- Short communication

Phytochemical and Biological Investigations of *Acokanthera spectabilis*

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The *n*-hexane, carbon tetrachloride and choloroform soluble fractions of a methanol extract of *Acokanthera spectabilis* were subjected to antimicrobial screening and brine shrimp lethality bioassay. All of the partitionates showed moderate to strong inhibitory activity to microbial growth, while the carbon tetrachloride soluble fraction demonstrated highest cytotoxicity having LC_{50} 6.16 µg/ml.

Acokanthera spectabilis (Family- Apocynaceae) is a fragrant ornamental shrub. The plant has reputation for its cardiotonic activities.[1] Previous phytochemical investigation with A. *spectabilis* led to the isolation of acobioside A and 14-*O*-acetylacovenodose C.[2] In the present study, we report the presence of a cardenolide as well as antimicrobial activity and cytotoxicity of the extractives of *A. spectabilis* growing in Bangladesh.

The ^{1}H NMR spectra were recorded using a Bruker AMX-400 (400 MHz) instrument and the NMR spectra were acquired in $CDCl_3$ and the δ values for ^{1}H spectra were referenced to the residual nondeuterated solvent signal.

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The leaves and stem of *A. spectabilis* were collected from Baldah garden, Dhaka in the month of September 2005. A voucher specimen for this collection has been deposited in the herbarium of the Department of Botany, University of Dhaka.

Fresh leaves and stem of *A. spectabilis* were collected, dried and ground to a coarse powder. The powdered sample (149 g) was subjected to cold extraction with methanol for about 7 days and filtered through a cotton plug followed by Whatman filter paper number 1. The extract was then concentrated by using a rotary evaporator. A portion (5 mg) of the concentrated methanol extract was fractionated by the modified Kupchan partitioning method[3] into *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions. Evaporation of solvents afforded *n*-hexane (0.50 g), carbon tetrachloride (0.65 g), chloroform (1.30 g) and aqueous soluble (1.20 g) materials.

The carbon tetrachloride soluble fraction (300 mg) was fractionated over Sephadex LH-20. The column was eluted with *n*-hexane - dichloromethane - methanol (2:5:1) mixtures to provide 25 fractions, each 10 ml. Preparative thin layer chromatography (stationary phase- silica gel F_{254}, mobile phase - 10 % ethylacetate in toluene, thickness of plates-0.5 mm) of fractions 5-8 afforded a pure compound.

The ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of the purified compound indicated the presence of a cardenolide type glycoside.[8] However, due to poor spectral resolution and insufficient data, the structure of the compound could not be established.

The antimicrobial activity of the Kupchan fractions was determined by the disc diffusion method.[4] The bacterial strains listed in Table-1 were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh. The samples were dissolved separately in chloroform and applied to sterile filter paper discs at a concentration of 400 and 200 µg/ disc for crude extractives and isolated cardenolide, respectively. Discs were then carefully dried to evaporate the residual solvent. Standard kanamycin (30 µg/disc) was used as the positive control in the experiment.

For cytotoxicity screening, DMSO solutions of the plant extractives were applied against *Artemia salina* in a 1-day *in vivo* assay, the experimental details of which could be found elsewhere.[5-7] For the experiment, 4 mg of each of the Kupchan fraction was dissolved in DMSO and solutions of varying concentrations (400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.781 µg/ ml) were obtained by serial dilution technique.

For each of the extractives, three samples were prepared for each of the bioassay. The zone of inhibition and LC_{50} were calculated as mean ± SD (n=3) for the antimicrobial screening and brine shrimp lethality bioassay, respectively.

