



## Antibacterial and antifungal screening on various leaf extracts of *Barringtonia acutangula*

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### ABSTRACT

The various extracts of leaves of *Barringtonia acutangula* (Lecythidaceae) viz., n-hexane, chloroform, ethyl acetate and ethanol were subjected to preliminary phytochemical screening and screened for their antibacterial activity against gram-positive (*Staphylococcus aureus*, *Enterococci*, *Coagulase staphylococci*) and gram-negative bacteria (*Escherichia coli*, *Klebsiella*, *Citrobacter*, *Aceneto bacter*, *Pseudomonas*, *Salmonella typhi* and *Salmonella paratyphi*) using Minimum Inhibitory Concentration (MIC) and zone of inhibition by Agar Disc Diffusion method. The results of the preliminary investigation revealed the presence of terpenoids, steroids, tannins, saponins, flavanoids and glycosides. Among the crude extracts, n-Hexane extract showed good antibacterial activity against all tested organisms followed by chloroform (MIC = 100 µg/ml), Ethyl acetate (MIC = 100 µg/ml), ethanol and aqueous extracts (MIC = 166.67 µg/ml). Results on the zone of inhibition (mm) revealed n-Hexane extract showed the maximum antibacterial potential followed by ethyl acetate, ethanol, aqueous and chloroform. The extracts were subjected to antifungal activity using MIC method against *Candida albicans*, *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus niger*. The n-Hexane extract inhibited growth of pathogenic fungi at a lesser concentration followed by aqueous, ethanol, chloroform and ethyl acetate. The results reveal that the *Barringtonia acutangula* leaves possess potential antibacterial and antifungal activity. Further study can be extrapolated towards the isolation of the active constituents.

**Keywords:** *Barringtonia acutangula*; antibacterial; antifungal; preliminary phytochemical studies.

### 1. INTRODUCTION

*Barringtonia acutangula* is an evergreen tree of moderate size, called by Sanskrit writers as Hijja or Hijjala. The fruit is spoken of as Samudra-phala and Dhātriphala or "nurse's fruit", and is one of the best known domestic remedies for cholera, asthma and dysentery. This tree has long been used for medicine, timber and as a fish poison. In traditional medicine, when children suffer from a cold, the seed is rubbed down on a stone with water and applied over the sternum, and if there is much dyspnoea a few grains with or without the juice of fresh ginger are administered internally. This induces vomiting and the expulsion of mucus from the air passages. More recently it has become the focus of research for pain-killing constituents (Parrotta, 2001). Considering the vast potentiality of plants as sources for antimicrobial drugs, with reference to antibacterial and antifungal agents, a systematic investigation was undertaken to screen the various

extracts of leaves of *Barringtonia acutangula*.

### 2. MATERIALS AND METHODS

#### 2.1. Plant collection and authentication

Fresh leaves of *Barringtonia acutangula* were collected from Madras Medical College premises, Chennai, Tamil Nadu. The plant was identified and authenticated by comparing with an authentic specimen by a botanist Dr. P. Jayaraman, Plant Anatomical Research Centre, Tambaram, Chennai.

#### 2.2. Plant preparation and extraction

The dried, coarsely powdered leaves were extracted successively with n-Hexane, Chloroform, Ethyl acetate and Ethyl alcohol using Soxhlet's apparatus by hot percolation method for 24 hrs respectively and aqueous extract was prepared by maceration with Chloroform water. The concentrated extracts were dried on a water bath and preserved in a vacuum desiccator for further studies. The percentage yields of extracts were noted.

#### 2.3. Antibacterial activity

Clinical strains of human pathogenic bacteria comprising 3 Gram-positive (*Staphylococcus aureus*, *Enterococci*, *Coagulase negative staphylococci*) and 7 Gram-negative bacteria (*Escherichia coli*, *Klebsiella*, *Citrobac-*

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ter, *Aceneto bacter*, *Pseudomonas*, *Salmonella typhi* and *Salmonella paratyphi*) were used for the antibacterial assay, while for the antifungal assay, *Candida albicans*, *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus niger* were used for the studies. All the microorganisms were obtained from the laboratory stock of the Department of Microbiology, Madras Medical College, Chennai, India.

### 2.3.1. Media preparation

Muller Hinton agar (MH agar) (38gm) was weighed and dissolved in 1000ml of distilled water and adjusted to pH 7.3±0.2, sterilized by autoclaving at 121°C for 15min at 15 psi pressure and was used for sensitivity tests (Ananthanarayanan *et al.*, 2000)

### 2.3.2. Preparation of Bacterial Cultures

Few colonies of the bacterial strains selected for study were picked from the agar slopes and inoculated into 4ml peptone water in a test tube. These tubes were incubated for 2-4 hrs to produce suspensions. The suspensions were then diluted, if necessary with saline to a density visually equivalent to that of standard prepared by adding 0.5ml of 1% barium chloride to 99.5ml of 1% sulphuric acid. These suspensions were used for inoculation.

