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Antimicrobial Investigation of Leaves of *Barringtonia acutangula* Linn.

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ABSTRACT

Methanolic and aqueous extracts of the leaves of *Barringtonia acutangula* Linn. belonging to the family Barringtoniaceae, commonly known as *Hinjal*, were screened for their antibacterial activity against some selected organisms causing urinary and gastrointestinal tract infection, fish-virulent pathogens, human and plant pathogenic fungi. The methanolic extract, which exhibited potent antimicrobial activity, was subjected to high performance thin layer chromatography (HPTLC). Chromatography of the methanolic extract was carried out with an acetone-hexane (4:6) solvent system. HPTLC fingerprinting patterns were developed at three different wave lengths viz. 254, 366 and 650 nm. Antimicrobial activity was carried out against selected bacterial and fungal strains by a disc diffusion assay method at a concentration of 1000 µg/ml and minimum inhibitory concentration values were calculated by a two-fold serial dilution method at a concentration range of 7.8-1000 µg/ml. The yield (%) of methanolic and aqueous extracts was found to be 12.6 and 16.1% (w/w), respectively for the air-dried plant leaves. Antimicrobial screening revealed that the extract showed potent activity against bacterial and fungal strains, namely *Enterococcus faecalis* (22.9 ± 0.23 mm, MIC 31.25 µg/ml), *Aspergillus niger* (27.2 ± 0.15 mm, MIC 62.5 µg/ml) and *Candida albicans* (23.3 ± 0.87 mm, MIC 31.25 µg/ml).

Keywords: antimicrobial activity, disc diffusion method, *Hinjal*, HPTLC, MIC
Abbreviations: HPTLC, High Performance Thin Layer Chromatography; **MIC**, minimum inhibitory concentrations

INTRODUCTION

Barringtonia acutangula (L.) Gaertn belonging to the family Barringtoniaceae, locally known as *Hinjal*, is a medium-sized glabrous tree found throughout India in deciduous and evergreen forests, mostly along the bank of rivers (Kapoor 1990) and streams (Warrier *et al*. 1994) attaining a height of around 10–15 m. It is used in folklore in vitiated conditions of *kapha* and *pitta*, leprosy, arthralgia, dysmenorrhea, skin diseases (Sahoo *et al*. 2008), diarrhoea (Yusuf *et al*. 1994), inflammation, flatulence, hallucinations (Warrier *et al*. 1994), hemorrhoids, as an anthelmintic (Satapathy and Brahmam 1994). Despite the vast traditional uses, only a 50% ethanolic extract of *B. acutangula* has been investigated for hypoglycemic and antiprotozoal properties (Dhar *et al.* 1968). The plant is reported to have anti-implantation activity in female albino rats (Mathur *et al*. 1983). Seeds are used as fish poison (Chakraborty *et al*. 1972). It is reported to cure various common veterinary diseases of Warangal district of Andrapradesh, India (Reddy *et al*. 1998). Phytochemical screening revealed that it contains triterpenopid glucoside (Pal *et al*. 1991), saponin (Pal *et al*. 1994), barringtonin, starch, protein, cellulose, fat, caoutchouc, alkaline salts (Nadkarni 1976), etc. In Ayurveda, its preparations include powder and pastes.

The present study was intended to evaluate the antimicrobial potency of the extracts of *B. acutangula* leaves against some selected bacterial and fungal pathogens and compare it with potent standard antibacterial (Bauenfiend 1997) and antifungal agents (Fostel *et al*. 2000).

MATERIALS AND METHODS

Collection and processing of plant material

The plant was collected from the coastal area of Jajpur district, Orissa, India in the month of September. It was authenticated by the taxonomist of the Institute of Minerals and Materials Technology, Bhubaneswar, Orissa, India and a voucher specimen (10001/RRL(B)) was deposited in the herbarium. Leaves of the plant were bulked and washed with tap water to remove soil and dirt, then shade dried and powdered with a mechanical grinder. The powder was passed through a sieve number 40 and stored under laboratory conditions at 20 ± 4°C with 36 ± 3% of relative humidity in an air-tight container for further use.

