Reprint **ISSN 1997-2571 (Web Version)**

Journal of Innovation & Development Strategy (JIDS)

(J. Innov. Dev. Strategy)

Volume: 5 **Issue: 2** **August 2011**

J. Innov. Dev. Strategy 5(2):62-69(August 2011)

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http://ggfagro.com/ejournals/current issues

JIDS** issn 1997-2571, HQ:19-10 central place, saskatoon, saskatchewan, s7n 2s2, Canada

ISSN-1997-2571 (Online)
J. Innov. Dev. Strategy 5(2):62-69(August 2011)

INVESTIGATION ON ANTIMICROBIAL ACTIVITIES OF THE TWO SELECTED SHRUBS FROM THE SUNDARBANS (*Clerodendrum inerme* and *Caesalpinia crista*)

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Accepted for publication on 30 July 2011

ABSTRACT

Sabrin F, Hasan MN, Rahman MM, Islam KD, Billah MM (2011) Investigation on antimicrobial activities of the two selected shrubs from the sundarbans (*Clerodendrum inerme* and *Caesalpinia crista*). *J. Innov. Dev. Strategy* 5(2), 62-69.

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 various ailments. The aim of the study was to evaluate the antimicrobial activity of *Clerodendrum inerme* (Family: Verbenaceae) and *Caesalpinia crista* (Family: Leguminosaceae), two widely distributed shrubs of the Sundarbans mangrove forest. The *in vitro* antimicrobial activity was tested against *E. coli, Shigella flexneri, Shigella dysenteriae, Vibrio cholerae, Salmonella paratyphi, Proteus* spp., *Staphylococcus aureus* and *Staphylococcus epidermis* using disc diffusion assay where the chloroformic bark extract of of *C. inerme* showed excellent performance against *E. coli*. The chloroformic extracts of bark of *C. crista* also showed significant activity against *V. cholera*, *S. dysenteriae* and *E. coli*.

Key words: *antimicrobial activity, Clerodendrum inerme, Caesalpinia crista, extract, disc diffusion method, medicinal plant*

INTRODUCTION

The name Clerodendrum is derived from the Greek word kleros, meaning chance or fate, and dendron, meaning tree, in reference to the uncertain medicinal qualities of some of the plants. *Clerodendrum inerme* is known as Glory Bower, Indian privet, Seaside clerodendrum, Wild Jasmine, Sorcerers Bush across the world (Fig. 1). *C. inerme* are reported to be used for treating coughs, serofulous infection, buboes problem, venereal infections, skin diseases and as a vermifuge, febrifuge and also for treating beriberi disease (Anonymous 1992; Kanchanapoom *et al*. 2001). It has also been described that tribal people use *C. inerme* as an antidote of poisoning from fish, crabs and toads (Kanchanapoom *et al.* 2001; Pandey *et al.* 2003).

Crista is sometimes seen growing wild along the seashore and back mangroves. It also known as 'Kuku tupai' means 'squirrel's claws' in Malay and aptly describes an encounter with this shrub (Fig. 2). The seeds, root, bark and the leaves are used for medicinal purpose. The shrub is used both internally as well as externally. The external use includes the paste of its leaves to relieve the pain and edema. The massage with its seed-oil particularly helps in rheumatic disorders and arthritis.

Fig. 1. Parts of *Clerodendrum inerme*

The wounds and ulcers should be dressed with the paste of its seed mashed in water. Internally, *crista* is used in vast range of diseases. It is the best panacea for abdominal pain due to flatulence. The powder of its roasted seeds with butter mitigates the condition and relieves the pain. A combination recommended for malaria is the powders of marica and *crista*. The splenic enlargement due to malaria, responds well to *crista*. The leaves fried in butter, eliminate pain and relieve constipation, hence valuable in piles.

Fig. 2. Parts of *Caesalpinia crista*

The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants (Duraipandiyan *et al.* 2006). Natural products and related drugs are used to treat 87% of all categorized human diseases including bacterial infection, cancer and immunological disorders (Newman *et al.* 2007). 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 2003). However, the majority of these plants have not yet undergone chemical, pharmacological and toxicological studies to investigate their bioactive compound(s) (Ghani 2003). Traditional records and ecological diversity indicate that Bangladeshi plants represent an exciting resource for possible lead structures in drug design. Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Mojab *et al.* 2003). Plant secondary metabolites have been used for mankind as remedies since the beginning of civilization. At present, they play an important role in health care for about 80% of the world's population. The presence of diverse bioactive metabolites in plants has formed the therapeutic basis of herbal medication. Thus, emphasis is given on *C. inerme* and *C. crista* to evaluate their antimicrobial activities and cytotoxic effects for further exploration of their active constituents. The present study aims at evaluating the antimicrobial activity of these two plants.

