

A Study on Antimicrobial Activity of *Nephrolepis Biserrata* from Eastern Ghats of India, Visakhapatnam District, Andhra Pradesh

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Abstract *Nephrolepis biserrata* (Giant Sword Fern) is evaluated for antimicrobial activity against six bacterial strains and four fungal pathogens by Agar well diffusion method. Distilled Water, Chloroform, Methanol and Ethyl Acetate extracts of different concentrations are used in the study. From the study, it is observed that the methanol extracts of the plant species showed higher zone of inhibition against *Bacillus subtilis* (gram positive), *Klebsiella pneumonia* (gram negative) and *Candida albicans* (Fungal strains). Hence, *Nephrolepis biserrata* can be used in the treatment of ailments caused by the resistant pathogenic micro organisms.

Keywords: *Nephrolepis biserrata*, Leaf Extracts, Antimicrobial activity, Zone of inhibition, Micro organisms, Agar well diffusion method.

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I. Introduction

India has rich variety of plants with applications in medicine and treatment of several ailments. Many native plant species are being used as traditional medicine locally but, they are not reported globally. Hence, it is essential to evaluate their antimicrobial activity which will be helpful in the preparation of improved herbal or drug formulations. Among various plants, Pteridophytic flora is one such plant species which are resistant to pathogenic micro organisms. This is one of the important factors influencing the longer periods of survival of pteridophytes¹.

Several researchers have conducted studies on antimicrobial activity of various Pteridophytes. Based on antimicrobial studies on *Polypodium vulgare*, Hafler² and Wafa³ et al., it was reported that it can be effectively used in manufacture of medicines and treatment of cough, colds, adenoids etc. Farzana⁴ et al. reported that *Dryopteris* species, *Pteris vittata* and *Nigella sativa* would be helpful in treating antibiotic resistant gram positive bacteria. The maximum antimicrobial activity was exhibited by the methanolic extract of *Adiantum venustum* followed by *Adiantum capillus-veneris*, *Adiantum peruvianum* and *Adiantum caudatum*⁵. Most of the *Asplenium* fern species showed strong antibacterial activity against gram negative bacteria and used for treating cold, cough, cancer^{6,7} etc .

Dolly Rani⁸ et al. reported that *Nephrolepis biserrata* of water extract was less inhibitory to the growth of *Enterobacter aerogenes* and most inhibitory against *Escherichia coli*. *Nephrolepis biserrata* exhibited an inhibition zone of 8.2mm and 8.3mm against *Aeromonas hydrophila* and *Vibrio parahaemolyticus* respectively in methanolic extracts of 50mg/mL. Bioactive compounds from the extract of *Nephrolepis biserrata* are considered to significantly inhibit bacterial growth⁹. The studies on the antimicrobial activity of *Nephrolepis biserrata* at higher concentrations of organic solvent extracts are not carried out. Hence, the present study is carried out to evaluate antimicrobial activity of different solvent extracts of *Nephrolepis biserrata* at different concentrations of 100mg/ml, 150mg/ml and 200mg/ml against 3 gram positive, 3 gram negative and 4 fungi strains. The anti microbial studies are carried out as per NCCLS.^{10,11,12}

II. Material and Methods

For the anti-microbial activity study, *Nephrolepis biserrata* of *Nephrolepidaceae* family is collected from Galikonda hills of Visakhapatnam district, Eastern Ghats of India. Leaves and stem of *Nephrolepis biserrata* are dried under shade and finely grounded into powder. The fine powder is sequentially extracted by different solvents in a specific order based on increasing polarity through soxhlet extraction process.

Test micro organisms:

Antimicrobial activity is studied with respect to 4 fungi, 3 gram positive and 3 gram negative bacterial strains procured from Microbial Type Culture Collection and Gene Bank (MTCC). The fungal species considered for anti-microbial study are *Aspergillus fumigates*, *Candida albicans*, *Rhizopus Oryzae* and *Alternaria alternata*.

