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ANTIBACTERIAL ACTIVITY OF CHLOROFORMIC EXTRACTS OF *Xylocarpus mekongenesis* (Lamk.) M. Roem. and *Cerbera manghas*

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ABSTRACT

Sabrin F, Khatun MA, Alam SMN, Islam KMD, Billah MM (2012) Antibacterial activity of chloroformic extracts of *Xylocarpus mekongenesis* (Lamk.) M. Roem. and *Cerbera manghas*. *J. Innov. Dev. Strategy.* 6(2), 13-18.

Xylocarpus mekongenesis (Family: Meliaceae) and *Cerbera manghas* (Family: Apocynaceae) are two widely distributed woody plants of the Sundarbans mangrove forest. In the present study, efforts were undertaken to determine the antimicrobial effect of chloroformic stem and leaf extracts of *X. mekongenesis* and *C. manghas* using disc diffusion method. A number of both gram positive and gram negative bacteria were cultured on Petri dishes (120 mm in diameter) containing nutrient agar media for this antimicrobial screening. Tested extracts in different concentration diffused from the discs to the surrounding medium of the plate. The plates were maintained at 37°C for 18 hours for optimum growth of the microorganisms. The antibacterial activity of the extracts was determined by measuring the diameter of the zone of inhibition in terms of millimetre. Antimicrobial screening showed that the crude chloroformic stem and leaf extracts of *X. mekongenesis* and *C. manghas* possessed antimicrobial activity against most of the test organisms depending upon the nature of their active ingredients in the extract and capacity of diffusion into the agar medium. Among the test organisms, the extract showed moderate activity against *Escherichia coli, Shigella flexneri, Shigella dysenteriae, Vibrio cholerae, Salmonella typhi, Salmonella paratyphi, Pseudomonas spp, Proteus spp, Staphylococcus aureus* and *Staphylococcus epidermis*. The experimental findings provide a support for their uses in ethnomedicine and show rationale for further investigation.

Key words: *antibacterial activity, chloroform extracts, Xylocarpus mekongenesis, Cerbera manghas, mangrove plants, the Sundarbans*

INTRODUCTION

Bioactive natural products are chemical compounds produced by living organisms with a possible biological effect on other organisms. This includes therapeutic activity for diseases of humans and animals, toxic activity responsible for causing human and animal diseases, and selective, biodegradable toxicity in combating pests that may adversely affect our endeavors to feed and otherwise services for human population (Colegate and Moleneux, 2008).

The Sundarbans mangrove forest, located to the southern region of Bangladesh, offers a unique and uncommon diversity of flora. However, unlike other mangrove forests, the ecosystem of the Sundarbans has become threatened due to global warming, natural disasters and various man-made conditions. For adapting in an extreme environment, the plants of mangrove origin often produce unique bioactive compounds different from terrestrial plants. The compounds that have ability to interfere or inhibit the microbial growth and metabolism are called antimicrobial compounds (Pelczar *et al.* 1993) and the activity to inhibit microbial growth and metabolism is called antimicrobial activity. Antimicrobial agents are among the most dramatic examples of the advances of modern medicine. Many infectious diseases once considered incurable and lethal are now amenable to treatment. The remarkably powerful and specific activity of antimicrobial drugs arises from their selectivity for highly specific targets unique to microorganisms or much more important in them than in humans. Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, this necessitates the systematic screening of potent antimicrobial drugs for the treatment of infectious diseases from plants (Agarwal *et al*. 1996).

In vitro antimicrobial activity of plants can easily be detected by observing the growth response of various microorganisms to the plant extracts or their solvent fractions in contact with them. Three methods namely diffusion, dilution and bioautographic are used for this purpose. Diffusion and dilution methods have been employed to study the antimicrobial activity of medicinal plants. A number of modifications have been made in the technique in order to obtain better results. Since some factors (culture medium composition, microorganisms tested, extractive method, pH, solubility of the sample in the culture medium, etc.) can change results, it is difficult to standardize a procedure for the study of antimicrobial plants (Rios *et al.* 1988). Disk diffusion technique is widely acceptable for the preliminary screening of antimicrobial activity. It is essentially a qualitative or semi qualitative test indicating the sensitive or resistance of micro- organisms to the plant extracts. Diameter of zone of inhibition is used sometimes to measure the inhibition of growth of microorganisms. However, no distinction between bacteriostatic and bacteriocidal activity can be demonstrated by this method (Reiner 1982).

