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RESEARCH ARTICLE

DETERMINATION OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF ENDEMIC TREE FERN CYATHEA NILGIRENSIS PLANT EXTRACT

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ABSTRACT

Cyathea nilgirensis Holttum, a rare, southern Indian endemic tree fern is found in Kathalekan forest of Central Western Ghats. The preliminary phytochemical studies revealed the presence of carbohydrates, alkaloids, flavonoids. Secondary metabolites identified in the plant material have been reported to have an inhibitory action against pathogenic microorganisms. An experiment was conducted to determine the antibacterial and antifungal activities of *Cyathea nilgirensis.* The antibacterial activity of plant extracts against the test organisms with varying zones of inhibition ranging from 7.00±1.00 to 10.00 ± 1.00 has revealed the antibacterial potency of the plant. The zone of inhibition of fungi ranging from 7.00 ± 0.50 to 12.50± 0.50, indicates that the plant exhibit antifungal potency.

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INTRODUCTION

Nature worship is an integral part of most human societies since pre-historic era. Central Western Ghats known for its numerous sacred forests and sacred sites of pre-colonial village communities in the Uttar Kannada district of Karnataka state, part of the state reserve forests continue to shelter rare elements of biodiversity. The Myristica swamps, confined to merely few remnant patches in the southern hills of the district. Interestingly, one among them is *Cyathea nilgirensis. Cyathea nilgirensis* Holttum, a southern Indian endemic tree fern (Fraser - Jenkins 2008) was seen growing in the deep shade of the swampy forest of Uttar Kannada. The tree genus Cyathea belongs to the family Cyatheaceae which has 241 species and four of them are distributed throughout the mountainous regions of the world. (Khare *et al*., 2009). India has 11 species of Cyathea. (Dixit, 1984) of which *Cyathea nilgirensis* was listed 'Endangered' (Walter *et al.,* 1998). The plant is scattered in restricted pockets in the shade and damp stream side forests of Kerala, Karnataka, Tamil Nadu, and Andhra Pradesh.

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It was accounted from Kemmangundi and Charmadi Ghats of Chikmagalur district, Mercara- Bhagamandala Talacaveri stretch in Kodagu district. (Rajagopal *et al*., 1998) and also from the south of Uttar Kannada, Central Western Ghats. Red listing of Indian pteridophytes and as a lone population of a few individuals of *Cyathea nilgirensis* survives precariously threatened swamps of Kathalekan in Uttar Kannada.

Description of the plant

The plant belongs to the family Cyatheaceae. It is endemic to South India and 13 cm in diameter, it is unbranched ; bearing crown of fronds at the apex and scales densely covering the younger fronds. Stipes up to 40 cm long swollen at base bearing small hairs. Lamina bipinnate, oblong, lanceolate, pinnae about 12 pairs, alternate, distinct petiolate, pale green below, dark green above, texture herbaceous. Sori situated in the vein forks of the lower half of the segments, exindusiate, paraphyses intermingled with sporangia, spores trilete. Grows as terrestrial species along shaded stream banks (Manickam and Irudayaraj 1992).

MATERIALS AND METHODS

Herbarium

Cyathea nilgirensis Holttum an endemic tree fern is collected from Kathalekan (kathale-dark, Kan-sacred forest) relic forest

in Uttar Kannada district of Central Western Ghats in Karnataka. The herbarium is archived at the JCB herbarium Centre for Ecological Sciences, Indian Institute of Science, Bangalore. The allotted number of herbarium specimens are HJCB 189 (a), HJCB189 (b).

Phytochemical analysis

The phytochemical screening of the crude extract was carried out in order to ascertain the presence of secondary metabolites such as carbohydrate, saponins, alkaloids, flavonoids, steroids, tannins, glycosides, terpenoids by following the standard method described by Trease and Evans (1987) and Harborne (1973).

Collection of test organisms

The antibacterial activity was tested against *Klebsiella pneumoniae* MTCC 7407, *Staphylococcus aureus* MTCC 7443, *Escherichia coli* MTCC 7410, *Bacillus subtilis* MTCC 121 and the antifungal activity was tested against *Candida albicans* MTCC 183, *Fusarium solani* MTCC 4289, *Aspergillus ochraceus* MTCC 1877, *Fusarium verticillioides* MTCC 10726.

