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Study on Antimicrobial Activity of *Barringtonia acutangula* and *Premna corymbosa,* two widely distributed Shrubs of the Sundarbans

Md. Jalal Uddin[1] and Rony Kumar Banik[2]

[1]Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna-9208, Bangladesh

[2]Department of Pharmacy, University of Science and Technology Chittagong, Chittagong-4202, Bangladesh

ABSTRACT: Use of plant based drugs and chemicals for curing various ailments and personal adornment is as old as human civilization. Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various ailments. The aim of the study was to evaluate the antimicrobial activity of Barringtonia acutangula (Family: Lecythidaceae) and Premna corymbosa (Family: Verbenaceae), two widely distributed shrubs of the Sundarbans mangrove forest by disc diffusion assay. The in vitro antimicrobial activity was tested against E. coli, S. flexneri, S. dysenteriae, V. cholerae , S. paratyphi, Proteus spp, S. aureus and S. epidermis using disc diffusion assay where the chloroformic extract of bark of B. acutangula showed excellent performance against E. coli and the zone of inhibition was recorded as 10mm and 13mm for 500µg and 1000µg consistently. The chloroformic extracts of bark of P. corymbosa also showed significant activity against V. cholera, S. dysenteriae and E. coli respectively. In this investigation Kanamycin (30 µg / disc) standard disc were used as the reference.

KEYWORDS: Shrubs, Antimicrobial Activity, Zone of Inhibition, Disc Diffusion, Barringtonia acutangula and Premna corymbosa.

1 INTRODUCTION

Medicine is the wonder of the world and blessings for mankind. From the very ancient time men used various plant parts as their wound healing. Medicinal Plants have been used for centuries as a popular remedy against several health disorders. In the field of natural product biology, ethnopharmacology, as well as bioprospecting approaches, have received renewed attention in recent years (Bremner, P.*et al*., 2002; Geetha *et al*., 2002). Natural products and herbal remedies used in traditional folk medicine have been the source of many medically beneficial drugs (Battle *et al*., 2005) as many of the medicinal plants have been shown to present interesting biological and pharmacological activities and are used as therapeutic agents (Gertsch *et al*., 2003). Natural products and related drugs are used to treat 87% of all categorized human diseases including bacterial infection, cancer and immunological disorders (Newman, D. J. *et al.,* 2007). About 25% of prescribed drugs in the world originate from plants (Rates, S.M. K. 2001) and over 3000 species of plants have been reported to have anticancer properties (Graham, J. G. *et al*; 2000). About 80% of the populations in developing countries rely on traditional plant based medicines for their primary health care needs (FAO, 2004). Bangladesh has a rich and prestigious heritage of herbal medicines among the South Asian countries. More than 500 species of medicinal plants are estimated as growing in Bangladesh and about 250 species of them are used for the preparation of traditional medicines (Ghani, A. 2003). However, the majority of these plants have not yet undergone chemical, pharmacological and toxicological studies to investigate their bioactive compound(s) (Ghani, A. 2003). Thus, this research work was designed to evaluate the antimicrobial activity of *B. acutangula and P. corymbosa* by disc diffusion assay for further exploration of their bioactive constituents. In vitro antibacterial activity of plants can be detected by observing the growth response of various microorganisms to those plant extracts or their solvent fractions that are placed in contact with them. This can be done in three ways (diffusion, dilution, and bioautographic methods) and a great number of factors viz. the extraction method (Nadir *et al*., 1986), inoculate volume, culture medium composition (Bauer *et al,* 1966), pH (Leven *et al.,* 1979), and incubation temperature (Lorian, 1991)

Corresponding Author: Md. Jalal Uddin

can influence the results. There is no standardized method for expressing the result of antibacterial screening (Ayafor *et al.,* 1982). Diameter of zone of inhibition is used sometimes to measure the inhibition of growth of microorganism. Disc diffusion technique is widely acceptable for the preliminary screening of antibacterial activity. The leaves and barks extract of *B. acutangula and P. corymbosa* were tested with eight species of pathogenic bacteria to investigate whether they have any antibacterial activity or not and the result is reported in this chapter.

