Asian J. Exp. Sci., Vol. 25, No. 1, 2011; 73-80

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

M. Manjunath*, G. Lavanya, R. Sivajyothi and O.V.S. Reddy
Department of Biochemistry
S.V. University,
Tirupati (A.P.); India

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% (IC_{50}) 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 vitilated 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, β-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

*** Corresponding author :** Dr. M. Manjunath, Department of Biochemistry, S.V.University, Tirupati-517502 (A.P.); India; Cell: 09966291895; E-mail : *Manubolu69@gmail.com*

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, ethyl acetate 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 90-95ºC. 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 (10^8 cells/mL) washed in Tris-HCl buffer (20mM, pH 7.0) with $CuCl_2$ (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 37ºC 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 ìg/** 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:

$$\text{DPPH Scavenged}(\%) = \frac{(A_{cont} - A_{test})}{A_{cont}} \times 100$$

Where A_{cont} is the absorbance of the control reaction and A_{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 NB**T (50 ìM) solution, 1 mL NADH (78ìM) solution and a sample solution of plant extracts (200-1000 ìg/**mL) in water. The reaction started by adding 1 **mL of phenazine methosulphate (PMS) solution (10 ìM) to the mixture. The reaction mixture was incubated at**

100°C for 5 min, and the absorbance at 560 nm was measured against blank samples. L-ascorbic acid was used as a standard. Decreased absorbance of the reaction mixture indicates increased super oxide anion scavenging activity. The % inhibition of superoxide anion generation by the extract was calculated using the formula, as stated in the methodology of DPPH radical scavenging activity.

Evaluation of the hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was carried out by measuring the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system. Attack of the hydroxyl radical on deoxyribose led to TBARS (thiobarbituric acid-reactive substances) formation (Halliwell and Gutteridge, 1981). The formed TBARS were measured by given by Ohkawa method (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} $FeCl_3$, 104 µmol L^{-1} EDTA, 100 µmol L^{-1} ascorbic acid, 1 mmol L^{-1} H_2O_2 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 test tube and incubated at 100°C for 20 min. After cooling, absorbance was measured at 532 nm against a blank containing deoxyribose and buffer. Reactions were carried out in triplicate. In the series of control experiments, reference compound, ascorbic acid (100 µg mL^{-1} in phosphate buffer pH 7.4) was used instead of the extract solution. The reaction mixture was incubated at 37°C for 1 hr. The capability of the extract to scavenge hydroxyl radical was calculated, using the above stated formula.

Metal chelating activity on ferrous ions

The chelating effect of ferrous ions by hexane, chloroform, ethylacetate, ethanol and aqueous extracts of *A. radiata* and standards were estimated by Dinis method (Dinis *et al*., 1994). Briefly, different concentrations of hexane, chloroform, ethylacetate, ethanol and aqueous extracts of *A. radiata* (200-1000 µg/ mL) were added to a solution of 2mM $FeCl_2$ (0.05 mL).The reaction was initiated by the addition of 5mM ferrozine (0.2 mL) and the mixture was shaken vigorously and left standing at room temperature for 10 minutes. Then, the absorbance of the solution was measured spectrophotometrically at 562 nm. EDTA was used as standard. The percent of inhibition of ferrozine-Fe^{2+} 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 [$K_3Fe(CN)_6$] (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 $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer. Ascorbic acid was used as 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 Whattman filter paper No.1. Thus, the filtrates obtained were used as test solutions for the screening. Alkaloids were identified by Iodine test, Dragendroff's test and Wagner's test, Flavonoids by pews test, Shinda test, and NaOH test, Glycosides by Conc.H_2SO_4 test, Keller-Kiliani test, and Molisch test, Phenols 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).

