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Research Article

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In vitro antibacterial activity of the fern, *Actiniopteris radiata* (Sw.) Link. inhabiting the Shervaroyan Hills, the Eastern Ghats.

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ABSTRACT

The present study was undertaken to investigate the antibacterial activity of the fern, Actenopteris radiata by well diffusion method. The whole plant was extracted successively with certain alcoholic and aqueous solvents (petroleum ether, chloroform, acetone, methanol and water) by using soxhlet apparatus. Different concentrations of crude extracts such as 100, 200 and 300 µg/mL were prepared to found antibacterial activity. Chloramphenicol was used as a positive control. The results of the study revealed that water and methanolic extracts of the plant had strong antibacterial activity in all the concentrations than the other solvents used. Therefore, the plant extract may be used to control the diseases caused by pathogenic bacteria.

Key words: *Actiniopteris radiata*, Shervaroyan hills, Eastern Ghats, antibacterial activity**.**

INTRODUCTION

The use of plants as medicines is as old as human civilization itself. Many of the existing medicinal systems such as ayurveda, unani, homeopathy, naturopathy, siddha and other alternative medicinal systems have been utilizing plants as effective source of medicines to cure many diseases. According to World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs [1]. It is reported that more than rocky crevices 400, 000 plant species of tropical origin have medicinal properties [2,3]. About 80 % of individuals in developed countries are using traditional medicine, of plant origin. Therefore, such plants should be investigated to understand their properties, safety and efficacy and for a search of new potent antimicrobial compounds and fractions [4]. As a result of microbial resistance to available antibiotic and increasing popularity of traditional medicine, researchers around the globe are investigating the antibacterial compounds in different plants species [5,6,7].

Actiniopteris radiata is a tiny terrestrial fern found throughout India. It is of limited distribution, and in areas where it occurs is restricted to depleted walls and of steep slopes of exposed hilly areas, up to the altitude of 1200 m above msl [8]. This species has great medicinal value and according to the ayurvedic text it is used as styptic and anthelmentic [9], for the treatment of bronchitis and gynecological disorders and tuberculosis [10]. It is also having the properties of astringent, antiinflmmatory, antipyretic and alleviates vitiated blood, cough, asthma and bronchitis [11,12] and also increases fertility in women and in spermatorrhoea. Phytochemically, the species contains the bioactive compounds like quarcetin-3-rutinoside, hentriacontane, hentriacontanol, β-sitosterol palmitate, β-sitosterol, β-sitosterol-D-glucoside, alkaloids, glycosides, flavonoids, tannins, phenol, etc. [13].

EXPERIMENTAL SECTION

Collection of the plant material

Whole plant of the study species, *Actenopteris radiata* was collected from the Shervaroyan Hills, Coimbatore, Tamil Nadu and was washed under running tap water, air dried and homogenized to fine powder and stored in air tight bottles.

Preparation of plant extracts

50 g of air-dried powder was subjected to 250 mL of methanol in soxhlet extraction for 8 hours (50-65°C) by using successive solvent method (petroleum ether, chloroform, acetone, methanol and water). Then the extracts were evaporated to dryness.

Aqueous extract

For aqueous extraction 100 gms of air dried powder of *Actenopteris radiata* was immersed in 300 mL of distilled water in a beaker and kept for maceration for 7 days with occasional shaking. At the end of seventh day it was filtered through Buchner funnel and allowed to evaporate. The percentage yield of aqueous extract was 31.1% w/w.

Bacterial strains

Four Gram-positive bacteria viz., *Bacillus subtilis, Streptococcus faecalis, Enterococcus faecalis* and *Staphylococcus aureus* and four Gram-negative bacteria viz., *Escherichia coli, Salmonella paratyphii, Pseudomonas aeruginosa,* and *Serratia marcescens* were collected from Department of Microbiology, Hindusthan College of Arts and Science, Coimbatore.

Assay for antibacterial activity

The antibacterial activity of the extracts was systematically performed against the eight different strains of fore mentioned bacteria by following agar well diffusion method. About 30 mL of nutrient agar medium inoculated with the respective strain of bacteria (6 mL of inoculums to 300 mL of nutrient agar medium) was transferred aseptically into each sterilized Petriplate (10 cm diameter). The plates were left at room temperature to allow solidification. In each plate 5 wells of 8mm diameter were made with a sterile borer. Accurately 100, 200 and 300 µg/mL of the test solution was added separately to the wells aseptically and labeled accordingly. After incubation of the plates at 37±1°C for 24hrs, the diameter of the zone of inhibition surrounding each of the well was noted. The effects were compared with the standard drug, Chloramphenicol (5µg/mL).

Statistical analysis

The antibacterial activity of *A. radiata* whole plant extract was indicated by clear zones of growth inhibition. All experiments were performed in triplicates and the results are presented as mean ± SD (Standard Deviation). The significancy in the difference of mean was determined according to New Duncan's Multiple Range Test [14].

