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Research Article

EVALUATION OF *IN VITRO* ANTIOXIDANT POTENTIAL OF METHANOLIC EXTRACTS OF THE FERNS, *ACTINIOPTERIS RADIATA* (SW) LINK. AND *EQUISETUM RAMOSISSIMUM* DESF.

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ABSTRACT

Present study reports the antioxidant activities of methanolic extracts of *Actiniopteris radiata* and *Equisetum ramosissimum*. The analyses carried out were DPPH radical scavenging, $ABTS^{\cdot+}$, reducing power, and ferrous ion chelating tests. From the analyses, *Actiniopteris radiata* and *Equisetum ramosissimum*, were found to have potent antioxidant activity against DPPH with the IC^{50} value of 93.48 and 78.58 respectively. *Actiniopteris radiata* had the highest values for $ABTS^{\cdot+}$ radical scavenging activity (2523.11µ TE/g) and reducing power assay (0.853 absorbance at 700µg/ml). However, the fern, *Equisetum ramosissimum* exhibited higher ferrous iron chelating activity (41.18% at 5000µg/ml) than *Actiniopteris radiata*. Thus the results obtained in the present study indicate that these plants have the potential as natural source of antioxidants, capable of protecting against free radical mediated damage and may have applications in preventing and curing various diseases.

Key words: *Actiniopteris radiata, Equisetum ramosissimum,* antioxidant activity.

INTRODUCTION

Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Oxidative stress has been linked to cancer, aging, atherosclerosis, ischemic injury, inflammation and neurodegenerative diseases (Parkinson's and Alzheimer's)[1]. Antioxidants can delay, inhibit or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress [2,3]. Natural antioxidants have been studied extensively for decades in order to find compounds protecting against a number of diseases related to oxidative stress and free radical-induced damage. To date, many plants have been claimed to pose beneficial health effects such as antioxidant properties [4,5]. However, the investigation of ferns for antioxidant activity is meagre. *Actiniopteris radiata,* and *Equisetum ramosissimum* are the two ferns reported to have importance in folklore medical practice in Shavaroyan hills. As there is no scientific validation of these species for their medicinal uses, the present study was undertaken to bringout their applications in terms of antioxidant activities.

Materials and methods

Plant material

The plant materials of *Actiniopteris radiata,* and *Equisetum ramosissimum* were collected from Shevaroyan Hills, Salem district, Tamil Nadu. Collected plant materials were washed thoroughly in tap water and then shade dried.

Preparation of extract

About 50g of coarsely powdered whole plant of *Actiniopteris radiata,* and *Equisetum ramosissimum* was extracted with 250mL of methanol through soxhlet apparatus separately for 8 to 10 hours. The extracts obtained were then concentrated and finally dried to a constant weight.

In vitro antioxidant activities

DPPH radical scavenging activity

The 2, 2-diphenyl-picryl-1-picryl-hydrazyl radical (DPPH) scavenging activity was measured according to the method of Blois [6]. Methanol extract of the samples at various concentrations (50, 100, 150,200 and 250µg/mL,) was added separately to each 5mL of 0.1mM methanolic solution of DPPH and allowed to stand for 20min. Absorbance at 517nm using spectrophotometer was measured. BHT was used as standard. The corresponding blank reading was also taken and DPPH radical scavenging activity was calculated by using the following formula:

DPPH radical scavenging activity (%) = Control OD-Sample OD/Control OD×100

IC_{50} value is the concentration of the sample required to scavenge 50% DPPH free radical / OH^+ radical which has been determined by using the software SPSS v.16.

Antioxidant activity by the $ABTS^{\bullet+}$ assay

The total antioxidant activity of the samples was measured by $ABTS^{\bullet+}$ [2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)] radical cation decolorization assay according to the method of Re *et al.* [7]. $ABTS^{\bullet+}$ was produced by reacting 7mM ABTS aqueous solution with 2.4 mM potassium persulfate in the dark for 12–16 h at room temperature. Prior to assay, this solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 30 °C to give an absorbance of 0.700 ± 0.02 at 734 nm. The stock solution of the sample extracts was diluted such that after introduction of 10 l aliquots into the assay, which have been produced between 20% and 80% inhibition of the blank absorbance. After the addition of 1 ml of diluted ABTS solution to 10 l of sample or trolox standards (final concentration 0–15 M) in ethanol, absorbance was measured at 30 °C exactly 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay. Triplicate determinations were made at each dilution of the standard, and the percentage inhibition was calculated from the blank absorbance at 734 nm and then it was plotted as a function of trolox concentration. The unit of total antioxidant activity (TAA) is defined as the concentration of trolox having equivalent antioxidant activity expressed as mol/g sample extract on dry matter.