The bacterial species used in the studies on pteridophytes are *Klebsiella Pneumoniae* (gram negative), *Salmonella enteric* (gram negative), *Shigella boydii* (gram negative), *Streptococcus mutans* (gram positive), *Clostridium Perfringens* (gram positive) and *Basillus Subtilis* (gram positive).

In this study, the finely ground powder of *Nephrolepis biserrata* is dissolved in Distilled Water, Chloroform, Methanol and Ethyl Acetate extracts of different concentrations 100mg/ml, 150mg/ml and 200mg/ml is analyzed for its antimicrobial activity. Antimicrobial studies are performed as per ICC.

Antibacterial activity of different solvent extracts of *Nephrolepis biserrata* is evaluated using Agar well diffusion method^{13,14}. Nutrient agar medium is sterilized and inoculated with 3µL aliquots of culture (10⁵ CFU/ml) of test organisms and transferred into sterile petri dishes. It is allowed to set at room temperature for 10 minutes and then placed in refrigerator for 30 minutes. After setting, a number 3-cup borer (6mm) diameter is properly sterilized by flaming and used to make three to five uniform cups/wells in each Petri dish. A drop of molten nutrient agar was used to seal the base of each cup. The cups/wells were filled with 50µl of the extract concentration of 100mg/ml, 150 mg/ml, 200 mg/ml and allowed to diffuse for 45 minutes. The plates are then incubated at 37°C for 24hours for bacteria and at 25°C for 48 hours for fungi. The agar plates are then examined for inhibition zones and the zones are measured in millimetres.

In the present study, Ciprofloxacin (10µg/ml) and Fluconazole are used as standard drugs for antibacterial and antifungal studies respectively. 0.1% Dimethyl Sulfoxide (DMSO) is used as control.

III. Results and Discussion

The zones of inhibition measured from antimicrobial studies of *N. Biserarta* at concentrations of 100mg/l, 150mg/l and 200 mg/l for bacterial and fungal strains under study are presented in Tables 1 to 6 and Figs. 1 to 6.

Table no 1: Antibacterial activity of *Nephrolepis biserrata* at 100mg/ml concentration

Organism	Extract			
	Methanol	Ethyl Acetate	Chloroform	Distilled Water
<i>Bacillus subtilis</i>	7	2	-	-
<i>Shigella boydii</i>	--	2	-	2
<i>Salmonella enterica</i>	--	3	-	-
<i>Streptococcus mutans</i>	--	2	-	2
<i>Clostridium perfringens</i>	--	2	-	2
<i>Klebsiella pneumoniae</i>	10	3	2	-

Table no 2: Antibacterial activity of *Nephrolepis biserrata* at 150 mg/ml concentration

Organism	Extract			
	Methanol	Ethyl Acetate	Chloroform	Distilled Water
<i>Bacillus subtilis</i>	9	3	-	3
<i>Shigella boydii</i>	7	2	-	2
<i>Salmonella enterica</i>	--	3	3	2
<i>Streptococcus mutans</i>	7	3	2	3
<i>Clostridium perfringens</i>	--	2	-	2
<i>Klebsiella pneumoniae</i>	12	3	3	2

Table no 3: Antibacterial activity of *Nephrolepis biserrata* at 200 mg/ml concentration

Organism	Extract			
	Methanol	Ethyl Acetate	Chloroform	Distilled Water
<i>Bacillus subtilis</i>	13	4	4	4
<i>Shigella boydii</i>	11	3	3	3
<i>Salmonella enterica</i>	7	4	4	3
<i>Streptococcus mutans</i>	8	4	3	4
<i>Clostridium perfringens</i>	10	3	3	3

<i>Klebsiella pneumoniae</i>	13	4	4	3
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Table no 4: Antifungal activity of *Nephrolepis biserrata* at 100 mg/ml concentration

Organism	Extract			
	Methanol	Ethyl Acetate	Chloroform	Distilled Water
<i>Alternaria alternata</i>	11	2	-	2
<i>Rhizopus oryzae</i>	14	2	-	-
<i>Aspergillus fumigatus</i>	10	3	2	2
<i>Candida albicans</i>	11	2	-	-

Table no 5: Antifungal activity of *Nephrolepis biserrata* at 150 mg/ml concentration

