

ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY OF *Barleria acuminata* Ness IN ETHANOLIC EXTRACT ON DIFFERENT PATHOGENS

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ABSTRACT

Barleria acuminata Ness belongs to family Acanthaceae. The objective of the present work was to identify the study of antibacterial and antifungal activity of extract of *Barleria acuminata*. Recently, natural products have been evaluated as sources of antimicrobial and antifungal agents with efficacies against a variety of microorganisms. Present study was designed to evaluate the antibacterial and antifungal activity of *Barleria acuminata*. The extract was prepared using ethanol. Antimicrobial activity was tested against two gram positive bacteria and gram negative bacteria while antifungal activity was tested against two fungi. Evaluations were based on the zone of inhibition using Agar well diffusion assay. The inhibitory activity was

found to be dose dependent. This study represents that ethanol extracts of Leaf, Stem and Root of *Barleria acuminata* may be utilize as a potential source of antimicrobial and antifungal agents.

KEYWORDS: *Barleria acumiata*, Antibacterial, Antifungal, Extraction.

INTRODUCTION

Man always been surrounded by countless microorganisms. The disease producing microbes are playing a very important role in human life. Pathogenic microorganisms are always trying to develop resistance to the various antimicrobial agents used for their control. Therefore, the chemotherapy of communicable diseases has proved to be a continuous great effort. Scientists are forever in exploring of new antimicrobial agents to run the ever increasing menace of the microbes. Thus it is of very importance for the microbiologists to develop new

resistant strains. Therefore, medicinal plants are gifts of nature to cure limitless number of diseases among human beings.^[1]

Infectious Diseases constitute a major proportion of the Global Disease burden and continues to be the foremost leading cause of mortality in the developing countries.^[2] Over the past few decades tremendous advancements in the scientific field have led to the development of many antibiotics. However most of the available drugs have their own drawbacks in terms of adverse effects and high cost. The emergence of antibiotic resistance also poses a major threat to the patients and the treating physicians.^[3] Thus, strategies to develop better and newer antibiotics is the need of the hour. This has forced the attention of researchers towards plant products. Plants have long since been used as potential sources of many drugs including antibiotics. Several studies have indicated that plants have active metabolites such as alkaloids, flavanoids, tannins and phenolic compounds which show good antimicrobial activity.^[4,5,6] Plant derived drugs are generally well tolerated, with minimal side effects and can be sourced more easily.^[7] *Barleria acuminata* is an ornamental shrub belonging to Acanthaceae family widely found in Central and South India. It has been used as a traditional herbal medicine for treating various disorders including anaemia, toothache, cough, fever, asthma, bronchitis and diabetes.

B. acuminata grows as a shrub up to 3m tall. The leaves are dark green on the upper surface and pale green on the lower surface. They are elliptic to narrowly ovate. The flowers are about 5 cm long, funnel-shaped in violet, pink, or white color. Leaves to 4-5 x 2.5-3 cm, ovate-orbicular, apex acute, apiculate, base rounded, tomentose; petiole to 3.5 cm, pubescent. Racemes axillary and terminal; bracts 1.3 cm long, lanceolate, glandular, pubescent, acute; outer calyx lobes larger, 13 x 2 mm; inner smaller to 6 x 2 mm, lanceolate, pubescent; corolla tube to 3 cm, lobes 1 cm, obovate, obtuse, imbricate; filaments 7 mm; ovary 3 mm, ovoid, style 3.5 cm long, hairy at the base. Cultivated as an ornamental plant in villages and gardens. The shrub grows also as a ruderal species along roadsides and disturbed areas from near sea level to about 100 m. Found along the forest edges and scrub jungles from plains to 600m. Common. Peninsular India. The present study was emphasized the antimicrobial activity constituent of ethanolic extracts from the various parts of *B. acuminata*.

MATERIALS AND METHODS

Collection of Plant Material

The medicinal plant *Barleria acuminata* was collected from Pachaimalai, Tiruchirappalli District, Tamilnadu, India. The plant was identified and authenticated (BSI/SRC/5/23/2014-15/Tech/539) by Dr. G.V.S. Murthy, Scientist "F" & Head of Office, Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamilnadu, India. The leaf, stem and root were separated and washed thoroughly in running tap water to remove soil particles and adhered debris and then finally washed with sterile distilled water. The parts leaf, stem and root of *B. acuminata*. Were shade dried separately and grind well into powder. The powdered materials were stored in air tight containers at 4°C.

Preparation of Extract: The powdered leaf material (50 g) was subjected to successive solvent extraction (250ml) with ethanol and water using soxhlet apparatus and the extract was filtered using what man No.1 filter paper. The crude extract was further concentrated and used for further studies.

