WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES

SJIF Impact Factor 6.647

Volume 6, Issue 8, 1034-1042 Research Article ISSN 2278 – 4357

ANTIOXIDANT POTENTIAL OF *ADIANTUM AETHIOPICUM* L.

***Abhijit S. Limaye and Shankar L. Laware**

*PG Department of Botany, Nowrosjee Wadia College, Pune, S. P. Pune University, M. S. India.

Principal, ACS College, Sonai, Ahmednagar, S. P. Pune University, M. S. India.

Article Received on
23 May 2017,

Revised on 13 June 2017,
Accepted on 04 July 2017,

DOI: 10.20959/wjpps20178-9677

***Corresponding Author**
Dr. Abhijit S. Limaye
PG Department of Botany, Nowrosjee Wadia College, Pune, S. P. Pune University, M. S. India.

ABSTRACT

Naturally occurring secondary metabolites produced in plants form an integral component of the 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. Fronds of *Adiantum aethiopicum* L. were collected at two different growth stages *viz.* vegetative and reproductive, from local area of Pune. The methanolic extracts were prepared and the crude extract was used for its antioxidant potential. The methanolic extracts showed considerable amounts of total phenol, tannins, flavonoids and ascorbic acid content. The reproductive fronds exhibited significantly higher values than vegetative fronds for aforesaid metabolites. The methanolic crude extracts possessed free radical scavenging activity ($IC_{50:}$ 53.34 ± 0.09 µg / ml) as revealed by DPPH assay. The ferric reducing antioxidant power (FRAP) was carried out for the plant sample showed significant results at 500 µg / ml concentration. Hydroxyl radical scavenging activity was performed using calf thymus DNA and the results proved that frond extract protect DNA from oxidative damage. *Adiantum* is one of the important pteridophytes mentioned in Ayurvedic system of medicine. The literature has provided many uses of *Adiantum aethiopicum* L. as a traditional medicinal fern. But, this is first *in vitro* report showing *A. aethiopicum* L. as potent source of natural antioxidants. Hence, the present investigation suggests *A. aethiopicum* L. as promising plant source in food, health and cosmetic industries.

KEYWORDS: *Adiantum*, Antioxidant, DPPH, hydroxyl radical oxidative damage.

INTRODUCTION

Many phytochemicals are mentioned as radical scavengers to overcome the problem of stress. Normally free radicals of different form are generated in low level in the cells to help modulation of several physiological functions and are reduced by integrated antioxidants system in the body. However, produced in large amount can lead to cancer and other degenerative diseases.[1] The most common form of free radicals is reactive oxygen species (ROS), which try to steal electrons from other molecules, causing damage to even DNA and other molecules. Once formed, free radicals can start a chain reaction leading to formation of more free radicals. 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. [2] To combat these oxidative stresses plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, ascorbic acid, polyphenols, flavonoids and vitamins as well as enzymes. [3] Many plant extracts and phytochemicals especially polyphenols and vitamins have been shown to have antioxidant or free radical scavenging activity. [4]

There are many ferns known to have medicinal properties. *Adiantum* is one of the important among them and mentioned in Ayurveda. As very scanty literature is available with reference to secondary metabolites produced by *Adiantum aethiopicum* L. Hence the attempt was made to estimate non-enzymatic metabolites with antioxidant potential.

MATERIALS AND METHODS

Chemicals: Liquid reagents such as Ethanol, Methanol, and Ammonium hydroxide, aluminium chloride ferric Chloride, Folin-Ciocalteau reagent and Folin-Dennis reagent, Potassium acetate, Sodium carbonate, were purchased from Qualigen whereas, DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) was obtained from Hi-media.

Plant material: Fronds of *Adiantum aethiopicum* L. at vegetative and reproductive stage were collected from the local area of Pune and was authenticated from BSI, Western Circle, Pune. The material was shade-dried and extracted by boiling for two hours in methanol. The extract was centrifuged at 10,000 xg for 20 minutes and the supernatant was evaporated so as to get crude extract, which was further used as sample for various analyses explained below:

Estimation of total phenols

Total phenols were assessed as per the method given by Farkas and Kiraly. [5] The dried fronds were cut into small pieces. One gram tissue was extracted with 10 ml of 80% hot ethanol. The extract was condensed on hot water bath and volume of the supernatant was adjusted to 10 ml with distilled water. From this 0.2 ml aliquot was used for estimation. Tannic acid at the concentration of 100 µg ml $^{-1}$ was used to prepare the standard curve.