Organism	Extract			
	Methanol	Ethyl Acetate	Chloroform	Distilled Water
<i>Alternaria alternata</i>	12	3	-	2
<i>Rhizopus oryzae</i>	15	2	3	2
<i>Aspergillus fumigatus</i>	12	3	3	2
<i>Candida albicans</i>	14	3	2	-

Table no 6: Antifungal activity of *Nephrolepis biserrata* at 200 mg/ml concentration

Organism	Extract			
	Methanol	Ethyl Acetate	Chloroform	Distilled Water
<i>Alternaria alternata</i>	13	4	4	4
<i>Rhizopus oryzae</i>	16	3	4	3
<i>Aspergillus fumigatus</i>	14	4	3	3
<i>Candida albicans</i>	18	4	3	-

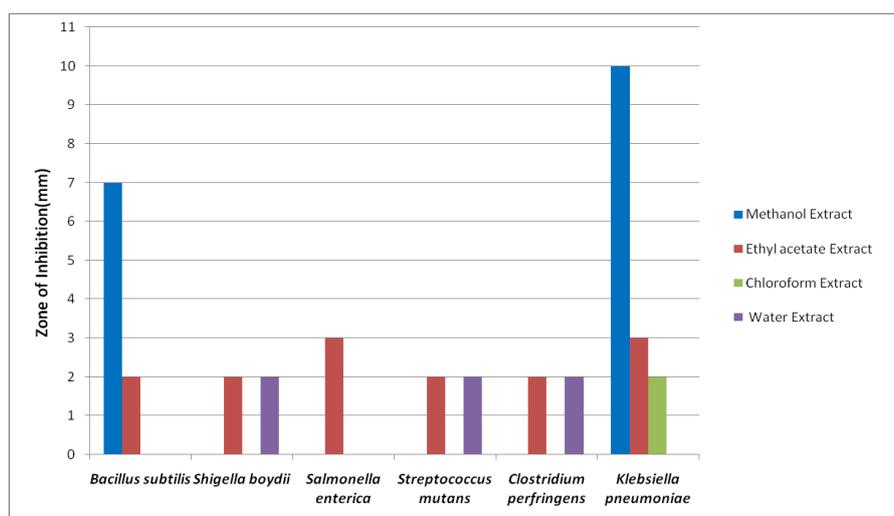


Fig.1 Antibacterial activity of different extracts of *Nephrolepis biserrata* at 100mg/ml concentration

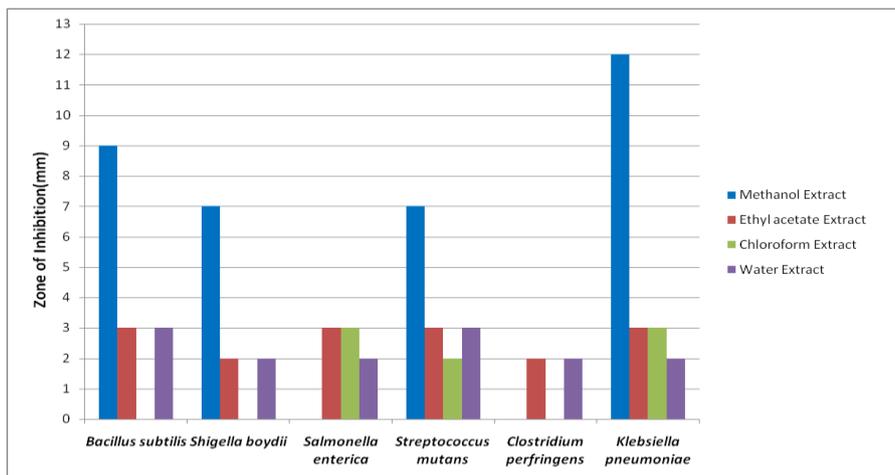


Fig.2 Antibacterial activity of different extracts of *Nephrolepis biserrata* at 150mg/ml concentration

