCTION

Since ancient times fern have been used by mankind as food and medicine[1] Ferns mainly belong to the bioactive components of phenolic, flavonoid, alkaloid and terpenoids families [2]The potent antioxidants have been demonstrated Flavonoids and other phenolic compounds [3] Hence, one of ferns functional properties of that are pertinent to human health and their antioxidant activities[4] The antioxidants supports from the capacity damaging oxidative stress, which is a conclusion of an imbalance between creation of Reactive Oxygen Species (ROS) and the antioxidant defense body. The protective system of antioxidants possesses a Living cells which prevents excessive formation and make able the inactivation of ROS[5] The evidence indicates a role of growing amount of reactive oxygen species (ROS) such as peroxyl radicals , hydroxyl radical , superoxide anion and singlet oxygen in the different degenerative diseases such as cancer, cardiovascular diseases, Alzheimer's disease and Parkinson's disease and pathophysiology of aging (Davies, 2000; Fenkel & Holbrook, 2000) Antioxidants stabilize or deactivate free radicals, They frequently attack targets in biological cells[6] Essential antioxidative mechanism has an Human body and mostly the biological functions such as the anti-carcinogenic, antimutagenic, and anti-aging responses originate from this property.[7,8,9] Antioxidant properties have been investigated from number of medicinal plants and antioxidants are the naturally form of raw extracts and their chemical constituents and they are very affective to prevent the destructive processes caused by oxidative stress. Despite numerous advantages, recently interest in naturally occurring antioxidants has considerably increased for use in food, cosmetic and pharmaceutical products, because they possess multifacetedness in their multitude and magnitude of activity and provide enormous scope in correcting imbalance [10,11]. The purpose of this study was to investigate the antioxidant potentials of *Cheilanthes* sp.whole plant with rhizome. The antioxidant properties of CSWPR extracts were tested for their total antioxidants capacity with 3-different methods.

MATERIALS AND METHODS

Plant Material

Plant materials of four *Cheilanthes* species were obtained from different localities of Northern Western Ghats of India (*Cheilanthes farinosa* (Forssk.) Kaulf. — Molem locality, GPS: 15^0 $22^{l}09^{ll}$ N',74^0 12^{l} 44^{ll} E; *Cheilanthes anceps* Sw.— Mahabaleshwar locality, GPS: 17^0 55^{l} 31^{ll} N,73^0 39^{l} 45^{ll} E; *Cheilanthes tenuifolia*(Burm.f.) Sw. — Gaganbawada locality, GPS: 16^031^{l} 58^{ll}N',73^0 49^{l} 5^{ll} E; *Cheilanthes albomarginata* Clarke.— Amboli locality, GPS: 15^0 57^{l} 42^{ll} N,73^0 59^{l} 48^{ll} E;).Specimens were authentically identified with help of Dr. Manisha kale (Associate Professor Department of Botany, Jaysingpur College Jaysingpur, Maharashtra, India. The Whole Plant with rhizome of *Cheilanthes* sp. was collected from Northern Western Ghats, Maharashtra, India. The *Cheilanthes* species were cleaned and separated into dry powder form. The CSWPR was stored in a freezer (−20°C) until further analysis.[30]

Chemicals

Entire chemicals and solvents were of analytical grade and purchased from Native suppliers., 1, 1-diphenyl-2-picrylhydrazyl

(DPPH), Folin-Ciocalteu reagent, Ascorbic acid, aluminum trichloride ,potassium phosphate (monobasic and dibasic), ferric chloride, sodium carbonate, 2,4,6-tripyridyls- triazine (TPTZ) and trichloroacetic acid were obtained from Sigma Chemical Co., USA. N, N-dimethyl-p-phenylendiamine (DMPD) was buying from Fluka (Schweiz, Switzerland). Methanol (HPLC grade) and Glacial acetic acid (HPLC grade) were obtained from Merck Trolox. (6- Hydroxy 2,5,7,8 tetramethyl chroman 2-carboxylic acid) was acquired from Aldrich Chemical Co, USA.

