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ANTIMICROBIAL EFFICACY OF *BLECHNUM ORIENTALE* L.

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ABSTRACT

The antimicrobial property in the extracts of *Blechnum orientale* L. has been evaluated on clinically isolated bacterial pathogens such as *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi, Mycobacterium luteus, Pseudomonas aeruginosa* and *Escherichia coli.* Also the antifungal activity was tested on *Candida albicans* and *Aspergillus flavus.* The pet-ether, chloroform, methanol and aqueous extracts were used for the study of antimicrobial activity by agar well diffusion, micro titre method and tube dilution methods. Results indicated that all the four extracts were effective against *E. coli*, *P. aeruginosa* and *C. Albicans,* . Aqueous extract showed relatively higher zone of inhibition against *E. coli, P. aeruginosa* and *C. albicans* while *S. aureus, B. subtilis, S. typhi, M. luteus* and *A. flavus* were not found to be sensitive to all the extracts.

KEYWORDS: ***Blechnum orientale,*** **Solvent extraction,Bacteria,Fungi**

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INTRODUCTION

Pteridophytes by virtue of their possessing great plant variety and fascinating foliage have drawn the attention and admiration of horticulturists and botanists for centuries. Recently, Fraser Jenkins revised pteridophytic numbers to be 1000 species in Indian origin[5,6]. Medicinal value of Pteridophytes is known to man for more than 2000 years. After the introduction of ethnobotanical survey, many attempts are made on scientific research regarding the medicinal importance of pteridophytes [18]. A number of angiosperms with significant antimicrobial activity have been reported in literatures[7,8]. However, very less information is available at present in the literature regarding antimicrobial activity of pteridophytes. Now it is aimed to explore scientifically the antimicrobial potential frond of *Blechnum orientale* L. and substantiate the folklore claims. *Blechnum orientale* is belongs to family Blechnaceae and distributed in North Western Himalaya, Eastern Himalaya and Peninsular India. It has very good medicinal values used in the treatment of diarrhoea and stomach problems[20, 22]. The plant used as poultice for boils by tribal communities in central India[17]. The rhizome showed antibacterial activity especially in acetone extract[19]. The present study therefore aims to determine the antimicrobial potential of *B. orientale.*

MATERIALS AND METHODS

(i) The extraction of B. orientale

Fronds of *B. orientale* were collected from Tarikere taluk of Chikmagalur district, Karnataka during the period of 2010-2011. Specimen was identified by referring to available literature and Pteridophytic Floras[1,2,3,12]. The species identity was kindly confirmed by Prof. emeritus C R Fraser-Jenkins, Kathmandu, Nepal. The collected specimen was properly processed as herbarium specimen (KU/ST/09-DPN06), which has been deposited in the Department of Applied Botany, Kuvempu University. The fronds were shade dried, powdered and subjected to Soxhlet extraction using pet-ether, chloroform, methanol and aqueous successively[21]. The resulting extracts were evaporated in vacuum to dryness and stored in desiccators for future use.

(ii) Microorganisms tested

The microbial cultures were *Staphylococci aureus* (NCCS-2200), *Mycobacterium luteus* (NCCS-2169), *Bacillus subtilis* (NCCS-2079), *Pseudomonas aeruginosa* (NCCS-2079), *Escherichia coli* (NCCS-265), *Salmonella typhi* (NCCS-2079), *Candida albicans* (NCCS-3471) and *Aspergillus flavus* (NCCS-1196) procured from National Centre for Industrial Microorganisms (NCIM), Pune, India.

(iii) Minimum Inhibition zone

Sterile NA plates were prepared and 0.1 ml of the inoculum from standardized culture of test organism was spread uniformly. Wells were prepared by using a sterile borer of diameter 10mm and 100µl (To get the final concentration of 1000 and 500 µg/well) of the test substance, standard antibiotic were added in each well separately. A standard antibiotic, ciprofloxacin was tested against bacteria and Fluconozole for fungi. The plates were incubated at a temperature optimal for the test organism and for a period of time sufficient for the growth of at least 10 to15 generations (usually 24 hours at 37^0C). The zone of inhibition of microbial growth around the well was measured in mm[13,16].

(iv) Minimal Inhibitory Concentration (MIC) for Bacteria

A sterile 96 well plates were labelled. A volume of 100 µl of test material in DMSO was pipetted into the first row of the plate. To all other wells 50 µl of sterile broth was added. Tips were discarded after use such that each well had 50 µl of the test material in serially descending concentrations. To each well 10 µl of resazurin indicator solution was added. Using a pipette 30 µl of sterile broth was added. Finally, 10 µl of microbial suspension (0.5 Macfarland) was added to each well. Each plate was wrapped loosely with cling film to ensure that cultures did not

become dehydrated. Each plate has a set of positive, negative and a standard. The plates were prepared and placed in an incubator at 37 °C for 18–24 h/ 28 °C for 48 h. The colour change was then assessed visually. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value[15].

