ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 4, pp: (170-173), Month: October - December 2018, Available at: www.researchpublish.com

Evaluation of *In-vitro* Antibacterial activity of *Actiniopteris radiata*

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Abstract: **The current research work was aimed to evaluate the antibacterial activity of *Actiniopteris radiata,* which have been using for treatment for different ailment. The root was chosen for the preparation of extracts using maceration process with hexane, ethyl acetate and hydro-alcoholic solvents successively. The antibacterial activity was evaluated using cup plate methods on different infectious bacterial species (gram +ve and gram –ve) of prepared extracts at different concentrations. The results were showed as zones of inhibition (in mm) for each contraction. The extracts showed more activity on gram –ve species analyzed with gram +ve and hydro-alcoholic extract showed better inhibition on tested bacteria compared to hexane and ethyl acetate extracts. The extracts possess more inhibition on *Escheichia coli* and *Staphylococcus aureus.* The results of the current study supports the traditional use of *Actiniopteris radiata* and evidence for inhibition if infectious bacterial strains. Further studies are worth full in identification of phytochemical profile and their mechanism of action.**

Keywords: Actiniopteris radiata, **Bacteria, Roots, Zone of inhibition.**

I. INTRODUCTION

Now a days, the use of antibiotic drugs became in adequate manner for treatment of different diseases (Eggleston *et al.*, 2010). This situation is not good for the society because adequate use of antibiotic leading to resistance to diseases including microbial infections and causing the unusual side-effects (Hintz *et al.*, 2015). The microorganisms becoming more resistance to currently using drugs and becoming more untreatable (Levy, 2002). The current status stipulating scientist to identify new drugs for treating diseases with fewer or no side effects from different natural resources (Davies and Davies, 2010). Herbal medicine is oldest healing system around the world like Ayurveda and Unani medicines of India, Chinese herbal medicine etc., (Cowman, 1999). The use of medicinal plants in the treatment of diseases is becoming popular because of synthetic drugs resistance and more side effects (Alviano and Alviano, 2009). In recent decade different medicinal plants were reported scientifically about their medicinal use and scientists are isolating different biological active metabolites from them and are became source for synthesis of new drugs in treatment of diseases (Rajananda Swamy and Ganga Rao, 2018). In this point of view the present work was carried out on to evaluate the antibacterial activity of *Actiniopteris radiata* root part.

Actiniopteris radiata is a fern plants growing around the India belongs to the family Pteridaceae. The plants have posses' different medicinal values like antifertility, anti-tubercular but there is very less scientific evidence (The Wealth of India, 2006). So, the present work was carried out to identify antibacterial activity of *Actiniopteris radiata* root.

II. MATERIALS AND METHODS

Chemicals:

The solvents and chemicals used in current research were analytical grade and used standard drug ciprofloxacin was procured from local market (make Dr. Reddy's Laboratories).

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Plant material collection and extracts preparation:

The plant material *Actiniopteris radiata* was collected near Guntur region, Andhra Pradesh, India and authenticated by Dr. Prayaga Murthy. Pragada, Govt. Degree College, Yeleswaram, E. Godavari, A.P. India. The root parts were separated from freshly collected plant material and washed under running tap water to remove clay particles. The cleaned root parts were shade dried up to complete dry. The dried root part was made into granulated powder, then powder used for successive extraction with hexane, ethyl acetate and hydro-alcoholic solvents using maceration process. The extracted solvents were concentrated using rota vapor (Buchi) to get dry extract. The dried extracts were stored in desiccator for further use.

Selected bacterial strains:

Gram positive and gram negative bacterial strains were tested in the current *In-vitro* antibacterial activity of *A. radiate* study (Table I). The bacterial strains were taken from National Chemical Laboratory (NCL), Pune.

