Antioxidant and Radical Scavenging Activity of *Actiniopteris radiata* (Sw.) Link.

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**Abstract**: Medicinal plants are recognized as sources of natural antioxidants that can protect biological system from oxidative stress. In the present, study, the antioxidative potential of different solvent extracts (n-hexane, chloroform and ethyl acetate, ethanol and aqueous) of *Actiniopteris radiata* (Sw.) link were evaluated using different *in vitro* methods. Among all the solvent extracts, ethanol extract of *A. radiata*, showed potent activity and the inhibitory concentrations of extract at 50% (IC₅₀) were 742.11, 670.80, 505.902, 372.43 and 425.23 µg/mL for DPPH, superoxide and hydroxyl radical scavenging activities, metal chelating and anti-lipid peroxidation activities, respectively and were nearly similar to that of the standard antioxidant tested. Moreover, ethanol extract of *A. radiata* showed strong reducing power, which denotes the antioxidant capacity of the extract. The results indicated that the ethanolic extract of *A. radiata* is a good source of natural antioxidants.

**Key words**: *In vitro* antioxidant activity, Medicinal herb, *Actiniopteris radiata*.

**Introduction**

Antioxidants are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals or their actions (Cadenas and Packer, 1996). Free radicals have been implicated in the etiology of several major human ailments, including cancer, cardiovascular diseases, neural disorders, diabetes and arthritis (Sies, 1996; Yoshikawa *et al*., 2000; Devasagayam *et al*., 2004). Phytomedicines, as an alternative to synthetic drugs, have played an important role at the level of basic, public health care in various countries, especially in Asia. During the past several years, a broad range of various natural products from plants have been characterized as useful as pharmaceuticals or nutraceuticals, and some of them have drastically increased in market availability and public usage worldwide, e.g., *Echinacea* spp. and *Ginkgo biloba* plant extracts. However, the potential of higher plants as sources for new drugs is still largely unexplored. Hence, studies involving the use of plants as therapeutic agents should be emphasized, especially those related to the control of free radicals, which are generated during oxidative stress. A great number of aromatic, spicy, medicinal and other plants contain chemical compounds exhibiting antioxidant properties. A number of reports suggest that phenolics (flavonoids, phenolic acids, stilbenes, lignans, lignin, tannins etc.) are the major antioxidant constituents of plant materials (Sanchez Moreno *et al*., 1999). The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, metal chelating agents, hydrogen donors and singlet oxygen quenchers (Kumar *et al*., 2005).

*Actiniopteris radiata* (Sw.) Link. belonging to *Actiniopteridaceae* family is an herb with great medicinal value. According to Ayurvedic texts *Mayurashikhaa* (*A. radiata*) is used as astringent, anti-inflammatory, tonic to genitourinary tract, alleviates vitilaged blood, indicated in cough, bronchitis, asthma, diarrhoea, dysentery, dysuria, used internally as well as externally for infected wounds, ulcers, erysipelas (Khare, 2004). The leaves and stem of the herb has been reported to possess rutin, hentriacontane, hentriacontinol, ß-sitosterol palmitate, as well as antibiotic property (Taneja and Tiwari, 1974). The fronds juice of this herb was also reported for its anti-diabetic effect. *A. radiata* has been reported to possess anti-helminthic and styptic property. A polyherbal patent formulation, traditionally inherited from the families of Gonupalli village (Nellore District, Andhra Pradesh, India) for decades, contains 20 ingredients of rare plant species, and *A. radiata* is one of the principal ingredients in the formulation. It has been found to be more active at the stage that the herb contains spores (Gokhale *et al*., 1993). Anti-bacterial activity of *A. radiata* also has been reported earlier from our lab (Manjunath *et al*., 2008). But, no scientific data is available on the antioxidant activity of *A. radiata* till date. Therefore, we hypothesized that the beneficial effect of this plant might be due to its antioxidant properties. Hence the present study is focused to evaluate the antioxidant

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potential of the A. radiata by employing various in vitro models.