MATERIALS AND METHODS

Collection of the plant samples

Plants selected for this study work were *C. inerme* and *C. crista*. These plants were collected from Ghagramaree, Chadpai range, Forest Department, the Sundarbans East Division on 25th March, 2011.

Identification

Collected plants samples were identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka as *inerme* and *crista*.

Preparation (drying and grinding)

Collected plant samples were washed by distilled water (DW) to remove undesirable materials and excess 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 and dried for few days under sunlight with shade. Then the dried samples were powdered by grinding and finally stored into the air-tight plastic bags separately**.**

Preparation of crude extract

The precise mode of extraction naturally depends on the texture and water content of the plant material being extracted and on the type of substance being isolated. Generally, two types of procedures were used for obtaining organic constituents, i.e. cold extraction and hot extraction.

Cold Extraction with ethanol

About 5.4 gm powdered leaf of *inerme* were taken into different clean, flat-bottomed glass containers and soaked in 200 ml of 95% ethanol. The container with its contents was 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.

Extraction with methanol

About 5.4 gm powdered leaf of *inerme* and 12.8 gm powdered bark of *crista* were taken into different clean, flat-bottomed glass containers and soaked in 200 ml of absolute methanol. The container with its contents was 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.

Extraction with chloroform

About 5.4 gm powdered leaf and 10.72 gm powdered bark of *inerme* and 12.8 gm powdered bark of *crista* were taken into different clean, flat-bottomed glass container and soaked in 200 ml of absolute chloroform. The container with its contents was 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.

Evaporation and Storage

The filtrates (95% ethanol, methanol and choloroformic extracts) 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 *inerme* and *crista*. Then the dried crystal forms of crude extracts were kept in refrigerator for further experiment.

Test material

Ethanolic, methanolic and chloroformic extracts of leaves and chloroformic extract of barks of *inerme,* methanolic and chloroformic extracts of barks of *crista* are used as test materials in this study.

Microorganisms used for the test

Both gram positive and gram-negative bacterial strains were taken for the test. These bacterial strains used for the investigation are listed in Table 1. These organisms were collected from the different laboratories of Khulna University, Bangladesh.

Table 1. List of bacteria used in studying the antimicrobial properties of the extracts

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

Three types of discs were used for antibacterial screening: Sample discs, Standard discs and Blank discs.

Sample discs

Sterile filter paper discs (5 mm in diameter) were taken in a blank petridish. Sample solution of the desired concentration (500 & 1000µg) was applied on the discs with the help of a micropipette in aseptic condition. These discs were left for few minutes in the same condition for complete removal of solvent.

- **Standard discs**

These were used as positive control to ensure the activity of standard antibiotics against the test organisms as well as for comparison of the response produced by the known antibacterial agent with that produced by test samples. In this present study, Kanamycin standard disc (30 µg / disc) were used as the reference.

- **Blank discs**

These were used as negative control. They ensure that the residual solvents (left over the discs even after air drying) and the filter paper were not active themselves.

Preparation of test samples

Fifty mg of ethanolic, methanolic and chloroformic extracts of leaves and chloroformic extract of bark of *inerme*, methanolic and chloroformic extracts of barks of *crista* were dissolved in 2ml of ethanol, methanol and chloroform respectively to produce final concentration of 25µg/µl.

Assay for antimicrobial activity

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 forcep 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 terms of millimeter with a transparent scale.

RESULTS AND DISCUSSION

Ethanolic, methanolic and chloroformic extracts of leaves and chloroformic extract of barks of *inerme*, methanolic and chloroformic extracts of barks of *crista* were tested for antibacterial activity against a number of pathogenic bacteria. Standard antibiotic discs of Kanamycin (30 µg / disc) were used as standard.