2 MATERIALS AND METHOD

Test Materials

Selected *Barringtonia acutangula and Premna corymbosa* were collected on 25th March, 2011 and collected plants samples were identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka. This research work was carried out from 26th March to 15th September, 2011 in Molecular Biology and Animal Cell Culture Laboratory of Khulna University, Khulna, Bangladesh.

Experimental Materials

Filter paper discs, Nutrient agar medium, Petri dishes, Inoculating loop, Sterile cloth, Sterileforceps ,Sterile scissors, Tissue paper, Micropipette, Chloroform / Dimethyl sulfoxide, 70% ethanol, Test tubes, Beaker, Nose masks and hand gloves, Standard antibiotic discs (Kanamycin 30µg/disc), Laminar air flow, UV lamp, 10 ml volumetric flask, Incubator Autoclave, Water Bath, Digital Balance and Refrigerator.

Preparation of Materials

Collected plant samples were washed by distilled water (DW) to remove undesirable materials and excess of water was drained off. The leaves and stems were separated from each other and they were sliced into small pieces. The sliced materials were weighed by balance. The sliced leaves and stems were dried for few days under sunlight with shadow. After that the dried samples were powdered separately by grinding machine and then 5.4 gm powdered leaf of *B. acutangula* was taken into clean flat-bottomed glass container and soaked in 200 ml of 95% ethanol, 5.4 gm powdered leaf of *B. acutangula* and 12.8 gm powdered bark of *P. corymbosa* was taken into clean flat-bottomed glass container and soaked in 200 ml of absolute methanol and 5.4 gm powdered leaf and 10.72 gm powdered bark of *B. acutangula* and 12.8 gm powdered bark of *P. corymbosa* was taken into clean flat-bottomed glass container and soaked in 200 ml of absolute chloroform. The containers with its contents were sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper. The filtrates obtained were evaporated under ceiling fan and in a water- bath until dried. It rendered concentrates of greenish, brownish, reddish and sometimes blackish in color. The concentrates were designated as crude extract of 95% ethanol, methanol and chloroform of *B. acutangula and P. corymbosa*. Then 50mg of each ethanolic, methanolic and chloroformic extract of leaves and chloroformic extract of bark of *B. acutangula*, methanolic and chloroformic extract of barks of *P. corymbosa* were dissolved in 2ml of ethanol, methanol and chloroform respectively to produce final concentration of 25µg/µl.

Preparation of Media

Nutrient agar media were prepared by adding water to a dehydrated product, which contains all the ingredients. Practically all media are available commercially in powdered form (Pelczar *et al,* 1986).

Table 1. Composition of nutrient agar media

Ingredients	Amounts (gram/liter)
Peptone A	5.0
Beef extract	3.0
Sodium chloride	8.0
Agar A	15
Distilled water	1L
pH	7.3 (approximately)

Preparation of the Seeded Test Plates

With the help of an inoculating loop, the test organisms from the pure cultures were transferred to the agar slants in an aseptic condition using laminar air hood. The inoculated slants were then incubated at 37°C for 18-24 hours (for bacteria) to assure the growth of test organisms. This culture was used within two days. Each of the test organisms were transferred from the subculture to the test tube containing 16 ml autoclaved media with the help of the sterilized inoculating loop at 45°C under laminar air follow. The test tubes were shaken by rotation to get a uniform suspension of organism. The bacterial suspensions were immediately transferred to the sterile Petri dishes aseptically. The Petri dishes were rotated several times, first clockwise and then anticlockwise, to assure homogeneous distribution of the test organisms.

Table 2. List of Microorganisms used in this study

Gram positive(+ve)	Gram negative(-ve)
	Shigella flexneri
	Shigella dysenteriae
Staphylococcus aureus	*Vibrio cholerae*
Staphylococcus epidermis	*Salmonella paratyphi*
	Escherichia coli
	Proteus spp.