Estimation of Tannins

Total amount of tannins were estimated by the method given by Polshettiwar *et al.* [6] using Folin-Denis reagent. The dried 0.5 gm powdered material was boiled with five ml distilled water. One 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). Tannic acid at the concentration of 100 µg ml^{-1} was used to prepare a standard curve.

Estimation of Flavonoids

Colorimetric assay was used to estimate total flavonoids as per the method given by Chang *et al.* [7] One gram powder 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 made as five ml with distilled water. Quercetin solution at the concentration of 12.5 mg ml^{-1} was used for 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 volume of filtrate was made 100 ml with 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 (100 µg ml^{-1}) was titrated with DCPIP dye in the same way so as to get standard curve.

Assay of free radical scavenging activity by DPPH

Determination of the scavenging effect on DPPH was carried out 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. An aliquot (100 µl) of the extract was added to 100 µl of freshly prepared DPPH solution (39.5 mg ml^{-100} of methanol) and final volume was adjusted to 3.0 ml with methanol. After 30 minutes of incubation 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]

Radical scavenging activity (%) = $[(A_B – A_A)/ A_B] \times 100$

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

Ferric reducing antioxidant potential (FRAP)

The ferric reducing antioxidant potential assay was carried out as per the method given by Oyaizu [11] with little 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 was added to it. The reaction mixture was incubated at 50^0C 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 absorbance 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 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 extracts of mature fronds at concentration of 500 mg ml^{-1} were 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 characters, radical

scavenging activity and reducing power capacity of said species of *Adiantum* were processed statistically using student *t*-test. The values for $p < 0.05$ were regarded as significant.

RESULTS AND DISCUSSION

Naturally occurring plant phenolics include several groups of compounds that have health protecting properties. These are not the metabolic by-products but generally they are involved in the growth and metabolism. [13] They may act as antioxidants, reducing the risk of coronary heart diseases as well as they may protect against some forms of cancer. [14] The phenols also increase in response to abiotic stresses. These compounds are also famous for their chelating properties. [15] Interactions between ascorbic acid and glutathione and ascorbic acid and phenolics are well known. [16] Thus phenolics are not a single factor, which is responsible for radical scavenging activity.

Table 1: Important non-enzymic antioxidants of *Adiantum aethiopicum* 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 $^{-1}$**
A. aethiopicum L.	Vegetative	375.00 ± 2.06	2.16 ± 0.05	1.46 ± 0.11	47.00 ± 0.29
	Reproductive	437.00 ± 1.72	3.53 ± 0.04	2.47 ± 0.04	54.30 ± 0.29
	P (T <= t) at 5%	1.78×10^{-12}	3.88×10^{-13}	1.11×10^{-10}	4.05×10^{-12}

It has been recognized that flavonoid show 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, 18] The results of the present investigation showed that the extract of *A. aethiopicum* L. which contain highest amount of flavonoid (54.30 ± 0.29 mg g^{-1}) but moderate amount of phenolic compounds, exhibited the maximum radical scavenging activity.

Radical scavenging activity by DPPH

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

Sr. No	**Growth stage**	**(IC_{50}) value in µg ml $^{-1}$**
01	Vegetative stage	63.99 ± 0.21
02	Reproductive stage	53.34 ± 0.09

This investigation was based on the measurement of the relative inhibitory activity of extract tested at different concentrations. The radical scavenging activity of all samples on the DPPH radical was strongly dependent on the extract concentration. Some authors found a correlation between the phenolic content and antioxidant activity while other found no such relationship.[19] Pawar *et al.*[20] solated total phenolics from *Caesalpinia pulcherrima* wood and studied the antioxidant and cytotoxic activity in *in-vitro* models. They observed that wood contain high amount of total phenolics, tannins and flavonoids. They have studied the antioxidant activity of methanolic extract and observed that methanolic extract scavenge DPPH, Nitric oxide and superoxide radicals. They have reported that antioxidant activity might be due to total phenolics and tannins present in the extract.

