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ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACTS OF THE LEAVES AND STEM OF *Amoora cucullata*

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ABSTRACT

Ferdoushi A, Sabrin F, Hasan MN, Islam KD, Billah MM (2012) Antibacterial activity of methanolic extracts of the leaves and stem of *Amoora cucullata*. *J. Innov. Dev. Strategy*. 6(2), 35-38.

Amoora cucullata, a typical mangrove plant is traditionally used as a therapeutic agent to treat diarrhoea and inflammatory diseases. The juice prepared from the leaves is extensively used for the treatment of dysentery and skin diseases. In order to find out the scientific basis of such traditional uses, the present study was undertaken to determine the antimicrobial potential of the extracts obtained from leaves and stems of this plant. Disc diffusion method was employed to test the antibacterial activity of methanolic extracts of *A. cucullata* leaves and stems and these extracts showed potent antimicrobial activity against eight out of ten tested bacterial strains. These extracts showed less activity against gram positive bacteria. However, strong antimicrobial activity was observed against *E. coli*. These experimental findings validate the use of this plant in ethno-medicine.

Key words: *antibacterial activity, methanolic extract, Amoora cucullata, mangrove plants*

INTRODUCTION

The agent responsible for either interfering or inhibiting the growth and metabolism of the microbes is called antimicrobial agent (Pelczar *et al.* 1993). The capacity of this agent or substance to inhibit microbial growth is called antimicrobial activity. *In vitro* antimicrobial activity of plants can easily be determined by observing the growth response of various microorganisms to the plant extracts or their solvent fractions if placed altogether or in contact with each other. There are three ways of accomplishing this- diffusion, dilution and bioautographic methods. Diffusion and dilution methods are routinely used in studying the antimicrobial activity of medicinal plants. A number of modifications have been made in these techniques to obtain better results and control. 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. Bioautography is another method for studying antimicrobial activity (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 test materials. Diameter obtained from zone of inhibition is used sometimes to measure the inhibition of growth for micro organisms. However, no distinction between bacteriostatic and bacteriocidal activity can be demonstrated by this method (Roland 1982)**.**

Amur (*Amoora cucullata*) is a rare and endangered tree species of the Sundarbans. Traditionally, *A. cucullata* is used for the treatment of diarrhoea and inflammation in folk medicine (Boonyapraphat and Chockchaicharaenphorn, 1998). It was reported that juice prepared from the leaves of this plant possessed antibacterial activity and was extensively used for the treatment of dysentery, skin and cardiac diseases (Kirtikar and Basu, 1999). Related species *A. rohituka* and *A. chittagonga* were reported to show considerable antitumor and antibacterial properties (Chan *et al.* 2011). The stem of *A. rohituka* particularly exhibited significant *in vitro* antibacterial activity. The extracts also demonstrated mild antifungal effect **(**Chowdhury *et al.* 2003). However, the plant parts have been evaluated for few bioactivities and therefore, entail the possibility of determining the antimicrobial potential with two different types of extracts.

MATERIALS AND METHODS

Test of antibacterial activity of A. cucullata

Antibacterial activity of the crude extract was determined by disc diffusion method (Bauer *et al.* 1966).

Principle of disk diffusion method

Measured amount of the test sample is dissolved in definite volumes of solvent to prepare solutions of known concentration (µg/µl). Then sterile Whatman filter paper discs are impregnated with known amount of test samples using a micropipette and left for drying. Standard antibiotic discs and blank discs on which the solvent are used to dissolve the samples are adsorbed and dried. They are used as positive and negative controls, respectively. These discs are then placed into the Petri dishes (120 mm in diameter) containing a suitable agar medium seeded with the test organisms using a sterile transfer loop for antibacterial screening. The plates are then kept at 37ºC facilitating maximum diffusion. A number of events take place simultaneously including

- The dried discs absorb water from the agar medium and the test samples become dissolved.

- The test sample diffuses from the discs into the surrounding medium. The diffusion takes place according to the physical law that controls the diffusion of molecules through agar gel.
- There is a gradual change of test sample concentration in the agar surrounding each disc. The plates are then kept in an incubator (37˚C) for 12-18 h to allow the growth of the microorganisms. If the test material has any antimicrobial activity, it will inhibit the growth of microorganism giving a clear, distinct zone called "zone of inhibition". The antibacterial activity of the test agent is determined by measuring the diameter of the zone of inhibition in term of millimeters. The experiments are carried out at least three times and the mean of the readings are recorded.

Test material

Methanolic extracts were prepared from the leaves and stems of *A. cucullata* for evaluation of antimicrobial activity.

Bacterial strains and their maintenance

Antibacterial screening was carried out using ten pathogenic bacteria (Table 1) responsible for common bacterial diseases in Bangladesh. These pathogenic bacteria were collected and maintained at Animal Cell Culture Laboratory, Biotechnology & Genetic engineering Discipline, Khulna University, Bangladesh.

Table 1. List of bacteria used in the antimicrobial screening and diseases caused by them

Gram Negative	Diseases	Gram Positive	Diseases
Escherichia coli	Gastroenterities, urinary tract infections and neonatal meningitis	*Staphylococcus aureus*	Scaled skin syndrome
Vibrio cholerae	Cholera		
Salmonella typhi	Typhoid		
Salmonella paratyphi	Paratyphoid A	*Staphylococcus epidermis*	Endocarditis and grow as biofilms on catheters
Shigella dysenteriae	Bacillary dysentery		
S*higella flexneri*	Shigellosis		
Pseudomonas spp.	Flourishes in hospital environments		
Proteus spp.	Urinary tract infections		

For routine culture and maintenance, Trypton Soy Broth (TSB), McConkey Agar and Nutrient Agar (NA) media were used.