Preparation of Discs

Sterile filter paper discs (5 mm in diameter) were taken in a blank Petri dish. Sample solution of the desired concentration (500/1000µg) was applied on the discs with the help of a micropipette in an aseptic condition. These discs were left for few minutes in aseptic condition for complete removal of solvent. Kanamycin (30 µg / disc) standard disc were used as positive control and blank disc were used as negative control which ensure the residual solvents (left over the discs even after air drying) and the filter paper were not activated themselves.

Procedure

Sample impregnated discs (500/1000µg), standard antibiotic discs (Kanamycin) and negative control discs were placed gently on the solidified agar plates, freshly seeded with the test organisms with the help of a sterile forceps to assure complete contact with medium surface. The spatial arrangement of the discs was such that the discs were not closer than 15mm to the edge of the plate and far enough apart to prevent overlapping the zones of inhibition. The plates were then inverted and kept in refrigeration for about 24 hours at 4°C. This was sufficient for the material to diffuse into a considerable area of the medium. Finally the plates were incubated upside down at 37°C for 12-18 hours. After incubation, the antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition in term of millimeter with a transparent scale.

3 RESULTS AND DISCUSSIONS

Antibacterial activity of ethanolic, methanolic and chloroformic extracts of bark and leaf of *Barringtonia acutangula* and *Premna corymbosa* were examined and found to exhibit activities against the test bacteria. In this regard, disk diffusion method was applied in a dose dependent manner. Commercial kanamycin discs were used to compare the extract of test sample. The result of the antibacterial activity of the ethanolic, methanolic and chloroformic extracts were measured in terms of diameter of zone of inhibition in millimeter and shown in Table 3, 4, 5, 6, 7, 8, 9 and 10.

Table 3. Study on antimicrobial activity of B. acutangula and P. corymbosa extracts against S. aureus

Name of the plant	Type of extract	Solvent	Diameter of zone of inhibition in mm			
			Test Sample		Kanamycin	
			250 (µg/disc)	500 (µg/disc)	Positive Control	Negative Control
Barringtonia acutangula	Leaf	Methanol	__	__	37	__
	Leaf	Ethanol	8	11	35	__
	Leaf	Chloroform	9	12	37	__
	Bark	Chloroform	9	10	32	__
Premna corymbosa	Bark	Methanol	11	14	34	__
	Bark	Chloroform	12	16	33	__

Table 4. Study on antimicrobial activity of B. acutangula and P. corymbosa extracts against Proteus spp.

Name of the plant	Type of extract	Solvent	Diameter of zone of inhibition in mm			
			Test Sample		Kanamycin	
			500 (µg/disc)	1000 (µg/disc)	Positive Control	Negative Control
Barringtonia acutangula	Leaf	Methanol	__	3	30	__
	Leaf	Ethanol	6	8	28	__
	Leaf	Chloroform	4	7	30	__
	Bark	Chloroform	5	7	30	__
Premna corymbosa	Bark	Methanol	__	5	27	__
	Bark	Chloroform	12	15	29	__

Table 5. Study on antimicrobial activity of B. acutangula and P. corymbosa extracts against S. dysenteriae

Name of the plant	Type of extract	Solvent	Diameter of zone of inhibition in mm			
			Test Sample		Kanamycin	
			500 (µg/disc)	1000 (µg/disc)	Positive Control	Negative Control
Barringtonia acutangula	Leaf	Methanol	__	__	17	__
	Leaf	Ethanol	__	__	17	__
	Leaf	Chloroform	8	9	18	__
	Bark	Chloroform	9	11	17	__
Premna corymbosa	Bark	Methanol	6	8	19	__
	Bark	Chloroform	10	14	17	__

Table 6. Study on antimicrobial activity of B. acutangula and P. corymbosa extracts against V. cholerae

Name of the plant	Type of extract	Solvent	Diameter of zone of inhibition in mm			
			Test Sample		Kanamycin	
			500 (µg/disc)	1000 (µg/disc)	Positive Control	Negative Control
Barringtonia acutangula	Leaf	Methanol	4	7	28	__
	Leaf	Ethanol	__	5	31	__
	Leaf	Chloroform	9	12	30	__
	Bark	Chloroform	8	11	29	__
Premna corymbosa	Bark	Methanol	6	10	30	__
	Bark	Chloroform	14	18	28	__