### 2.3.3. Minimum Inhibitory Concentration

Dilution methods are used to determine the minimum inhibitory concentrations (MICs) of antimicrobial agents and are the reference methods for antimicrobial susceptibility testing against which other methods, such as disc diffusion, are calibrated. MIC methods are widely used in the comparative testing of new agents (Ericsson *et al.*, 1971). In clinical laboratories they are used to establish the susceptibility of organisms that give equivocal results in disc tests, for tests on organisms where disk tests may be unreliable, and when a more accurate result is required for clinical management. In dilution tests, microorganisms are tested for their ability to produce visible growth on a series of agar plates (agar dilution). The lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism is known as the MIC.

### 2.3.4. Preparation of agar plates

The test solutions were introduced aseptically into sterilized Petri dishes to get final concentrations ranging from 33.33µg/mL, 66.67 µg/mL, 100µg/mL, 133.33 µg/mL, and 166.67 µg/mL and the volume was made up to 15ml by adding MH media and the Petri dishes were swirled until the agar begins to set.

### 2.3.5. Test procedure

The plates with different test solutions of various dilutions were inoculated with a loopful of cultures at the labeled spots. These plates were incubated at 37°C for 24 hrs. The results were read by the presence or absence of growth of the organisms.

### 2.3.6. Antibiotic Disc Diffusion Technique

The discs of 6mm diameter were prepared from Whatmann filter paper No.1 and were sterilized in a hot air oven at 160°C for 1 hr. The discs were then impregnated with the (MIC) test solutions and solvent Dimethyl formamide (DMF). Ciprofloxacin discs were used as standard. Each disc of ciprofloxacin contained 5µgm of the drug (Agarwal, 1974; Collee *et al.*, 1989 & Kirby *et al.*, 1966).

The pathogenic strains were then seeded on the MH agar media in a Petri dish by streaking the plate with the help of a sterile swab. Care was taken for the even distribution of culture all over the plate. The seeded plates were allowed to dry and then the ciprofloxacin, test solutions and DMF discs were placed on the seeded medium plates and maintained at 4°C for 30 min allow perfusion of drugs being tested. The plates were then incubated at 37°C for 24 hrs. The results were read by presence or absence of zone of inhibition. The zone of inhibition was then measured.

### 2.4. Anti-fungal activity

Media used for the growth of fungi was Sabouraud's Dextrose Agar. It contains Mycological peptone – 10 g/L, Dextrose – 40 g/L, Agar – 15 g/L and distilled water - 1000ml. 65g of Sabouraud's Dextrose Agar was weighed and dissolved in 1000ml of distilled water by boiling. The pH was adjusted to 5.6 ± 0.2 and sterilized by autoclaving at 121°C at 15 lb pressure for 15 min.

#### 2.4.1. Preparation of SDA Slopes

The test solutions were introduced aseptically into sterilized tubes to get final concentrations of 500, 1000, 2000 µg/ml and made up to 3ml with SDA and the slopes were prepared by slanting the tubes.

#### 2.4.2. Preparation of fungal cultures

The fungi were maintained on SDA slopes in the Institute of Microbiology, Madras Medical College, Chennai.

#### 2.4.3. Determination of minimum inhibitory concentration

The slants were prepared and allowed to set. The various mentioned fungi were inoculated into the plain Sabouraud's Dextrose Agar. All the slants were incubated at 37°C in an incubator for the duration of 1 week to 4 weeks. The presence or the absence of fungi in the test tube and Minimum Inhibitory Concentration were noted and recorded in Table.

## 3. RESULTS

The extraction of *Barringtonia acutangula* with n-Hexane, Chloroform, Ethyl acetate, Ethanol and water gave yields of 2.17%, 2.43%, 1.11%, 3.97%, and 6.39% respectively (Harborne, 1984). The results of the phytochemical screening (Khandelwal, 2006) indicated the presence of Terpenoids, Steroids, Tannins, Saponins,

**Table 1: Minimum inhibitory concentration of different extracts of leaves of *Barringtonia acutangula* against various bacteria**