Xylocarpus mekongenesis (*X. mekongenesis*) (Lamk.) M. Roem. (Meliaceae) and *Cerbera manghas* are two abundant woody species of the Sundarbans. *X. mekongenesis*, commonly known as Possur is a glabrous, medium-sized tree found in littoral forests of Bengal, Burma, the Andaman's, the Malay Peninsula and Archipelago, Australia, Fiji and Africa. In Bangladesh, this species are usually found in the north tract, remote from the sea, chiefly in low lying swampy locality, of the Sundarbans (Kirtikar and Basu, 1999). *X. mekongenesis* is traditionally used as an astringent and febrifuge and for the treatment of dysentery and diarrhoea (Ghani 1998). Xyloccensin has been isolated from the bark of *X. mekongenesis* (Bandaranayake 2002). The bark and pneumatophore of *X. mekongenesis* are reported to show antimalarial, antidiarrhoeal and antinociceptive activities (Bandaranayake 2002; Uddin *et al.* 2005, 2006). The traditional medicinal uses of *X. mekongensis* include the seeds for treating stomachaches, bark tannin for intestinal ailments (Giesen *et al.* 2006). Species belonging to the *Cerbera* (Apocynaceae) genus of plants are commonly found on the islands of Southeast Asia and Oceana, and on other lands surrounding the Indian Ocean. The two most frequently encountered species are *C. manghas* and *C. odollam*. They differ only in the color and shape of their fruits. Traditionally leaf juice of *C. manghas* is used in the treatment of rheumatism; the leaves are also used to treat skin diseases (Weiner 1984). Very few of the natural products from this plant were initially examined for biological activity. Therefore, it has been found that the plant parts of *X. mekongensis* and *C. manghas* have not been evaluated for potential bioactivities. However there remains the opportunity to determine systematically for possible bioactivities from these plants. The study was carried out to determine the antibacterial activity of crude chloroformic extracts of leaf and stem of *X. mekongensis* and *C. manghas*.

MATERIALS AND METHODS

Preparation of plant extracts

The selected *X. mekongensis* and *C. manghas* plant parts were collected from Ghagramaree, Chadpai range, East Sundarban Forest Department, Khulna, Bangladesh on 25 March, 2011. Collected plant samples were used to prepare the herbarium specimen and were later identified by the experts from Bangladesh National Herbarium, Dhaka as *X. mekongenesis* and *C. manghas* with proper accession numbers. Collected plant samples were also washed by distilled water (DW) to remove undesirable materials and excess of water was drained off. Then the leaves and stems were separated from each other and sliced into small pieces separately. The samples were then weighed and dried for few days under sunshade. Properly dried samples were ground into powder, weighed and finally stored into the separate air tight plastic bags**.**

The precise mode of extraction naturally depends on the texture and water content of the plant material to be extracted and on the type of substance to be isolated. Generally, cold as well as hot extraction procedures are employed. In this study cold extraction procedure was chosen to do the extraction with chloroform. About 16.386gm powdered stem and 3.27gm powdered leaf of *C. manghas* as well as 40.48 gm powdered stem and 8.58gm powdered leaf of *X. mekongenesis* were taken into separate 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 was subjected to a coarse filtration by a piece of clean, white cotton material followed by filtration with Whatman filter paper.

Antimicrobial activity by disc diffusion method

In the present study, the antibacterial activity of the chloroformic extracts was determined by disc diffusion method. Different amount of the stem and leaf extracts of these plants were dissolved into definite volumes of solvent to give solutions of known concentration (µg/ml). Then sterile Whatman filter paper discs were impregnated with known amount of the extracts using a micropipette and dried. Standard antibiotic discs and blank discs were absorbed into solvents and dried. These were used as positive and negative control, respectively. These discs were then placed inside the Petri dishes (120 mm in diameter) containing a suitable agar medium seeded with the test organisms using a sterile transfer loop. The plates were then maintained at 4ºC for several hours facilitating maximum diffusion. The plates were then kept in an incubator (37˚C) for 18 h to allow the growth of the microorganisms. If the extract possessed any antimicrobial activity, it would inhibit the growth of microorganisms giving a clear, distinct zone called "zone of inhibition". The antibacterial activity of the extracts was thus determined by measuring the diameter of the zone of inhibition in terms of millimeter. The experiments were carried out in triplicate and the average of the readings was recorded (Bauer *et al.* 1966).

Bacteria used for the activity test

Both Gram-positive and negative bacterial strains were taken into consideration for the evaluating the antimicrobial activity of the tested extracts. Gram negative bacteria included *Escherichia coli, Shigella flexneri, Shigella dysenteriae, Vibrio cholera, Salmonella typhi, Salmonella paratyphi, Pseudomonas* spp., *Proteus* spp. while *Staphylococcus aureus, Staphylococcus epidermis* were in the list of gram positive bacteria. These bacteria were collected from the Animal Cell Culture Laboratory, Biotechnology and Genetic Engineering Discipline, Khulna University where the culture of these bacteria was maintained.