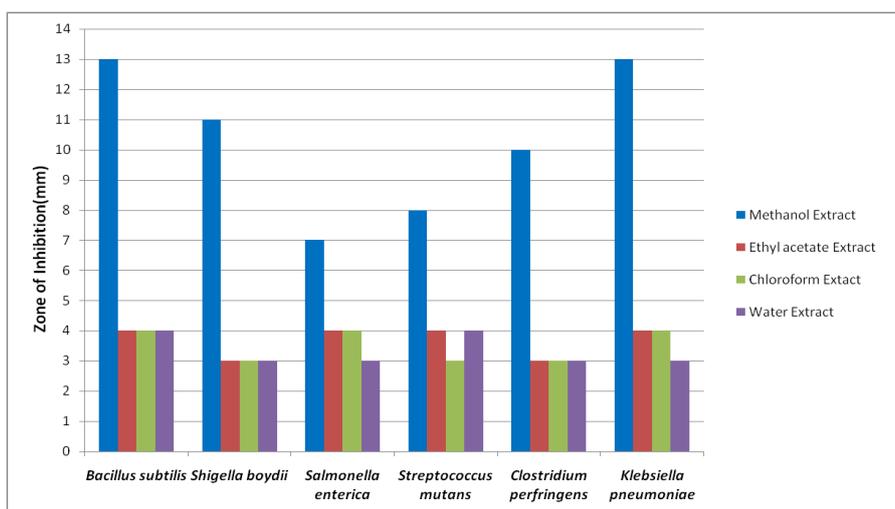


Fig.3 Antibacterial activity of different extracts of *Nephrolepis biserrata* at 200mg/ml concentration

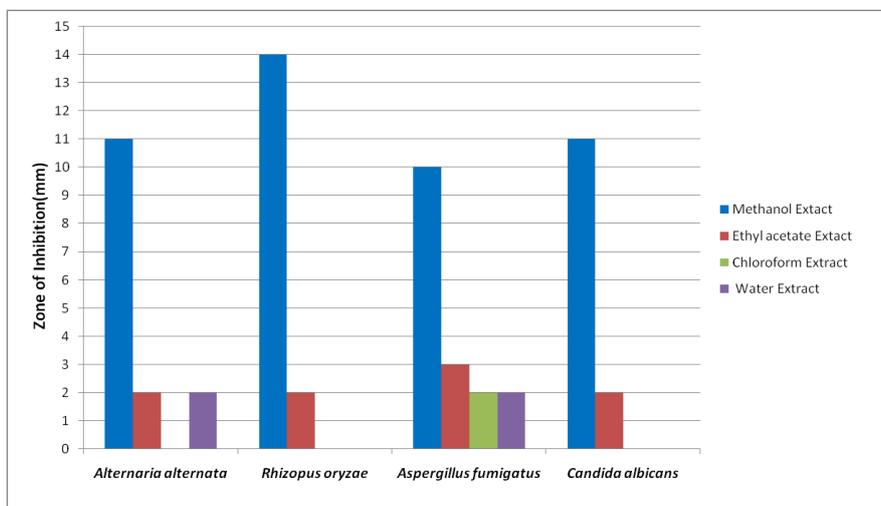


Fig.4 Antifungal activity of different extracts of *Nephrolepis biserrata* at 100mg/ml concentration