Table 1. Antimicrobial activity of the extractives of *A. spectabilis*

Test microorganisms	Diameter of zone of inhibition (mm)				
	HX (400 µg/disc)	CT (400 µg/disc)	CF (400 µg/disc)	C (400 µg/disc)	Kan (200 µg/disc)
Gram positive bacteria					
Bacillus cereus	08.30 ± 1.21	11.33 ± 0.33	09.21 ± 1.36	09.12 ± 1.33	26.13 ± 1.83
Bacillus megaterium	11.21 ± 1.32	10.25 ± 1.57	--	--	29.21 ± 0.87
Bacillus subtilis	--	16.35 ± 1.34	13.25 ± 1.37	--	23.33 ± 1.11
Staphylococcus aureus	10.14 ± 0.94	14.25 ± 1.34	12.22 ± 1.19	08.16 ± 1.38	25.33 ± 1.67
Sarcina lutea	--	12.34 ± 2.02	11.45 ± 1.26	10.28 ± 1.37	24.47 ± 0.33
Gram negative bacteria					
Escherichia coli	09.25 ± 1.33	13.46 ± 1.44	11.50 ± 1.65	--	25.33 ± 1.13
Pseudomonas aeruginosa	08.34 ± 0.97	11.36 ± 1.26	09.34 ± 1.74	--	25.21 ± 0.54
Salmonella paratyphi	08.16 ± 1.67	11.11 ± 1.54	--	09.30 ± 1.48	20.27 ± 0.33
Salmonella typhi	11.41 ± 2.34	13.32 ± 1.29	17.02 ± 1.37	09.29 ± 0.87	25.24 ± 0.42
Shigella dysenteriae	09.35 ± 1.26	15.43 ± 1.64	12.35 ± 1.09	-	24.18 ± 1.33
Vibrio mimicus	10.29 ± 1.57	12.26 ± 0.33	13.29 ± 1.31	11.23 ± 1.11	26.17 ± 1.12
Vibrio parahemolyticus	10.31 ± 1.24	10.16 ± 1.32	15.22 ± 0.92	09.33 ± 1.23	25.26 ± 0.61
Fungi					
Candida albicans	11.34 ± 1.67	16.05 ± 1.15	11.11 ± 0.67	--	26.28 ± 0.91
Aspergillus niger	08.27 ± 1.36	11.25 ± 1.47	--	--	27.37 ± 1.02
Sacharomyces cerevaceae	--	15.41 ± 1.32	15.41 ± 1.06	--	27.26 ± 0.33

The diameters of zones of inhibition are expressed as mean ± SD (n=3); a diameter less than 8 mm was considered inactive; HX: *n*-hexane soluble fraction of the methanolic extract; CT: carbon tetrachloride soluble fraction of the methanolic extract; CF: chloroform soluble fraction of the methanolic extract; C: cardenolide; Kan: standard kanamycin

In the antimicrobial screening, the zones of inhibition produced by the *n*-hexane, carbontetra-chloride and chloroform soluble fractions were found to be 8-11 mm, 10-16 mm and 9-17 mm, respectively at a concentration of 400 µg/disc (Table 1). The zones of inhibition produced by cardenolide at a dose level of 200 µg/disc were 9-11 mm (Table 1).

Three crude extractives (*n*-hexane, carbon tetrachloride and chloroform soluble partitionates) were screened by brine shrimp lethality bioassay for probable cytotoxic activity. The LC_{50} values of *n*-hexane, carbontetrachloride and chloroform fractions were found to be 26.91 µg/ml, 6.16 µg/ml and 12.58 µg/ml (Table 2), respectively. The positive

control, vincristine sulphate, showed LC_{50} of 0.32 µg/ml. From the results of the brine shrimp lethality bioassay it can be well predicted that the crude extracts have considerable cytotoxic potency. It is evident that carbon tetrachloride soluble materials has significant cytotoxic principles from which the cardenolide analog was isolated. But cytotoxic activity of the pure compound could not be performed due to lack of the test sample. Further study is warranted to identify the bioactive principle from *A. spectabilis.*

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

Samples	LC_{50} (µg/ml)
Vincristine sulphate	0.320 ± 0.11
HX	26.91 ± 1.21
CT	06.16 ± 0.33
CF	12.58 ± 2.10

The values of LC_{50} are expressed as mean ± SD (n=3). VS: vincristine sulphate (Std.); HX: *n*-hexane soluble fraction of the methanolic extract; CT: carbontetrachloride soluble fraction of the methanolic extract; CF: chloroform soluble fraction of the methanolic extract

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