Extraction

The air-dried powered materials of the leaves were extracted with methanol in a Soxhlet apparatus (Pulok 2002) and then water. The solvent was completely removed under reduced pressure and stored in a vacuum dessicator.

Antimicrobial evaluation

In-vitro screening was carried out using selected urinary tract (UT) and gastrointestinal tract (GIT) infection-causing pathogens, *Pseudomonas aeruginosa* 1035, *Escherichia coli* 118, *Proteus mirabilis* 743, *Klebsiella pneumoniae* 109, *Staphylococcus aureus* *1430, *Enterococcus faecalis* 2729 and *Propionibacteri acnes* *1951 procured from the Microbial Type Culture Collection Centre (MTCC) and Gene Bank, Chandigarh, India. *Streptococcus mitis* *2695 and *S. equi* were collected from MTCC and clinical specimens of patients with diseased periodontium and dental caries. Some virulent strains of fish viz. *Aeromonas hydrophila* (two isolates), *Edward-*

Received: 16 June, 2008. Accepted: 6 December, 2008.

Original Research Paper

siella tarda, *Pseudomonas aureginosa*, *Flavobacterium branchiophylum* were collected from the Central Institute of Freshwater Aquaculture (CIFA), Kausalyaganga, Bhubaneswar, Orissa, India. Fungal strains belonging to important pathogenic genera *Candida*, *Aspergillus*, few dermatophytes and plant pathogens causing candidiasis, vulvovaginitis (Charmaine *et al*. 2005), paronychia, aspergillosis (Ho and Yuen 2003), dermatophytosis (Shams-Ghahfarokhi *et al*. 2006) and plant diseases included *Candida albicans* MTCC 3017, *Candida tropicalis*, *Candida krusei*, *Aspergillus niger* MTCC 1344, *Aspergillus flavus*, *Cryptococcus neoformans* and *Rizopus orizae*. The remaining strains included *Salmonella typhi*, *Vibrio cholerae* and *Micrococcus luteus* which were obtained from the Post Graduate Department of Microbiology, Orissa University of Agriculture and Technology (OUAT), Bhubaneswar, Orissa, India. These organisms were identified by standard microbiological methods (Murray *et al*. 1995). The antibacterial and antifungal screening of the extracts was carried out by determining the zone of inhibition using the disc diffusion method (Sahoo *et al*. 2008). The minimum inhibitory concentration (MIC) was studied by two-fold serial dilution method (Barry 1976). The methanol free extract was dissolved in DMF (N, N'-dimethyl formamide) and estimation of zone of inhibition and MIC determination was carried out using DMF as control which exhibited no zone of inhibition against all organisms under test.

HPTLC study

The methanolic extracts were subjected to the development of HPTLC finger printing patterns. Chromatography of methanolic extract was carried out with an acetone-hexane (4:6) solvent system. The sample solution for chromatography was prepared by dissolving 50 mg of crude extract in 25 mL of methanol. A 60 µL of sample solution was applied on Silica gel $60F_{254}$ pre-coated aluminum sheet by using Linomat 5 applicator as 6 mm band. The plate was developed with acetone-hexane (4:6, v/v) as mobile phase and allowed to run up to 80 mm under laboratory conditions of 31 ± 3°C with relative humidity of 35 ± 4%. The chromatograms were scanned densitometrically using Camag TLC scanner 3 with winCATS software (version 1.3.4) in absorbance-reflectance scan mode at different wavelength and developed fingerprinting patterns. The scanner slit was set at 5.0 × 0.45 mm (micro) and 20 mm/s scanning speed with 100 µm per step data resolution were employed.

Statistics

The values of the zone of inhibitions are given in terms of mean ± SD of three determinations. The statistical significance was assessed using one way analysis of variance (ANOVA) followed by Dunnett's test and $p<0.05$ was considered as statistically significant.

Table 2 Minimum inhibitory concentrations of methanol extract of *B. acutangula* leaves by two fold serial dilution method.

