ISSN: 0976-4607
Research Article

International Journal of Innovative Pharmaceutical Research

Journal homepage: www.ijipr.com

Polyphenols, Vitamin-E Estimation and *In Vitro* Antioxidant Activity of *Adiantum Capillus-Veneris*

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ABSTRACT

In the present study to evaluate the polyphenols, vitamin E and *in vitro* antioxidant activity of methanol and petroleum ether extract of whole plant parts of *Adiantum capillus-veneris.* Total phenolic content (TPC) was measured using the Folin-Ciocalteu method and Vitamin E estimation dry weight. *In vitro* antioxidant actives were determined by the DPPH (1, 1-diphenylpicryl- 1-picryl-hydrazyl) radical scavenging, (ABTS) 2, 2'azinobis (3-ethyl-benzothiozoline-6-sulfonic acid) and Metal chelating assays. The Results were showed that the methanolic extracts had higher antioxidant potential and polyphenol contents than the petroleum ether extracts. The total phenol content in methanol extracts was determination as 19.05 ± 4.60 mg/g. Total flavonoid contents were measured between 7.90 ± 0.90 mg g^{-1} and tannin content were 23.57±7.75 mg/g tannic acid equivalent. In conclusion, the whole plant extract of *Adiantum capillus-veneris* is capable for free radical scavenging molecules and it can be used as a potential source of natural antioxidants and nutrients.

Key words: *Adiantum capillus-veneris*, antioxidant, polyphenol, vitamin E, methanol extract.

INTRODUCTION

Adiantum capillus-veneris (Family: Adiantaceae) is one of the most common species with potential importance for medicinal and nutritive purpose. Adiantaceae generally occur in the mountainous region of throughout India; in plains they grow on rocks, inhabiting in shady places near swamps and on slopes of lower hills (Chandra, 2000). In traditional herbal medicinal system, *Adiantum capillus-veneris* is used as expectorant, diuretic, febrifuge, hair tonic, chest diseases, catarrhal infection and anti cancer (Puri and Arora, 1961; Singh, *et al.*, 1989; Jain *et al.*, 1992; Kumar *et al.*, 2003). Free radicals can be scavenged through utilizing natural antioxidant compounds present in medicinal plants. Some pteridophytic medicinal plants have been showed that the chemopreventive and therapeutic effects on human diseases (Sabu and Kuttan, 2002).

In folklore medicine, the Pteridophytes which constitutes ferns and ferns allies, have been known to man for more than 2000 years, and also been mentioned in ancient literature (Chopra, *et al*., 1958; Kumar and Roy, 1972; Watt, 1972; Dixit and Bhatt, 1975). In India it is profusely rich in the history of medicinal plants and its 75% folk population is still using herbal preparations in the form of powder, extracts and decoction because these are easily available in nature and the natives have stronger faith on traditional knowledge (Dixit, 1974). Vitamin E is an important component of antioxidant assay. It is considered as a master of antioxidant because: (Papas, 1999; Papas, 1998) it inhibit the bad cholesterol (LDL) - which is believed to be the first step in atherosclerosis. The main objective of the study is to investigate the determination of polyphenols, vitamin E and antioxidant activity of entire plant of *Adiantum capillus- veneris* in methanolic extract and petroleum ether extract.

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MATERIAL AND METHODS

Plant material

The whole plant of *Adiantum capillus-veneris* were collected from field at foothills of Valparai hills Western Ghats of Coimbatore district, of southern India. The samples of plants were identified self and binomially by Botanical Survey of India (Southern part Coimbatore, Tamilnadu, India) and voucher specimens were deposited at the Herbarium Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamilnadu, India.

Extraction of plant material

Healthy fresh plant of *Adiantum capillus-veneris* was collected from Valparai region of Western Ghats of Coimbatore district. 50g of fine powder was packed with No.1 Whatman filter paper and placed in soxhlet apparatus along with solvent, petroleum ether followed by methanol. The residues were collected and dried at room temperature, 30˚C after that the yield was weighed and then performed to activity.

Estimation of total polyphenols

Total phenol

Total polyphenols content was measured using Folin–Ciocalteu spectrophotometric method described previously by Gao *et al.* (2000). Plant extracts (100 µl) were mixed with 0.2 ml of Folin–Ciocalteu reagent and 2 ml of H_2O and incubated at room temperature for 3 min. Following the addition of 1 ml of 20% sodium carbonate to the mixture, total polyphenols were determined after 1 h of incubation at room temperature. The absorbance of the samples was measured in UV spectrophotometer at 765 nm against reagent as blank. Quantification was done with respect to the standard curve of gallic acid. The results were expressed as gallic acid equivalents (GAE), mg per 100 g of dry weight. All determinations were performed in triplicate (*n=3*).

