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Preliminary antimicrobial and cytotoxic activities of *Amoora cucullata* extractives

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SUMMARY

Amoora cucullata (Meliaceae), a mangrove plant, has folkloric reputation as a medicinal agent in Bangladesh. In this study, the n-hexane, ethyl acetate and methanolic extracts of the stem bark of this plant were subjected to microbiological investigation and brine shrimp lethality bioassay. In case of antimicrobial screening, the ethyl acetate and methanolic extracts appeared to be potent in terms of both zone of inhibition and spectrum of activity showing the average zones of inhibition 8 - 14 mm and 9 - 16 mm, respectively. In the brine shrimp lethality bioassay, the methanolic extract demonstrated highest cytotoxicity having LC_{50} of 0.549 μg/ml, whereas the ethyl acetate and n-hexane extract showed LC_{50} of 7.943 and 17.180 μg/ml, respectively.

Key words: *Amoora cucullata*; Antimicrobial; Cytotoxicity; Disc diffusion; Brine shrimp lethality bioassay

INTRODUCTION

Amoora cucullata (Bengali name- Amoor; Family- Meliaceae) is a mangrove plant distributed in the coastal forest of Bangladesh, India, Burma and Malay Peninsula (Kirtikar and Basu, 1980; Boonyaprapha t and Chockchaicharaenphorn, 1998; Hassan, 2000). It is a medium sized evergreen tree and typically grows where salinity is low. The flowers of racemose inflorescence are small and white in color which produces fruit with 1 to 3 conspicuous red seeds (Hassan, 2000). The plant is used for the treatment of diarrhea (Chumkaew *et al*., 2006). The leaves are locally used to reduce inflammation (Basak *et al*., 1996) and were reported to show CNS depressant activity (Das *et al*., 2005). Previous phytochemical investigations revealed the occurrences of 1-O-formylrocagloic acid, 3'-hydroxy rocagloic acid, rocaglaol, rocagloic acid, 3'-hydroxymethylrocaglate, 1-O-formylmethyl rocaglate, methyl-rocaglate (Chumkaew *et al*., 2006), fridelin, stigmasterol, β-sitosterol, betulinic acid and caffeic acid (Rahman *et al*., 2005). We, herein, report the results of our preliminary antimicrobial and cytotoxicity screenings.

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MATERIALS AND METHODS

Plant materials

The stem bark was collected from Sundarban, a mangrove forest of Bangladesh in Khulna Division. This plant is very renowned by its local name - "amoor". Local people helped to collect the plant. The identification of the plant was taxonomically

confirmed by Dhaka University Herbarium, Department of Botany. It was compared with its previously preserved sample in the herbarium. Besides, extensive studies were conducted on plant parts (flowers, fruits, leaves, roots, bark etc.). In addition to these, literature description was also found to be identical. The plant was also compared with an artificially grown *A. cucullata* at the Dhaka Botanical Garden. For the collection of bark, feasible places were searched on the stem of the matured plants. After marking the places, a portion of the surrounding bark was collected as a slice in August, 2004. Sufficient portion of the bark was kept in the plant to maintain its viability. These collected barks were then cut in to small pieces, sun-dried and ground to a fine powder.

Extraction

The powdered bark (575 g) of *A. cucullata* was successively extracted using 1 l of n-hexane which was followed by ethyl acetate (1 l) and methanol (1 l). All three extracts were filtered separately through a fresh cotton plug and finally with a Whatman No.1 filter paper. The filtrates were then evaporated individually under reduced pressure at 40°C using a Buchii rotary evaporator to have concentrate of the n-hexane extract (2.1 g), ethyl acetate extract (2.7 g) and methanol extract (4.5 g).

Bioassays

The antimicrobial activity of the extractives was determined by the disc diffusion method (Bauer *et al*., 1966; Inouye *et al*., 2001; Kelman *et al*., 2006; Mbwambo *et al*., 2007; Mayachiew and Devahastin, 2008; Rahman and Rashid, 2008). The samples were dissolved separately in a suitable solrent (chloroform or methanol) and applied to sterile discs at a concentration of 300 μg/disc and carefully dried to evaporate the residual solvent.

For cytotoxicity screening, DMSO solutions of the plant extracts were applied against *Artemia salina* in a 1-day *in vivo* assay, the experimental details of which could be found elsewhere (Persoone, 1980; Meyer *et al*., 1982; McLaughlin *et al*., 1998; Rahman *et al*., 2006; Rahman and Rashid, 2008). For the experiment, 4 mg of each of the extracts was dissolved in DMSO. Solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.781 μg/ml were obtained by serial dilution technique. The median lethal concentration LC_{50} of the test samples after 24 h was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration.

Statistical analysis

All the bioassays were conducted in triplicate. The zone of inhibition and LC_{50} were calculated as mean ± S.D. (n = 3) for the antimicrobial screening and brine shrimp lethality bioassay, respectively.

RESULTS

The extractives of *A. cucullata* demonstrated varying degrees of inhibition to the growth of microorganisms and strong cytotoxic activity against *A. salina*. The n-hexane, ethyl acetate and methanol extracts were subjected to screening for inhibition of microbial growth against 13 bacteria and 3 fungal strains. Table 1 represents the summary of the antimicrobial activity of the samples with respect to each of the test organisms. The ethyl acetate and methanol extracts showed moderate to strong antimicrobial activity against the tested microorganisms by showing the zones of inhibition 8 - 14 mm and 9 - 16 mm, respectively (Table 1). The n-hexane extract showed very poor inhibitory activity (data not shown on table). On the other hand, the cytotoxcity of the samples was evaluated against *A. salina* (Table 2) where n-hexane, ethyl acetate and methanol extracts showed LC_{50} of 17.18, 7.943 and 0.549 μg/ml, respectively.