Extractions

CSWPR Dried, ground plant extracts were obtained by homogenizing. The CSWPR in four different solvents (acetone, methanol, ethanol, and water) keeping CSWPR to solvent ratio of 1:10. Extraction was carried out on an orbital shaker for 24 h at room temperatures. The homogenates were centrifuged at 15,000 rpm at 4°C for 10 min and the supernatants were recovered and stored at −20°C.

Determination of Total Phenolic Content (TPC)

The total phenolic content in the Whole Plant with rhizome of *Cheilanthes* species (CSWPR) extracts was determined spectrophotometrically utilizing the Folin-Ciocalteu method. A portion of a total amount of a solution of 125µl extract mixed with 1.8 ml of Folin Ciocalteu reagent and which is distilled water diluted 10-fold with previously. Before adding 1.2 ml of 15% sodium Carbonate solution in distilled water the solution was allowed to stand at 25°C for 5 min and absorbance was measured at 765 nm using a spectrophotometer. After 90 min at room temperature, this was compared as expressed mg of Gallic acid equivalents per g (mg GAE g−1) of dry powder to standard curve of gallic acid concentrations. [12].

Determination of Total Flavonoids Content (TFC)

The colorimetric method was measured by Total flavonoid contents of all four extracts [13]. *Cheilanthes* species extracts (0.5 ml) were mixed with methanol (1.5 ml), to which 10% aluminum chloride (0.1 ml), 1 M potassium acetate (0.1 ml) and distilled water (2.8 ml) were added. The solution was vortexed, allowed to stand for 30 min at room temperature. The UV–vis spectroscopic analysis using an absorbance of reaction mixture was measured at 415 nm (Hitachi U-2800; Hitachi, Tokyo, Japan). According to the standard curve prepared for rutin and the concentration of flavonoids was reported as mg of rutin equivalents per g (mg RE g−1) of sample was quantified the total flavonoids content.

DPPH Radical Scavenging Activity

Potential of Antioxidant activity of the *Cheilanthes* species extracts was evaluated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay [12] 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 farinosa* 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).

% RSA = (A control − A sample) ×100 = 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

The Ferric Reducing/Antioxidant Power assay was completed by Benzie and Strain as before described [14] 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· 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). 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 cation 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 cation was stable. 50 μl antioxidant solutions and 1 µl of DMPD+ solution were mixed 10 min at 25°C endlessly. This solution was occupied at 505 nm after mixing of the absorbance [15]. The potential of antioxidative was evaluated the four Constituents as display for the DPPH assay.

Statistical Analysis

In this study, Three analyses of every sample were made and all experiment was carried out in triplicate i. e. (n=3).The obtained data were calculated from the mean value and standard deviation. Determined by linear regression analysis of obtained RSA (Microsoft Excel programmer for Windows XP) wherever values signifying the concentration of examined extracts that cause 50% of inhibition (IC_{50}).

RESULTS AND DISCUSSION

Total Phenolic Content (TPC)

The Total Phenolic Content (TPC) amount of determined in different solvent extracts of *Cheilanthes* species is shown in Table 1 and 2. In phenolic compounds extracting results discovered that ethanol was the best solvent for followed by water, methanol, and acetone. Total phenolics (0.44 mg GAE g−1) of *Cheilanthes* species contained amounts. There are time to time variations in the TPC of the different plants and some ferns and mangrove plants Antioxidant Capacity and Phenol Content reported from different authors in Mangrove Infoline Database as well as Fern Ethnomedicinal plant database [16, 27, 28and 29]. Due to the complex nature of these groups of compounds the methods of extraction and analysis [17]. It was shown that the plants phenolic content of are influenced by extrinsic (agronomic, environmental, handling and storage) factors and a number of intrinsic (genus, species, cultivar) [18, 19].