Materials and Methods

Chemicals

L-Ascorbic acid, quercetin, gallic acid, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma Chemical Co. (St Louis, MO, USA). Potassium ferricyanide, ethylene-diamine-tetra acetic acid (EDTA), nitro blue tetrazolium (NBT), phenazine methosulphate (PMS), nicotinamide adenine dinucleotide (NADH), thiobarbituric acid (TBA), trichloroacetic acid (TCA), 2-deoxy ribose, ammonium thiocyanate and ferric chloride were purchased from Hi-media Chemical Co. Mumbai, India. All other chemicals and reagents used were of analytical grade.

Plant material and extraction

A. radiata were collected from in & around Tirumala Hills, Andhra Pradesh, India and authenticated through Department of Botany, Sri Venkateswara University, Tirupati. One hundred gram of the air dried and powdered A. radiata whole herb were extracted with hexane first. The remaining material was dried and extracted with chloroform; in a similar way extraction with ethyl acetate and then with ethanol was performed for 10 hr using soxhlet apparatus (Suffness and Douros, 1979). The solvents were completely evaporated at 40ºC using a rotary vacuum evaporator. The residues were designated as hexane extract, chloroform extract, ethylacetate extract and ethanol extract (HEAR, ChEAR, EaEAR and EEAR respectively). The remaining material after solvent extraction was suspended in 1 L distilled water and boiled for 1h at 90-95 oC. The supernatant removed and the extraction was repeated once again. The supernatants thus obtained were combined and filtered through Whatmann No.1 filter paper. The filtrate was concentrated at low temperature and finally the concentrate was lyophilized. The residue was designated as aqueous extract (AEAR). The samples were stored at 4ºC and used for studies.

Assay of Lipid peroxidation

Lipid peroxidation (LPO) was induced by Fe 2+-ascorbate system in human red blood cells (RBC) and estimated as thiobarbituric acid reacting substances (TBARS) by Buege and Aust method (Buege and Aust, 1978). The reaction mixture contained RBC packed cell (10⁶ cells/mL) washed in Tris-HCl buffer (20mM, pH 7.0) with CuCl₂ (2mM), ascorbic acid (10mM) and different concentrations of hexane, chloroform, ethylacetate, ethanol and aqueous extracts of A. radiata (200-1000 µg/mL) at final volume (1mL). The reaction mixture was incubated at 37°C for 1 hr. Lipid peroxidation was measured as malondialdehyde (MDA) equivalent using trichloroacetic acid (TCA), thiobarbituric acid (TBA) and HCl (TBA-TCA reagent: 0.375% w/v TBA, 15%w/v TCA and 0.25N HCl) (Halliwell et al.,1987). The incubated reaction mixture was mixed with 2 mL of TBA-TCA reagent and heated in a boiling water bath for 15 min. After cooling the flocculants precipitate was removed by centrifugation at 1000g for 10 min. Finally malondialdehyde concentration in the supernatant fraction was determined spectrophotometrically at 535nm. Ascorbic acid was used as standard.

Determination of DPPH radical scavenging activity

The free radical scavenging activity of the plant extracts and ascorbic acid was measured by Blois method (Blois, 1958). 0.1 mM solution of the stable radical DPPH in ethanol was prepared and 1 mL of this solution was added to 3 mL of plant extract solutions in water at different concentrations (200-1000 µg/mL). Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

\[
\text{DPPH Scavenged(%) = } \left( \frac{A_{\text{cont}} - A_{\text{test}}}{A_{\text{cont}}} \right) \times 100
\]

Where \(A_{\text{cont}}\) is the absorbance of the control reaction and \(A_{\text{test}}\) is the absorbance in the presence of the sample of the extracts.