(v) Minimal Inhibitory Concentration (MIC) for Fungi

For this assay[9], a series of assay tubes were prepared containing uniform volume (1ml) of sterile SD broth and equal volume of known concentration of test substance was added. The test substance in the first tube was serially diluted in twofold decreasing concentrations through the sixth tube and seventh tube was left without test substance as positive control. The tubes with the test substance i.e. from one to seventh were inoculated with 1 ml of inoculum ($1x10^6$ CFU per ml). The final concentration of test substance ranged from 1000 to 31.25 µg per ml. Solvent control and sterility controls were maintained in the experiment. The tubes were incubated at 28^0C for 48 h. Standard antibiotic; Fluconozole was tested as standard drug at concentrations ranging from 100 to 3.12 µg per ml. The tubes were inspected visually to determine the growth of the organism as indicated by turbidity, the tubes in which the antibiotic is present in concentration sufficient to inhibit fungal growth remain clear. In experimental terms the MIC is the concentration of the drug present in the last clear tube, i.e. in the tube having the lowest concentration in which growth is not observed.

RESULTS AND DISCUSSION

The frond extract of *B. orientale* had tested *in-vitro* potential of antimicrobial activities against six bacterial including gram positive and gram negative and two fungal species tested[8]. The extracts were tested at 1000 and 500 µg/ml concentration against both bacterial and fungal strains. The data obtained from the Agar well diffusion method (Table 1) indicated that all the four extracts showed high degree of antimicrobial activity on *E. coli*, *P. aeruginosa* and *C. albicans.* However, the plant did not show inhibition activity towards *S. aureus, B. subtilis, S. typhi, M. luteus* and *A. flavus.* The Chloroform and aqueous extract showed maximum effect against *E. coli, P. aeruginosa* and *C. albicans* with the strongest inhibition zones at 1000 µg/ml fallowed by pet-ether and methanol extract. In addition, the aqueous extract was also effective against *E. coli* with inhibition zone than Chloroform, Methanol and pet-ether extract at 500 µg/ml. Present result concerning the strongest antimicrobial activity in aqueous extract. Similarly, Dahot[4] showed that the aqueous extract of plant leaves investigated showed significant antimicrobial activity compare to the other organic counter parts. The results of minimum inhibitory concentration (MIC) were shown in Table 2. The result indicated that all the four extracts showed sensitive against *E. coli, P. aeruginosa* and *C. albicans.* While Chloroform and aqueous extracts showed great sensitivity against *E. coli* at 250 µg/ml. *P. aeruginosa* was maximum inhibited by all extracts at 250 µg/ml level. *Staphylococcus aureus, B. subtilis, S. typhi, M. luteus* and *A. flavus* were not sensitive against all the four extract because the MIC values not detected up to 1000 µg/ml. The inactivity against all Gram-positive bacteria tested could be due to the impermeable nature of the components in outer membrane[14]. When compared with earlier reports on the antibacterial activity of *B. orientale,* Lai et al[11], reported no activity against *E. coli* and *P. aeruginosa* while present study showed strong inhibition activity against those organisms.

Table 1
Zone of inhibition by frond extract of Blechnum orientale L. (mm)

Solvent extract	Gram +ve strains			Gram –ve strains			Fungal strains	
	Mi	Sa	Bs	Ec	Pa	St	Ca	Af
Methanol	-	-	-	21.7±0.16[a] (18±0.06)[b]	12.0±0.15[a]	-	12.0±0.06[a]	-
Pet-ether	-	-	-	22.0±0.12[a] (16.13±0.09)[b]	12.13±0.08[a]	-	11.13±0.15[a]	-
Chloroform	-	-	-	24.0±0.09[a] (20.2±0.17)[b]	11.0±0.06[a]	-	11.0±0.09[a]	-
Aqueous	-	-	-	24.0±0.18[a] (20.0±0.06)[b]	14.13±0.09[a]	-	12.13±0.09[a]	-
Ciprofloxacin	40.0	40.1	42.1	36.12	36.12	46.0	-	-
Fluconozole	-	-	-	-	-	-	25.12	19.0

Note: Values are mean±S.D of the triplicate, ' [a]' Value at 1000 µg/ml, ' [b]' Value at 500 µg/ml, '-' Zero inhibition, Ml: Mycobacterium luteus; Sa: Staphylococcus aureus; Bs: Bacillus subtilis; Ec: Escherichia coli; Pa: Pseudomonas aeruginosa; St: Salmonella typhi; Ca: Candida albicans; Af: Aspergillus flavus.

Table 2
The MIC value for the different solvent extract of Blechnum orientale L. (µg/ml)

Sl. No.	Solvent extract	Gram +ve strains			Gram –ve strains			Fungal strains	
		Mi	Sa	Bs	Ec	Pa	St	Ca	Af
1	Methanol	>1000	>1000	>1000	500	250	>1000	1000	>1000
2	Pet-ether	>1000	>1000	>1000	500	250	>1000	1000	>1000
3	Chloroform	>1000	>1000	>1000	250	250	>1000	1000	>1000
4	Aqueous	>1000	>1000	>1000	250	250	>1000	1000	>1000

Ml: Mycobacterium luteus; Sa: Staphylococcus aureus; Bs: Bacillus subtilis; Ec: Escherichia coli; Pa: Pseudomonas aeruginosa; St: Salmonella typhi; Ca: Candida albicans; Af: Aspergillus flavus.

CONCLUSION

Results of this study indicated that all four extracts have almost similar against the limited organisms. Thus in search of novel broad spectrum antimicrobial agent for particular pathogens, the formulation comprising different proportions of these extracts may be proved good. This study has shown the scientific basis for some of the therapeutic uses of traditional plant, *B. orientale* and supported its ethnobotanical importance.

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