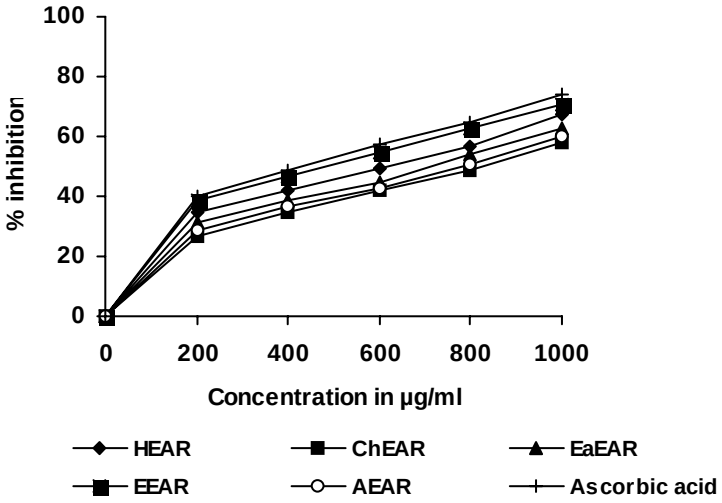
Statistical analysis

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

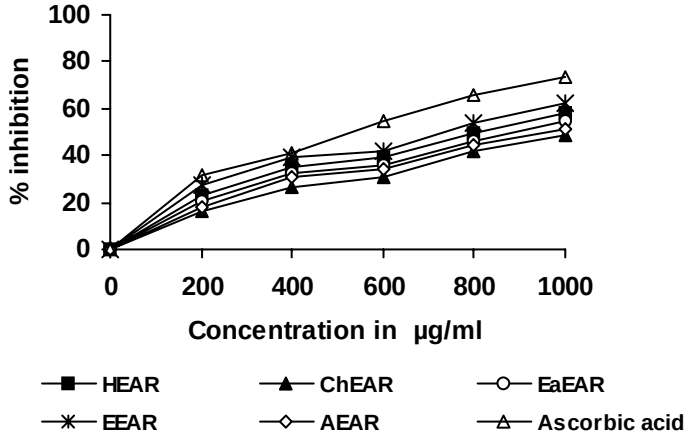
Inhibition of lipid peroxidation

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



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.

Fig. 1: Anti-lipid peroxidation activity of hexane, chloroform, ethyl acetate, ethanol and aqueous extracts of *A.radiata*.



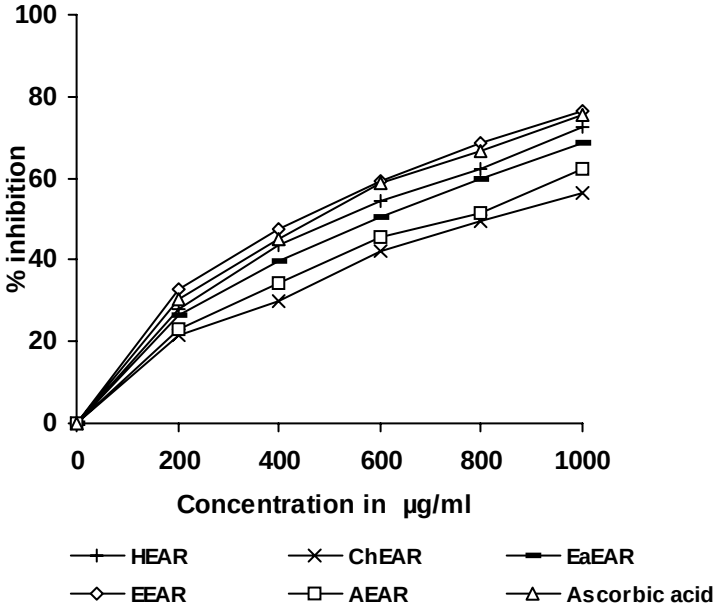
Values are expressed as mean ± S.E. Mean values at all concentrations of all extracts are compared with standard by Dunnet's test at 0.01 level. HE = Hexane extract; ChE = Chloroform extract; EaE = Ethyl acetate extract; EE = Ethanol extract; AE = Aqueous extract; AR = Actiniopteris radiata.

Fig. 2: DPPH radical scavenging activities of hexane, chloroform, ethyl acetate, ethanol and aqueous extracts of *A.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 ($p<0.05$).

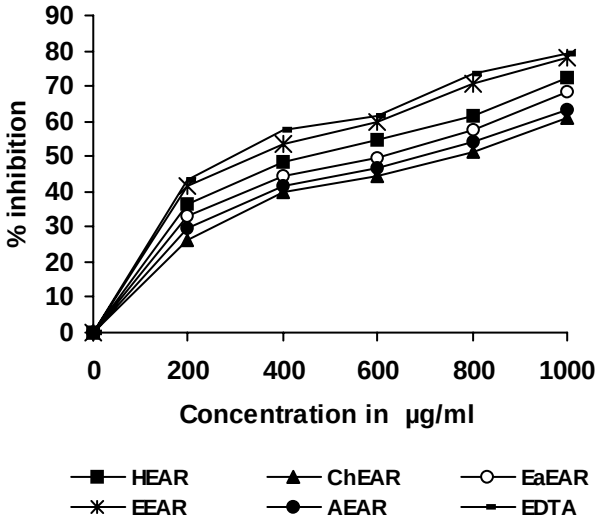
Inhibition of DPPH radical

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.



