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Assessment of *Adiantum trapeziforme* L. for antioxidant activities

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ABSTRACT

A plant-based diet reduces the risk for development of several chronic diseases. It is a fact that antioxidants contribute to this protection. As dietary plants contain several hundred different antioxidants, it would be useful to know the total concentration of electron donating antioxidants (i.e., reluctants) in individual plants. The present investigation was based on assessment of total antioxidant activity from fronds of Adiantum trapeziforme L. Fronds of Adiantum trapeziforme L. were collected at two different growth stages viz. vegetative and reproductive. The methanolic extracts were prepared and the crude extract was used for its antioxidant potential. Ascorbic acid content, total phenolic compounds, tannins and flavonoids were estimated. The antioxidant activities were determined by free radical scavenging DPPH method, by ferric reducing antioxidant power (FRAP) and hydroxyl mediated DNA damage. Adiantum is one of the important pteridophytes mentioned in Ayurvedic system of medicine. It has a long history of traditional medicinal uses in many countries. The results presented in this investigation confirm the potential use of this plant in pharmaceutics. Thus the systematic analysis presented here will assist research into the nutritional role of the effect of antioxidants in human diet.

KEY WORDS: *Adiantum, ascorbic acid, antioxidant, DPPH, hydroxyl radical DNA damage.*

INTRODUCTION

India has a rich cultural heritage of traditional medicines which chiefly comprised of Ayurvedic system of medicines since ancient times. The crude drugs always available easily in abundance, comparative cheaper with negligible side effects. They have been prescribed for the patients of all age groups [1]. Plants are the rich source of naturally occurring secondary metabolites form vital component in human diet. Many of the compounds belonging to phenolic group are potent antioxidants that suggest a direct correlation between high flavonoids intake and decreased risk of cardiovascular disease, cancer and other age-related diseases.

Stress is a major factor that constitutes every individuals daily life. Excess stress leads to suppression in physical endurance as well as mental capability for logical thinking. It also suppresses immunity leading to pathological conditions and hampers the normal functioning of body [2]. In today's era of fast-track life, normal individuals are subjected to innumerable stressful situations. One of the earliest responses to stress is generation of reactive oxygen species (ROS) in large quantity that can cause oxidative stress [3]. The reactive oxygen species produced in cells include free radicals such as the hydroxyl radical (OH^-) and the superoxide anions (O_2^-) and non-free radicals like H_2O_2 [4].

Various species of *Adiantum* are being used traditionally for varieties of ethno medicinal purposes. *Adiantum* is one of the important medicinal plants mentioned in Ayurveda. Ethnomedicinally, the genus is important and popularly known as "*Hansraj*" or "*Hanspadi*" in Ayurvedic system of medicine. It grows up to the height of 1 meter and very common in Western Ghats. It generally prefers humus rich, moist, well-drained sites ranging from bottomland soils to vertical rock walls. It is the most popular among ferns because of the beauty and delicacy of their foliage.

Hence, detailed study of *Adiantum trapeziforme* L. was planned with reference to important secondary metabolites and their antioxidant capacities become worthwhile both from fundamental and applied angles.interviewed for knowing of their ethnobotanical knowledge.

The collected plant specimens were identified by using standard floras [5-10].

MATERIAL AND METHODS

Chemicals

Liquid reagents such as Ethanol, Methanol, were procured from Qualigens, a division of Glaxo India Ltd. Dr. Annie

Besant Road, Mumbai 400 025. Ferric Chloride, Folin-Ciocalteau reagent and Folin-Dennis reagent, ammonium hydroxide, Potassium acetate, aluminium chloride, Sodium carbonate, were purchased from Qualigen, a division of Glaxo India Ltd. Dr. Annie Besant Road, Mumbai 400 025. DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) was obtained from Hi-media.

Plant material

Fronds of *Adiantum trapeziforme* L. at vegetative and reproductive stage were collected from local area of Pune. It was authenticated from BSI, Western Circle, Pune. The shade-dried material was extracted by boiling for two hours in methanol. The extract was centrifuged at 10,000 x g for 20 minutes and the supernatant was evaporated so as to get crude extract, which was further used as sample for radical scavenging and reducing power assays.

Estimation of total phenols

Total phenols were estimated as per the method given by Farkas and Kiraly [5]. One gram of the dried fronds was homogenized in 10 ml of 80% hot ethanol. The extract was condensed on hot water bath to approximately 1.0 ml and then centrifuged at 10,000 ×g for 10 min. Volume of the supernatant was adjusted to 10 ml with distilled water. From this 0.2 ml aliquot was used for estimation. The blue colour developed in reaction mixture was read at 650 nm on UV-visible spectrophotometer (Shimadzu-1700). Tannic acid was used to prepare the standard curve.