RESULTS AND DISCUSSION

The data obtained revealed that crude whole plant methanol and water extracts of *A. radiata* inhibits effectively the growth of the Gram-positive bacteria *viz., Bacillus subtilis* and *Staphylococcus aureus* and Gram-negative bacteria *viz., Escherichia coli* and *Salmonella paratyphii* at all concentrations (100 to 300µg/mL) than that of other solvent extracts used (Table 1). Similar trend of results of suppressing the growth of certain pathogenic bacteria by methanol and water extracts of the medicinal plant species *viz., Ocimum gratissimum, Vernonia amygdalina* and *Aframomum melegueta* was reported by Alo *et al.* [15] and *Acalypha alnifolia* by Evanjelene and Natarajan [16]. Wang *et al.* [17] and Jahan *et al.* [18] explained that increasing of extract concentration for many species also enhancing the inhibitory effect against the growth of microbes to certain extent. The growth response of bacterial strains to the plant extract in the present study varied widely. Higher zone of inhibition was noted in water extract of the study species, *Actenopteris radiata* against the Gram-positive bacteria *Pseudomonas aeruginosa* (24mm) and *Enterococcus faecalis* (23mm) at 300µg/mL and the minimum inhibition zone (5mm) was noticed in 100µg/mL against the bacterium *Bacillus subtilis.* The methanolic leaf extract of the study species also inhibited effectively the growth of the two bacteria *Bacillus subtilis* (16mm diameter of inhibition zone) and *Staphylococcus aureus* (15mm diameter of inhibition zone). However, some bacterial species were highly resistant and not inhibited by the plant extract at any concentration in the present study. The two Gram-negative bacteria *viz.,Pseudomonas aeruginosa* and *Serratia marcescens* were recorded no inhibition of growth in all the extracts of the study species, *A. radiata.* Acetone extract inhibit *Bacillus subtilis* and *Streptococcus faecalis* at 300µg/mL. Usha *et al.* [19] reported that acetone extract had moderate antibacterial activity against certain bacteria. The chloroform and petroleum ether extracts showed no activity against both Gram-positive and negative bacteria at all concentrations attempted. The antibacterial activity against Gram-negative bacteria showed only in methanol and water extracts against

Escherichia coli and *Salmonella paratyphii.* It showed the higher resistance of Gram-negative bacteria against the plant extracts.

From the above results, it was noticed that the antibacterial activity of *Actenopteris radiata* specific to Gram-positive bacteria than the Gram-negative bacteria. Gram-negative bacteria are more resistant than the Gram-positive bacteria [,20, 21, 22]. Generally it has been recorded that the Gram-positive bacterial strains are more susceptible than the Gram-negative bacterial strains to the plant extracts [23, 24]. This might be attributed to the fact that these two groups differ in their structure of the cell wall components and by the presence of their thick murein layer which prevents the entry of inhibitors. Further, the Gram-positive bacteria have single layered cell wall with higher amino sugar content, very little lipid and few amino acids where as the Gram-negative bacteria have two layered cell wall with lesser amount of amino sugars, and the lipid forming a major constituent along with variety of amino acids which characterized the cell wall resistant against any extract or organic chemicals [25]. In addition, unlike the Gram-positive strain, Gram-negative strains have an outer phospholipid membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes.

Table 1. Antibacterial activity of *Actenopteris radiata* using agar well method.

S. No.	Microorganisms	Zone of inhibition (mm)															
		Pet. Ether (µg/mL)			Chloroform (µg/mL)			Acetone (µg/mL)			Methanol (µg/mL)			Water (µg/mL)			*Control (µg/mL)
		100	200	300	100	200	300	100	200	300	100	200	300	100	200	300	5
Gram Positive																	
1.	*Bacillus subtilis*	-	-	-	-	-	-	-	-	8±0.21	7±0.08	10±0.41	16±0.04	5±0.23	-	10±0.05	28±0.31
2.	*Staphylococcus aureus*	-	-	-	-	-	-	-	-	-	7±0.34	10±0.16	15±0.10	7±0.11	9±0.31	24±0.34	30±0.27
3.	*Streptococcus faecalis*	-	-	-	-	-	-	-	-	8±0.09	-	-	-	6±0.05	8±0.50	18±0.17	27±0.31
4.	*Enterococcus faecalis*	-	-	-	-	-	-	-	-	-	-	-	-	7±0.60	10±0.18	23±0.22	25±0.50
Gram Negative																	
5.	*Escherichia coli*	-	-	-	-	-	-	-	-	-	6±0.08	10±0.27	15±0.13	5±0.31	9±0.41	18±0.18	18±0.64
6.	*Salmonella paratyphi*	-	-	-	-	-	-	-	-	-	7±0.41	16±0.10	16±0.18	8±0.29	10±0.15	20±0.34	20±0.15
7.	*Pseudomonas aeruginosa*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	30±0.27
8.	*Serratia marcescens*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20±0.34

** Chloramphenicol .*

Values were performed in triplicates and represented as mean ±SD.

Mean values followed by different superscripts in a horizontal row are significantly different (p<0.05).

CONCLUSION

The results of the study revealed that the water and methanol extracts of the fern, species *Actiniopteris radiata* exhibited higher antibacterial activity against Gram-positive bacteria. Further phytochemical studies are needed to elucidate the components responsible for antibacterial activity of these extracts against bacteria.

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