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.

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

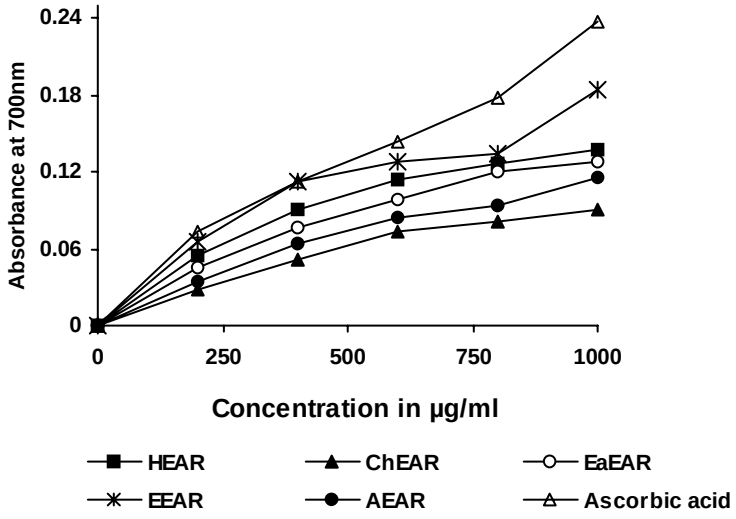


Values are expressed as mean ± S.E. Mean values at all concentrations of all extracts are compared with standard by Dunnet's test at 0.01 level. HE = Hexane extract; ChE = Chloroform extract; EaE = Ethyl acetate extract; EE = Ethanol extract; AE = Aqueous extract; AR = Actiniopteris radiata.

Fig. 5: Metal chelating activities of hexane, chloroform, ethyl acetate, ethanol and aqueous extracts of *A.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 ($p<0.01$).



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.

Fig. 6: Reducing power of hexane, chloroform, ethyl acetate, ethanol and aqueous extracts of *A.radiata.*

Inhibition of hydroxyl radical

All solvent extracts of *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 *A. radiata* exhibits a marked capacity for iron binding when compared to all other solvent extracts of *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 *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 *A. radiata* are represented in Table 1. Ethanol extract of *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 *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 $O_2^{\cdot-}$, which reduces NBT, resulting in the formation of formazan

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

Secondary metabolites	Name of the test	H	Ch	Ea	E	A
Alkaloids	Iodine	-	-	-	-	-
	Dragendorff's	-	-	-	-	-
	Wagners	-	-	-	-	-
	Mayer's	-	-	-	-	-
Flavonoids	Pews	-	-	+	+	+
	Shinda	-	-	+	+	+
	NaOH	-	-	-	+	+
Glycosides	Keller-Kiliani	+	-	-	+	-
	Conc. H_2SO_4	+	-	-	+	-
	Molisch	+	-	-	+	-
Phenols	Ellagic acid	-	-	+	+	+
	Phenols	-	-	+	+	+
Saponins	Foam	+	+	-	-	-
Sterols	Liberman-Buchard	+	+	-	-	-
	Salkowski	+	+	-	-	-
Tannins	Gelatin	-	+	+	+	+

(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*

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 DPPH, 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

Beauchamp C. and Fridovich I. (1971): Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.*, **44**, 276–287.

Blois M.S. (1958): Antioxidant determination by the use of a stable free radical. *Nature*., **181**, 1199–1200.

Buege J. and Aust D.S. (1978): Microsomal lipid peroxidation. In: *Methods in enzymology.*, Academic press, New York: 302-310.

Cadenas E. and Packer L. (1996): *Hand Book of Antioxidants*, Plenum, New York.

Dastmalchi K., Dorman H.J., Kosar M. and Hiltunen R. (2007): Chemical composition and *in vitro* antioxidant evaluation of a water soluble Moldavian balm (Dracocephalum moldavica L.) extract. *LWT*., **40**, 239–248.

Devasagayam T.P.A., Tilak J.C., Boloor K.K., Sane K.S., Ghaskadbi S. and Lele R.D. (2004): Free radicals and antioxidants in human health: current status and future prospects. *J Assoc Physicians India*., 794–804.

Dey P.M. and Horborne J.B. (1989): Methods in Plant Biochemistry. Plant phenolics. Academic Press, London: 180-250.