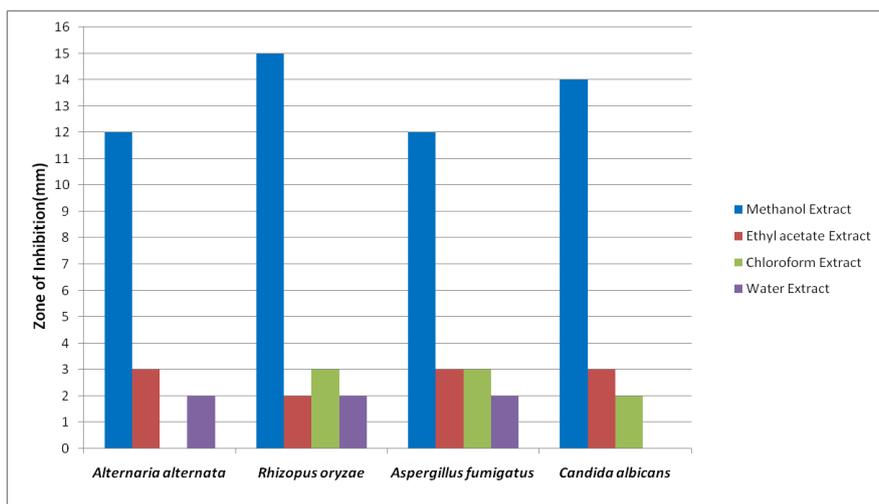


Fig.5 Antifungal activity of different extracts of *Nephrolepis biserrata* at 150mg/ml concentration

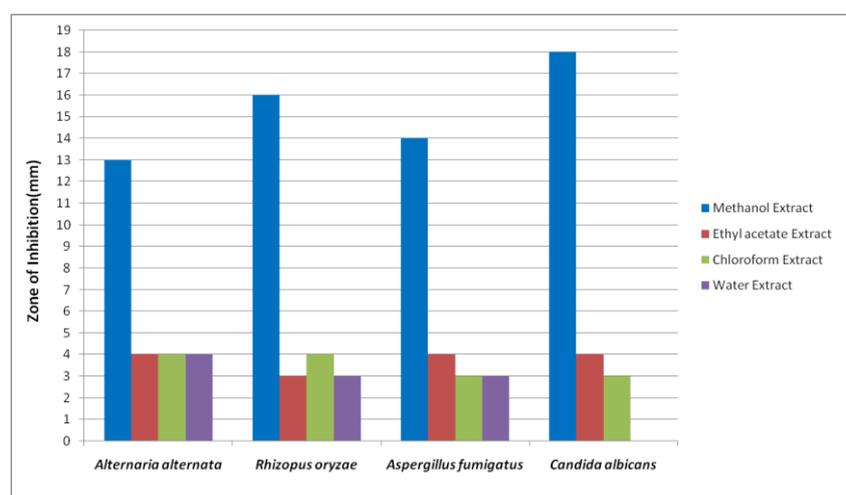


Fig.6 Antifungal activity of different extracts of *Nephrolepis biserrata* at 200mg/ml concentration

The antibacterial activity is rated (Alves¹⁵ et al.) based on the value of zone of inhibition as given below.

- <9 mm, inactive
- 9–12 mm, less active
- 13–18 mm, active
- >18 mm, very active

From the results it is observed that the antimicrobial activity increased with increase in concentration of the extracts. Methanol extract exhibited moderate to high inhibition zones (activity) while Distilled water, Ethyl acetate and Chloroform extracts showed only mild to no activity. Among the four extracts chloroform extract of *Nephrolepis biserrata* showed no activity at lower concentrations of the extract.

Nephrolepis biserrata at 200mg/ml of Methanol extract exhibited maximum antibacterial activity in *Bacillus subtilis* and *Klebsiella pneumoniae* (13 mm) followed by *Shigella boydii* (11 mm), *Clostridium perfringens* (10 mm), *Streptococcus mutans* (8 mm) and least activity was observed in *Salmonella enteric* (7 mm). It did not show any antibacterial activity against *Shigella boydii*, *Salmonella enteric*, *Streptococcus mutans* and *Clostridium perfringens* bacterial strains in Methanol extract of 100mg/ml. Significant zone of inhibition was observed for both gram positive and gram negative bacterial strains.

Nephrolepis biserrata at 200mg/ml of Methanol extract showed maximum activity on fungi *Candida albicans* (18 mm) followed by *Rhizopus oryzae* (16 mm), *Aspergillus fumigates* (14 mm) and least activity is observed in *Alternaria alternate* (13 mm). At 100mg/ml and 150mg/ml concentrations of Methanol extracts, *Rhizopus oryzae* exhibited higher zone of inhibition of 14-15mm while *Candida albicans* exhibited inhibition zones of 11-14mm.

As methanol extract of *Nephrolepis biserrata* exhibited maximum activity against *Candida albicans* and *Klebsiella pneumoniae*; it can be used in the treatment of urinary tract infections, pneumonia and bloodstream infections.

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