Reducing power assay

Reducing power assay was determined according to the method of Yildirim *et al.*[8] Different concentrations of methanolic extracts of the two study species (300, 400, 500, 600 and 700 µg/mL) were mixed with 2.5mL of 200mM sodium potassium ferric cyanide separately and incubated at 50°C for 20 min. After adding 2.5mL of 10% trichloro acetic acid, the mixture was centrifuged at 3000rpm for 10min. The supernatant was taken out and immediately mixed with 5mL of distilled water and 0.5mL of 1% ferric chloride. After incubation for 10min. the absorbance was measured at 700nm. Higher absorbance of the reaction mixture indicates reductive potential of the extract.

Ferrous ion chelating assay

The chelating of ferrous ions by whole plant methanolic extracts of the two study species were estimated by the method of Dinis *et al.*[9]. Briefly the extract samples (250 l) were added to a solution of 2 mmol/L $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mmol/L ferrozine (0.2 ml) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The chelating activity of the extracts was evaluated using EDTA as standard. The results were expressed as mg EDTA equivalent/g extract.

Statistical analysis

All the analysis was done in triplicate and results were expressed as mean±SD. The data were subjected to one way analysis of variance (ANOVA) and the significance between mean was determined by Duncan's Multiple Range test with significance level, $P<0.05$. ANOVA was performed using the statistical software SPSS (SPSS Inc. Chicago, USA).

RESULTS AND DISCUSSION

In vitro antioxidant activity

DPPH radical scavenging assay

The free radical scavenging activity of the extracts is related with hydrogen atom or electron donation abilities and the

conformations of the antioxidant compounds of the extracts. DPPH, a stable free radical with a characteristic absorption at 517 nm, was used as free radical to study the radical scavenging effects of some natural products[10]. Free radical scavenging capacities of the extracts were tested by DPPH. The free radical scavenging activity of both the study species, *Actiniopteris radiata, Equisetum ramosissimum* was increased with the increase of concentrations (Table 1). *Actiniopteris radiata,* and *Equisetum ramosissimum* extracts showed significantly scavenging the free radicals (IC_{50} value 93.48 and 78.58 respectively). The DPPH radical scavenging activity was detected and compared with the standard, BHT (IC_{50} 22.83).

Reducing power assay

Table 3 shows the reductive capabilities of different concentrations of methanolic extracts of *Actiniopteris radiata*, *Equisetum ramosissimum* in comparison to that of the standard, ascorbic acid. It was found that the reducing power increased with the increasing of the concentrations of the extracts. In the present study, *Actiniopteris radiata* extract showed the highest reducing ability (absorbance 0.853 at 700µg/ml) than the other fern, *Equisetum ramosissimum* extract (absorbance 0.084 at 700µg/ml). However, the activity was lesser than the standard, ascorbic acid (absorbance 1.05 at 700µg/ml). Presence of reducers

Table 1. DPPH radical scavenging activity of methanol extracts of *Actiniopteris radiata*, *Equisetum ramosissimum* and the standard BHT.

S.No	Sample Concentration (µg/mL)	Percentage inhibition		
		BHT	*Actiniopteris radiata*	*Equisetum ramosissimum*
1	50	$67.24^{a} \pm 0.21$	$52.11^{a} \pm 0.56$	$41.00^{a} \pm 0.68$
2	100	$74.21^{b} \pm 0.35$	$59.63^{b} \pm 0.57$	$55.21^{b} \pm 0.49$
3	150	$83.27^{c} \pm 0.36$	$65.82^{c} \pm 0.49$	$61.68^{c} \pm 1.16$
4	200	$87.16^{d} \pm 0.51$	$71.35^{c} \pm 0.36$	$67.32^{d} \pm 0.49$
5	250	$89.42^{e} \pm 0.12$	$74.10^{d} \pm 0.43$	$73.46^{e} \pm 0.14$
IC_{50} Value		22.83	93.48	78.58

Values are expressed as mean ± SD of three parallel measurements.

Values within a column followed by different letter(s) are significantly different ($P<0.05$).

Table 2. $ABTS^{\cdot+}$ radical scavenging activity of methanolic extract of *Actiniopteris radiata*, *Equisetum ramosissimum*.

Sample	Total antioxidant activity (µm of TE/g DW)
Actiniopteris radiata	2523.11±1.73
Equisetum ramosissimum	1946.36±2.12

Total antioxidant activity (µmol equivalent trolox performed by using $ABTS^{\cdot+}$ radical cation).