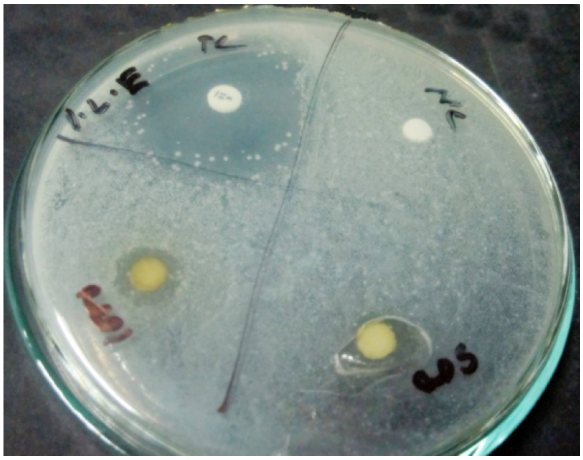

Figure 1. Zone of Inhibition of Barringtonia acutangula leaf against S. aureus

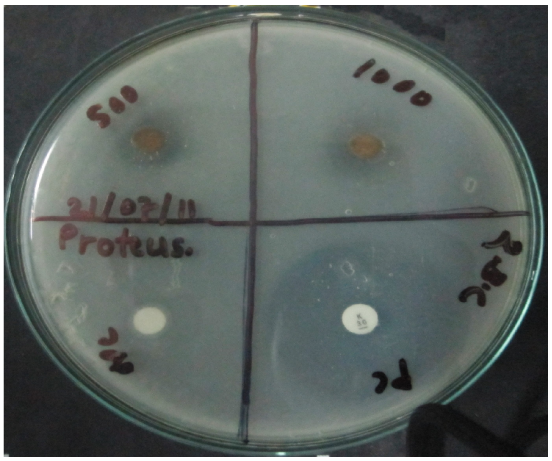

Figure 2. Zone of Inhibition of Premna corymbosa bark against V. cholerae

Table 7. Study on antimicrobial activity of B. acutangula and P. corymbosa extracts against S. paratyphi

Name of the plant	Type of extract	Solvent	Diameter of zone of inhibition in mm			
			Test Sample		Kanamycin	
			500 (µg/disc)	1000 (µg/disc)	Positive Control	Negative Control
Barringtonia acutangula	Leaf	Methanol	8	11	32	__
	Leaf	Ethanol	16	8	30	7
	Leaf	Chloroform	12	14	30	13
	Bark	Chloroform	8	11	29	8
Premna corymbosa	Bark	Methanol	7	10	28	__
	Bark	Chloroform	11	14	30	__

Table 8. Study on antimicrobial activity of B. acutangula and P. corymbosa extracts against E. coli

Name of the plant	Type of extract	Solvent	Diameter of zone of inhibition in mm			
			Test Sample		Kanamycin	
			500 (µg/disc)	1000 (µg/disc)	Positive Control	Negative Control
Barringtonia acutangula	Leaf	Methanol	8.5	11	13	__
	Leaf	Ethanol	8	10	14	__
	Leaf	Chloroform	7	10	13	__
	Bark	Chloroform	10	13	12	__
Premna corymbosa	Bark	Methanol	7	9.5	13	__
	Bark	Chloroform	7	12	12	__

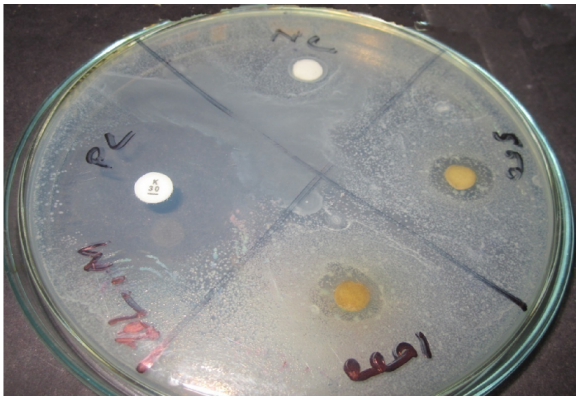

Figure 3. Zone of Inhibition of Barringtonia acutangula leaf against S. paratyphi