DISCUSSION

In the antimicrobial susceptibility test by the disc diffusion method, the ethyl acetate extract (300 μg/

Table 1. Antimicrobial activity of the extractives of A. cucullata at 300 μg/disc

Test microorganisms	Diameter of zone of inhibition (mm)		
	EA	ME	KAN
Gram positive bacteria			
Bacillus cereus	12.06 ± 1.36	13.33 ± 1.40	24.42 ± 1.37
Bacillus megaterium	11.33 ± 1.45	12.41 ± 1.59	25.55 ± 1.51
Bacillus subtilis	13.34 ± 0.85	14.35 ± 1.14	23.08 ± 1.45
Staphylococcus aureus	10.19 ± 1.74	12.34 ± 1.29	25.22 ± 1.10
Sarcina lutea	13.29 ± 1.15	12.31 ± 1.57	23.33 ± 1.14
Gram negative bacteria			
Escherichia coli	12.37 ± 1.64	10.26 ± 1.39	24.66 ± 1.20
Pseudomonas aeruginosa	11.23 ± 2.10	09.28 ± 1.64	25.67 ± 1.20
Salmonella paratyphi	09.25 ± 1.34	11.35 ± 1.38	24.25 ± 1.34
Salmonella typhi	08.36 ± 1.26	12.22 ± 1.85	23.33 ± 1.67
Shigella boydii	11.11 ± 1.18	13.24 ± 1.87	22.34 ± 1.28
Shigella dysenteriae	12.28 ± 1.84	14.37 ± 1.54	24.61 ± 1.38
Vibrio mimicus	14.45 ± 1.15	16.23 ± 1.15	23.91 ± 1.29
Vibrio parahemolyticus	09.27 ± 1.34	14.06 ± 1.25	24.54 ± 0.97
Fungi			
Candida albicans	10.25 ± 1.37	11.33 ± 1.52	23.37 ± 1.63
Aspergillus niger	08.37 ± 1.55	10.14 ± 2.10	22.54 ± 1.42
Saccharomyces cerevaceae	09.49 ± 1.58	12.34 ± 1.36	23.26 ± 1.13

The diameters of zones of inhibition are expressed as mean ± S.D. (n = 3); EA: ethyl acetate extract of the stem bark; ME: methanolic extract of the stem bark; Kan: standard kanamycin (30 μg/disc).

Table 2. LC_{50} data of test samples of *A. cucullata*

Samples	LC_{50} (μg/ml)
Vincristine sulphate (positive control)	0.223 ± 1.37
n-hexane extract	17.180 ± 1.62
Ethyl acetate extract	7.943 ± 1.31
Methanol extract	0.549 ± 1.28

The values of LC_{50} are expressed as mean ± S.D. (n = 3).

disc) revealed strongest inhibitory activity against *V. mimicus* having the zone of inhibition 14.45 mm. The growth of *B. subtilis* (13.34 mm), *S. lutea* (13.29 mm), *E. coli* (12.37 mm), *S. dysenteriae* (12.28 mm) and *B. cereus* (12.06 mm) was also moderately inhibited by this extract. Besides, a mild inhibitory activity was noticed against the growth of *B. megaterium* (11.33 mm), *P. aeruginosa* (11.23 mm) and *S. aureus* (10.19 mm). In case of fungi, this extract showed weak inhibitory activity. At the same time, the growth of *V. mimicus* (16.23 mm), *S. dysenteriae* (14.37 mm), *B. subtilis* (14.35 mm) and *V. parahemolyticus* (14.06 mm) was strongly inhibited by the methanolic extract. It also showed moderate inhibitory activity against *B. cereus* (13.33 mm), *S. boydii* (13.24 mm), *B. megaterium* (12.41 mm), *S. aureus* (12.34 mm), *S. lutea* (12.31 mm) and *S. typhi* (12.22 mm). The growth of *E. coli, P. aeruginosa* and *S. paratyphi* was weakly inhibited. This extract also showed mild to moderate activity against fungal growth having the zone of inhibition 10 - 12 mm.

For the brine shrimp lethality bioassay, the n-hexane, ethyl acetate and methanol extracts were studied. Table 2 shows the results of the brine shrimp lethality after 24 h exposure of brine shrimps to all the samples and the positive control, vincristine sulphate. The degree of lethality was directly proportional to the concentration of the extract ranging from the lowest concentration (0.781 μg/ml)

to the highest concentration (400 μg/ml). Maximum mortality took place at a concentration of 400 μg/ml, whereas least mortality was at 0.781 μg/ml concentration. In other words, mortality increased gradually with the increase in concentration of the test samples. LC_{50} obtained from the best-fit line slope were 0.223, 17.18, 7.943 and 0.549 μg/ml for vincristine sulphate, n-hexane, ethyl acetate and methanol extracts, respectively. In comparison with the positive control (vincristine sulphate), the cytotoxicity exhibited by the methanolic extract was promising.

The results of antimicrobial study and cytotoxicity screening exhibited by the extractives of *A. cucullata* indicate the presence of bioactive principles in the extractives, especially consistent with its traditional uses in treating diseases like diarrhea and inflammation as well as showing CNS depressant activity.

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