WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 9, 873-879. Research Article ISSN 2277– 7105

ANTIOXIDANT, ANTI-DIABETIC AND ANTIMICROBIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *ACTINOPTERIS RADIATA* LINK

K. Rupnath[1], M. Lakshmi Narasu[1] and K. Mallikarjuna*[2]

[1]Centre for Biotechnology, Jawaharlal Nehru Technological University, Hyderabad.
[2]Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur, AP.

Article Received on
29 June 2016,
Revised on 19 July 2016,
Accepted on 09 August 2016
DOI: 10.20959/wjpr20169-6893

***Corresponding Author**
Dr. K. Mallikarjuna
Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur, AP.

ABSTRACT

Medicinal plants used in Indian ayurveda were having potential for antimicrobial and antioxidant and anti-diabetic properties. Here different solvent extracts of *Actinopteris radiata* plant were studied for antioxidant, anti-diabetic and antimicrobial activities. Ethanolic extract of plant at a dose of 100µg/ml was found to be having more DPPH inhibition activity than 50µg/ml concentration of standard Ascorbic Acid. Anti diabetic activity was found to be more in aqueous extract. More than 70% amylase inhibition was observed for aqueous extract. Ethanolic extract has shown more anti-microbial activity on bacteria (*B subtilis, S aureus, P aeruginosa, S typhi,*) and fungi (*A niger* and *C albicans).* Further, study need to be carried for the determination of active components responsible for these biological activities.

KEYWORDS: *Actinopteris radiata*, biological activities, antioxidant activity, anti-diabetic activity, antimicrobial activity.

INTRODUCTION

The *Actinopteris radiata* (commonly known as Nemaliadugu in Telugu) belonging to Actinopteridaceae family is a plant with great medicinal value.[1,2] *A radiata* is a small fern growing up to 10 - 15cm tall from a short creeping rhizome. The fronds have fan-shaped leaves.[3] Figure 1 showing the areal parts of the plant *A radiata*. The plant is sometimes harvested from the wild for local medicinal use. It is often grown as an ornamental in gardens. The plant *A radiata* is having large medicinal importance. According to ayurveda, *A radiata* is having anti helmentic activity.[4]

Different researchers proved different medicinal activities of plant. The plant *A radiata* is used for treatment of bronchitis and gynecological disorders,[5] tuberculosis, astringent, anti-inflammatory, antipyretic, alleviates vitiated blood, cough, asthma and bronchitis[6,7,8,9] and also increases fertility in woman and spermatorrhoea.[10] Though many pharmacological activities has been screened on this medicinal plant, activities pertaining to anti diabetic, anti oxidant and anti microbial are lacking. Hence in the present paper, we are reporting above activities.

MATERIALS AND METHODS

Instrumentation

Double beam UV visible spectrophotometer (TECHOMP –UV 2301) with 10mm path length, standard quartz cuvets and HITACHI UV solutions software was used for spectral analysis.

Chemicals and reagents

All the chemicals used for the present study were of laboratory reagent grade and were purchased form Merck chemicals private limited, Mumbai, Fisher scientific, Mumbai and SD fine chemicals Mumbai.

Extraction and quantification of phytochemicals

The phytochemicals were extracted using soxhelt extraction method with different solvents like chloroform, ethanol and water in successive mode respectively until clear solution was observed. The solvents were evaporated and powdered extract was collected. The phytochemical screening and quantitative estimation of phytochemicals were investigated using standard methods in our previous work, **(**Rupnath et al., 2016: Screening and quantification of phytochemicals in different solvent extracts of *Actinopteris radiata* Link; Science Spectrum).

Measurement of Antioxidant Activity using DPPH method

The antioxidant activity of the different solvent extracts of *Actinopteris radiata* was determined on the basis of their scavenging activity of the stable 1, 1- dipheny1-2-picryl hydrazyl (DPPH) free radical. DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. l ml of each solution of different concentrations (1-500gg/m1) of the extracts was added to 3 ml of 0.004% ethanolic DPPH free radical solution. After 30 minutes, the absorbance of the preparations were taken at 517 nm by a UV spectrophotometer which was

compared with the corresponding absorbance of standard ascorbic acid concentrations (1-500µg/ml). The method described by Hatano et al (1989), was used to measure the absorbance with some modifications. Then the % inhibition was calculated by the following equation:

$$\% \text{ Radical Scavenging Activity} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