Preparation of stock solution from the extracts

25mg chlorofomic extracts from both stem and leaf of *Xylocarpus mekongenesis* and *Cerbera manghas* were accurately measured and placed into one drum vial. Then 1ml chloroform was added to the extracts, mixed thoroughly and finally, concentration was prepared 25µg /µl.

Preparation of disks

Three types of discs (Sample discs, Standard discs and Blank discs) were used for antibacterial screening. For preparation of sample discs**, s**terile filter paper discs (5 mm in diameter) were taken into a blank Petri dish. Sample solutions of 20µl and 40µl volume were applied into the discs to achieve desired concentration (500, 1000µg) with the help of a micropipette in an aseptic condition from the stock solution. These discs were left for few minutes in aseptic condition for complete removal of solvent. In case of standard discs, Kanamycin (30µg/ disc) was used. They were used as positive control to ensure the activity of standard antibiotic against the test microorganisms as well as for comparison of the response produced by the known antibacterial agent with that produced by the extracts. Blank discs were used as negative control. It was ensured that the residual solvents (left over the discs even after air drying) and the filter paper were not active themselves. All these types of discs were placed gently on the solidified agar plates, freshly seeded with the test microorganisms 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 ensured sufficient time for the material to diffuse into a considerable area of the medium. Finally the plates were incubated upside down at 37°C for 18 h.

Determination of antimicrobial activity

After proper incubation, the antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition in termd of millimeter with a transparent scale.

RESULTS AND DISCUSSION

Ten common pathogenic bacteria such as *E. coli, S. flexneri, S. dysenteriae, V. cholerae, S. typhi, S. paratyphi, Pseudomonas* spp., *Proteus* spp., *S. aureus* and *S. epidermis* were used in the present investigation. Antibacterial activity of chloroformic stem and leaf extracts of *X. mekongenesis* and *C. manghas* were examined using disc diffusion method and it was found that the extracts showed responses in varying levels. The result of the antibacterial activity of the chloroformic extracts was measured from the diameters from the zone of inhibition expressed as mm and presented in Table 1, 2, 3 and 4.

Table 1. *In vitro* antibacterial activity of chloroformic extract of *X. mekongenesis* stem

Bacterial strains	Diameter of zone of inhibition in mm				
	Chloroformic extract (500µg/disc)		Chloroformic extract (1000µg/disc)		Kanamycin (30µg/disc)
	mm	Efficiency	mm	Efficiency	
Gram negative					
E. coli	7	+++	9	+++	13
S. flexneri	8	++	7	+	30
S. dysenteriae	7	++	9	++	19
V. cholerae	9	++	11	++	28
S. typhi	7	++	8	++	20
S. paratyphi	__	__	__	__	32
Pseudomonas spp.	__	__	__	__	22
Proteus spp.	8	++	9	++	32
Gram positive					
S. aureus	9	+	11	++	38
S. epidermis	6	+	7	+	32

Here, "+++" represents very good, "++"represents good, "+" represents fair, "_" represents no inhibition

Table 2. *In vitro* Antibacterial activity of chloroformic extract of *X. mekongenesis* leaf

Bacterial strains	Diameter of zone of inhibition in mm				
	Chlorophormic extract (500µg/disc)		Chlorophormic extract (1000µg/disc)		Kanamycin (30µg/disc)
	mm	Efficiency	mm	Efficiency	
Gram negative					
E. coli	7	+++	8	+++	13
S. flexneri	9	++	10	++	30
S. dysenteriae	8	++	10	+++	19
V. cholerae	11	++	12	++	28
S. typhi	__	__	7	++	20
S. paratyphi	12	++	14	++	32
Pseudomonas spp.	12	+++	14	+++	22
Proteus spp.	7		7		32
Gram positive					
S. aureus	12	++	14	++	38
S. epidermis	8	++	11	++	32

Here, "+++" represents very good, "++"represents good, "+" represents fair, "_" represents no inhibition

Table 3. *In vitro* antibacterial activity of chloroformic extract of *C. manghas* stem

Bacterial strains	Diameter of zone of inhibition in mm				
	Chloroformic extract (500µg/disc)		Chloroformic extract (1000µg/disc)		Kanamycin (30µg/disc)
	mm	Efficiency	mm	Efficiency	
Gram negative					
E. coli	8	+++	10	+++	13
S. flexneri	9	++	10	++	30
S. dysenteriae	6.5	++	7	++	19
V. cholerae	7	++	12	++	28
S. typhi	__	__	__	__	20
S. paratyphi	10	++	14	++	32
Pseudomonas spp.	__	__	__	__	22
Proteus spp.	12	++	13	++	32
Gram positive					
S. aureus	10	++	11	++	38
S. epidermis	6	+	8	++	32