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

Name of the plant	Conc. in µg ml^{-1}	Vegetative stage (OD at 700 nm)	Reproductive stage (OD at 700 nm)
***A. aethiopicum* L.**	100	0.662 ± 0.012	0.965±0.016
	200	0.886± 0.018	1.256±0.025
	300	1.107± 0.021	1.478±0.027
	400	1.425± 0.026	1.985±0.031
	500	1.760± 0.029	2.205±0.034

Reducing power assay

Total antioxidant activity of *Adiantum aethiopicum* L. was also determined using ferric reducing antioxidant power (FRAP). The data expressed in tanninc acid and Fe (II) equivalent per gram. Chang *et al.* [7] reported that reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Various secondary metabolites play key role because of their ability to reduce Fe^{3+} to Fe^{2+} transformation. Reducing power of tannins prevents liver injury by inhibiting the formation of lipid peroxidase. [21] The strong correlation between the content of total phenolics and reducing power was shown by Amarowicz *et al.* [22] in selected plants from Canadian prairies. In the present investigation *A. aethiopicum* L. supports the conclusion drawn from DPPH radical scavenging assay.

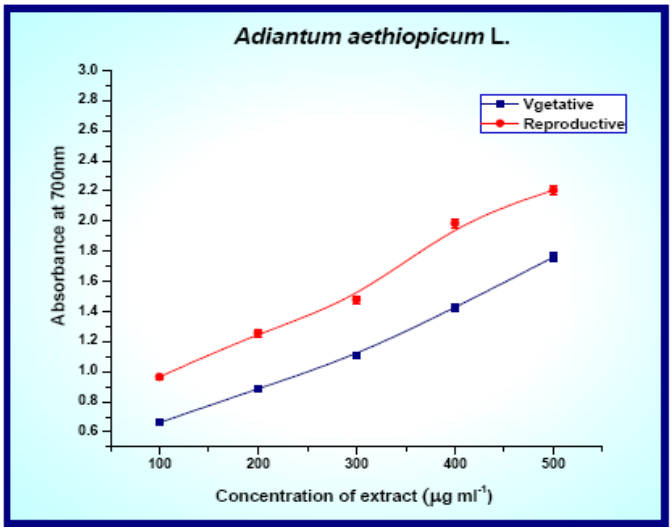
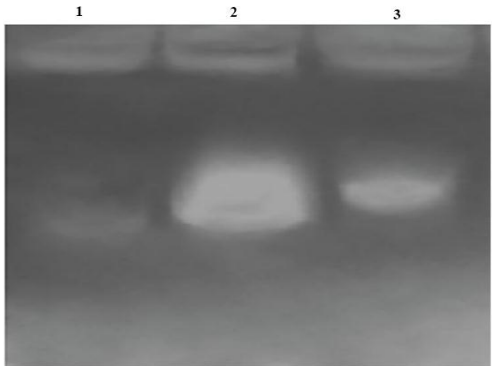


Fig. 1: Reducing power assay of frond extracts of *Adiantum aethiopicum* L. at vegetative and reproductive stage.

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 (Halliwell and Gutteridge, 1989). 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. The results found on agarose gel represent a good ability of frond extract of *A. aethiopicum* L. against hydroxyl radical damage to DNA.



Lane 1: DNA + Hydrogen peroxide

Lane 2: DNA

Lane 3: DNA + Hydrogen peroxide + *Adiantum aethiopicum* L. frond extract

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

ACKNOWLEDGEMENT

We are grateful to Principal, Dr. Ravindrasinh Pardeshi, Fergusson College and Dr. S. S. Gadekar, Head, Department of Botany, Nowrosjee Wadia College, Pune, for providing all essential library and laboratory facilities required for this research.