Anti-diabetic activity by inhibition of alpha-amylase enzyme method

To 1ml of alpha amylase and 1 ml of plant extract was added in a test tube and incubated at 37◦C for 10 min. After pre-incubation, 1ml of 1% (v/v) starch solution was added to each tube and incubated at 37◦C for 15min. The reaction was terminated with 2 ml DNSA reagent, placed in boiling water bath for 5min, cool to room temperature, diluted and the absorbance measured at 546 nm. The control reaction representing 100% enzyme activity did not contain any plant extract. To eliminate the absorbance produced by plant extract, appropriate extract controls were also including. % inhibition of alpha amylase by each plant extract can be calculated using the formula:

$$\% \text{ Indibition} = \frac{(\text{Enzyme activity of control} - \text{Enzyme activity of extract}}{\text{Enzyme activity of control}} \times 100$$

Anti-microbial Activity using well diffusion method

Antimicrobial activity of ethanolic extract of *Actinopteris radiata,* was determined using six strains of micro-organisms including 4 bacteria (*B subtilis, S aureus, P aeruginosa* and *S typhi*) and 2 fungi (*A niger* and *C albicans*) were studied. Agar plate well diffusion method was followed for determination of anti-microbial Activity. Cell suspension of the microbial cell were inoculated in nutrient agar medium for *B subtilis, S aureus* and *P aeruginosa*, Mueller Hinton agar for *S typhi*, Potato dextrose Agar medium for *A niger* and Czapek Dox Agar medium for *C albicans* were used. The wells were punched over the agar plates using sterile gel puncher. Various concentrations of each plant extract were added to the wells. The plates were incubated for 24 hours for bacteria and 48 hours for fungi at 37^{o}C. After incubation, the diameter of inhibitory zones formed around each discs were measured in mm and recorded. Standard drug ampicillin for bacteria and fluconazole for fungi were used as +ve controls for antimicrobial activity. As negative controls, pure solvents were used.

RESULTS AND DISCUSSIONS

Though medicinal uses of plants are known but their phytochemical principles and in vitro/ in vivo biological activities are not known. Hence in the present study, DPPH free radical

activity, antimicrobial and in-vitro anti diabetic activity of different solvent extracts of *A. radiata* was studied.

Antioxidants play an important role as health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Hence anti-oxidant activity of *A. radiata* was studied by inhibition the stable free radical DPPH. Ascorbic Acid was used as standard antioxidant for DPPH activity. Figure 1 and table 1 showing the DPPH activity results of *A. radiata*. Among the solvents in the study, ethanolic extract was proved to be having best antioxidant activity and the activity was found to be very similar (more than 90%) for standard Ascorbic Acid. At a high concentration of 500µg/ml, ethanol extract shows more than 90% inhibition activity. Chloroform extract shows 40.86%, aqueous extract shows 84% inhibition. Hence results proved that ethanolic extract was found to be having very high percentage of DPPH inhibition activity.

Table 1: DPPH free radical screening activity of *A. radiata*

S NO	Concentration in µg/ml	% DPPH inhibition			
		Ascorbic Acid	Chloroform	Ethanol	Water
1	1	14.65	…	8.66	…
2	10	34.33	8.24	22.57	10.27
3	25	54.22	12.94	40.32	31.66
4	50	76.15	16.68	53.05	42.67
5	75	97.01	32.83	77.11	52.62
6	100	97.75	37.86	81.93	75.83
7	500	97.97	40.86	92.73	84.28

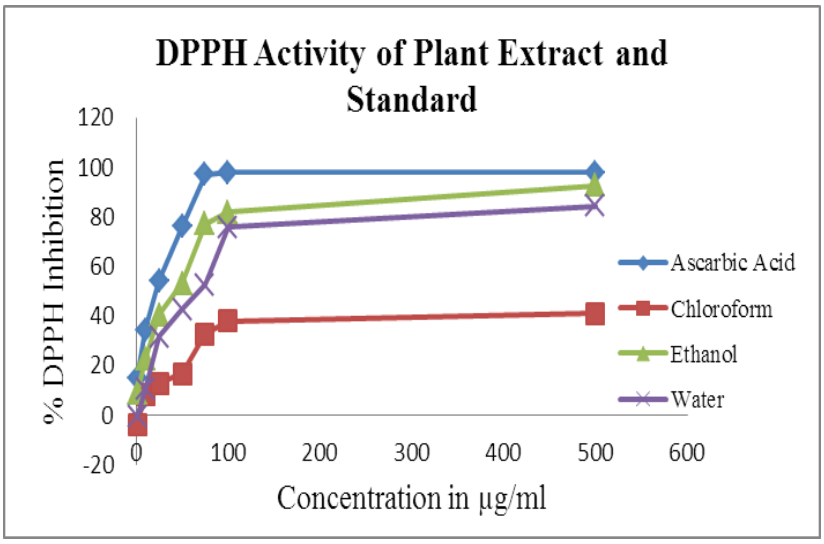

Figure 1: Comparative DPPH free radical screening activity results for *A. radiate*