Estimation of Tannins

Tannins were estimated by Folin-Denis reagent given by Polshettiwar *et al.* [6] Dried 0.5 gm powdered sample was boiled with 5 ml distilled water. It was then centrifuged at 5, 000 ×g for 10 minutes. Supernatant was collected and 1 ml of sample was added with 0.5 ml Folin-Denis reagent and 1 ml of sodium carbonate solution. The final volume was adjusted to 10 ml with distilled water. The reaction mixture was incubated for 30 minutes at room temperature and absorbance was read at 775 nm on UV-visible spectrophotometer (Shimadzu-1700). Standard curve was prepared by using tannic acid.

Estimation of Flavonoids

Total flavonoids were estimated by colorimetric assay by aluminium chloride method given by Chang *et al.* [7]. One gram of dried powdered material was extracted with methanol (1:10 w/v). For reaction, 0.5 ml of extract was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride and 0.1 ml of 1M potassium acetate. The final volume of reaction mixture was adjusted to 5 ml with distilled water. It was incubated for 30 minutes at room temperature and the absorbance was read at 415 nm on UV-visible spectrophotometer (Shimadzu-1700). Quercetin was used for the standard curve.

Estimation of ascorbic acid content

Ascorbic acid content was estimated by titrimetric method suggested by Ghosh *et al.* [8]. Five grams fresh fronds were extracted with 4% oxalic acid. The extract was filtered and the filtrate was transferred and the volume was made 100 ml with 4% oxalic acid. Five ml of filtrate was added to 10 ml 4% oxalic acid and titrated against 2, 6-dichlorophenol indophenol (DCPIP). Colourless to faint pink colour was considered as end point. The standard ascorbic acid solution was titrated so as to get standard curve.

Assay of free radical scavenging activity by DPPH

Free radical scavenging activity was determined by DPPH treated with methanolic extracts prepared from plant material. In this method a commercially available and stable free radical DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) was used. It is soluble in methanol and produce violet colour. DPPH has absorption maxima at 515 nm or 517 nm when dissolved in methanol (in its radical form.) It gets disappeared on reduction by an antioxidant compound. An aliquot (100 µl) of the extract was added to 100 µl of freshly prepared DPPH solution and final volume was adjusted to 3.0 ml with methanol. Incubate the reaction mixture for 30 minutes at room temperature; the absorbance of a reaction mixture was measured at 515 nm on UV-visible spectrophotometer (Shimadzu-1700). The percent free radical scavenging activity was calculated according to Motalleb *et al.* [9] and compared with L-Ascorbic acid, which was used as standard antioxidant [10].

$$\textbf{Radical scavenging activity (\%)} = [(A_B - A_A)/ A_B] \times 100$$

Where, A_B = Absorbance of blank, A_A = Absorbance of the test solution.

Total ferric reducing antioxidant reducing power

The ferric reducing power assay (FRAP) was carried out by Oyaizu method [11] with some modifications. A 0.25 ml aliquot of plant extract was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml 1% potassium

ferricyanide. After incubation at 50^{0}C for 20 min; 2.5 ml of 10% TCA was added to the mixture and then it was centrifuged at 2000 g for 10 min. A 5 ml of upper layer was mixed with 5 ml of distilled water and 1 ml of 0.1 % ferric chloride was added. The green colour developed was measured at 700 nm with UV-visible spectrophotometer (Shimadzu-1700). A higher absorbance indicated the higher reducing power.

Determination of hydroxyl radical scavenging assay

Hydroxyl radicals were generated on the basis of Fenton reaction. Hydroxyl radical mediated DNA damage was studied according to Sharma *et al*. [12]. The reaction mixture contains $FeSO_4$, H_2O_2, and DNA. The frond extract of *Adiantum trapeziforme* L. at concentration of 500 µg ml^{-1} were analyzed for protection against hydroxyl radical mediated oxidative damage to DNA. The 1 ml reaction volumes contained 100 µM $FeSO_4$, 1 mM H_2O_2 and 1 mg Calf thymus DNA in phosphate buffered saline. A control DNA was maintained without $FeSO_4$ and H_2O_2 in buffer. The extract of mature fronds at concentration of 500 µg ml^{-1} was added in the reaction mixtures to evaluate DNA protection action of the extract against hydroxyl radicals. After incubation period of 10 minutes, 50 µl of reaction mixtures along with control were loaded on 1% agarose gel and visualized after staining the DNA with ethidium bromide on UV illuminator.

Statistical Analyses

The experiments were conducted in ten replicates and experimental results were mean of ± SD of ten parallel measurements. The data in respect to various non-enzymatic antioxidants, radical scavenging activity and reducing power capacity of *Adiantum trapeziforme* L. were processed statistically using student *t*-test. The values for $p< 0.05$ were regarded as significant.