Total flavonoids contents

Flavonoids contents were determined according to the method of Zhishen *et al*., (1999). An aliquot (250µl) of each extract or standard solution was mixed with 1.25 ml of distilled water and 75 µl of 5% $NaNO_2$ solution. After 6 min, 150 µl of 10% $AlCl_3.H_2O$ solution were added. After 5 min, 0.5 ml of 1 M NaOH solution were added and then the total volume was made up to 2.5 ml with distilled water. Following thorough mixing of the solution, the absorbance against blank was determined at 510 nm. Gallic acid was utilized for constructing the standard curve. The results were expressed as mg gallic acid equivalents (GAE)/g extracts (DW).

Condensed tannin contents

Condensed tannins were determined according to the method of Julkunen-Titto (1985). An aliquot (50 µl) of each extract or standard solution was mixed with 1.5 ml of 4% vanillin (prepared with MeOH) and then 750 µl of concentrated HCl were added. The well-mixed solution was incubated at ambient temperature in the dark for 20 min. The absorbance against blank was read at 500 nm. The results were expressed as mg tannic acid equivalents (TA)/g extracts (DW).

Determination of vitamin E content

Dried plant material (0.5 g) was immersed in 20 ml of ethanol for 30 min in a water bath at 85˚C. The solution was allowed to cool and then filtered into a separating funnel. Heptane (10 ml) was added, and the solution was shaken for 5 min. Then, 20 ml of 1.25% sodium sulfate was added and the solution was shaken again for 2 min, and allowed to separate into layers. Total tocopherols were determined by a reaction with cupric ions and complexation with 2, 2'-biquinoline (cuproine) according to Contreras-Guzma´n and Strong (1982). A volume of 0.5 ml of a-tocopherols in ethanol was processed in the same way as a sample, and used as a standard.

***In vitro* antioxidant activity**

DPPH· radical scavenging activity

The 2, 2-diphenylpicryl- 1-picryl-hydrazyl (DPPH) radical scavenging activity of entire plant extracts were measured according to the method (Blios, 1958). IC50 values of the extract i.e., concentration of extract necessary to decrease the initial concentration of DPPH by 50% was calculated.

Total antioxidant activity by the ABTS·+ assay

The 2, 2'azinobis (3-ethyl-benzothiozoline-6-sulfonic acid) disodium salt (ABTS) was dissolved in water to a 7Mm concentration. ABTS radical cation (ABTS·+) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. Prior to assay, the solution was diluted in ethanol (about 1:89 v/v) and equilibrated 30ºC to give an absorbance at 734 nm of 0.70±0.02 in a 1-cm cuvette (Re, *et al.,* 1999). The concentration of the extracts that produced between 20-80% inhibitions of the blank absorbance was determined and adapted. After the addition of 1 mL of diluted ABTS·+ solution to 10µL of entire extracts or Trolox standards (Final concentration 0-15 µM) in ethanol, optical density (OD) was taken at 30ºC exactly 30 minutes after the initial mixing. The unit of total antioxidant activity (TAA) is defined as the concentration of Trolox having equivalent antioxidant activity expressed as µmol/g sample extracts on dry matter.

Metal chelating activity

The metal chelating effect on ferrous ion was determined according to the method of Dinis *et al* (1994). The different concentration of plant extracts were mixed with 0.05 ml of 2 mM. Solution of $FeCl_2$ and this followed by the addition of 0.2ml of mM ferrozine, which was left to react at room temperature for 10 min and the absorbance of the mixture was react at 562 nm. The metal chelating activity of the extracts was evaluated using (Ethylene Diamine Tetra acetic acid) as standard. The results were expressed as mg EDTA equivalent/g extracts.

Statistical analysis

All analyses were carried out in triplicate and the data were reported as means's. Where there were significance of the difference between means was determined by Duncan's multiple range test ($p<0.05$) using statistical.

RESULTS AND DISCUSSION

Phenol compound

Phenol compound is ubiquitous bioactive compounds and a diverse group of secondary metabolites

universally present in higher plants (Liu *et al.*, 2009). The phenolic compounds may contribute directly to ant oxidative action. The methanolic extracts showed that the highest phenolic content 19.05 mg/g and petroleum ether was least at 9.79 mg/g. The methanol is good solvent for *Adiantum capillus-veneris* as large amount of phenolics compounds. The Phenolic compounds are known as powerful chain breaking antioxidants (Shahidi *et al.*, 1992).

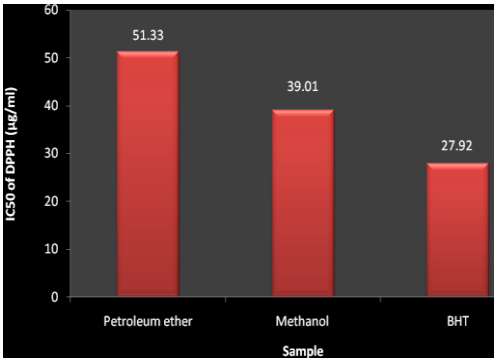
Table.1 Estimation of total polyphenols and Vitamin E content of whole plant parts extract of *Adiantum capillus-veneris*

