- Short communication

Antimicrobial Activity and Cytotoxicity of *Clausena suffruticosa*

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The Crude methanolic extract of *Clausena suffruticosa* bark and its *n*-hexane, chloroform soluble fractions along with some leaf extractives were subjected to antimicrobial screening and brine shrimp lethality bioassay. In case of antimicrobial screening the *n*-hexane soluble fraction of the bark exhibited strongest antibacterial activity in term of zone of inhibition. In the brine shrimp lethality bioassay, the crude methanol extract of the bark revealed significant cytotoxicity with LC_{50} of 1.55 µg/ml.

Clausena suffruticosa (Bengali name – Kalomoricha) is an under shrub belonging to the Rutaceae family. It is widely distributed in Chittagong and Sylhet districts.[1] The plants of this genus are known to be useful in paralysis, ulcerated nose, colic, stomach trouble, fever and headache, muscular pain, malarial fever.[2,3] They are also reputed as diuretic, astringent, insecticide, tonic and vermifuge.[4] Previous phytochemical studies with *Clausena* led to the isolation of carbazole alkaloids,[2] coumarins[5] and limonoids.[6]

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The plant *C. suffruticosa* was collected from Ramakalanga, Sylhet district in October 2005. It was identified by Dr. Mahbuba Khanam, Principal Scientific Officer, Bangladesh National Herbarium, Ministry of Environment and Forest, Dhaka, Bangladesh, where a voucher specimen is maintained representing the collection (accession no. DACD-31233). The stem bark and leaves of the plant were cut into small pieces, cleaned, dried and pulverized.

The powdered bark (171 gm) of *C. suffruticosa* was soaked in 500 ml methanol, filtered and concentrated using a rotary evaporator at low temperature (36-40ºC) and reduced pressure. A portion (4 gm) of the concentrated methanol extract was fractionated by the modified kupchan partitioning method[7] into *n*-hexane (1.5 gm), carbon tetrachloride (300 mg), chloroform (1.3 gm) and aquous soluble fractions (800 mg). The air dried powdered leaves (124.5 gm) were successively extracted with a soxhlet apparatus using *n*-hexane, ethyl acetate and methanol to yield 6.1 gm, 6.15 gm and 8.0 gm of extracts, respectively.

Antimicrobial activity of the extracts were determined by the disc diffusion method.[8] Measured amount of the test samples were dissolved in definite volumes of solvent (chloroform or methanol) and applied to sterile discs at a concentration of 600

µg/disc and carefully dried to evaporate the residual solvent. The extracts were tested for antimicrobial activity against a number of gram positive and gram negative bacteria and fungi (Table 1). The bacterial and fungal strains used for the experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka. In this investigation, Kanamycin (30 µg/disc) standard disc was used as a reference standard.

Table 1. Antimicrobial activity of the test samples of *Clausena suffruticosa* extractives *(*600 µg/disc) and Kanamycin (30 µg/disc).

Test microorganisms	Diameter of zone of inhibition (mm)						
	MEB	HXB	CFB	HXL	EAL	MEL	Kanamycin
Gram positive bacteria							
Bacillus cereus	-	11	-	10	10	-	26
B. megaterium	-	10	-	10	08	-	24
B. subtilis	-	10	08	08	10	-	25
Sarcina lutea	-	12	-	10	08	-	23
Staphylococcus aureus	08	12	08	12	10	-	22
Gram negative bacteria							
Escherichia coli	08	13	08	13	10	-	25
Pseudomonas aeruginosa	-	16	-	13	10	-	23
Salmonella paratyphi	-	12	-	12	10	-	25
Salmonella typhi	10	16	08	13	10	09	25
Shigella boydii	09	13	08	12	10	-	23
Shigella dysenteriae	-	--	-	13	08	-	25
Vibro mimicus	08	13	-	10	08	-	24
V. parahemolyticus	-	13	-	12	08	-	25
Fungus							
Candida albicans	-	10	-	10	10	-	25
Aspergillus niger	08	10	-	09	--	-	25
Sacharomyces cerevaceae	-	20	-	16	10	-	23

MEB: Crude methanol extract of the stem bark; HXB: *n*-hexane soluble fraction of the methanol extract; CFB: Chloroform soluble fraction of the methanol extract; HXL: *n*-hexane extract of the leaf; EAL: Ethyl acetate extract of the leaf; MEL: Methanol extract of the leaf; "-" indicates no activity.

For cytotoxicity screening, DMSO solutions of the plant extracts were applied against *Artemia Salina* for 24 hours in a *in vivo* simplified assay.[9,10] For the experiment 4 mg of the plant extractives were dissolved separately in DMSO and by serial dilution technique, solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781 µg/ml were obtained. Then each of this test solution was added to test tubes containing 10 shrimps in 5 ml of simulated brine water. After 24 hrs, the median lethal concentration LC_{50} of the test samples was obtained by a plot of percentage the shrimps killed against the logarithm of the sample concentration.

The crude methanolic extract of the bark, its *n*-hexane and chloroform soluble fractions showed the average zone of inhibition by 8-10 mm, 10-20 mm and 8 mm, respectively. At the same time, the *n*-hexane, ethyl acetate and methanol extracts of the leaves revealed varying degrees of inhibitory activity showing the average zone of inhibition of 8-16, 8-10, and 9 mm, respectively. The *n*-hexane soluble fraction of the methanol extract of the bark strongly inhibited the growth of *P. aeruginosa* and *S. typhi* having the zone of inhibition 16 mm each. The growth of *E. coli* (13 mm), *S. boydii* (13 mm), *V. mimicus* (13 mm), *V. parahemolyticus* (13 mm), *S. aureus* (12 mm), *S. lutea* (12 mm), and *S. paratyphi* (12 mm) was also moderately inhibited. On the other hand, the crude methanol extract of the bark and its chloroform soluble fraction showed mild inhibitory activity against most of the test microorganisms. However, the crude methanol extract showed its strongest inhibitory activity against *S. typhi* (10 mm). The *n*-hexane extract of the leaf moderately inhibited the growth of *E. coli* (13 mm), *P. aeruginosa* (13 mm), *S. dysenteriae* (13 mm), *S. aureus* (12 mm), *S paratyphi* (12 mm), *S boydii* (12 mm), and *V. parahemolyticus* (12 mm). The growth of *B. cereus* (10 mm), *B. magaterium* (10 mm), *S. lutea* (10 mm), *V. mimicus* (10 mm) and *B. subtilis* (8 mm) was also

inhibited. Again, the ethyl acetate extract of the leaf showed very weak growth inhibitory activity against all of the tested microorganisms having maximum zone of inhibition 10 mm. The methanol extract remained insensitive to microbial growth except *S. typh*i (9 mm). In case of fungal strains, the *n*-hexane soluble fraction of the methanol extract of the bark showed strongest inhibition against the growth of *S. cerevacae* (20 mm) whereas the *n*-hexane extract of the leaf strongly inhibited the growth of *S. cerevacae* with zone of inhibition 16 mm.

In case of brine shrimp lethality bioassay, the crude methanol extract of the bark, its *n*-hexane and chloroform soluble fractions and ethyl acetate extract of the leaf were studied and the LC_{50} values were found for them 1.25, 1.78, 3.16 and 3.72 µg/ml , respectively. The standard vincristine sulfate showed the LC_{50} value 0.23 µg/ml. From the experiment, it is clearly evident that the crude methanol extract of the bark and its *n*-hexane soluble fractions were highly cytotoxic. Antimicrobial activity and cytotoxicity demonstrated by various extractives of *C. suffruticosa* substantiate the folk uses of this plant in various diseases.

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