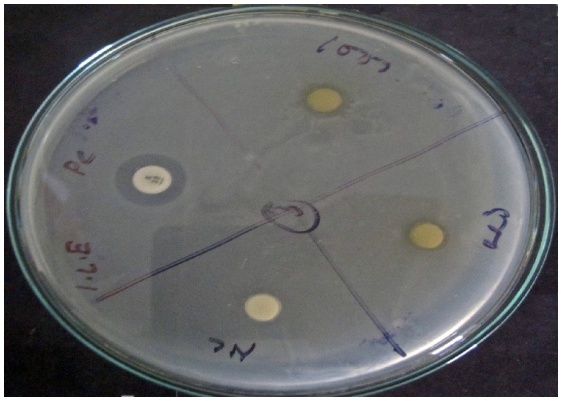

Figure 4. Zone of Inhibition of Barringtonia acutangula leaf against E. coli.

Table 9. Study on antimicrobial activity of B. acutangula and P. corymbosa extracts against S. flexneri

Name of the plant	Type of extract	Solvent	Diameter of zone of inhibition in mm			
			Test Sample		Kanamycin	
			500 (µg/disc)	1000 (µg/disc)	Positive Control	Negative Control
Barringtonia acutangula	Leaf	Methanol	10	14	28	__
	Leaf	Ethanol	10	12	30	__
	Leaf	Chloroform	12	15	31	__
	Bark	Chloroform	9	__	29	__
Premna corymbosa	Bark	Methanol	__	7	27	__
	Bark	Chloroform	9	14	27	__

Table 10. Study on antimicrobial activity of B. acutangula and P. corymbosa extracts against S. epidermis

Name of the plant	Type of extract	Solvent	Diameter of zone of inhibition in mm			
			Test Sample		Kanamycin	
			500 (µg/disc)	1000 (µg/disc)	Positive Control	Negative Control
Barringtonia acutangula	Leaf	Methanol	8	15	30	__
	Leaf	Ethanol	7	10	29	__
	Leaf	Chloroform	7	9	30	__
	Bark	Chloroform	8	11	31	__
Premna corymbosa	Bark	Methanol	10	12	31	__
	Bark	Chloroform	12	14	30	__

From these tabulated vales it is seen that the chloroformic extracts of bark of *Premna corymbosa* has significant antimicrobial activity against *S. dysenteriae* (Table 5) and gave zone of inhibition of 14 mm for 1000 µg/disc and 10 mm for 500 µg/disc*,* where commercial antibiotic disc kanamycin gave zone of inhibition of 17 mm. On the other hand, chloroformic extract of bark of *Barringtonia acutangula* shows an outstanding performance against *E. coli* (Table 8) and gave zone of inhibition of 10 mm for 500 µg/disc and 13 mm for 1000 µg/disc, where kanamycin gave 12 mm. Besides these, the methanolic, ethanolic and chloroformic extracts of barks and leaves of *B. acutangula and P. corymbosa* shows significant antimicrobial activity especially against *E. coli* and a mild response against *S.paratyphi*, *S.flexneri* and *S.epidermis.* But in case of other bacterial strains no satisfactory results are found especially on *S.aureus*, *Proteus Spp.* and *V. cholera.*

4 CONCLUSION

Barringtonia acutangula and *Premna corymbosa* traditionally been used as therapeutic agents to treat piles. tumors, eye infection, fever, flu, skin rash, womb cleaning, rheumatic disorders and arthritis, diarrhea, dysentery and colitis. This study proved that the ethanolic, methanolic, and chloroformic extracts of *B. acutangula and P. corymbosa* exert potent antibacterial activity against *E. coli, S. flexneri, S. dysenteriae, V. cholerae, S. paratyphi, Proteus spp, S. aureus* and *S. epidermis* where chloroformic extract of bark of *Barringtonia acutangula* showed an outstanding performance against *E. coli*.

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