Academic Sciences

International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 4, Issue 2, 2012

Research Article

ANTIMICROBIAL EFFICACY OF SELECTED FERNS OF WESTERN GHATS, SOUTH INDIA

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Received: 10 January 2012, Revised and Accepted: 19 February 2012

ABSTRACT

The objective of this research was to investigate the *in vitro* antimicrobial activity of the ethanolic extracts of *Cyathea nilgiriensis* Holttum, *Cyathea crinita* (Hook.) Copel, *Leptochilus lanceolatus* Fee and *Osmunda hugeliana* Presl against five different microbial pathogens. Antibacterial and antifungal activity was determined using the paper disc diffusion assay with three different concentrations viz., 25, 50 and 100 µg/ml. The results revealed that the plant extracts showed significant antimicrobial activity. The maximum size of inhibition zone (2.2 cm) was observed in *C. crinita* against *Pseudomonas aureus* and *Klebsiella pneumonia* and followed by *L. lanceolatus* against *K. pneumonia* and *Aspergillus niger.* All the selected ferns show the inhibitory effect on four pathogens viz., *P. aureus, K. pneumonia, A. niger* and *Fusarium* sp. with the maximum inhibition in the highest concentration (100 µg/ml). *Streptococcus* sp. is resistant to all the tested plant extracts. The next step is to isolate the pure compounds and to screen the bioactivity of individual compounds which will lead to the development of new pharmaceuticals for therapeutic needs.

Keywords: Antibacterial, Antifungal, Inhibition zone, Pathogens

INTRODUCTION

Plants have a great potential for producing new drugs of large benefit to mankind[1]. The World Health Organization (WHO) estimates that 80% of the world's inhabitants rely mainly on traditional medicines for their health care. The tribal and rural people of various parts of India are highly depending on medicinal plant therapy for meeting their health care needs [2, 3]. This attracts the attention of several botanists and plant scientists who directing vigorous researches towards the discovery or rediscovery of several medicinal plants along with their medicinal remedies for various diseases [4, 5]. Many medicinal plants have proved to successfully aid in various ailments leading to mass screening for their therapeutic components. Today, the search for natural compounds rich in antimicrobial properties is escalating due to their medicinal importance in controlling many diseases [6]. The rapid emergence of multiple drug resistant strains of pathogens to current antimicrobial agents has generated an urgent intensive search for new antibiotics from medicinal plants [7-9]. Human infections constitute a serious problem especially in tropical and subtropical countries. The use of antibiotics and chemically synthesized medicines cures microbial infections very fast but they may also disturb the natural immunity of the body and cause variety of side effects. This has aroused interest in plant products which may partially support or substitute synthetic drugs. Medical communities are now trying to resolve the aforesaid problems from plant-based medicines [10-12].

The rich diversity of Indian medicinal ferns has been screened extensively for their antimicrobial potential worldwide [13-17]. The Pteridophytes are mostly distributed in the high altitude mountainous regions such as Himalayas, Western Ghats and Eastern Ghats. More than 300 species of ferns and fern allies are reported from the Western Ghats, South India [18]. Ferns show various economic values towards food and fodder indicators, biofertilizers, insect repellents, medicine and folk medicines [19]. The tree fern *Cyathea nilgirensis,* which is endemic to South India, has analgesic and antidiabetic activities[20]. The related species *Cyathea gigantea* (Wall. ex Hook.) Holttum has free radical scavenging activity, anti-inflammatory and hepatoprotective effects [21-23]. *Osmunda regalis* is also used as medicinal herb. The root is astringent, diuretic, tonic and vulnerary. It is useful in the treatment of jaundice and removing obstructions of the viscera. The fronds are used to make compresses for external application to wounds and rheumatic joints - for which purposes they are fairly effective. An infusion of the fronds, combined with wild ginger roots has been used in the treatment of children with convulsions caused by intestinal worms [24]. *Osmunda regalis* is locally considered a highly efficient remedy. The rhizome has been traditionally employed in Cantabria mainly for the treatment of bone fractures, joint disorders and rheumatic and arthritic pain[25]. With this, knowledge, the present study was aimed to investigate the antibacterial and antifungal efficacy of selected ferns of Western Ghats, South India.

MATERIALS AND METHODS

Healthy, disease free ferns of *Cyathea nilgiriensis* Holttum, *Cyathea crinita* (Hook.) Copel., *Leptochilus lanceolatus* Fee and *Osmunda hugeliana* Presl were collected from Palni hills of Western Ghats, South India. The whole plants were air and shade dried for two weeks at room temperature and pulverized to powder using mortar and pestle. The powered materials (50 g) were extracted successively with 200 ml of ethanol by using Soxhlet extractor for 8 h at a temperature not exceeding the boiling point of the solvent. The ethanolic extracts were filtered using Whattman filter paper (No.1) and then concentrated in vacuum at 40°C using Rotary evaporator. The residues obtained were stored in a freezer -70° C until further tests [26]. Anti-bacterial and Anti-fungal studies were carried out by disc diffusion method [27, 28] against the pathogens viz., Proteus *aureus, Klebsiella pneumoniae*, *Streptococcus* sps*, Aspergillus niger* and *Fusarium* sps*.* Different concentrations of extracts ranging from 25, 50 and100 µg/ml were used for bacterial and fungal sensitivity test. The inhibition zone against the pathogenic bacteria and fungi were recorded. The experiments were repeated in triplicates and the results were documented.