Here, "+++" represents very good, "++"represents good, "+" represents fair, "_" represents no inhibition

Table 4. *In vitro* antibacterial activity of chloroformic extract of *C. manghas* leaf

Bacterial strains	Diameter of zone of inhibition in mm				
	Chloroformic extract (500µg/disc)		Chloroformic extract (1000µg/disc)		Kenamycin (30µg/disc)
	mm	Efficiency	mm	Efficiency	
Gram negative					
E. coli	7	+++	8.5	+++	13
S. flexneri	9	++	7	+	30
S. dysenteriae	__	++	__	++	19
V. cholerae	__	++	__	++	28
S. typhi	7	++	8	++	20
S. paratyphi	11	++	11	++	32
Pseudomonas spp.	__	__	__	__	22
Proteus spp.	__	__	__	__	32
Gram positive					
S. aureus	7	+	8	+	38
S. epidermis	_	__	__	__	32

Here, "+++" represents very good, "++"represents good, "+" represents fair, "-" represents no inhibition

Commercial antibiotic Kanamycin gave the highest zone of diameter (38 mm) against *S. aureus* among ten used bacterial strains. Chloroformic extract of leaf disc (1000µg/disc) of *X. mekongenesis* gave the highest 14 mm zone of inhibition against *S. paratyphi and Pseudomonas* spp. (Table 2) while the highest activity of chloroformic extracts of *mekongenesis* stem disc (1000µg/disc) was 11 mm (Table 1). In case of *C. manghas*, chloroformic extracts of stem disc (1000µg/disc) of gave the highest 14 mm zone of inhibition against *S. paratyphi* (Table 3) while leaf extracts showed the highest activity 11 mm in case of *S. paratyphi* (Table 4). No visible activity was observed against *S. paratyphi* and *Pseudomonas* spp. in the stem extract of *X. mekongenesis* in both the concentrations (Table 1) while moderate activity was observed in the remaining stains. Similarly in case of leaf extracts of *X. mekongenesis* showed no response against *S. typhi* and *Proteus* spp. (Table 2) while minimum activity was observed in the remaining strains. Chloroformic stem extract of *C. manghas* showed least activity against *S. dysenteriae* (7mm) and potent activity against *Proteus* spp. (13mm) and *V. cholerae* (12mm) (Table 3) while the performance of chloroform leaf extract of *C. manghas* was not noteworthy. Differences in the performances of antibacterial activity of the extracts and Kanamycin do not necessarily indicate poor efficiency or potency of the extracts. Antibiotic discs are highly pure whereas the sample discs are diluted and greater zone of inhibition is possible to get by increasing the concentration of sample per disc. Sometimes, the choice of solvents for extraction dictates the process as polar compounds may not be extracted with non-polar solvents and vice-versa. Alam *et al.* (2006) conducted antibacterial activity of the crude ethanolic extract of in a related species *X. granatum* stem barks and found that the extracts showed significant activity *S. epidermis*, *S. aureus*, *S. boydii*, and *Proteus* spp., moderate activity against *E. coli*, *S. pyogenes*, and no activity against *S. dysentery*, *Enterococci*, and *S. typhi.* Another report by Rao *et al.* (2003) showed that the alcoholic extracts (100 mg/ml and 300 mg/ml) of bark and leaf of *X. granatum* possessed significant ($P<0.01$) antimicrobial activity against *Bacillus subtilis*, *B. pumilus*, *S. aureus*, *E. coli*, *Candida albicans* and *Saccharomyces cerevisiae*. They also found that the alcoholic extract of bark of *X. granatum* showed better antimicrobial activity. Shahid-ud-daula and Basher (2009) reported that the primary methanolic extract of *X. granatum* demonstrated antibacterial activity against the gram positive bacteria *S. aureus* and *B. subtilis* and the gram negative bacteria *Proteus vulgaris*. The primary methanolic extract was found to be inactive against *E. coli* and *Pseudomonas aeruginosa*. The primary methanolic extract was more active against gram-positive bacteria than gram-negative bacteria. In these findings, it was observed that most of the extraction was carried out with polar solvent systems and the antibacterial activity originated from the compounds extracted in them.

CONCLUSION

The present study of determining antibacterial activity of chloroformic stem and leaf extracts of *X. mekongensis* and *C. manghas* provides the opportunity to explore new compounds from the plant. Although the extracts did not show potent activity against all tested microorganisms, different components with better antibacterial activity could still be isolated using different solvent systems. Therefore, further studies could identify the pure component and establish the therapeutic uses of these plants and particularly with its active principles.

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