The experiment was conducted with eight different species of bacteria, namely- *Escherichia coli, Shigella flexneri, Shigella dysenteriae*, *Vibrio cholerae*, *S. paratyphi, Proteus* spp., *Staphylococcus aureus* and *Staphylococcus epidermis*. Antibacterial activity of ethanolic, methanolic and chloroformic extracts of bark and leaf of *Clerodendrum inerme* and *Caesalpinia crista* were examined and found to exhibit activities against the test bacteria. In this regard, disk diffusion method was applied in a dose dependent manner. The result of the antibacterial activity of the ethanolic, methanolic and chloroformic extracts were measured in terms of diameter of zone of inhibition in mm and shown in Table 2, 3, 4, 5, 6, 7, 8, and 9 respectively.

From these tabulated values it is seen that the chloroformic extracts of bark of *C. crista* has significant antimicrobial activity against *S. dysenteriae* (Table 4) and gave zone of inhibition of 12 mm for 1000 µg/disc and 8 mm for 500 µg/disc, where commercial antibiotic disc kanamycin gave zone of inhibition of 19 mm. On the other hand, chloroformic extract of bark of *Clerodendrum inerme* shows an outstanding performance against *E. coli* (Table 9) and gave zone of inhibition of 11 mm for 500 µg/disc and 12 mm for 1000 µg/disc, where kanamycin gave 14 mm. Besides these, the methanolic, ethanolic and chloroformic extracts of barks and leaves of *C. inerme* and *C. crista* 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.* Representative illustrations of zones of inhibition for both *C. Inerme* and *C. crista* as indicative of antimicrobial activity are shown in Fig. 3, 4 and 5 respectively.

Table 2. Study on antimicrobial activity of *C. inerme* and *C. crista* extracts against *S. aureus*

Name of the plant	Type of extract	Solvent	Diameter of zone of inhibition in mm				Performance (µg/disc)	
			Test Sample (µg/disc)		Kanamycin			
			250	500	(+) ve Control	(-)ve Control	250	500
C. inerme	Leaf	Methanol	—	—	38	—	—	—
	Leaf	Ethanol	8	10	38	—	+	++
	Leaf	Chloroform	8	10	38	—	+	++
C. crista	Bark	Chloroform	8	12	38	—	+	++
	Bark	Methanol	10	13	40	—	++	++
	Bark	Chloroform	11	15	38	—	++	++

"+"= Fair "++"= Good "+++" = Very good "++++"= Excellent

Table 3. Study on antimicrobial activity of *C. inerme* and *C. crista* extracts against *Proteus* spp.

Name of the plant	Type of extract	Solvent	Diameter of zone of inhibition in mm				Performance	
			Test Sample		Kanamycin			
			500 (µg/disc)	1000 (µg/disc)	Positive Control	Negative Control	500 (µg/disc)	1000 (µg/disc)
C. inerme	Leaf	Methanol	—	—	32	—	—	—
	Leaf	Ethanol	—	—	32	—	—	—
	Leaf	Chloroform	—	—	32	—	—	—
C. crista	Bark	Chloroform	—	—	32	—	—	—
	Bark	Methanol	—	—	32	—	—	—
	Bark	Chloroform	13	14	32	—	++	++

Fig. 3. Zone of inhibition of *C. crista* against *Proteus* Spp. and *C. inerme* against *S. aureus*

Table 4. Study on antimicrobial activity of *C. inerme* and *C. crista* extracts against *S. dysenteriae*

Name of the plant	Type of extract	Solvent	Diameter of zone of inhibition in mm				Performance	
			Test Sample		Kanamycin			
			500 (µg/disc)	1000 (µg/disc)	Positive Control	Negative Control	500 (µg/disc)	1000 (µg/disc)
C. inerme	Leaf	Methanol	—	—	19	—	—	—
	Leaf	Ethanol	—	—	19	—	—	—
	Leaf	Chloroform	6	7	19	—	++	++
	Bark	Chloroform	7	9	19	—	++	++
C. crista	Bark	Methanol	7	9	19	—	++	++
	Bark	Chloroform	8	12	19	—	++	+++

"+"= Fair "++"= Good "+++" = Very good "++++"= Excellent

Table 5. Study on antimicrobial activity of *C. inerme* and *C. crista* extracts against *V. cholerae*