Determination of superoxide anion radical scavenging activity

Measurement of superoxide anion scavenging activity of the hexane, chloroform, ethylacetate, ethanol and aqueous extracts of A. radiata was based on the method described by Liu et al. (1997) with slight modification. Superoxide radicals are generated in PMS-NADH systems by oxidation of NADH and assayed by the reduction of nitro blue tetrazolium (NBT). In this experiment, the superoxide radicals were generated in 3.0 mL of Tris-HCl buffer (16mM, pH 8.0) containing 1 mL of NBT (50 iM) solution, 1 mL NADH (78iM) solution and a sample solution of plant extracts (200-1000 µg/mL) in water. The reaction started by adding 1 mL of phenazine methosulphate (PMS) solution (10 iM) to the mixture. The reaction mixture was incubated at...
were measured by the method of Ohkawa et al. (1979). Different concentrations of hexane, chloroform, ethylacetate, ethanol and aqueous extracts of A. radiata (200-1000 µg/mL) were added to the reaction mixture containing 2.8 mmol L–1 deoxyribose, 100 µmol L–1 FeCl₃, 104 µmol L–1 EDTA, 100 µmol L–1 ascorbic acid, 1 mmol L–1 H₂O₂ and 230 mmol L–1 phosphate buffer (pH 7.4), making a final volume of 1.0 mL. 1 mL of thiobarbituric acid TBA (1%) and 1.0 mL trichloroacetic acid (TCA 2.8%) were added to the mixture. The mixture was incubated at 50°C for 20 min. 2.5 mL of 10% trichloroacetic acid was added to the mixture, which was then centrifuged for 10 min at 1000g. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and FeCl₃ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer. Ascorbic acid was used as a standard. Higher absorbance of the reaction mixture indicated greater reducing power.

**Qualitative analysis of secondary metabolites:**

Secondary metabolites are identified in the hexane, chloroform, ethylacetate, ethanol and aqueous extracts of A. radiata by various methods. 500mg of each extract was dissolved in 100 mL of the respective solvent and filtered through Whatman filter paper No.1. Thus, the filtrates obtained were used as test solutions for the screening. Alkaloids were identified by Iodine test, Dragendorff’s test and Wagner’s test, Flavonoids by pew’s test, Shinda test, and NaOH test, Glycosides by Dragendroff’s test and Wagner’s test, Flavonoids by Ellagic acid test and Phenols test, Saponins by Foam test, Sterols by Liberman-Buchard test and Salkowski test, Tannins by Gelatin test (Gibbs, 1974; Dey and Harborne, 1989; Evans, 1989; Harborne, 1998).

**Statistical analysis**

Experimental results were mean ± S.E. of three measurements. The data obtained was analyzed statistically using one way ANOVA followed by Dunnet’s test using the SPSS statistical software. The values p < 0.05 were regarded as significant.

**Results**

**Inhibition of lipid peroxidation**

At 200 µg/ mL concentration of hexane, chloroform, ethylacetate, ethanol and aqueous extracts of A. radiata and ascorbic acid inhibited the lipid peroxidation by 34.66, 26.86, 31.53, 38.53, 28.9 and 40.24 % respectively. The anti-lipid peroxidation activity Fe²⁺ complex formation was calculated using the same formula employed in calculating DPPH radical scavenging activity.

**Determination of reducing power**

The reducing power of hexane, chloroform, ethylacetate and ethanol extracts of A. radiata was determined according to the method of Oyaizu (1986). Different concentrations of hexane, chloroform, ethylacetate, ethanol and aqueous extracts of A. radiata (200-1000µg/mL) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [K₃Fe(CN)₆] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. 2.5 mL of 10% trichloroacetic acid was added to the mixture, which was then centrifuged for 10 min at 1000g. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and FeCl₃ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer. Ascorbic acid was used as a standard. Higher absorbance of the reaction mixture indicated greater reducing power.
Fig. 1: Anti-lipid peroxidation activity of hexane, chloroform, ethyl acetate, ethanol and aqueous extracts of *A. radiata*.

Fig. 2: DPPH radical scavenging activities of hexane, chloroform, ethyl acetate, ethanol and aqueous extracts of *A. radiata*.

Values are expressed as mean ± S.E. Mean values at all concentrations of all extracts are compared with standard by Dunnet’s test at 0.01 level. HE = Hexane extract; ChE = Chloroform extract; EaE = Ethyl acetate extract; EE = Ethanol extract; AE = Aqueous extract; AR = Actiniopteris radiata.

of hexane, chloroform, ethylacetate, ethanol and aqueous extracts of *A. radiata* and ascorbic acid are in the following decreasing order of L-ascorbic acid > EEAR > HEAR > EaEAR > AEAR > ChEAR respectively (Fig.1). When compared to all solvent extracts of *A. radiata*, ethanol extract of *A. radiata* showed highest anti-lipid peroxidation activity and the results are statistically significant (p<0.05).