Microorganisms	MIC (µg/ml)
Staphylococcus aureus	62.5
Klebsiella pneumoniae	250
Proteus mirabilis	62.5
Pseudomonas aeruginosa	62.5
Streptococcus mitis	500
Micrococcus luteus	62.5
Salmonella typhi	125
Vibrio cholerae	250
Streptococcus equi	250
Escherichia coli	250
Enterococcus faecalis	31.25
Aeromonas hydrophila 1	n.f.
Edwardsiella tarda	n.f.
Aeromonas hydrophila 2	500
Flavobacterium branchiophylum	500
Aspergillus niger	62.5
Candida albicans	31.25
Candida tropicalis	62.5
Candida krusei	250
Aspergillus flavus	125
Rizopus orizae	125
Cryptococcus neoformans	500
Propionibacteri acnes	125

MIC indicates minimum inhibitory concentration.
n.f – MIC not detected at the concentration range from 7.8-1000 µg/ml.

Table 1 Antimicrobial activity of *B. acutangula* leaf extracts.

Microorganisms		Zone of Inhibition (in mm)		
		ME	AQ	Standard
Staphylococcus aureus	B	23.4 ± 1.16	18.9 ± 0.9	35.2 ± 1.5 (CF)
Klebsiella pneumoniae	B	17.2 ± 0.84	21.6 ± 0.72	35.8 ± 1.8 (CF)
Proteus mirabilis	B	24.7 ± 0.25	16.8 ± 0.36	30.7 ± 0.65 (CF)
Pseudomonas aeruginosa	B	23.1 ± 0.73	18.9 ± 0.82	36.9 ± 1.0 (CF)
Streptococcus mitis	B	13.5 ± 0.3	10.6 ± 1.0	32.5 ± 0.5 (CF)
Micrococcus luteus	B	24.6 ± 0.91	15.5 ± 0.5	20.7 ± 0.2 (CF)
Salmonella typhi	B	20.9 ± 1.32	19.3 ± 0.9	33.5 ± 0.45 (CF)
Vibrio cholerae	B	16.4 ± 1.0	17.3 ± 0.45	24.6 ± 0.8 (CF)
Streptococcus equi	B	21.0 ± 0.5	18.2 ± 1.32	23.5 ± 0.2 (CF)
Escherichia coli	B	20.9 ± 0.3	21.9 ± 0.85	31.5 ± 0.5 (CF)
Enterococcus faecalis	B	22.9 ± 0.23	20.7 ± 0.92	28.1 ± 0.82 (CF)
Aeromonas hydrophila 1	B	- [ns]	- [ns]	22.5 ± 1.5 (CF)
Edwardsiella tarda	B	10.3 ± 0.6	11.4 ± 1.2	25.3 ± 0.93 (CF)
Aeromonas hydrophila 2	B	9.7 ± 0.63	7.0 ± 0.5	26.5 ± 0.45 (CF)
Flavobacterium branchiophylum	B	11.3 ± 0.7	8.1 ± 0.9	28.3 ± 0.54 (CF)
Propionibacteri acnes	B	19.2 ± 0.8	17.8 ± 0.53	27.3 ± 1.3 (CF)
Aspergillus niger	F	27.2 ± 0.57	21.9 ± 0.4	22.1 ± 1.1 (CL)
Candida albicans	F	23.3 ± 1.2	21.9 ± 1.1	21.6 ± 0.5 (FLU)
Candida tropicalis	F	20.8 ± 0.8	16.0 ± 0.5	22.2 ± 0.2 (FLU)
Candida krusei	F	15.5 ± 0.6	17.1 ± 0.2	13.7 ± 1.32 (FLU)
Aspergillus flavus	F	21.8 ± 1.4	20.0 ± 1.0	33.6 ± 1.4 (CL)
Rizopus orizae	F	19.9 ± 0.3	21.7 ± 0.84	26.5 ± 0.5 (AP)
Cryptococcus neoformans	F	15.7 ± 0.62	18.9 ± 0.5	14.7 ± 0.72 (AP)
Dimethyl formamide (DMF)	Control	-	-	-

- Indicates no zone of inhibition. B and F indicate bacterial and fungal pathogens. ME and AQ stands for methanol and aqueous extracts of *B. acutangula* leaves at concentration of 1000 µg/disc respectively. CF, FLU, AP and CL stand for ciprofloxacin (25 µg/disc), fluconazole (10 µg/disc), amphotericin B (100 units/disc) and clotrimazole (10 µg/disc).
Values are given in mean ± SD and significantly different when compared to control ($p<0.01$).
[ns] statistically not significant.