RESULTS AND DISCUSSION

Stress is a major factor that constitutes every individuals daily life. This leads to suppression in physical endurance as well as mental capability for logical thinking. It also suppresses immunity leading to pathological conditions and cardiovascular diseases by hampering the normal functioning of the body [2]. Our bodies are actually battle grounds for infections and diseases. Normal body functions such as breathing, physical activities and other lifestyle habits such as smocking and all diseases produce substances called "free radicals", which attack healthy cells. When healthy cells are weakened, they are more susceptible to cardiovascular diseases and certain types of cancers. An antioxidant activity of several plant materials has recently been reported [1]. Scientific evidences suggest that antioxidants reduce the risk for chronic diseases including cancer, diabetes and heart disease [13].

The results of present investigation showed that the frond extracts of *A. trapeziforme* L. contain high amount of ascorbic acid i.e. 1520.24 ± 6.61 mg g^{-1} and 1770.80 ± 0.23 mg g^{-1} at vegetative and reproductive stages respectively. Ascorbic acid is supposed to be the best antioxidant. It interacts with glutathione and phenolics thus protect the cell damage from free radicals [14, 15]. Several researchers have reported the strong relationship between phenolic compounds and antioxidant activity. Velioglu *et al.* [16] have shown a relationship between total phenolic content and antioxidant activity of selected fruits, vegetables and grain products.

Table 1 Important non-enzymic antioxidants of *Adiantum trapeziforme* L. at vegetative and reproductive stages

***Adiantum* species**	**Growth stage**	**Ascorbic acid mg g^{-1}**	**Total Polyphenols mg g^{-1}**	**Tannins mg g^{-1}**	**Flavonoids mg g^{-1}**
Adiantum trapeziforme L.	Vegetative	1520.24 ± 6.61	4.30 ± 0.05	2.89 ± 0.06	25.15 ± 0.15
	Reproductive	1770.88 ± 0.23	9.60 ± 0.05	6.45 ± 0.27	31.30 ± 0.23
	P (T ? t) at 5%	1.47×10^{-22}	1.39×10^{-17}	1.55×10^{-14}	8.89×10^{-16}

Apart from phenolic compounds; other secondary metabolites like, tannins and flavonoids have been reported to possess the antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process [17]. The results of present investigation revealed the considerable amounts of tannins (2.89 ± 0.06 mg $g^{-1}$and 6.45 ± 0.27 mg g^{-1} at vegetative and reproductive stages of fronds respectively) and flavonoids (25.15 ± 0.15 mg g^{-1}) at vegetative stage and (31.30 ± 0.23 mg g^{-1}) at reproductive stage of fronds. Many phenolics and flavonoids have been shown to have antioxidant activity. Surinut *et al.* [18] studied many fruit extracts and showed the correlation between phenolic contents, flavonoids and radical scavenging activity.

Table 2 Radical scavenging activity of *Adiantum trapeziforme* L. at vegetative and reproductive stage by DPPH assay

Sr. No	Growth stage	Radical scavenging activity in (%)	(IC_{50}) value in µg ml^{-1}
01	Vegetative stage	50.86 ± 1.14	83.99 ± 0.21
02	Reproductive stage	60.11 ± 1.27	74.34 ± 0.09

The reducing power of the crude methanolic extracts of fronds was examined (Table 3 and Fig. 1). In this assay, the yellow colour of the reaction mixture changed to various shades of green depending upon the capacity of extract to reduce Fe^{+++} to Fe^{++} Reducing power is similar to antioxidant activity. A higher absorbance at 700 nm indicated the higher reducing power. In present investigation (Table 3), fronds harvested at reproductive stage (1.220 ± 0.024) exhibited significant higher reducing power than that of vegetative stage (1.032 ± 0.024). In this study, our findings showed a good linear correlation between reducing power and the total phenolic content in frond extracts. These results indicated that the reducing power of frond extracts might be related to the concentration of hydroxyl hydrogen. Phenolics could easily donate their hydroxyl hydrogen due to resonance stabilization [19].