**Inhibition of DPPH radical**

The radical scavenging effect of hexane, chloroform, ethylacetate, ethanol and aqueous extracts of *A. radiata* and ascorbic acid on the DPPH radical is in the decreasing order of L-ascorbic acid > EEAR > HEAR > EaEAR > AEAR > ChEAR and the percentage of inhibitions were found to be 73.2, 62.63, 58.3, 55.1, 51.3 and 48.66 % (Fig. 2), respectively at the concentration of 1 mg/ mL.
The superoxide radical scavenging effects of the hexane, chloroform, ethylacetate, ethanol and aqueous extracts of *A. radiata* and ascorbic acid are in the following order: Ascorbic acid (71.266%) > EEAR (68.33%) > HEAR (59.95%) > EaEAR (53.82%) > AEAR (51.55%) > ChEAR (49.35%) at the dose of 1 mg/mL (Fig. 3). The results obtained were found to be statistically significant (p<0.01).

**Fig. 4**: Hydroxyl radical scavenging of hexane, chloroform, ethyl acetate, ethanol and aqueous extracts of *A. radiata*.

Values are expressed as mean ± S.E. Mean values at all concentrations of all extracts are compared with standard by Dunnet's test at 0.01 level. HE = Hexane extract; ChE = Chloroform extract; EaE = Ethyl acetate extract; EE = Ethanol extract; AE = Aqueous extract; AR = Actiniopteris radiata.

**Inhibition of superoxide anion radical**

The superoxide radical scavenging effects of the hexane, chloroform, ethylacetate, ethanol and aqueous extracts of *A. radiata* and ascorbic acid are in the following order: Ascorbic acid (71.266%) > EEAR (68.33%) > HEAR (59.95%) > EaEAR (53.82%) > AEAR (51.55%) > ChEAR (49.35%) at the dose of 1 mg/mL (Fig. 3). The results obtained were found to be statistically significant (p<0.01).
Inhibition of hydroxyl radical

All solvent extracts of \textit{A. radiata} was capable of protecting 2-deoxy-D-ribose from oxidative degradation by scavenging hydroxyl radicals and did so in a concentration-dependent fashion (Fig. 4). The most effective hydroxyl radical scavenger was ascorbic acid followed by EEAR, HEAR, EaEAR, AEAR and ChEAR. The results obtained were found to be statistically significant (p< 0.01).

Metal Chelating Activity

The percent of metal chelating capacity at 1000 µg/mL concentration of EEAR and EDTA were found to be 77.79% and 79.23% respectively. The data analysis (Fig. 5) reveals that ethanol extract of \textit{A. radiata} exhibits a marked capacity for iron binding when compared to all other solvent extracts of \textit{A. radiata}, suggesting that its action as peroxidation protector may be related to its iron binding capacity.

Reducing Power

The reducing powers of hexane, chloroform, ethyl acetate, ethanol and aqueous extracts of \textit{A. radiata} are in the following order of L-ascorbic acid > EEAR > HEAR > EaEAR > AEAR > ChEAR (Fig. 6). In the present study, EEAR showed higher reducing power when compared to the other solvent extracts.

Qualitative analysis of secondary metabolites

Qualitative analysis of secondary metabolites of hexane, chloroform, ethylacetate, ethanol and aqueous extracts of \textit{A. radiata} are represented in Table 1. Ethanol extract of \textit{A. radiata} revealed the presence of phenols, flavonoids, glycosides, tannins, and saponins.

Discussion

There are numerous antioxidant methods for evaluation of antioxidant activity. Of these, anti-lipid peroxidation activity, DPPH scavenging assay, active oxygen species scavenging assays, metal chelating activity and reducing power are most commonly used methods to determine the antioxidant activities of extracts.