Anti diabetic activity of *A. radiata* was studied by α-amylase inhibition assay. α-amylase inhibition was carried out by quantifying the reducing sugar (maltose equivalent) liberated under the assay conditions. The enzyme inhibitory activity was expressed as a decrease in units of maltose liberated. A modified dinitrosalicylic acid (DNS) method was adopted to estimate the maltose equivalent. Among the three solvents in the study, aqueous extract shows more anti-diabetic activity. More than 80% inhibition was observed for aqueous extract at a concentration of 400µg/ml of plant extract. At this concentration, 65.23% inhibition was observed for ethanolic extract and 21.94% inhibition was observed for chloroform extract. Hence aqueous extract was found to be having very high anti-diabetic activity than two other extracts in the plant *A. radiate*. α-amylase inhibition activity results were given in table 2 and comparative results were given in figure 2.

Table 2: anti-diabetic activity of *A radiata*

S.No	Concentration of plant extract	Chloroform Extract		Ethanolic Extract		Aqueous Extract	
		Absorbance	% inhibition	Absorbance	% inhibition	Absorbance	% inhibition
1	50µg/ml	0.793	7.4679	0.129	15.052	0.662	22.754
2	100µg/ml	0.714	16.686	0.156	18.203	0.561	34.539
3	150µg/ml	0.696	18.786	0.236	27.538	0.457	46.674
4	200µg/ml	0.659	23.104	0.321	37.456	0.326	61.960
5	250µg/ml	0.612	28.588	0.383	44.691	0.219	74.446
6	300µg/ml	0.611	28.705	0.488	56.943	0.178	79.230
7	350µg/ml	0.653	23.804	0.539	62.894	0.171	80.047
8	400µg/ml	0.669	21.937	0.559	65.228	0.168	80.397

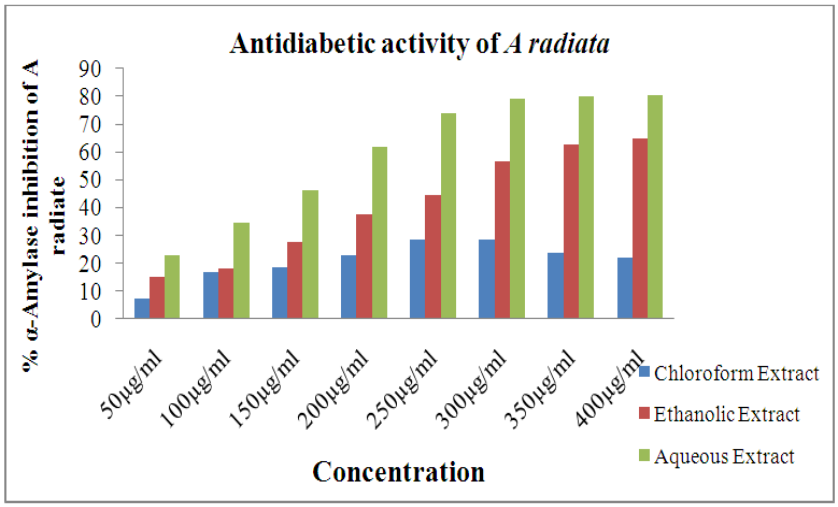

Figure 2: Comparative anti-diabetic activity of *A radiate*

Antimicrobial activity was carried for ethanolic extract of plant due to high quantitative presence of phytochemicals and high antioxidant and α-amylase inhibition activity.

Antimicrobial activity was studied against different pathogenic bacteria and fungi on agar plate well diffusion method. Dose dependent antimicrobial activity was observed for the extract. Results of the antimicrobial activity were compared with standard drugs statistically. The plant extract was found to be inhibiting the growth of the total micro organism in the study. Among all the bacteria, extract shows more activity against the growth of *S typhi* (10mm at 100µg/ml dose), less activity was observed against *S aureus*. Among the fungi, the extract was found to be inhibiting *A niger* than *C albicans*. The results of anti microbial activity in terms of zone of inhibition were given in table 3.