Test Organisms: The anti-microbial activity for the given sample was carried out by Disc Diffusion Technique (Indian Pharmacopoeia 1996, Vol II A-105). The test microorganisms of Bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus vulgaris* *Klebsiella aerogenes*, and Fungus *Candida albicans*, *Aspergillus niger* were obtained from National Chemical Laboratory (NCL) Pune and maintained by periodical sub culturing on Nutrient agar and Sabouraud dextrose agar medium for bacteria and Fungi respectively. The effect produced by the sample was compared with the effect produced by the positive control (Reference standard Ciprofloxacin 5 µg/disc for bacteria; Nystatin 100 µg/disc for fungi).

Antibacterial Activity

The NCIM numbered strains bought from National Chemical Laboratory (NCL) Pune was periodically sub cultured in Nutrient agar Zone of inhibition between 6 mm to 12 mm Intermediate, Zone of inhibition below 6 mm Resistent, Zone of inhibition more 12 mm Sensitive and maintained in the laboratory. The Strains namely *Proteus vulgaris*, *Klebsiella aerogenes*, *Staphylacoccus aureus*, and *Bacillus subilities* were brought to the active phase by sub culturing in Nutrient broth and incubated at 37 °c for 18 hours. The Standardized inoculam about 0.1 ml was inoculated on Muller hinton agar (Hi media) uniformly. The sterile disc watt man No. 2 of 6 mm diameter was placed at equal interval on uniformly inoculated plate and a standard disc Ciprofloxacin 5 mcg/disc was also placed by aseptic technique. The test

sample about 100 μ l was loaded to the sterile disc by using aseptic precautions. The plates were incubated at 37 $^{\circ}$ C for 24 hours. During this period the drug diffuse through the agar and inhibit the growth if the drug is potent. Muller Hinton Agar plating medium. This medium is recommended for the disc diffusion method of antimicrobial susceptibility testing of bacteria (Kirby-Bauer method). When enriched with blood (chocolate) it can be used for Neisseria and Haemophilus species as well. Beef extract 300 g, Peptone 7.5 g, Starch 1.5 g, Agar 17 g, Weigh and suspend the ingredients in 1000 ml of cold distilled water, heat to boiling. Adjust PH to 7.4 Sterilize by autoclaving (121 $^{\circ}$ c for 10 minutes) Cool to 50 $^{\circ}$ c before pouring. For preparing chocolate blood agar plates, read the procedure given earlier. Nutrient Broth Sub culturing medium Nutrient broth is made from commercial meat extract. It is used to cultivate those bacteria which are not nutritionally fastidious. Peptone 5 g, Beef extract 3 g, Sodium chloride 8 g, Distilled water 1000 ml Weigh out all in the ingredients in an Erlenmeyer flask (2000 ml) suitable container that can stand heating. Dissolve by heating and constant stirring. When cool adjust the pH to 7.4 to 7.6. Distribute in tubes, flasks and sterilize by autoclaving at 121 $^{\circ}$ c for 15 minutes.

Antifungal Activity

The Strains namely *Aspergillus niger* & *Candida albicans* were brought to the active phase by sub culturing in Sabouraud dextrose broth and incubated at room temperature for 4 days. The Standardized inoculum about 0.1 ml was inoculated on Sabouraud dextrose agar uniformly. The sterile disc (watt man No. 2 of 6 mm diameter was placed at equal interval on uniformly inoculated plate and a standard disc Nystatin 100 units/disc was also placed By aseptic technique. The test sample about 100 μ l was loaded to the sterile disc by using aseptic precautions. The plates were incubated at room temperature for 2 to 4 days. During this period the drug diffuse through the agar and inhibit the growth if the drug is potent. The diameter of inhibiting zone around the disc was measured by using the Zone of inhibition between 6 mm to 12 mm Intermediate, Zone of inhibition below 6 mm Resistent, Zone of inhibition more 12 mm Sensitive. Sabouraud Dextrose Agar Plating medium. This is the most useful selective medium for the culture of mycotic agents, particularly the filamentous moulds. With the addition of antibiotics (chloramphenicol or cycloheximide, or a combination of pencillin and streptomycin), growth of bacterial contaminants can be prevented. Ingredients of Dextrose 40 g, Peptone 10 g, Agar 15 g, distilled water 1000 ml. Dissolve agar in 1000 ml of distilled water by heating. While hot, add peptone and dextrose Boil gently until dissolved. Adjust the PH 6.0 .Dispense into culture tubes (20-ml) with

cotton plugs or caps. Sterilize by autoclaving (121⁰ c for 15 minutes). Cool the culture medium in slants. When the temperature of the medium reaches 50⁰ c, pour in sterilized plates. Sabouraud Dextrose Broth sub culturing medium Ingredients of Dextrose 40 g, Peptone 10 g, and Distilled water 1000 ml. Dissolve the ingredients with gentle heating and stirring. Dispense in 10 ml amounts in culture tubes. Autoclave (121⁰ c for 10 minutes).

Table 1: Effect of *Barleria acuminata* Ness extract on growth of bacteria *in vitro*. Zone of inhibition (mm).