In the present study, all solvent extracts exhibited different extents of anti-lipid peroxidation and DPPH radical scavenging activities. DPPH radical is considered to be a model of lipophilic radical and is usually used as a substrate to evaluate antioxidative activity of antioxidants (Oyaizu, 1986). Among all solvent extracts of \textit{A. radiata}, EEAR showed highest anti-lipid peroxidation and DPPH radical scavenging activities and this may be due to the greater ability of EEAR to scavenge the free radicals generated during lipid peroxidation by acting as a potent hydrogen donor.

Superoxide radical is known to be a very harmful species to cellular components as a precursor of more reactive oxygen species (Halliwell and Gutteridge, 1985). Photochemical reduction of flavins generates $O_2^-$, which reduces NBT, resulting in the formation of formazan.
(Beauchamp and Fridovich, 1971). The hexane, chloroform, ethylacetate ethanol and aqueous extracts of *A. radiata* were found to be efficient scavengers of superoxide radical generated in PMS–NADH–NBT system in vitro and their activities are incomparable to that of ascorbic acid. From this experiment using different solvent extracts of *A. radiata*; it is noted that the inhibition of the formation of formazan and also the percentage inhibition are directly proportional to the concentration of the plant extracts.

The hydroxyl radicals fragment the 2-deoxy-D-ribose substrate into 2-thiobarbituric acid reactive substances (TBARS), a sample which is able to inhibit the formation of TBARS in this assay may be described as hydroxyl radical scavenger, capable of protecting carbohydrates from oxidative degradation (Dastmalchi *et al*., 2007). The result clearly indicates that the tested extracts have a noticeable effect on scavenging superoxide and hydroxyl radicals and it is evident that EEAR has highest capacity to scavenge superoxide and hydroxyl radicals when compared to all other solvent extracts of *A. radiata*.

Metal chelating capacity is claimed as one of the antioxidant mechanisms, since it reduces the concentration of the catalyzing transition metal in lipid peroxidation. (Diplock, 1997). The results of this assay revealed that all extracts of *A. radiata* and standard metal chelating compound (EDTA) interfered with the formation of ferrous and ferrozine complexes, suggesting that they have chelating activity and capture ferrous ion before ferrozine.

Reducing power assay is often used to evaluate the ability of natural antioxidant to donate electron (Dorman *et al*., 2003). Many reports have revealed that there is a direct correlation between antioxidant activities and reducing power of certain plant extracts (Yildirim *et al*., 2001). The reducing power of all solvent extracts of *A. radiata* was increased with the increasing concentrations. According to the results in the present study, it is suggested that ethanol extract of *A. radiata* has a remarkable potency to donate electron to reactive free radicals, converting them into more stable non-reactive species and terminating the free radical chain reaction.

The medicinal values of the plants lie in their component phytochemicals, which produce definite physiological actions on the human body. Phenols, flavonoids, glycosides, tannins, saponins and sterols contribute to the antimicrobial and antioxidant activities of drugs (Ya *et al*., 1998). In the present study, phytochemicals were qualitatively analyzed and the results revealed that the ethanol extract of *A. radiata*

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### Table 1: Qualitative analysis of secondary metabolites of *A. radiata*.

<table>
<thead>
<tr>
<th>Secondary metabolites</th>
<th>Name of the test</th>
<th>H</th>
<th>Ch</th>
<th>Ea</th>
<th>E</th>
<th>A</th>
</tr>
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<td></td>
<td>Conc. H$_2$SO$_4$</td>
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contain most of the secondary metabolites analyzed when compared to the other extracts, which may be the reason for its potent in vitro antioxidant activity.

Conclusion

The ethanol extract of *A. radiata* showed strong antioxidant activity by inhibiting lipid peroxidation, and by scavenging DPH, superoxide anion and hydroxyl radicals and also through exhibiting strong metal chelating activity and reducing power when compared with other solvent extracts of *A. radiata*. The antioxidant activity of the extracts is compared with standard antioxidant i.e. ascorbic acid and standard metal chelator (EDTA). In addition, EEAR found to contain a noticeable phytochemicals (phenols, flavonoids, glycosides, tannins, and saponins), which play a role in scavenging free radicals. From the results of the present study one can consider that EEAR can be used as an easily accessible source of natural antioxidant in pharmaceutical industry.

References


