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PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY OF *ACTINIOPTERIS RADIATA* LINN.-AN IMPORTANT PTERIDOPHYTIC MEDICINAL PLANT OF GULBARGA REGION

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ABSTRACT

The present study was under taken to evaluate the *In vitro* antioxidant property and phytochemical constituents of different solvent extracts of *Actiniopteris radiata*. The scavenging activity of DPPH, H_2O_2 and reducing power including phenols, flavonoids, alkaloids and tannin contents were determined. The extracts exhibited scavenging activity towards all radicals tested due to the presence of relatively high alkaloids and flavonoids content. The present study suggests that *Actiniopteris radiata* is endowed with antioxidant phytochemicals and could serve as a base for future drugs.

KEYWORDS: Phytochemical, Antioxidant, DPPH, *Actiniopteris*, BHA, BHT

INTRODUCTION

Phytochemicals are bioactive chemical compounds occur naturally in plants. They are regarded as secondary metabolites because the plants that manufacture them may have little need for them. Phytochemicals such as alkaloids, flavonoids, vitamins, terpenoids, phenolic acids, tannins, coumarins and other metabolites are rich in antioxidant activity (Cai Y Z and Sun M Corke H 2003), (Zheng W and Wang S Y, 2001).

Antioxidant is a molecule, which terminate the chain reaction by removing free radical intermediates. Free radicals are types of reactive oxygen species (ROS). They are chemically unstable atoms which include all highly reactive oxygen containing molecule. Types of ROS include the hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen and various lipid peroxidase, free radicals may either be produced by physiological or biochemical process or by pollution and other endogenous sources. All these free radicals are capable of reacting with membrane lipids, nucleic acids, proteins, enzymes and other small molecules resulting in cellular damage (Aiyegoro O A and Okoh A I 2010).

Several synthetic antioxidants are commercially available but have been reported to be toxic (Madhavi D L and Salunkhe D K 1995). The most commonly used antioxidant at the present time are butylated hydroxyanisol (BHA), butylated hydroxytolune (BHT), Propyl gallate (PG) and tetra-butylhydroquinone (TBHQ) (Sherwin E R et al., 1990). However BHA and BHT have suspected of being responsible for liver damage and carcinogenesis (Grice H C 1988). Therefore development and utilization of more effective antioxidant of natural origin are desired (Oktay M *et al*., 2003).

Many naturally occurring antioxidants from plant sources have been identified as free radical scavengers or active oxygen scavenger. Currently there is a great interest in the study of antioxidant substances because antioxidants prevent the human system by neutralizing the free radical scavenging molecules such as vitamins, terpenoids, phenolic acids, lignins, tannins, flavanoids, alkaloids and other metabolites which are rich in antioxidant activity (Lata N and Dubey V 2010) .

Actiniopteris radiata (Swartz) Link. belongs to the family Actiniopteridaceae. It is found throughout India, especially the peninsula, in dry rocky places, below 4,000ft. The plants are 8-25cm high rooting in the crevices of rocks or in between the joints of brick walls in moist and shady places. The rhizome is oblique to horizontal 1.5 to 2.0 cm in length, densely covered with wiry roots, the young leaves show circinate venation but the lamina become flat early stage of development. The lamine are stiff and rough to tough. The sporangia are sub-marginal on an intermarginal vein covering almost the entire abaxial surface of segment. The plant *Actiniopteris radiata* is described in the Indigeneous system of medicine for its utility as astringent, anti-inflammatory, useful in treating cough, bronchitis, asthma, diarrhea, dysentery, dysuria, used internally and externally for infected wounds and ulcers (Khare C P 2004), Quercetin-3- rutinoside (Taneja S C and Tiwari H P 1972), B-sisterol (Reddy O V S et al., 2008), detected in the different extracts of the plant. Plant known to possess antioxidant (Manjunath M et al., 2009). Antibacterial (Parihar P and Parihar L 2006) ; antifertility (Dixit R D 1974), activity.

The present work is focused on exploration of secondary metabolites and also to evaluate the antioxidant potential of the *A. radiata* which were collected from Gulbarga region.

MATERIALS AND METHODS

Plant Material

Actiniopteris radiata was collected from Gulbarga region. Then plant is identified and authenticated through Natinal Ayurveda Dietetics Research Institute, Banglore. The collected plant materials were thouroughly washed, shade dried and powdered, powder materials were subjected to Soxhlet extractor by using different solvents such as chloroform, ethanol, methanol and distilled water (aqueous).