Dinis T.C.P., Maderia V.M.C. and Almeida L.M. (1994): Action of phenolic derivatives (acetaminophen, salicylate and aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch Biochem Biophys*., **315**, 161-169.

Diplock A.T. (1997): Will the 'good fairies' please proves to us that vitamin E lessens human degenerative of disease? *Free Rad Res*., **27**, 511-532.

Dorman H.J.D., Peltoketo A., Hiltunen R. and Tikkanen M.J. (2003): Characterization of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae Herbs. *Food Chem.*, **83**, 255–262.

Evans W.C. (1989): Trease and Evans. Pharmacognosy, 13th edn. Bailliere Tindall. London: 830.

Gibbs R.D. (1974): Chemotaxonomy on flowering plants. Mc Gill Queens University Press, Montreal and London.

Gokhale S.B., Kokate C.K. and Purohit A.P. (1993): A Text Book of Pharmacognosy. Published by Nirali Prakashan, Pune, India.

Halliwell B. and Gutteridge J.M.C. (1985): In: Free radicals, ageing, and disease, free radicals in biology and medicine. 2nd edn., Clarendron Press, Oxford: 279–315.

Halliwell B., Gutteridge J.M.L. and Aruoma O.I. (1987): The deoxyribose method: A sample "test tube" assay for determination of rate constants for reactions of hydroxyl radicals. *Anal Biochem*., **165**, 215-219.

Halliwell B. and Gutteridge J. (1981): Formation of a thiobarbituric-acid-reactive substance from deoxyribose in the presence of iron salts. The role of superoxide and hydroxyl radicals. *FEBS Lett*., **128**, 347–352.

Harborne J.B. (1998): Phytochemical Methods: A guide to modern techniques of plant analysis, 3rd edn. Chapman and Hall, Madras: 302.

Khare C.P. (2004): Indian herbal remedies: Rational Western Therapy, Ayurvedic and other. Springer link Publishers, 21- 22.

Kumar R.S., Siva Kumar T., Sunderam R.S., Gupta M., Mazumdar U.K., Gomathi P., Rajeshwar Y., Saravanan S., Kumar M.S., Murugesh K. and Kumar, K.A. (2005): Antioxidant and antimicrobial activities of *Bauhinia racemosa* L. stem bark. *Braz J of Med Biol Res*., **38**, 1015-1024.

Liu F., Ooi V.E.C. and Chang S.T. (1997): Free radical scavenging activity of mushroom polysaccharide extracts. *Life Sci*., **60**, 763–771.

Manjunath M., Sharma P.V.G.K. and Reddy O.V.S. (2008): *In-Vitro* evaluation of antibacterial activity of *Actiniopteris radiata* whole plant. *J Pharm Chem*., **2**, 112-117.

Ohkawa H., Ohishi N. and Yagi K. (1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*., **95**, 351–358.

Oyaizu M. (1986): Studies on product of browning reaction prepared from glucose amine. *Japn J Nutr*., **44**, 307-315.

Sies H. (1996): Antioxidants in Disease, Mechanisms and Therapy, Academic Press, New York.

Sanchez-Moreno C., Larrauri J.A. and Saura-Calixto F. (1999). Free radical scavenging capacity and inhibition of lipid oxidation of wines, grape juices and related polyphenolic constituents. *Food Res Int*., **32**, 407–412.

Suffness M. and Douros J. (1979): Methods in Cancer Research, vol. XVI. Academic Press, New York: 116.

Taneja S.C. and Tiwari H.P. (1974): Chemical constituents of *Actiniopteris radiata* (SW.) Link. *Curr Sci*., **43**, 749-750.

Ya C., Gaffney S.H., Lilley T.H. and Haslam E. (1998): In: Hemingway R.W. and Karchesy J.J. (Eds.,) Chemistry and Significance of Condensed Tannins, Plenum Press, New York: 553.

Yamaguchi F., Ariga T., Yoshimura Y. and Nakazawa H. (2000): Antioxidative and anti-glycation activity of garcinol from Garcinia indica fruit rind. *J Agric Food Chem*., **48**, 180-185.

Yoshikawa T. (2000): Free Radicals in Chemistry, Biology and Medicine, OICA International, London.

Yildirim A., Mavi A. and Kara A.A. (2001): Determination of antioxidant and antimicrobial activities of Rumex crispus L. extracts. *J Agric Food Chem*., **49**, 4083–4089.