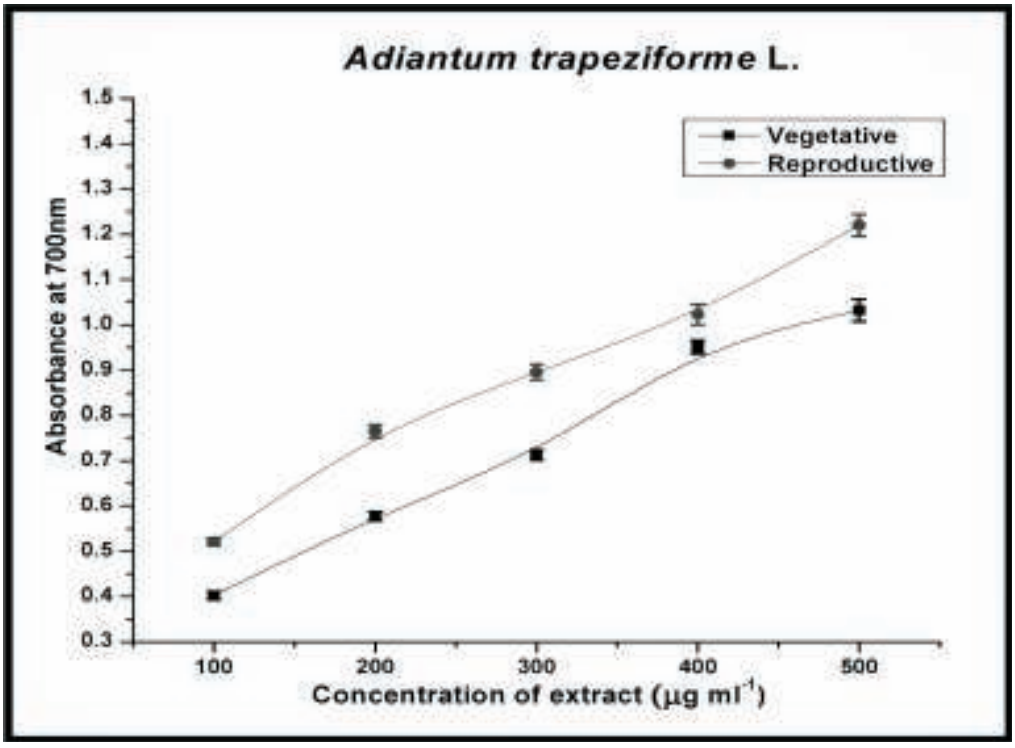

Fig. 1: Reducing power assay of frond extracts of *Adiantum trapeziforme* L. at vegetative and reproductive stage.

Table 3 Reducing power assay of frond extracts of *Adiantum trapeziforme* L. at vegetative and reproductive stage

Name of the plant	Concentration (µg ml^{-1})	Vegetative stage (OD at 700 nm)	Reproductive stage (OD at 700 nm)
***A. trapeziforme* L.**	100	0.402± 0.009	0.521±0.009
	200	0.578± 0.010	0.765±0.015
	300	0.713± 0.011	0.896±0.017
	400	0.951± 0.015	1.023±0.023
	500	1.032± 0.024	1.220±0.024

Hydroxyl radical mediated DNA damage

Oxidative DNA damage has been implicated to be involved in various degenerative diseases including Alzheimer's disease, Parkinson's disease, Hodgkin's disease and Bloom's disease [20]. In view to make ascertain hydroxyl radical scavenging activity of frond extract, Fenton reaction generated hydroxyl radical damaging effect to calf thymus DNA was investigated. It is evident from the comparison of DNA-electrophoresis, that DNA with hydroxyl radicals showed degradation however, frond extract protects DNA from oxidative damage by scavenging free hydroxyl radicals (Fig. 2). Thus, the results of the electrophoretic studies represent a substantial ability of frond extract of *A. trapeziforme* L. against hydroxyl radical damage to DNA. The results are in accordance with Sharma *et al.* [12]. They showed the effect of *Euphorbia hirta* extract on hydroxyl radical mediated DNA damage.

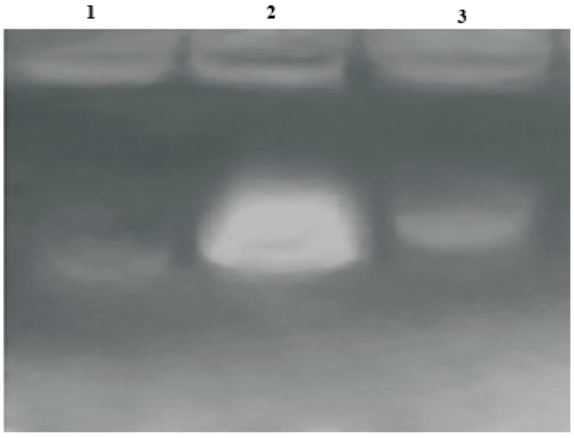

Fig. 2 Protection effect of frond extract of *Adiantum trapeziforme* L. against hydroxyl radical mediated DNA damage.

Lane 1: DNA + Hydrogen peroxide
Lane 2: DNA
Lane 3: DNA + Hydrogen peroxide + *Adiantum trapeziforme* L. frond extract

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