REFERENCES

1. Halliwell, B. (1998). Free radicals, antioxidants and human diseases: curiosity, cause and consequence, Lancet. 344: 721-724.
2. Melov, S. (2002). Animal models of oxidative stress, ageing and therapeutic antioxidants interventions. Int J Biochem Cell Biol. 34: 1395-1400.
3. Ganapaty, S.; Chandrashekhar, V.M.; Chimate, H.R. and Lakashmi Narsu M. (2007). Free radical scavenging activity of gossypin and nadensin: an in vitro evaluation. Indian J Pharmacol 39(6): 281-283.
4. Larson, R.A. (1988). The antioxidants of higher plants. Phytochemistry. 27: 969-978.
5. Farkas, G. L. and Kiraly, Z. (1962). Role of phenolic compounds in the physiology of plant disease and disease resistance. Phytopathol 44: 105-150.
6. Polshettiwar, S. A.; Ganjiwale, R. O.; Wadher, S. J. and Yeole, P. G. (2007). Spectrophotometric estimation of total tannins in some Ayurvedic eye drops. Indian journal of pharmaceutical sc., 69(4): 574-576.
7. Chang, C., Yang, M., Wen, H., Chern, J. (2002). Estimation of total flavonoid content in Propilis by two complementary colorimetric methods. J. Food Drug Analysis. 10: 178-182.
8. Ghosh, A. K.; Bhargava, S. N. and Tandon, R. N. (1966). Studies on fungal diseases of some tropical fruits. VI. Post infection change in ascorbic acid contents of mango and papaya. Indian Phytopath. 19: 262-266.
9. Motalleb, G.; P. Hanachi; S. H. Kua; O. Fauziah and R. Asmah (2005). Evaluation of phenolic content and total antioxidant activity in Berberis vulgaris fruit extract. J. Biol. Sci., 5: 648-653.
10. Aquino, R. Morelli, S., Laura, M. R., Abdo, S., Saija, A., Tomaino, A. (2001). Phenolic constituents and antioxidant activity of an extract of Anthurium versicular leaves. J. Natl Prod. 64: 1019-1023.
11. Oyaizu, M. (1986). Studies on products of browning reaction-antioxidative activities of products of browning reaction prepared from glucosamine. Japnese Journal of Nutrition. 44: 307-315.

12. Sharma, N. K., Dey, S. and Prasad, R. (2007). InVitro Antioxidant potential evaluation of Euphorbia hirta L. Phamacologyonline. 1: 91-98.
13. Harborne, J. B. (1988). Plant phenolics. In: Encyclopedia of plant physiology, New series, rol.8 (secondary plant products) (eds.) E-A. Bell and B. R. Charlwood, Springer Verlag, Berlin pp. 329-402.
14. Emmons, C. L. and D. M. Peterson (2001). Antioxidant activity and phenolic content of oats as affected by cultivar and location. Crop Sci., 41: 1676–1681.
15. Clemetson, C. A. B. and Anderson, L. (1966). Plant polyphenols as antioxidants for ascorbic acid. Ann. N. Y. Acad. Sci., 136: 339-378.
16. Blokhina, O., Virolainen, E. and Fagerstedt, K. V. (2003). Antioxidants, Oxidative damage, and oxygen deprivation stress: A Review. Ann. Bot. 91: 179-194.
17. Kessler, M.; Ubeaud G. and Jung, L. (2003). Anti- and pro-oxidant activity of rutin and quercetin derivatives. J. Pharm and Pharmacol 55: 131- 142.
18. Cook, N.C. and Samman, S. (1996). Flavonoids- chemistry, metabolism, cardio-protective effects, and dietary sources. Nutritional Biochemistry 7: 66- 76.
19. Sawadogo, W. R., Meda, A., Lamien, C. E., Kiendrebeogo, M., Guissou, I. P. and Nacoulma, O. G. (2006). Phenolic content and antioxidant activity of six acanthaceae from Burkina. Faso. J. Biol Sci., 6: 249-252.
20. Pawar, C. R., Mutha, R. E.., Landge, A. D., Jadhav, R. B. and Surana, S. J. (2009). Antioxidant and cytotoxic activities of Caesalpinia pulcherrima Wood. Indian Journal of Biochemistry and Biophysics. 46: 198-200.
21. Okuda, T., Kimura, Y., Yoshida, T., Hatano,T., Okuda, H. and Arichi, S. (1983). Studies on the activity and related compounds from medicinal plants and drugs. I. Inhibitory effects on lipid peroxidation on mitochondria and microsomes of liver. Chem. Pharm. Bull. 31: 1625-1631.
22. Amarowicz, R., Pegg, R. B., Rahimi-Moghaddam, P., Barl, B. and Well J. A. (2004b). Free radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chemistry. 84: 551-562.
23. Halliwell, B. and Gtteridge, J. M. C. (1989). Free radical aging and diseases. In: Free radicals in biology and Medicine. 2nd edn, Oxford, Clarendon Press. pp 416-417.