RESULTS

The results of antibacterial and antifungal studies on ethanolic extracts of selected ferns using disc diffusion method have been given in Table 1. The results revealed that the plant extracts showed significant antibacterial and antifungal activity with three different concentrations viz., 25, 50 and 100µg/ml. The maximum degree of zone of inhibition (2.2 cm) was observed in *C. crinita* against *P. aureus* and *K. pneumonia* and *L. lanceolatus* against *K. pneumonia* and *A. niger.* Different concentrations (25, 50 and 100 µg/ml) of ethanolic extracts of selected ferns show the inhibitory effect on four susceptible pathogens viz., *P. aureus, K. pneumonia, A. niger* and *Fusarium* sp. with the maximum inhibition in the highest concentration (100 µg/ml). *Streptococcus* sp. is resistant to all the tested plant extracts.

The ethanolic extract of *C. nilgirensis* showed the antibacterial activity against two pathogens viz., *P. aureus* and *K. pneumoniae* with the inhibition zones 2 and 1.8 cm and antifungal activity against two selected pathogens viz., *A. niger* and *Fusarium* sp. with 1.8 and 1.2 cm of inhibition zones respectively. *C. crinita* exhibited the antibacterial activity against *P. aureus* and *K.*

pneumoniae with the inhibition zone of 2.2 cm and antifungal activity with inhibition zones of 1.8 and 1.2 cm respectively against *A. niger* and *Fusarium* sp. The antibacterial activity of *L. lanceolatus* was observed against *P. aureus* and *K. pneumoniae* with the maximum zone of inhibition 1.8 and 2.2 cm respectively and antifungal activity only against *A. niger* with 2.2 cm of zone of inhibition. *Fusarium* sp. is resistant to ethanolic extract of *L. lanceolatus. O. hugeliana* exhibited the antibacterial activity with the maximum zone of inhibition 2 cm against *P. aureus* and *K. pneumonia*. The antifungal activity was observed against *A. niger* and *Fusarium* sp. with 1.6 and 1.2 cm of inhibition zones respectively.

Table 1: Anti-bacterial and Anti-fungal activity of selected species of Pteridophytes

Name of the Species	Conc. in µg/ml	Name of the Microorganisms (Zone of inhibition in Cms)			
		P. aureus	*K. pneumoniae*	*A. niger*	*Fusarium* sp.
Cyathea nilgiriensis	25	1.0	0.8	0.0	0.0
	50	1.5	1.2	1.0	0.0
	100	2.0	1.8	1.8	1.2
Cyathea crinita	25	1.2	1.0	0	0.0
	50	1.8	1.6	1.2	0.0
	100	2.2	2.2	1.8	1.2
Leptochilus lanceolatus	25	0.0	0.0	0	0.0
	50	1.2	1.4	1.4	0.0
	100	1.8	1.4	2.2	0.0
Osmunda hugeliana	25	1.1	0.8	0	0.0
	50	1.6	1.4	1.0	0.0
	100	2.0	2.2	1.6	1.2

DISCUSSION

Due to risk of adverse effects encountered with the use of synthetic antibiotics, medicinal plants may offer an alternative source for antimicrobial agent with significant activity against pathogenic and infective microorganisms. In addition, a number of antibiotics have lost their effectiveness due to the development of resistant strains, mostly through the expression of resistance genes [29]. Because of this reason, new antibiotics are discovered to control the infectious disease causing pathogens. In this regard, higher plants play an important role by providing antibiotic compounds which are rich in active principles and used as therapeutic drugs [30]. Very less work has been done on the antimicrobial activity of pteridophytes and ethnobotanical importance of these plants. They reported that these plants are of by great medicinal importance and are used by the tribal and local people for remedy against various ailments [31-37]. In the present study, the broad spectrum of *in vitro* antimicrobial activity of selected ferns may be due to the presence of some bioactive principles in the extract. These active compounds may act alone or in combination to inhibit microbial growth.

Antibacterial activity has been confirmed previously by Toji Thomas[38] in the Royal Fern, *Osmunda regalis*. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration values of acetone extract of 12.5mg/ml and 25mg/ml have been observed for *Pseudomonas aeruginosa* while MIC and MBC values of 25mg/ml and 50mg/ml have been observed for *Shigella sonnei.* The acetone extract of *Osmunda regalis* exhibited maximum activity compared to others. The plant did not show antibacterial activity towards *Serratia marcescens*, *Salmonella paratyphi, Klebsiella pneumoniae and Shigella dysentriae*. Acetone extract *showed* maximum antibacterial activity towards *Pseudomonas aeruginosa and Shigella sonnei* [38]. Flavonoids, phenols, steroids have been detected in acetone and methanol extracts of *Osmunda regalis*[38]. The rodlet-shaped wax crystals on fronds of *Osmunda regalis* consist of 139 compounds belonging to 14 homologous. They included typical plant wax constituents: alkanes (C_{25}–C_{33}), alkyl esters (C_{38}–C_{50}), primary alcohols (C_{22}–C_{32}), secondary alcohols (C_{27} and C_{29}), ketones (C_{27}–C_{33}), aldehydes (C_{24}–C_{34}), fatty acids (C_{24}–C_{32}), and b-sitosterol[39]. *Cyathea gigantea* and *Cyathea brunoniana* show the presence of steroid, flavonoid, and saponin[40].

All the above known along with unknown chemical compounds may have antibacterial and antifungal effects[41-42]. The overall study on antimicrobial activity of selected ferns suggests that it contains adequate variety of bioactive compounds to reduce or check the growth of microbial colonies. Further studies are recommended to purify the active compounds responsible for the formulation of new drugs while going for commercialization.

Conflict of interest statement

We declare that we have no conflict of interest.

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