TABLE I: LIST OF BACTERIAL STRAINS

S. NO	Gram Positive	Gram Negative
1.	*Streptococus pneumoniae*	*Pseudomonas aeruginosa*
2.	*Staphylococcus aureus*	*Yersinia enterocolotica*
3.	*Clostridium sporogenes*	*Escheichia coli*
4.	*Listeria monocytogenes*	*Salmonella typhimurium*

***In-vitro* antibacterial activity:**

The antibacterial activity of selected plant extracts were evaluated using agar well difusion method (Ganga Rao *et al.*, 2011; Ganga Rao *et al.*, 2012). The extracts' solutions were prepared in dimethyl sulfoxide at different concentrations i.e. 40, 80, 150 and 250 mg/mL. The agar plates were prepared with nutrient agar and was autoclaved, then cooled to room temperature. The cooled agar was equally poured in Petri dishes and refrigerated for solidification, then each plate was separately inoculated with testing bacterial strains as spread plate technique with sterilized spreader and with sterile steel borer (6mm) made wells on petri dish to equal distribution (100µl) as 4, 8, 15 and 25 mg/well and standard drug at 100µg/well was placed. Then, prepared plates were placed with no disturbance for transmission of placed samples in wells. Then petri plates were incubated for 24hrs at incubator (37±2°C). Finally, after 24hrs plates were examined for extracts' antibacterial activity by measuring their activity as zones of inhibition. The experiment was repeated for three time and results were showed as mean±SD.

III. RESULTS AND DISCUSSION

The selected plant extracts were found to be posses dosage dependent antibacterial activity and as concentration increased the activity was increased simultaneously on tested bacterial strains. The results for hexane, ethyl acetate and hydro-alcoholic extracts were showed in Tables 1, 2 and 3. The extracts showed low activity at low concentrations and no inhibition on bacterial growth. The hexane extract showed low activity on *Listeria monocytogenes*, more activity on *Salmonella typhimurium* (Table 1). The ethyl acetate extract do not showed inhibition on *Clostridium sporogenes* and *Yersinia enterocolotica* at 4mg/100µL concentration and more activity on *Escheichia coli* (Table 2). The hydro-alcoholic extract showed low activity on *Listeria monocytogenes* and more activity on *Escheichia coli* and *Staphylococcus aureus.* The antibacterial results of *Actiniopteris radiata* root extracts were comparable with standard drug ciprofloxacin, ciprofloxacin showed lower activity on *Listeria monocytogenes* and more activity on *Escheichia coli* and *Clostridium sporogenes.* The extracts of *A. radiata* showed more activity on gram -ve compared to gram +ve bacterial strains. On the whole, hydro-alcoholic extract showed better activity, hexane extract showed moderate and ethyl acetate extract showed low activity.

Now a days, different infectious diseases causing are major cause of mortality around the world, these infectious disease are decreasing the immunity and exposing the humans to easy side effects from other diseases (WHO, 2018). As said in introduction, the inadequate use of medicine (drugs) to control infections, the diseases and microorganisms getting resistance and becoming more powerful and leading to cause new diseases (Levy, 2002). So, there is a need to identify broad spectrum antibiotics to control different diseases with less side effects. This situation inducing the scientists to

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search for new antibiotics and there were many reports on antibacterial activity of different medicinal plants and some isolated bioactive compounds from them (Savoia, 2012). But, there were ample of traditional medicinal plants are not evaluated with scientific evidences about their biological activities including antibacterial activity. So, the current study was carried out on antibacterial activity of *A. radiata* root part and the results provides scientific evidence about its traditional use in traditional medicine and further research work will be worth full on its different biological activities and isolation of bioactive metabolites.