Sample extract	Total flavonoid (mg/g)	Phenol	Condensed tannin (mg/g) TAE	Vitamin E (mg/g)
Petroleum ether	5.44 ± .21	9.79 ± 1.19	4.97 ±2.64	-
Methanol	7.90 ± 0.90	19.05 ± 4.60	23.57±7.75	0.1870

Table.2 *In-vitro* antioxidant activity of entire plant parts of *Adiantum capillus- veneris*

Sample extract	Metal chelating ability (EDTA/mg equivalent)	TAA (µmol/g)
Petroleum ether	42.97	5251.04
Methanol	29.35	2852.36

Figure.1 Showing DPPH radical scavenging ability of *Adiantum capillus-veneris*



Flavonoids

The results showed that *Adiantum capillus-veneris* whole plant extracts contain methanol and petroleum ether extracts respectively. The methanolic extract has showed highest 7.90 mg/g than petroleum ether 5.44 mg/g (Table- 2). In various studies, antioxidant activities of the plant extracts were found to be fairly high which are rich in flavonoids (Cakir, 2003). Some flavonoids were reported to exhibit potential for anti–human immunodeficiency virus functions (Yao *et al.*, 2004).

Condensed tannin

The total content of condensed tannin was showed that the methanol extract contained 23.57 mg/g TAE and followed by petroleum ether extract showed 4.47 mg/g TAE.

Recovered Vitamin E content

Vitamin E functions primarily as an antioxidant in biological systems by trapping peroxyl free radicals (Combs, 1992; IOM, 2000). Vitamin E is synthesized only by plants therefore, it is found primarily in plant products, the richest sources being vegetable oils and to a lesser extent, seeds, nuts and cereal grains. The vitamins E has highest biopotency and nutritional importance α-a-tocopherol, were widely distributed in foods and is commonly found in leaves and other green parts of higher plants (Combs, 1992). In vitamin E deficiency, the oxidation of PUFA is more readily propagated along the cell membrane, leading to cell damage and symptoms, mainly neurological (Combs, 1992). Vitamin E and phenolic compounds are responsible for the antioxidant activity of some edible plants of Thailand (Chanwitheesuk, *et al.*, 2005).

DPPH radical scavenging activity

The results on DPPH• radical scavenging activity of Methanol and petroleum ether solvent extracts along with the reference standards Butylated hydroxyl anisole (BHA) are shown in (fig. 1). The model of stable DPPH free radicals can be used to evaluate the ant oxidative activities in a relatively short time. The absorbance decreases as a result of a color change from purple to yellow as radical is scavenged by antioxidants through the donation of hydrogen to form the stable DPPH molecule. Concentration of the sample necessary to decrease initial concentration of DPPH• by 50% (IC_{50}) under the experimental condition was determined. Therefore, the lower value of IC_{50} indicates a higher antioxidant activity. MeOH extract of (39.01µg/mL) plant extracts showed higher levels of free radical scavenging activity compared to the petroleum ether extract. The DPPH• radical scavenging activity was found to be the least in petroleum ether extracts (51.33µg/mL). DPPH stable free radical method is an easy, rapid and sensitive way to analyze the antioxidant activity of a specific compound or plant extracts (Koleva *et al.*, 2002). The compound such as flavonoids are responsible for the free radical scavenging effect in the plant crude extracts (Dass and perelra, 1990; Younes, 1981). The plant derived phytoconstituents are capable to to terminate free radical reaction and prevent our body from oxidative damage (Saikat *et al*., 2010) and the protection against chronic disease, neurodegenerative and cardio vascular disease (Prior *et al*., 2005). A potent scavenger of free radicals may serve as a possible preventative intervention for the diseases (Gyamfi *et al*., 1999).

$ABTS^+$ radical scavenging assay

The ability of the test sample to scavenging $ABTS^+$ radical cations was equivalent of Trolox solution, having total antioxidant ability (TAA) equivalent to 1g dry weight of the extract under the experimental investigation. The highest ABTS radical scavenging rate was found to be methanol extract 5251.04 µmol/g while, the lowest total scavenging potential was found for petroleum ether extract 2853.36 µmol/g. The scavenging activity of ABTS radical by the plant extract was found to be greater antioxidant potential.

Metal chelating assay

Metal chelating activity of whole plant parts of *Adiantum capillus-veneris* is shown Table-2. The methanolic extract was observed in maximum inhibition of 42.97 mg followed by 29.35 mg in petroleum ether. Metal chelating ability of plant crude extracts provides a strategy to avoid free radical and ion overload by chelating metal ions (Robert, *et al.,* 1985). It has been recognized that the flavonoids showed antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process (Kessler *et al.,* 2003, Cook and Samman, 1996). Phenolic compounds are a class of antioxidant agents which act as free radical terminators (Shahidi and Wanasundara, 1992).

CONCLUSION

Our present investigation showed that the whole plant crude extracts of *Adiantum capillus-veneris*, which contain rich in polyphenols, tannins and vitamin E compounds. These compounds could be responsible for antioxidant potentiality, may help in the treatment of various diseases like cardiovascular, cancer and reproduction failure, liver necrosis of vitamin E deficiency treatments.

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