S. No.	Name of the Microorganism	Zone of inhibition in mm				
		Leaf	Stem	Root	Solvent control	Standard
1.	<i>Staphylococcus aureus</i> (NCIM 2079)	28 mm	26 mm	20 mm	NIL	35 mm
2.	<i>Bacillus subtilis</i> (NCIM 2063)	16 mm	14 mm	10 mm	NIL	40 mm
3.	<i>Proteus vulgaris</i> (NCIM 2027)	14 mm	16 mm	12 mm	NIL	30 mm
4.	<i>Klebsiella aerogenes</i> (NCIM 2098)	12 mm	13 mm	08 mm	NIL	30 mm

Standard: Ciprofloxacin 5µg /disc for bacteria.

Solvent: DMSO.

Table 2: Effect of *Barleria acuminata* Ness extract on Pathogenic fungi Zone of inhibition (mm).

S. No.	Name of the Microorganism	Zone of inhibition in mm				
		Leaf	Stem	Root	Solvent control	Standard
1.	<i>Candida albicans</i> (NCIM 3102)	28 mm	28 mm	18 mm	NIL	32 mm
2.	<i>Aspergillus niger</i> (NCIM 105)	26 mm	28 mm	20 mm	NIL	35 mm

Standard: Nystatin 100 µg / disc for fungi.

Solvent: DMSO.

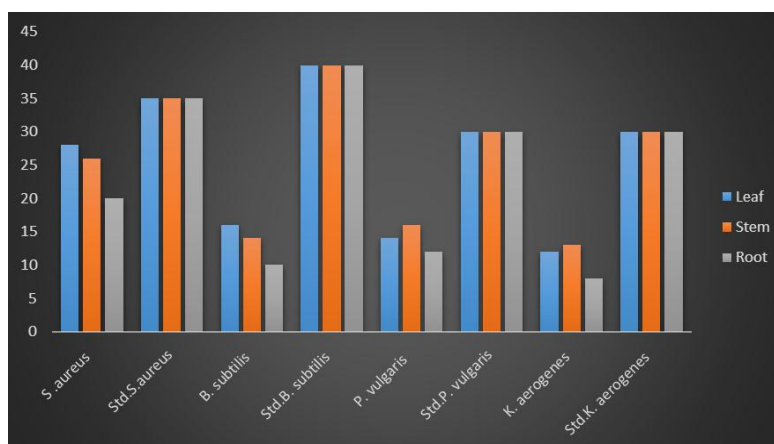


Figure 1: Effect of *Barleria acuminata* Ness extract on Pathogenic bacteria Zone of inhibition (mm).

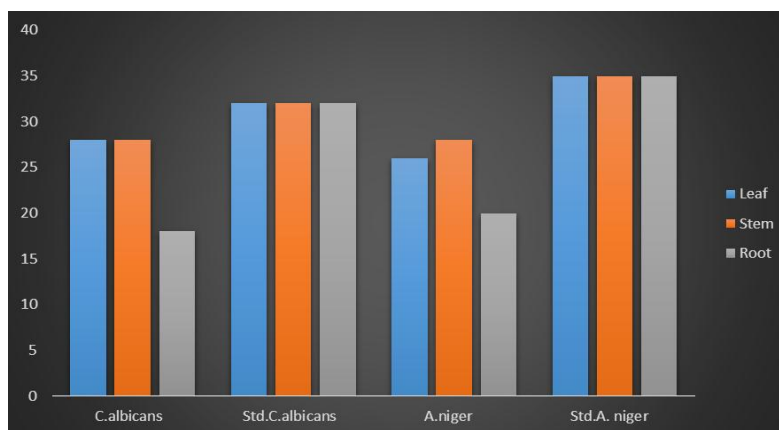


Figure 2: Effect of *Barleria acuminata* Ness extract on Pathogenic fungi Zone of inhibition (mm).

RESULTS AND DISCUSSTION

Barleria acuminata leaf on showed maximum zone of inhibition was against Gram positive bacteria *Staphylococcus aureus* (28mm) and Root on minimum against Gram negative bacteria *Klebsiella aerogenes* (8mm). *Barleria acuminata* Leaf and stem on showed maximum antifungal activity towards *Candida albicans* (28mm) and *Aspergillus flavus* (20mm). The occurrence of antibacterial and antifungal substances in the higher plants is well established. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicines can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines of it can be the base for the development of a medicine, a natural blueprint for the development of a drug. Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The results showed significant activity of *Barleria acuminata* and suggesting its use as natural antimicrobial agent. The result of present study indicated that ethanolic extract of *Barleria acuminata* shows potent antimicrobial and antifungal activity.

CONCLUSION

From the recent study it is concluded that, as dose of the *Barleria acuminata* increases the antimicrobial activity as well as antifungal activity increases. From the observations it clearly indicate that *Barleria acuminata* has potent antimicrobial activity as well as antifungal activity but it act by dose dependent manner.

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