Table 1: Zone of inhibitions of *Actiniopteris radiata* hexane extract

Name of the microorganism	Concentration of the extract (mg/100µL)				Ciprofloxacin (100µg/200µL)	DMSO (100µL)
	4	8	15	25		
	Zone of inhibition (in mm)					
Streptococus pneumoniae	2.00	3.67±0.33	5.67±0.67	8.33±0.33	20.67±0.3	-
Staphylococcus aureus	1.33±0.33	3.33±0.33	6.33±0.33	8.67±0.33	19.67±0.33	-
Clostridium sporogenes	2.00	4.00±0.58	6.00±0.58	9.00	23.33±0.33	-
Listeria monocytogenes	1.33±0.33	2.33±0.33	4.67±0.33	7.00±0.58	18.67±0.58	-
Pseudomonas aeruginosa	1.33±0.33	2.67±0.33	4.33±0.33	8.00	21.33±0.33	-
Yersinia enterocolotica	2.00±0.00	3.67±0.33	7.33±0.33	9.33±0.33	20.67±0.33	-
Escheichia coli	1.67±0.33	4.00	7.00±0.58	9.33±0.88	24.33±0.67	-
Salmonella typhimurium	1.67±0.33	4.00	7.67±0.33	10.00±0.58	21.33±0.67	-

Table 2: Zone of inhibitions of *Actiniopteris radiata* ethyl acetate extract

Name of the microorganism	Concentration of the extract (mg/100µL)				Ciprofloxacin (100µg/200µL)	DMSO (100µL)
	4	8	15	25		
	Zone of inhibition (in mm)					
Streptococus pneumoniae	2.00	3.67±0.33	8.33±0.33	12.00±0.58	20.67±0.3	-
Staphylococcus aureus	1.67±0.33	4.00	8.67±0.33	12.67±0.88	19.67±0.33	-
Clostridium sporogenes	0.00	1.33±0.33	3.67±0.33	6.33±0.33	23.33±0.33	-
Listeria monocytogenes	0.67±0.33	2.00	4.67±0.33	8.00±0.58	18.67±0.58	-
Pseudomonas aeruginosa	1.67±0.33	4.33±0.33	8.00±0.58	11.67±0.33	21.33±0.33	-
Yersinia enterocolotica	0.00	2.00	4.67±0.67	7.33±0.67	20.67±0.33	-
Escheichia coli	2.00	5.00	9.33±0.67	13.00±1.00	24.33±0.67	-
Salmonella typhimurium	1.67±0.33	4.67±0.33	9.33±0.33	12.67±0.33	21.33±0.67	-

Table 3: Zone of inhibitions of *Actiniopteris radiata* hydro-alcoholic extract

Name of the microorganism	Concentration of the extract (mg/100µL)				Ciprofloxacin (100µg/200µL)	DMSO (100µL)
	4	8	15	25		
	Zone of inhibition (in mm)					
Streptococus pneumoniae	1.67±0.33	4.33±0.33	9.33±0.67	14.33±0.33	20.67±0.3	-
Staphylococcus aureus	2.67±0.33	5.67±0.33	10.67±0.33	15.67±0.33	19.67±0.33	-
Clostridium sporogenes	2.33±0.33	4.67±0.33	8.67±0.33	12.67±0.33	23.33±0.33	-
Listeria monocytogenes	0.67±0.33	2.33±0.33	4.67±0.33	8.67±0.33	18.67±0.58	-
Pseudomonas aeruginosa	2.00	4.33±0.33	8.67±0.67	12.33±0.33	21.33±0.33	-
Yersinia enterocolotica	2.00	4.00	8.33±0.33	12.00±0.58	20.67±0.33	-
Escheichia coli	2.67±0.33	5.67±0.33	11.00	16.33±0.33	24.33±0.67	-
Salmonella typhimurium	2.33±0.33	5.00±0.58	9.00±0.58	13.33±0.88	21.33±0.67	-

IV. CONCLUSION

Our study reveals the antibacterial activity of *A. radiata* root part extracts and results were comparable with ciprofloxacin.

ACKNOWLEDGMENTS

The authors are thankful to MAM College of Pharmacy, Kesanupalli, Narasaraopet for providing the laboratory facilities.

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