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Short Communication

Preliminary Cytotoxicity and Antimicrobial Investigation of *Anthocaphalus chinensis*

M. A. Hossain[1], M. Z. Sultan[2], A. M. S. Chowdhury[1], C. M. Hasan[3, 4], and M. A. Rashid[3, 4*]

[1]Department of Applied Chemistry and Chemical Engineering, University of Dhaka, Dhaka-1000, Bangladesh

[2]Drug Research Laboratory, Centre for Advanced Research in Sciences, University of Dhaka, Dhaka-1000, Bangladesh

[3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh

[4]Centre for Biomedical Research, University of Dhaka, Dhaka-1000, Bangladesh

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Abstract

The methanol extract of the stem bark of *Anthocephalus chinensis* as well as its *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions were subjected to brine shrimp lethality bioassay and microbiological investigation. In the brine shrimp lethality bioassay, the aqueous soluble partitionate of the methanolic extract revealed the highest cytotoxicity having LC_{50} of 1.19 µg/ml while in case of antimicrobial screening, the chloroform soluble materials demonstrated moderate inhibition of growth of test organisms.

Keywords: Anthocephalus chinensis; Rubiaceae; Cytotoxicity; Antimicrobial.

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1. Inrtoduction

Anthocephalus chinensis (local name - Kadam, Kadamba; Family-Rubiaceae) is a moderate-sized to tall tree with broadly elliptic and strongly veined opposite leaves and beautiful globose pinkish white flower heads. It is found to grow wild in jungles in almost all areas of Bangladesh and also planted as a timber and shade tree [1]. Its bark is used as hypoglycemic, anthelmenthic, tonic, astringent and also in the treatment of snakebite and malarial fever. The decoction of leaves is used as a gargle in case of aphthae and stomatitis [1, 2]. Previous phytochemical investigations with *A. chinensis* led to the isolation cinchotannic, quinovic acid [2], cadambagenic acid, saponins, beta-sitosterol, fats and reducing sugars, a secoiridoid, 3-O-caffeoylsweroside and two phenolic

* *Corresponding author*: rashidma@univdhaka.edu; mdzakirsultan@yahoo.com

apioglucosides, kelampayosjde A and kelampayosjde B [3], indole alkaloids, cadambine and 3α-dihydrocadambine [4], cadamine, a glycosidal alkaloid, 3β-dihydrocadambine and 3β-isodihydrocadambine [5]; hentriacontanol and beta-sitosterol [6-8].

2. Materials and Methods

The stem bark of *A. chinensis* was collected from Boalmari, Faridpur and was identified at the Bangladesh National Herbarium, Mirpur, Dhaka (Accession no: DACB 31749). The bark was cut into small pieces, dried at room temperature for about 20 days and then ground to a coarse powder.

The powdered bark (350 g) of *A. chinensis* was soaked into 1.2 L of methanol for 10 days and then filtered through a cotton plug followed by Whatman filter paper no. 1. The extract was concentrated with a rotary evaporator. A portion (5.0 g) of the concentrated methanol extract (MSBE) was fractionated by the modified Kupchan partitioning method [9] into *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions. Subsequent evaporation of solvents afforded *n*-hexane (HXSP, 0.61 g), carbon tetrachloride (CTSP, 0.63 g), chloroform (CFSP, 0.92 g) and aqueous soluble materials (AQSP, 2.84 g).

For cytotoxicity screening, DMSO solutions of the plant extracts were applied against *Artemia salina* in a 1-day *in vivo* assay [10,11] using the established protocol. For the experiment, 1 mg of each of the Kupchan fractions was dissolved in DMSO and solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78 µg/ml were obtained by serial dilution technique. The median lethal concentration LC_{50} of the test samples after 24 hours of exposure was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration.

The antimicrobial activity of the extractives was determined by the disc diffusion method [12-14]. The samples were dissolved separately in solvent (methanol or chloroform) and applied to sterile discs at a concentration of 400 µg/disc and carefully dried to evaporate the residual solvent.

3. Results and Discussion

Following the procedure of Meyer [10], the lethality of the MSBE, HXSP, CTSP, CFSP and AQSP partitionates of the methanolic extract of *A. chinensis* was investigated against brine shrimp. It was found (Table 1) that the degree of lethality was directly proportional to the concentration of the extract ranging from the lowest concentration (0.78 µg/ml) to the highest concentration (400 µg/ ml). Maximum mortality was seen at 400 µg/ ml, whereas least mortality was observed at 0.78125 µg/ ml.

The LC_{50} obtained from the best-fit line slope were 0.23, 7.24, 5.25, 3.71, 3.50 and 1.19 µg/ml for Standard VS (vincristine sulfate), MSBE, HXSP, CTSP, CFSP and AQSP, respectively.

Table 1. LC_{50} data of test samples of *A. chinensis.*

Samples	LC_{50} (µg/ml)
VS	0.23
MSBE	7.24
HXSP	5.25
CTSP	3.71
CFSP	3.50
AQSP	1.19

VS: vincristine sulfate (std); MSBE: Methanol soluble bark extract; HXSP: *n*-Hexane soluble partitionate of MSBE; CTSP: Carbon tetrachloride soluble partitionate of MSBE; CFSP: Chloroform soluble partitionate of MSBE; AQSP: Aqueous soluble partitionate of MSBE.

Table 2. Antimicrobial activity of the *A. chinensis* extractives (400 µg/disc) and kanamycin (30 µg/disc).

Test microorganisms	Diameter of zone of inhibition (mm)		
	CFSP	AQSP	KAN
Gram positive bacteria			
Bacillus cereus	9	--	24
B. megaterium	9	8	27
B. subtilis	9	8	28
Staphylococcus aureus	8	8	25
Sarcina lutea	10	10	28
Gram negative bacteria			
Escherichia coli	--	--	28
Pseudomonas aeruginosa	9	8	27
Salmonella paratyphi	12	10	28
S. typhi	11	11	28
Shigella dysenteriae	9	8	33
S. boydii	8	--	23
Vibrio mimicus	10	8	27
V. parahemolyticus	--	8	28
Fungi			
Candida albicans	9	--	27
Aspergillus niger	9	8	23
Sacharomyces cerevaceae	10	8	28

KAN: standard kanamycin disc (30µg/disc); Diameter of zone of inhibition< 8 mm was considered inactive. Thus, the results of MSBE, HXSP, and CTSP were not shown in table.

For antimicrobial screening, the zones of inhibition produced by the chloroform soluble partitionate and aqueous soluble fraction of the methanolic extract of *A. chinensis* ranged from 8-12 mm and 8-11 mm, respectively at a concentration of 400 µg/disc (Table 2). The chloroform soluble extractive of the methanolic extract showed moderate inhibitory activity against *S. paratyphi* and *S. typhi* having the zone size 12 mm and 11 mm, respectively. This fraction also showed mild activity against *S. lutea* and *V. mimicus* (10 mm each). At the same time, the aqueous soluble fraction demonstrated mild inhibitory activity against *S. typhi* (11 mm), *S. paratyphi* and *S. lutea* (10 mm each). The methanolic extract of *A. chinensis* as well as its *n*-hexane and carbon tetrachloride soluble materials did not exhibit noticeable inhibition of growth of any microbe (data not shown). In case of fungal strains, the growth of *S. cerevaceae* was mildly inhibited by the chloroform soluble material. In comparison with positive control, the cytotoxicity exhibited by the extractives was promising. These bioactivities exhibited by the plant extractives substantiate the folk uses of the plant in several diseases.

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