Phytochemical Screening

The chloroform, ethanol, methanol and aqueous extracts of *Actiniopteris radiata* were used for preliminary phytochemical screening by following the method of Trease and Evans (1987) and Harborne (1973).

The ethanol, methanol, chloroform, and aqueous extracts of *Actiniopteris radiata* were subjected to the following chemical tests for the identification of various active constituents.

Estimation of Total Phenolic Content

The total phenols were estimated by following Folin-ciocalteau method (Malick C P 1980). In the experiment 500mg of the sample was crushed in a pestle and mortar with 100mL of 80% ethanol. The homogenate thus obtained was centrifuged at 10000rpm for 20 minute further the residue was extracted with 5mL 0f 80% ethanol and centrifuged and the supernatant was evaporated to dryness. Then the residue was dissolved in 5mL of distilled water and from this 0.5mL of the Folin-ciocalteaus reagent was added. After 3minutes 2mL of 20% NA_2CO_3 solution was mixed thoroughly and incubated for 1min on a boiling water bath further, the solution was cooled and the absorbance was measured at 650nm against reagent blank.

Estimation of Total Flavonoid Content

The total flavonoids were estimated by Swain and Hillis (1959) method, 500mg of plant material was homogenated with 10mL of methanol in a pestle and mortar and centrifuged at 3000rpm for 10min, the supernatant collected was evaporated to dryness keeping in a hot water bath (80^0c). thus, the residue obtained was redissolved in 5mL of distilled water and used for quantitative estimation of flavonoids.

0.1 and 0.2mL extracts were taken in test tube and diluted to 2mL with distilled water and to this 4mL vanillin reagent was added to each tube rapidly, exactly after 15min, the appeared brick red colour was read at 599nm against blank reagent. The standard curve was plotted using different concentration of phloroglucinol as the standard flavonoid. The amount of flavonoid present in the each sample was calculated with the help of the standard graph.

Estimation of Total Alkaloids

The alkaloid was estimated by the method of Harborne (1973). The acetic acid (5%) extract of plant material was warmed upto 70 ^{0}C and the pH 10 was made by NH_4OH and centrifuged at 5000rpm. The precipitate was dissolved in boiling methanol and evaporated. The alkaloid fraction was dissolved in ethanol (96%) and H_2SO_4 (20%). The alkaloid solution was mixed with 5mL of 60% H_2SO_4. After 5 minutes, 5Ml of solution of formaldehyde in H_2S0_4 was added. The solution was read at 565nm absorbance after 18minutes. The amount of alkaloids was calculated using the standard curve of Brucine.

Estimation of Total Tannins

The total tannins were estimated by Folin denis method (Schanderi,1970), 500mg of the dried plant material was transferred to a 250mL flask with 75mL of distilled water and was heated gently and boiled for 30min, further, the mixture is cooled and centrifuged at 2000rpm for 20min and supernatant was made upto the volume, then, 1mL of the sample extract was mixed with 75mL of water, 5mL of folin- denis reagent, 10mL of sodium carbonate solution and was diluted to 100mL with distilled water and shaken well. The solution was read at 700nm, absorbance after 30min. the total content of tannins was calculated using standard curve prepared using 0-100µg tannic acid (the colour was read at 700nm, against blank reagent). The standard curve was plotted using tannic acid at different concentrations (0.1, 0.2, 0.3, 0.4ml) from which the total tannin content of the plant material were calculated.

Reducing Power Activity

The reducing power of extract was determined by the method of yen and duh (1993). Different concentrations of extracts were mixed with 2.5mL of phosphate buffer (200mm, pH 6.6) and 2.5mL of 1% potassium ferricyanide. The mixtures followed by centrifugation at 650xg for 10min. the upper layer (5mL) of distilled water and 1mL of 0.1% ferric chloride and the absorbance of the resultant solutions were measured at 700nm.

Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide scavenging activity of extract was determined by the method of Ruch et al (1989). The extract was dissolved in 3.4mL of 0.1m phosphate buffer (pH7.4) and mixed with 600µL of 430nm solution of hydrogen peroxide, the absorbance value (at 230nm) of the reaction mixture was recorded at 10min intervals between zero and 40min for each concentration a separate blank sample was used for background subtraction.