Total Flavonoids Content (TFC)

Flavonoids are naturally present in mostly plants and flavonoid is a group of polyphenolic compounds. They constitute most of the yellow, red and blue colors in the fruit [20]. Currently wide studied as components flavonoids from the plants and fruits that has the capacity to provide multiple benefits of health. Clinical studies and Epidemiological have provided evidence of a capacity role for flavonoids in lowering the risk of cardiovascular diseases, free radical coronary heart disease prevention, osteoporosis, scavenging capacity, and neurodegenerative diseases [21, 22]. The amount of TFC in different solvent extracts of CSWPR is shown in Table 2. The results discovered that extract capacity of flavonoids was also affected by the solvent used. Extracting flavonoid compounds from the water are the best solvent for (0.28 mg of RE g−1). The methanol, ethanol and acetone were not good solvents for extraction of flavonoids from CSWPR Extract. Because of the flavonoids solubility

changed during growing process, the flavonoids widely held from under CSWPR were methanol soluble as well as the flavonoids from over CSWPR were water soluble [23].

Table 1 Total phenolic contents for the studied extracts of four species *Cheilanthes*.

Sr.No	Plant Parts	Solvent	Total phenolics (mg GAE/g)			
			C. farinosa	*C.anceps*	*C. tenuifolia*	C. *albomarginata*
1	Whole Plant with Rhizome	Water	0.24±0.016	0.28±0.014	0.22±0.016	0.26±0.013
2		Methanol	0.44±0.012	0.32±0.018	0.42±0.014	0.38±0.016
3		Ethanol	0.22±0.013	0.26±0.016	0.28±0.012	0.24±0.012
4		Acetone	0.24±0.013	0.22±0.012	0.26±0.013	0.24±0.014

Table 2 Total flavonoids contents for the studied extracts of four species *Cheilanthes*.

Sr.No	Plant Parts	Solvent	Total flavonoids (mg RE/g)			
			C. farinosa	*C.anceps*	*C. tenuifolia*	*C.albomarginata*
1	Whole Plant with Rhizome	Water	0.22±0.013	0.24±0.016	0.22±0.019	0.18±0.019
2		Methanol	0.24±0.017	0.22±0.012	0.23±0.013	0.18±0.019
3		Ethanol	0.26±0.019	0.32±0.013	0.24±0.017	0.22±0.017
4		Acetone	0.18±0.019	0.26±0.013	0.22±0.019	0.24±0.019

Radical Scavenging Activity on 1,

1-diphenyl-2-picrylhydrazyl (DPPH) Radical

The quantification of free radical scavenging activity is used by the DPPH method in worldwide, foreign to biological system and in vitro [22]. Antioxidant activity is unique mechanisms to investigate the scavenging result on proton radicals. In the 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 chromogens, which can be noticed spectrophotometrically at 517 nm. Type of solvents and Light, pH, Oxygen, are methods sensitive to used [23]. Extracts of CSWPR of all are the radical scavenging effects denoted in Fig. 1.

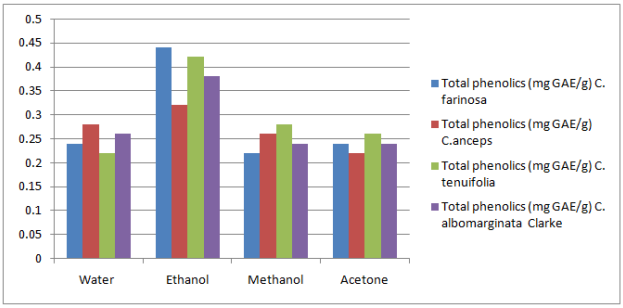

Fig.1 DPPH radical scavenging activity of different CSWPR extra cts, data expressed in mean ± SD, n=3

Entire extracts assessed were able to reduce the stable, purple colored DPPH· radical reaching 50% of reduction. The maximum as well as minimum IC_{50} value for methanolic extract 0.4 mg/ml and 0.7 mg/ml. It has been showed that flavonoids and phenolic compounds are present in the plants and responsible for essentially antioxidant activity [24].The methanolic and water Extracts of CSWPR evidenced their capacity as an antioxidant from the above results.