RESULTS AND DISCUSSION

Antimicrobial activity

The methanolic and aqueous extracts of *B. acutangula* leaves were subjected to extensive antimicrobial studies and the results of antibacterial and antifungal activity in terms of zone of inhibition is reported in **Table 1**. The extract showing highest antimicrobial activity i.e. methanolic extract was subjected for evaluation of its MIC value (**Table 2**).

The results of antibacterial activity indicated that the methanolic extract of *B. acutangula* exhibited better than the aqueous extract. The methanolic extract of *B. acutangula* leaves exhibited the highest zone of inhibition against *P. mirabilis* (24.7 ± 0.25 mm) and *M. luteus* (24.6 ± 0.91 mm) followed by *S. aureus* (23.4 ± 1.16 mm) and *P. aeruginosa* (23.1 ± 0.73 mm) and least inhibition against *S. mitis* (13.5 ± 0.3 mm). *A. hydrophila* 1 was found to be resistant; *E. tarda* and *A. hydrophila* 2 were weakly inhibited. Other bacterial strains were mostly sensitive to the methanolic extract. The aqueous extract of *B. acutangula* leaves showed a moderate effect against *E. coli* (21.9 ± 0.85 mm), *K. pneumoniae* (21.6 ± 0.72 mm) and *E. tarda* (11.4 ± 1.2 mm). However, the extracts did not possess promising inhibitory activity against the tested fish pathogens.

The results of antifungal activity revealed that the methanolic extract was more effective compared to the aqueous extract by significantly ($p<0.01$) inhibiting *A. niger* (27.2 ± 0.57 mm), *C. albicans* (23.3 ± 1.2 mm) and *A. flavus* (21.8 ± 1.4 mm). Mostly all fungal pathogens were inhibited by the methanol extract where aqueous extract showed significant inhibition in case of *A. niger* (21.9 ± 0.4 mm), *C. albicans* (21.9 ± 1.1 mm), *R. orizae* (21.7 ± 0.84 mm) and *A. flavus* (20.0 ± 1.0 mm).

The results of MIC indicated the potency of the methanol extract by significantly ($p<0.01$) inhibiting *E. faecalis*, *A. niger* and *C. albicans* at a lower concentration of 31.25, 62.5 and 31.25 µg/ml.

The phytoconstituents of the extracts of stem bark of *B. acutangula* were found to have potent antimicrobial effect (Rahman *et al*. 2005). Similar results were also reported by Sahoo *et al*. (2008) where ethanolic extract of *B. acutangula* seeds showed better inhibition against some human pathogenic fungi including *A. niger*.

Chromatographic studies of the crude extract

In general the HPTLC fingerprinting patterns of the methanolic extract of the leaves *B. acutangula* were developed at