DPPH Radical Scavenging Assay

The effect of extracts on DPPH radical was estimated using the method of Liyana-Pathirana and Shahidi (2005). A solution of 0.135mM DPPH in methanol was prepared and 1.0mL of this solution was mixed with 1.0mLof extract in methanol containing 0.02-0.1 mg of the extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30min. The absorbance of the mixture was measured spectrophotometrically at 517nm. Ascorbic acid and BHT were used as references. The ability to scavenge DPPH radical was calculated by the following equation: DPPH radical scavenging activity (%) = ($Abs_{control}$-Abs_{sample})/$Abs_{control}$ x100 where $Abs_{control}$ is the absorbance of DPPH radical + methanol; Abs_{sample} is the absorbance of zxDPPH radical + sample extract standard.

STATISTICAL ANALYSIS

All the data obtained were expressed as mean standard error. Differences in means were estimated by means of ANOVAs (Tukey) using Graph pad instant software. Results were considered significant at P *<0.05, ** < 001, ***<0.001.

Table 1: Priliminary Phytochemical Analysis of *Actiniopteris radiata*

Secondary Metabolites	Name of the Test	Chloroform	Methanol	Ethanol	Water
Phenols	Ellagic acid	+	+	-	+
	Phenol (ferric chloride)	-	+	+	+
Flavonoids	Shinoda	-	-	+	+
	Ferric chloride	+	+	-	+
	Lead aectate	+	+	+	+
	Zinc	-	-	-	-
Alkaloids	Mayer's	-	+	+	+
	Wagner's	+	+	+	+
	Dragendroff's	+	+	+	+
Tannins	Gelatin	-	+	+	-
	NaCl	-	+	+	-

Table 2: Quantitative Phytoichemical Analysis of Various Extracts of *Actiniopteris radiata* (mg/100g)

Phytochemicals	Chloroform	Methanol	Ethanol	Aqueous	Ascorbic Acid	Tannic Acid
Phenol	04.00±1.00	02.00±0.50	04.33±1.53	03.37±2.07	005.03±2.20	004.83±1.65
Flavonoid	81.00±4.00	78.67±3.06	91.67±5.03	74.00±2.00	166.67±3.06	029.67±4.04
Alkaloid	07.00±1.00	134.33±3.51	42.33±3.51	04.07±1.10	164.33±2.52	178.33±7.51
Tannin	02.07±0.90	010.27±1.62	9.00±0.00	08.03±0.55	015.33±3.51	023.33±5.51

Table 3: Reducing Power Activity of *Actiniopteris radiata*

Concentration (50µg/ml)	Chloroform	Methanol	Ethanol	Aqueous	BHA	BHT
Reducing Power	0.06±0.01	0.15±0.02 **	0.12±0.01 ***	0.04±0.00 *	0.61±0.37 ***	0.60±0.08 ***

Values are expressed as Mean ± SE for three trials, a values are significantly different compared to control when P *>0.05 ** >0.01 ***> 0.001.

Table 4: Hydrogen Peroxide-Scavenging Activity

Time (min)	Chloroform	Methanol	Ethanol	Aqueous	BHA	BHT
0	94.01±1.01	84.15±1.51 ***	85.92±2.56 ***	94.72±3.15	88.03±3.51 **	64.08±3.03 ***
5	90.10±2.05	84.82±0.01 **	85.48±0.45 **	94.39±0.58 *	89.11±0.75	67.000.44 ***
10	90.1±2.75	84.82±2.18 **	85.48±19.15 *	94.39±4.32 ***	89.11±2.00	67±1.00 ***
15	88.81±0.28	83.57±0.65 *	82.52±0.24 **	91.96±0.12	85.66±0.5	65.73±0.98 ***
20	90.54±0.89	82.43±3.54 *	83.11±3.00 *	93.24±2.11	85.81±5.15	70.27±1.98 ***
25	89.39±0.34	86.17±0.21	82.64±0.75 **	91.64±0.42	87.78±0.35 *	67.52±0.5 ***
30	88.81±1.09	83.57±0.92 **	82.52±5.14 ***	91.96±1.69	85.66±3.89	65.73±0.42 ***

Values are expressed as Mean ± SE for three trials, a values are significantly different compared to control when P *>0.05 ** >0.01 ***> 0.001.