$ABTS^{\cdot+}$ scavenging activity

In the present investigation, the methanolic extract of *Actiniopteris radiata* registered the highest total antioxidant activity (2523.11µmol/g) than the *Equisetum ramosissimum* extract (1923.36µmol/g). $ABTS^{\cdot+}$, a protonated radical has characteristic absorbance maxima at 734nm which decreases with the scavenging of the proton radicals. $ABTS^{\cdot+}$ was generated by incubating it with potassium persulfate. The presence of chemical compounds in the tested extracts that inhibit the potassium persulfate activity may reduce the production of $ABTS^{\cdot+}$.

causes the conversion of the Fe3+/ferric complex of antioxidants to the ferrous form. By measuring the formation of Pearl's Prussian blue at 700nm, it is possible to determine the concentration of Fe3+ ion.

Ferrous ion chelating assay

The chelating effect on the ferrous ions by methanolic extract of *Actiniopteris radiata*, and *Equisetum ramosissimum* is presented in Table 4. Both the sample exhibited the ability to chelate metal ions. Among the two extracts *Equisetum ramosissimum* showed higher activity (41.18% at 5000µg/ml) than that of the fern *Actiniopteris radiata*

Table 3. Reducing power assay of methanolic extract of *Actiniopteris radiata*, *Equisetum ramosissimum* and the standard ascorbic acid.

S.No.	Sample concentration (µg/mL)	Absorbance at 700nm		
		Ascorbic acid	*Actiniopteris radiata*	*Equisetum ramosissimum*
1.	300	$0.35^{a} \pm 0.02$	$0.651^{a} \pm 0.01$	$0.032^{a} \pm 0.51$
2.	400	$0.56^{b} \pm 0.03$	$0.776^{b} \pm 0.03$	$0.039^{b} \pm 0.12$
3.	500	$0.76^{c} \pm 0.13$	$0.779^{b} \pm 0.35$	$0.051^{c} \pm 0.56$
4.	600	$0.88^{d} \pm 0.12$	$0.789^{c} \pm 0.07$	$0.065^{d} \pm 0.45$
5.	700	$1.05^{e} \pm 0.34$	$0.853^{d} \pm 0.14$	$0.084^{e} \pm 0.34$

Values are expressed as mean ± SD of three parallel measurements.
Values within a column followed by different letters are significantly different ($P<0.05$).

Table 4. Ferrous ion chelating assay of methanolic extract of *Actiniopteris radiata, Equisetum ramosissimum* and the standard EDTA.

S.No.	Sample concentration (µg/mL)	Percentage inhibition		
		EDTA	*Actiniopteris radiata*	*Equisetum ramosissimum*
1.	1000	$52.18^{a} \pm 0.59$	$20.63^{a} \pm 0.54$	$30.45^{a} \pm 0.46$
2.	2000	$75.56^{b} \pm 0.23$	$28.48^{b} \pm 0.40$	$33.65^{b} \pm 0.64$
3.	3000	$81.26^{c} \pm 0.15$	$31.12^{c} \pm 0.78$	$33.55^{c} \pm 0.34$
4.	4000	$92.69^{d} \pm 0.11$	$32.41c^{d} \pm 0.61$	$38.18^{d} \pm 0.64$
5.	5000	$95.89^{e} \pm 0.19$	$34.30^{d} \pm 0.35$	$41.18^{e} \pm 0.46$

Values are expressed as mean ± SD of three parallel measurements.
Values within a column followed by different letters are significantly different ($P<0.05$).

extract at the same concentration (34.30%). Metal chelating capacity was significant as they reduced the concentration of catalyzing transition metal in lipid peroxidation[11]. Iron chelators mobilize tissue iron by forming soluble, stable complexes that are then excreted in the feces and/or urine. Chelation therapy reduces iron-related complications and thereby improves quality of life and overall survival. Therefore, it is important to characterize the extracts by the variety of antioxidant assays[12,13].

Conclusion

The results of the present study indicate that the two pteridophytes, *Actiniopteris radiata,* and *Equisetum ramosissimum* exhibit strong antioxidant activities. The scavenging activities noted against DPPH• and ABTS•+ radicals and reducing power and ferrous ion chelating tests lead us to propose these two species as promising natural sources of antioxidants suitable for application in nutritional/pharmaceutical fields, in the prevention of free radical-mediated diseases. It also confirms the traditional knowledge of these two fern species on medicinal importance.

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