Plant extraction

The collected plant material was shade dried and powdered using a commercial blender. The powdered plant material was sequentially extracted by using magnetic stirrer 200 RPM, using hexane, petroleum ether, chloroform, ethanol and methanol with increasing polarities, starting with the least polar solvent.

Determination of antimicrobial activity

Disc diffusion method, (Bauer *et al.,* 1966) The hot, sterile Muller Hinton agar and Sabouraud Dextrose agar medium were poured into the sterile petri plates to form a thick, uniform layer and allowed to solidify. Freshly grown test pathogen cultures were inoculated on Muller Hinton agar plates for bacterial and Sabouraud dextrose agar for fungal cultures.

Sterile Whatman No.1 discs of 6mm diameter were soaked with 10µL of 100 mg/mL and 200mg/mL of the solvent and were placed on Agar plates. Standard ciprofloxacin 10µL 1mg/ mL for bacteria and Fluconazole 10 µL of 10mg/ mL were used as a positive control for fungal cultures. The plates were incubated at 35^0 C for 24-48 hrs.

After the incubation period the plates were observed for the zone of inhibition around the discs, which indicates a positive antimicrobial activity of the test compounds. The zone of inhibition was measured. Each experiment was carried out in triplicates. The mean ±SD of the inhibition zone was taken for evaluating the antimicrobial activity of the plant extracts.

RESULTS AND DISCUSSION

Preliminary phytochemical studies revealed the presence of carbohydrates, alkaloids, flavonoids. Secondary metabolites identified in the plant material have been reported as having inhibitory action against pathogenic microorganisms, (Freeman *et al*., 2008; Xiao-tian *et al*., 2006). Alkaloids which are one of the largest of groups of phytochemicals in plants have greater effects on humans and this has led to the development of pain killer. Flavonoids have hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found in vitro to be effective anti-microbial substances against a wide array of microorganisms.

Table 1. Phytochemical constitute of the Hexane, Chloroform and Methanol extracts

Phytochemical	Hexane extract	Chloroform extract	Methanol plant extract
Carbohydrate	+	+	-
Alkaloids	_	+	+
Phlobatannins	_	_	_
Tannins	_	_	_
Saponins	_	_	_
Flavonoids	_	_	+
Terpenoids	_	_	_
Steroids	_	_	_
Glycosides	_	_	_

Table 2. Inhibitory activity of extracts on test organisms

Test Compound	Concentration (10µL, mg/mL)	Zone of Inhibition (in mm)			
		Test organisms			
		Klebsiella Pneumoniae	*Escherichia coli*	*Staphylococcus aureus*	*Bacillus subtilis*
Ethanol control		0.00	0.00	0.00	0.00
Methanol Control		0.00	0.00	0.00	0.00
Chloroform control		0.00	0.00	0.00	0.00
Petroleum ether control		0.00	0.00	0.00	0.00
CC	100	8.00 ± 1.00	0.00	0.00	0.00
CE	100	0.00	0.00	7.00 ± 1.00	10.00 ± 1.00
CM	100	0.00	0.00	0.00	0.00
CP	100	7.00 ± 1.00	0.00	8.00 ± 0.00	0.00
Ciprofloxacin	1	15.0 ± 1.00	12.0 ± 0.50	13.0 ± 1.00	35.0 ± 1.00

Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls (Kamalakannan *et al.,* 2012).