Culture media preparation

A mixture of nutrients used in the laboratory to support growth and multiplication of a culture (a population of microorganisms) is called culture medium (Pelczar *et al.* 1993). Nutrient Agar media was used for antimicrobial screening in the present study.

Sub-culture of test organisms

The test microorganisms from the pure culture (preserved at 4°C) were streaked onto Nutrient Agar plates with a sterile inoculating loop. To ensure proper growth of the test organisms, the inoculated plates were incubated at 37°C overnight (18-22 hours).

Preparation of test samples

The test sample was dissolved into specific volume of specific solvent to obtain the desired concentrations. In the present study, 50 mg of extracts were dissolved into 2ml of methanol to obtain a final concentration 25 µg/µl methanolic test extract.

Preparation of discs

Three types of discs were used for antimicrobial screening-sample, antibiotic and standard discs. Sterile filter paper discs (5 mm in diameter) were taken in a blank petridish. Twenty and fourty micro liter of the test sample were applied into the discs with a micropipette in an aseptic condition under the laminar air flow in order to get final concentration of 500 µg and 1000 µg per discs, respectively. In this investigation, commercial kanamycin antibiotic discs (30µg/disc) were used. Sterile filter paper discs (5 mm in diameter) were taken in a blank petridish. Appropriate volume of solvent was applied into them in the similar fashion under similar condition.

Disk diffusion assay for antibacterial activity

From the overnight culture, a small portion of fresh colony was transferred into test tube containing nutrient broth and incubated at 37°C until the growth reached log phase (4-6 h; 5×10 CFU/ml). After optimum growth, plates were seeded properly by pouring the culture broth with a Pasteur pipette to make bacterial lawn. The excess culture broth was withdrawn from the plates and allowed to dry for 5 min. Discs impregnated with

solvents were placed at proportionate distance from each other using a sterile needle. The plates were incubated overnight at 37°C and checked for zone of inhibition. A transparent scale was used to measure the zone diameter along with disc diameter in mm.

RESULTS AND DISCUSSION

Antimicrobial activity of any compound can be stated as capacity to inhibit or impede growth of a particular microorganism cultured in a suitable growth condition. Results of this study were taken by considering such observable fact and inhibitory action destined by numerical values.

Disk diffusion method was used to know the antibacterial activity of the methanolic stem and leaf extracts of *A. cucullata.* The methanolic extracts showed differential antimicrobial activity in regard to concentration of test samples with different bacteria. In this regard, disk diffusion method was carried out in a dose dependent manner with the methanolic extracts. As commercial kanamycin discs were used to compare activity of the test extracts, the antibacterial activity of the methanolic extracts of stem and leaf was measured in term of diameter of zone of inhibition in millimeters and compared with the zone of inhibition with kanamycin discs (Table 2 & 3).

Table 2. *In vitro* antibacterial activity of methanolic extract of *A. cucullata* stem

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

Here, "__" represents no inhibition, "+"= Fair, "++"= Good, "+++" = Very good, "++++"=Excellent

Table 3. *In vitro* antibacterial activity of methanolic extract of *A. cucullata* leaf

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

Here, "__" represents no inhibition, "+"= Fair, "++"= Good, "+++" = Very good, "++++"=Excellent

In case of kanamycin, the highest zone of diameter (38 mm) was obtained against *Staphylococcus aureus* in comparison with ten tested bacterial strains. The methanolic extracts of leaves (1000µg/disc) produced 13 mm zone of inhibition against *V. cholerae*. Alongside, the methanolic extracts of both leaves and stem showed potent antimicrobial activity against *E. coli*. The methanolic extract of leaves also showed potent activity against *S. dysenteriae* which was in agreement with the finding in which juice of the leaves were reported to be used for the treatment of dysentery, skin and cardiac diseases (Kirtikar and Basu, 1999). The methanolic extract of leaves

having potent antimicrobial activity was further supported by the report which showed that crude methanolic extracts of leaves possessed anti-inflammatory, antinociceptive, diuretic and central nervous system (CNS) depressant activities (Das *et al.* 2005). The overall response to Gram positive bacteria *S. aureus* and *S. epidermis* were not significant against the crude extracts while antimicrobial activity of the extracts against *S. paratyphi*, *V. cholera*, *S. dysenteriae*, and *E. coli* was promising. These findings were quite similar to the reports by Rahman and Rashid (2009). They found that the ethyl acetate and methanolic extracts of *A. cucullata* were potent in terms of both zone of inhibition and spectrum of activity was described as the average zones of inhibition between 8-14 mm and 9-16 mm, respectively for the extracts. In this present study, the variations of zone of inhibition between discs containing extracts and kanamycin do not necessarily prove to be less efficient and potent. It is because the antibiotic discs are highly pure and the discs containing extracts are diluted and larger zone of inhibition could be produced by concentrating the extracts in the discs. This test indicates that the extracts may have some bioactive compounds that are potent and further investigation, is needed to identify the pure component.

CONCLUSION

The methanolic extracts stem and leaf extracts of this mangrove plant showed activity against a number of pathogenic bacteria which is in good agreement of traditional use of the plant to treat diarrhoea and inflammatory diseases. Further pharmacological and toxicological studies are required to establish the therapeutic uses of the plant and particularly with its active principles.

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