Name of the plant	Type of extract	Solvent	Diameter of zone of inhibition in mm				Performance	
			Test Sample		Kanamycin			
			500 (µg/disc)	1000 (µg/disc)	Positive Control	Negative Control	500 (µg/disc)	1000 (µg/disc)
C. inerme	Leaf	Methanol	—	—	10	—	—	—
	Leaf	Ethanol	—	—	30	—	—	—
	Leaf	Chloroform	8	10	30	—	++	++
	Bark	Chloroform	7	7	20	—	++	++
C. crista	Bark	Methanol	—	—	30	—	—	—
	Bark	Chloroform	15	17	25	—	+++	+++

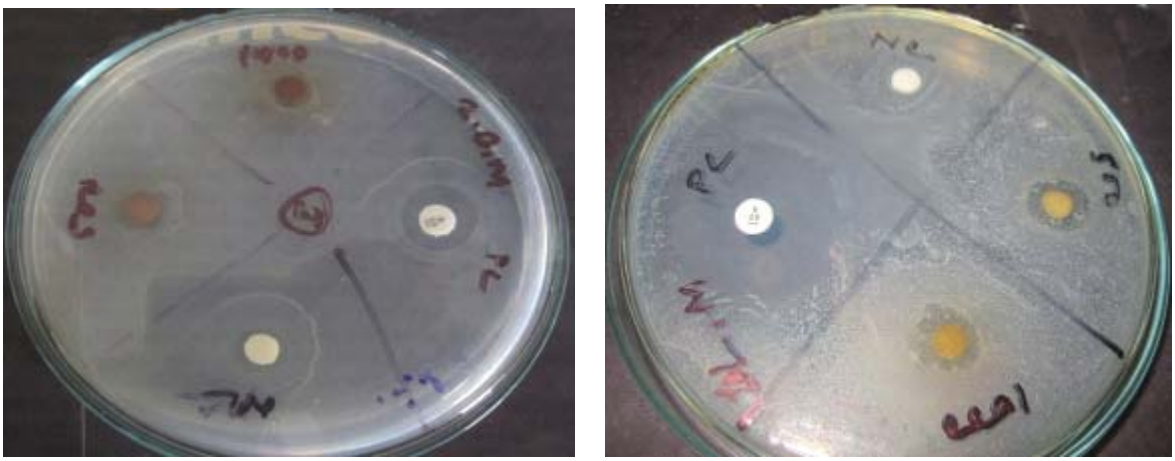

Fig. 4. Zone of inhibition of *C. crista* against *E. coli.* and *C. inerme* against *S. paratyphi*

Table 6. Study on antimicrobial activity of *C. inerme* and *C. crista* extracts against *S. paratyphi*

Name of the plant	Type of extract	Solvent	Diameter of zone of inhibition in mm				Performance	
			Test Sample		Kanamycin			
			500 (µg/disc)	1000 (µg/disc)	Positive Control	Negative Control	500 (µg/disc)	1000 (µg/disc)
C. inerme	Leaf	Methanol	9	7	32	—	++	+
	Leaf	Ethanol	15	7	32	7	++	+
	Leaf	Chloroform	11	12	32	13	++	++
	Bark	Chloroform	7	10	32	8	+	++
C. crista	Bark	Methanol	8	11	32	—	++	++
	Bark	Chloroform	10	12	32	—	++	++

"+"= Fair "++"= Good "+++" = Very good "++++"= Excellent

Table 7. Study on antimicrobial activity of *C. inerme* and *C. crista* extracts against *S. flexneri*

Name of the plant	Type of extract	Solvent	Diameter of zone of inhibition in mm				Performance	
			Test Sample		Kanamycin			
			500 (µg/disc)	1000 (µg/disc)	Positive Control	Negative Control	500 (µg/disc)	1000 (µg/disc)
C. inerme	Leaf	Methanol	11	12	30	—	++	++
	Leaf	Ethanol	9	10	30	—	++	++
	Leaf	Chloroform	11	12	30	—	++	++
	Bark	Chloroform	8	—	30	—	++	—
C. crista	Bark	Methanol	—	—	30	—	—	—
	Bark	Chloroform	8	12	30	—	++	++

Fig. 5. Zone of inhibition of *C. inerme* against *S. flexneri* and *C. inerme* against *E. coli*