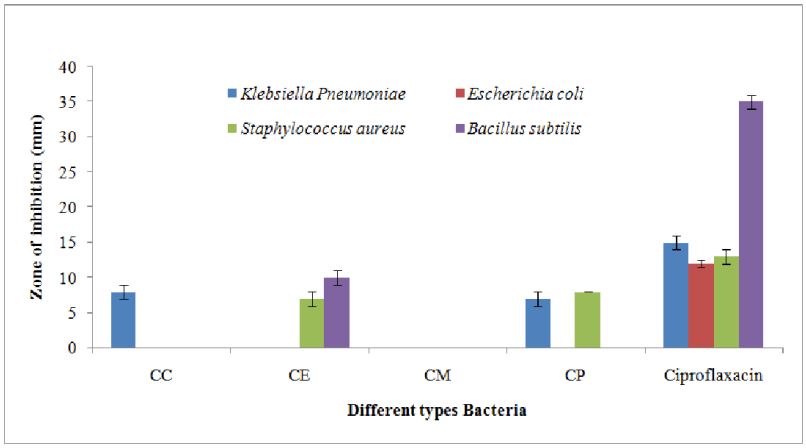

Fig. 1. Inhibitory activity of extracts on Different types of Bacteria

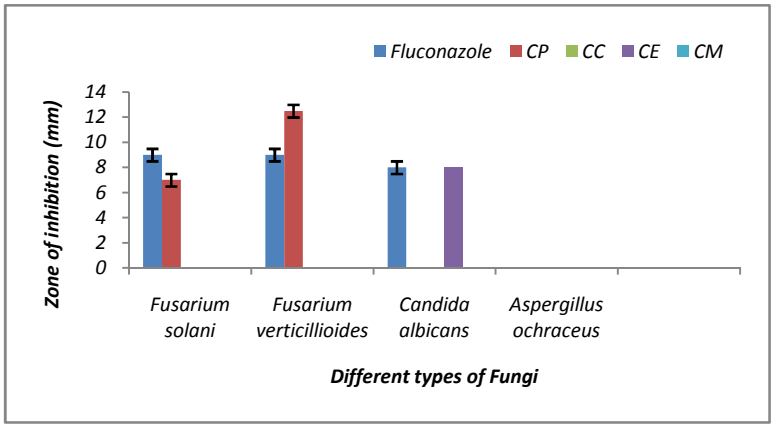

Fig. 2. Inhibitory activity of extracts on Different types of fungi

Table 3. Inhibitory activity of extracts on test organisms

Test Compound	Concentration (10µL, mg/mL)	Zone of Inhibition (in mm)			
		Test organisms			
		Fusarium solani	*Fusarium verticillioides*	*Candida albicans*	*Aspergillus ochraceus*
Ethanol control	10ul	0.00	0.00	0.00	0.00
Methanol Control	10ul	0.00	0.00	0.00	0.00
Chloroform control	10ul	0.00	0.00	0.00	0.00
Petroleum ether control	10ul	0.00	0.00	0.00	0.00
CC	100	0.00	0.00	0.00	0.00
CE	100	0.00	0.00	8.00± 0.00	0.00
CM	100	0.00	0.00	0.00	0.00
CP	100	7.00 ± 0.50	12.50 ± 0.50	0.00	0.00
Fluconazole	10	9.00 ± 0.50	9.00 ± 0.50	8.00 ± 0.50	0.00

The plant extract of *Cyathea niligernsis* posses both antibacterial as well as antifungal activity. The antibacterial activity of petroleum ether, chloroform ethanol and methanol extracts of *Cyathea nilgirensis* and standard ciprofloxacin were inspected against the selected experimental pathogens such as *Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Bacillus subtilis* by disc diffusion methods.

The results representing the antibacterial activity of plant extracts are reported in Table 2. *K. pneumoniae* was more affected by chloroform and petroleum ether, *S.aureus* was inhibited by extracts of ethanol and petroleum ether, *B. subtilis* was affected by ethanol plant extract, where as *E. coli* did not exhibit any inhibition.

Gram negative bacteria are frequently reported to have developed multi-drug resistance to many of the antibiotics currently available in the market of which *E. coli* is the most prominent, (Alonso *et al*., 2000: Sader *et al.,* 2002). Therefore, it is not surprising that *E .coli* was the least responding bacterial strain to the test plant extracts. The antibacterial activity of plant extracts against the test organisms with varying zones of inhibition ranging from 7.00±1.00 to 10.00 ± 1.00 has revealed the antibacterial potency of *Cyathea nilgirensis*. Antifungal activity of plant extracts of *Cyathea nilgirensis* and standard Fluconazole (10µL/mL) was determined against 4 pathogens, they are *Fusarium solani*, *F.verticillioides, Candida albicans* and *Aspergillus ochraceus*

by disc diffusion method. The results were summarized in Table 3. F. *solani and F. verticillioides* exhibited zone of inhibition only in the petroleum ether extract. *Candida albicans* showed zone of inhibition in ethanol extract, where as *A.ochraceus* was not affected by any of the extracts. The zone of inhibition ranging from 7.00 ± 0.50 to 12.50 ± 0.50, indicates that the plant possess antifungal potency.

Hence it can be concluded that the plant *Cyathea nilgirensis* exhibits antibacterial and antifungal activity. It can be used in the folk medicine and as a source of antibiotic for possible treatment of many diseases. However, to know the mechanism of action of the plant *Cyathea nilgirensis* extract, further studies with purified fractions / bioactive compounds are warranted.

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