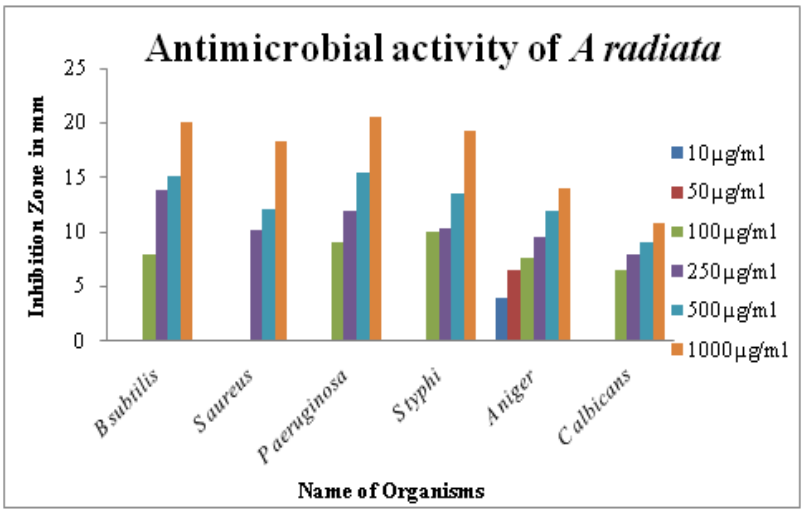

Figure 3: Comparative antimicrobial activity of *A radiate*

Table 3: Antimicrobial activity of ethanolic extract of *A. radiata*

S No	Name of the organism	Zone of inhibition in mm					
		10µg/ml	50µg/ml	100µg/ml	250µg/ml	500µg/ml	1000µg/ml
1	*B subtilis*	-	-	8	13.8	15.2	20
2	*S aureus*	-	-	-	10.2	12.1	18.3
3	*P aeruginosa*	-	-	9	12	15.5	20.6
4	*S typhi*	-	-	10	10.4	13.5	19.2
5	*A niger*	4	6.5	7.6	9.5	12	14
6	*C albicans*	-	-	6.5	7.9	9.1	10.8

CONCLUSION AND FUTURE PROSPECTS

Medicinal plants have served as a platform for ancient Ayurvedic system of medicine. In the present scenario, herbal therapeutics is gaining momentum in pharmacological applications and as molecular targets in the drug development. *A radiata* is a small fern having medicinal activities. Phytochemical screening proved that the plant is having large quantity of medicinally active compounds. The study of antioxidant activity, anti-diabetic activity and

antimicrobial activity results proved that the plant is having these biological activities in all the solvent extracts. Ethanolic extract of the plant is having high amount of these activities. Further studies was planned to prove the molecules responsible for these biological activities.

ACKNOWLEDGEMENTS

The authors are thankful to Ch.Vinay kumar and staff of RV labs, Guntur for providing lab facilities to complete the part of Ph.D dissertation work.

REFERENCES

1. Gibbs Russell GE, Welman WGM, Retief E, Immelman KL, Germishuizen G, Pienaar BJ, Van Wyk M and Nicholas A. List of species of southern African plants. Mem. Bot. Surv. S. Africa, 1987; 2(1–2): 1–152(pt. 1), 1–270(pt. 2).
2. "The Wealth of India". A Dictionary of Indian Raw Materials and industrial products, Council of Scientific and Industrial Research, New Delhi, India, 1969; 8: 256.
3. Burkil HM. The Useful Plants of West Tropical Africa, Royal Botanic Gardens; Kew, 1985 – 2004.
4. Dixit RD. Ferns-a much-neglected group of medicinal plants, J. Res. Indian. Med., 1974; 9: 74-90.
5. Parihar P. and Parihar L. Some of pterodophytes of medicinal importance from rajasthan, Nat. Prod. Rad., 2006; 4: 297-301.
6. Khare CP. Indian herbal remedies, Springer link publishers, Verlag Berlin, Heidelberg, 2004; 21.
7. Sharma BD and Vyas MS. Ethno botanical studies on the fern and fern allies of Rajasthan, Bull. Bot. Surv. India. 1985; 27: 90-91.
8. Bhattarchariee SK. Handbook of medicinal plants, Jaipur; Pointer publisher, 2003; 15.
9. Khare CP. Indian herbal remedies: an illustrated dictionary, Verlag Berlin, Heidelberg; Springer link publishers, 2007; 17.
10. Jain A, Katewa PK and Sharma P. Journal of Medicinal plant diversity of Sitamata wildlife sanctuary, Rajasthan, India; Ethno pharmacol, 2005; 102: 143-157.