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 Fig. 2

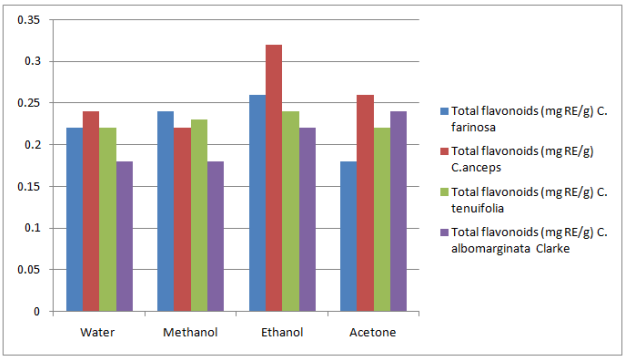

Fig.2 FRAP assay of different CFWP extracts, data expressed in mean ± SD, n=3

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+, 1.7 mM TEAC g−1 for methanolic and 1.4 mM TEAC g−1 for water extract. The methanol soluble factor is commonly responsible for reducing potential of the Extracts. The radical scavenging capacity of plant dry powder decreased with increasing growth status [23]. The essential role in determining the antioxidant properties which played a phenolic Phytochemicals exhibited redox properties [25]. Hence the ability of reducing extracts was strongly correlated with the phenolic and flavonoid content.

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

Several benefits of the **N**, N-dimethyl-p-phenylendiamine i.e.DMPD assay has as high stability of the end point, cost effective and fewer cumbersome rapid reaction times. DMPD is transformed to stable and colored 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 in antioxidant compounds [26].

Antioxidant activity (% RSA) of the diverse CSWPR extracts the Data in Fig. 3 showed and all the extracts demonstrated antioxidant activity and clearly indicated that Results in Fig. 3 and it was quantity dependent methanol > ethanol > water > acetone. The CSWPR extracts of concentrations that cause 50% inhibition (IC_{50}) were as follows: 3.43 mg/ml for methanolic extract, 3.6 mg/ml for ethanolic extract and 3.9 mg/ml for water extract. All extracts of CFWP exhibited that was experimental lower free radical scavenging activity than the standard ascorbic acid.

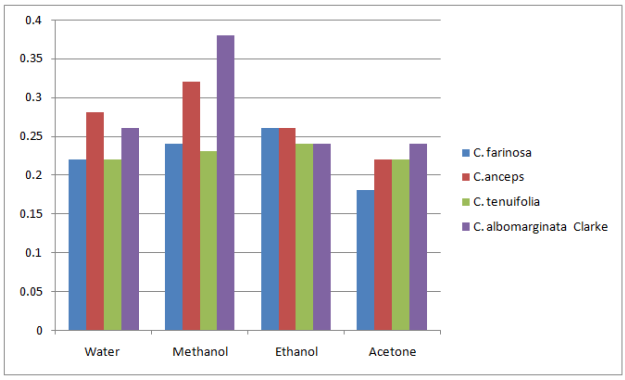

Fig. 3 DMPD radical scavenging activity of different CFWP extracts, data expressed in mean ± SD, n=3

CONCLUSION

In the current study, the *Cheilanthes* species from whole plant with rhizome powder extracts found strong antioxidant activity. The CSWPR extracts might be attributed to their free radical scavenging activity in the antioxidant mechanisms. The flavonoid and phenolic compounds appeared in addition, to be responsible for the antioxidant activity of CSWPR extracts. Here the results presented on the basis of *Cheilanthes* species from whole plant with rhizome powder possess good antioxidant properties. Potential of the total antioxidant capacity of Plants, vegetables and other fruit products and due to the multifaceted nature of Phytochemicals could not be performed accurately by any single method. We have stated in this study, their potential antioxidant capacity against DPPH radical, ferric reducing power and DMPD radical. The *Cheilanthes* species whole plant with rhizome is a rich source of phytochemicals as well as phenolic compounds and deals to the development of value-added products from *Cheilanthes* species whole plants with rhizome so enhance today's opportunities in nutraceuticals and food applications for Human Health.

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