Table 5: DPPH Radical Scavenging Activity (µg/ml)

Time (min)	Chloroform	Methanol	Ethanol	Aqueous	DPPH	BHT	BHA	Trehalose
5	24.59±1.64	84.79±2.54 ***	85.44±3.70 ***	33.33±0.83 ***	91.26±2.45 ***	72.41±0.23 ***	92.23±3.27 ***	91.26±3.27 ***
10	37.17±1.64	84.61±1.36 ***	84.93±0.82 ***	38.46±0.84	87.82±0.90 ***	91.98±0.42 ***	91.98±0.01 ***	91.98±0.86 ***
15	47.05±2.83	82.64±4.09 ***	82.33±0.83 ***	42.58±0.81	83.91±4.13 ***	91.37±0.19 ***	91.16±2.87 ***	90.54±2.46 **
20	59.68±3.28	81.87±0.91 ***	81.87±2.14	46.25±0.82 ***	80.32±3.62	92.82±2.05 ***	91.25±3.69 **	91.56±1.32
25	72.67±3.28	80.43±2.46 ***	81.67±0.31 ***	50.93±0.30 ***	76.71±0.36 **	92.56±3.07 ***	90.99±2.49 ***	97.20±0.10 ***
30	82.5±0.24	80.93±1.69	81.25±0.82	55.62±1.23 ***	75.31±0.83	96.15±0.82 ***	96.87±0.91 ***	95.93±0.39 ***

Values are expressed as Mean ± SE for three trials, a values are significantly different compared to control when P *>0.05 ** >0.01 ***> 0.001.

RESULTS AND DISCUSSIONS

The results of qualitative phytochemical analysis of *Actiniopteris radiata* have indicated that alkaloids, phenols, flavonoids and tannins were present in all the solvent extracts (Table 1).

The amount of phytochemicals which are found in the different extracts of *A. radiata* was quantitatively determined by standard procedures (Table 2). The amount of phenols, flavonoids, alkaloids and tannins contents were determined in chloroform, ethanol, methanol and aqueous extracts. High content of phenols and flavonoids were observed in ethanolic extracts compared to chloroform, methanol and aqueous extracts, similar studies have been carried out on the *Alpinia purpurata* (Subramanian V *et al.*, 2011)

The maximum content of alkaloids and tannins content were observed in methanolic extracts compared to chloroform, ethanol, and aqueous extracts. Similar results were reported by John de brito *et al.*, (2013).

The reducing powers of chloroform, methanol, ethanol, and aqueous extracts of *A. radiata* are in the following order of BHA>BHT>Methanol>Ethanol>Chloroform>Aqueous. In the present study, methanolic extract showed higher reducing power when compared to the other solvent extracts. Similar work has been carried out on the *A. radiata* (Manjunath *et al.*, 2011) and reported that ethanolic extract of *A. radiata* has a remarkable potency and followed by hexane extract>ethyl acetate>aqueous>chloroform (Table 3). According to the results in the present study it is suggested that methanol extract of *A. radiata* had a remarkable potency.

Hydrogen peroxide is an important reactive oxygen species because of its ability to penetrate biological membranes. scavenging activity of hydrogen peroxide in chloroform, methanol, ethanol, and aqueous extracts of *A . radiata* and BHA, BHT as reference compound was not remarkably different (Ranju pal *et al.*, 2011) reported ethanolic extract of *Morinda citrifolia* and BHT as reference compound was not remarkably different (Table 4).

The inhibition percentage (IP) values are considered to be a good measure of the antioxidant efficiency of pure compounds and extracts. The results of free radical scavenging capacity in chloroform, ethanol, methanol, and aqueous extracts of *Actiniopteris radiata* were evaluated (Table 5). The highest amount of % inhibition of DPPH activity is observed in methanol and ethanol extract of *A. radiata*. Similarly, highest percentage inhibition of DPPH activity have been reported in methanol and ethanol extracts of Wattakkaka volubilis and followed by chloroform (Madathupatti R U *et al*, 2012).

CONCLUSIONS

From the present study it can be concluded that the secondary metabolites such as alkaloids and flavonoids were found to be highest in ethanol and methanol extracts of *Actiniopteris radiata.* Similarly ethanol and methanol extracts were found to be efficient in antioxidant activity and results were very near to standard compounds (BHA, BHT, DPPH). Further from this study it can be concluded that, ethanol and methanol extracts of *A. radiata* can further may used for pharmacological studies.

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