Table 8. Study on antimicrobial activity of *C. inerme* and *C. crista* extracts against *S. epidermis*

Name of the plant	Type of extract	Solvent	Diameter of zone of inhibition in mm				Performance	
			Test Sample		Kanamycin			
			500 (µg/disc)	1000 (µg/disc)	Positive Control	Negative Control	500 (µg/disc)	1000 (µg/disc)
C. inerme	Leaf	Methanol	—	—	32	—	—	—
	Leaf	Ethanol	—	—	32	—	—	—
	Leaf	Chloroform	—	—	32	—	—	—
	Bark	Chloroform	7	11	32	—	+	++
C. crista	Bark	Methanol	9	11	34	—	++	++
	Bark	Chloroform	11	13	32	—	++	++

"+"= Fair "++"= Good "+++" = Very good "++++"= Excellent

Table 9. Study on antimicrobial activity of *C. inerme* and *C.crista* extracts against *E. coli*

Name of the plant	Type of extract	Solvent	Diameter of zone of inhibition in mm				Performance	
			Test Sample		Kanamycin			
			500 (µg/disc)	1000 (µg/disc)	Positive Control	Negative Control	500 (µg/disc)	1000 (µg/disc)
C. inerme	Leaf	Methanol	7.5	9	14	—	+++	+++
	Leaf	Ethanol	7	9	12	—	+++	+++
	Leaf	Chloroform	8	10	13	—	+++	+++
	Bark	Chloroform	11	12	14	—	++++	++++
C. crista	Bark	Methanol	7	8.5	13	—	+++	+++
	Bark	Chloroform	7	8	14	—	+++	+++

CONCLUSION

C. inerme (local name: sitki lota) belonging to Verbenaceae family and *C. crista* (local name: kutum kanta) belonging to Leguminosaceae family are two widely distributed shrubs of the mangrove forest Sundarbans. These shrubs have traditionally been used as therapeutic agents to treat eye infection, fever, flu, skin rash, womb cleaning, rheumatic disorders and arthritis, diarrhea, dysentery and colitis. The ethanolic, methanolic, and chloroformic extracts of *C. inerme* and *C. crista* showed 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 *C. inerme* showed an marked performance against *E. coli*. Besides these, the methanolic, ethanolic and chloroformic extracts of barks and leaves of *C. inerme* and *C. crista* 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*.

Therefore, further studies are required to identify, isolate and characterize their active principles responsible for the significant antimicrobial activity. The Sundarbans is the largest store house of different unique plants species and most of the plants are still untapped. So, extensive studies of other plants of the mangrove forest Sundarbans would lead to identification of novel anti-cancer, anti-tumor, anti-bacterial or pesticidal sources providing leads for the development of new drugs.

ACKNOWLEDGEMENT

This study was supported by an INSPIRE grant (no. SP_137, 2011–2013) from the British Council.

REFERENCES

Anynomous (1992) The Useful Plants of India, Publication and Information Directorate, CSIR, New Delhi, 132 pp.

Duraipandiyan V, Ayyanar M, Ignacimuthu S (2006) Antimicrobial Activity of Some Ethnomedical Plants Used by Paliyar Tribe from Tamil Nadu, India. BMC complementary and alternative medicine. pp 635.

Ghani A (2003) Medicinal Plants of Bangladesh with Chemical Constituents and Uses, Asiatic Society of Bangladesh, Dhaka, Bangladesh.

Kanchanapoom T, Kasaia R, Chumsric P, Hiragad Y, Yamasaki K (2001) Megastigmane and iridoid glucosides from Clerodendrum inerme. Phytochemistry 58, 333-336.

Mojab F, Kamalinejad M, Ghaderi N, Vahidipour H (2003) Phytochemical Screening of Some Iranian Plants. Iranian Journal of Pharmaceutical Research. pp 77-82.

Newman DJ, Cragg GM (2007) Natural products as sources of new drugs over the last 25 years," *Journal of Natural Products*, vol. 70, no. 3, pp. 461–477.

Pandey R, Verma RK, Singh SC, Gupta MM (2003) 4α-methyl-24β -ethyl- 5α-cholesta-14,25-dien-3β-ol and 24β -ethylcholesta-5, 9(11), 22e-trien-3β - ol, sterols from Clerodendrum inerme. Phytochemistry 63, 415-420.