Organism used	Minimum Inhibitory Concentration (µg/ml)				
	n-Hexane	Chloroform	Ethyl acetate	Ethanol	Aqueous
<i>Coagulase staphylococci</i>	66.67	66.67	66.67	66.67	66.67
<i>Entero cocci</i>	100.00	100.00	100.00	66.67	166.67
<i>Staphylococcus aureus</i>	66.67	100.00	100.00	166.67	66.67
<i>Aceneto bacter</i>	66.67	66.67	66.67	100.00	66.67
<i>Citrobacter</i>	66.67	66.67	66.67	66.67	66.67
<i>Escherichia coli</i>	66.67	66.67	66.67	100.00	66.67
<i>Klebsiella</i>	66.67	66.67	66.67	66.67	66.67
<i>Pseudomonas</i>	66.67	66.67	66.67	66.67	166.67
<i>Salmonella paratyphi</i>	66.67	66.67	66.67	66.67	66.67
<i>Salmonella typhi</i>	66.67	100.00	100.00	100.00	166.67

**Table 2: Zone of inhibition of different extracts of leaves of *Barringtonia acutangula* against various bacteria**

Extracts	Zone of Inhibition (mm)									
	Gram positive			Gram negative						
	1	2	3	4	5	6	7	8	9	10
n-Hexane	16	38	20	26	27	23	25	15	32	40
Chloroform	10	35	17	23	27	25	32	17	38	25
Ethyl acetate	13	40	18	28	28	23	30	19	34	30
Ethanol	12	39	14	29	26	22	36	18	27	34
Aqueous	15	38	16	26	19	22	34	15	31	35
Standard (Ciprofloxacin)	31	41	23	33	35	28	36	31	34	40
Solvent control (DMF)	--	--	--	--	--	--	--	--	--	--

**Table 3: Minimum inhibitory concentration of different extracts of leaves of *Barringtonia acutangula* against various pathogenic fungi**

Organisms	Minimum Inhibitory Concentration (µg/ml)				
	n-Hexane	Chloroform	Ethyl acetate	Ethanol	Aqueous
<i>Aspergillus flavus</i>	>1000	>1000	>1000	>2000	>1000
	<2000	<2000	<2000		<2000
<i>Aspergillus fumigates</i>	>1000	>2000	>2000	>2000	>500
	<2000				<1000
<i>Aspergillus niger</i>	<500	>1000	>2000	>1000	>2000
		<2000		< 2000	
<i>Candida albicans</i>	>1000	>2000	>2000	>1000	>2000
	<2000			<2000	

Flavonoids, Carbohydrates, Glycosides. For the antibacterial activity, the MIC (Table 1) and the Zone of Inhibition (Table 2) and for the antifungal activity MIC (Table 3) were studied and recorded.

#### 4. DISCUSSION AND CONCLUSION

Among the crude extracts, n-hexane extract showed good anti bacterial activity against all test organisms. The n-hexane extract (MIC = 66.67 µg/ml) was found to be very effective followed by chloroform (MIC = 100 µg/ml), ethyl acetate (MIC = 100 µg/ml), ethanol and aqueous extracts (MIC = 166.67 µg/ml). The Zone of Inhibition in mm for the tested organisms with successive extracts and the standard (Ciprofloxacin) by agar disc diffusion method was also studied. It was observed that all the extracts showed prominent antibacterial activity (Zone of inhibition between 10 mm to 40

mm) towards most of the microorganisms used in the study. The n-hexane extract showed the maximum antibacterial potential followed by ethyl acetate, ethanol, aqueous, and chloroform and also shown comparatively equivalent activity with the standard drug. The n-hexane extract inhibited growth of pathogenic fungi at a lesser concentration followed by aqueous, ethanol, chloroform and ethyl acetate. The results reveal that the *Barringtonia acutangula* leaves possess potential antibacterial and anti fungal activity. Further study can be extrapolated towards the isolation of the active constituents.

#### REFERENCES

Agarwal KC. Antibiotic Sensitivity Test by the Disc Diffusion Method – Standardisation and Interpretation. Ind. J. Path and Bact 1974; 17: pp. 149-152.

- Ananthanarayanan R, Paniker CJK. Text book of Microbiology. Chennai: Orient Longman Ltd, 6<sup>th</sup> edition, 2000, pp. 581-583.
- Collee PG, Duguid JP, Fraser AG, Marmion BP, Mackie, Cartney MC. Practical Medical Microbiology. 1989, pp. 161-80.
- Ericsson H, Sherris JC. Antibiotic sensitivity testing- Report of an international collaborative study. Acta. Pathol. Microbiol. Scand 1971; 2: pp 217
- Harborne JB. Methods of extraction and isolation. In: Phytochemical Methods, Chapman & Hall, London, 1984, pp 60-66.
- Khandelwal KR. Practical Pharmacognosy. Pune: Nirali Prakashan, 2006, pp 149-56.
- Kirby WM, Bauer AW, Sherris JC, Truck M. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol 1966; 45: pp. 493-496.
- Parrotta JA. Healing plants of peninsular India, UK: CA-BI publishing, 2001, pp 448-451.