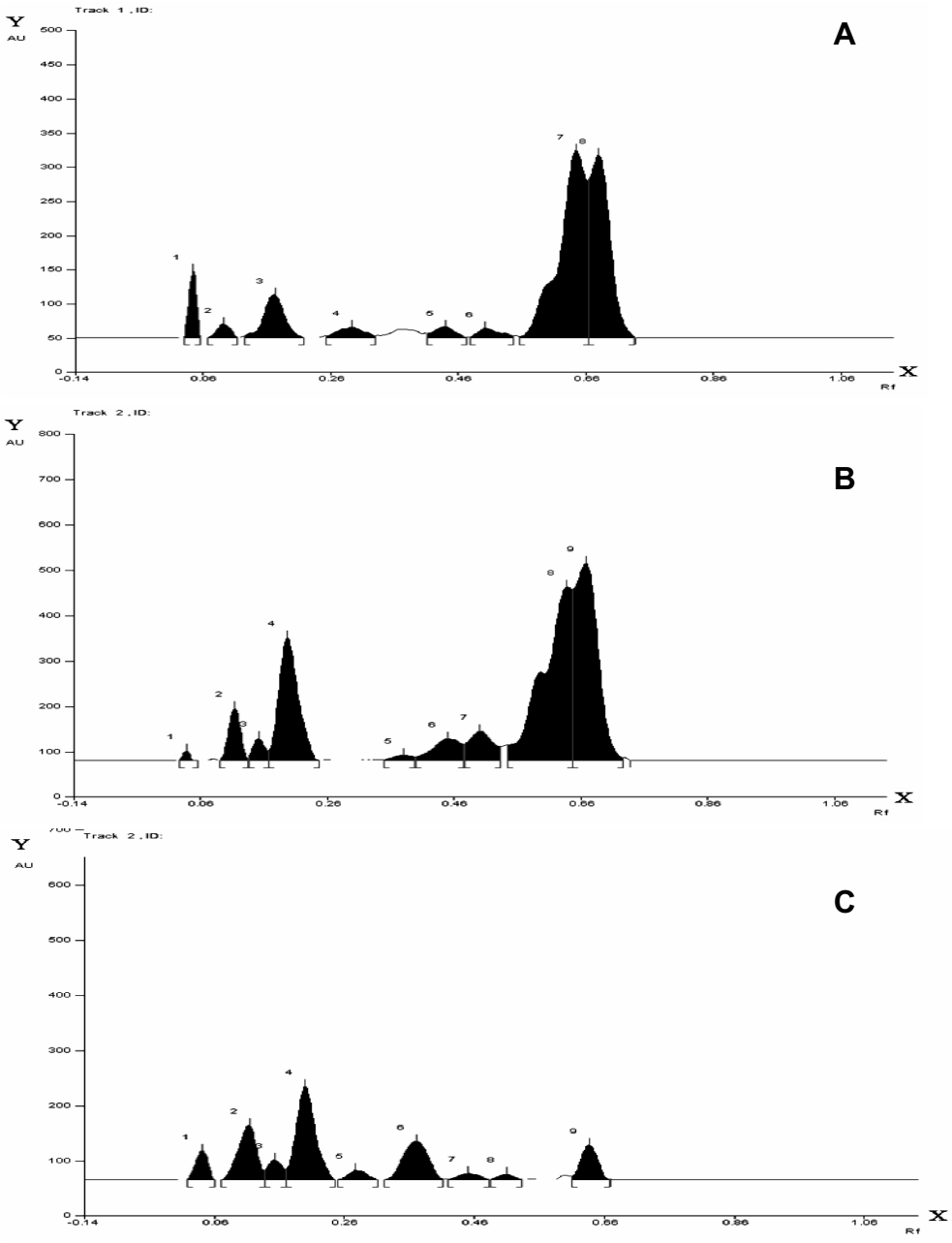

Fig. 1 HPTLC fingerprint of methanol extract of *B. acutangula* at different wavelengths. (**A**) 254 nm, (**B**) 366 nm, (**C**) 650 nm.

254, 366, and 650 nm (after derivatisation with anisaldehyde developing reagent). All three chromatograms differed from each other. Moreover chromatograms at 366 and 650 nm consisted of nine peaks where as chromatogram at 254 nm had eight. The components at R_f 0.17 is present in all chromatograms. Developed HPTLC plates of the methanolic extract of *B. acutangula* at different wavelengths is shown in **Fig. 1**. The HPTLC study revealed that the chromatographic fingerprint evaluation could be used efficiently for the identification, authentication and quality assessment of methanolic leaf extract of *B. acutangula* for its antimicrobial activity study.

ACKNOWLEDGEMENT

The authors are thankful to the H.O.D., U.D.P.S., Utkal University for providing laboratory facilities.

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