- Short communication

Phytochemical and Biological Studies of *Averrhoa carambola*

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p-Anisaldehyde (**1**) and β-sitosterol were isolated from carbon tetrachloride and chloroform soluble portion of the methanol extract of stem bark of *Averrhoa carambola.* The structures of the isolated compounds were elucidated by spectroscopic studies and by comparison with published data. Different partitionates of the methanol extract exhibited significant antimicrobial activities and varying degrees of cytotoxicity.

Averrhoa carambola (Bengali name- Kamranga; Family, Oxalidaceae) is medium sized tree. It is planted in all over the Bangladesh.[1] Fruits and its fruit juice are used as antioxidant, astringent, tonic also to treat diarrhoea, vomiting, dysentery, hepatic colic, bleeding piles, relieving thirst and febrile excitement. The leaves are antipruritic, antipyretic and anthelmintic and are also useful in scabies, fractured bones, and various types of poisoning, intermittent fevers and intestinal worms.[2] Previous phytochemical investigation led to the isolation of 5-hydroxymethyl-2-furfural,[1] volatile components,[3,4] L-ascorbic acid, (−)epicatechin and gallic acid[5] and dihydroabscissic alcohol.[6]

Plant sample of *A. carambola* was collected from Dhaka in February 2004 and identified at the Department of Botany, University of Dhaka. Stem bark of the plant was cut into small pieces and air-dried for several days. The pieces were then oven dried for 24 hours at considerably low temperature to effect grinding and was then ground into coarse powder.

The powdered bark (700 g) of *A. carambola* was soaked in 1.3 liter of methanol for 7 days and then filtered through a cotton plug followed by Whatman No. 1 filter paper. The extract was then concentrated with a rotary evaporator. An aliquot (5 g) of the concentrated methanol extract was fractionated by the modified Kupchan partitioning method[7] into petroleum ether, carbon tetrachloride, chloroform and aqueous soluble fractions. Subsequent evaporation of solvents afforded petroleum ether (0.25 g), carbon tetrachloride (0.36 g), chloroform (0.20 g) and aqueous soluble (4.10 g) materials.

The carbon tetrachloride soluble material was fractionated over silica gel (Kiesel gel 60, mesh 70-230) and the column was eluted with petroleum ether, ethyl acetate and methanol mixtures of increasing

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Dhaka Univ. J. Pharm. Sci. **6**(2): 125-128, 2007 (December)

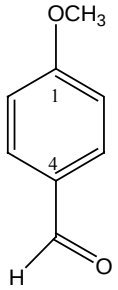
polarities. Compound **1** was isolated as white powder from the column fractions eluted with 20% ethyl acetate in petroleum ether upon re-chromatography over silica gel F_{254} in the solvent system toluene-ethyl acetate = 90:10, while fractions eluted with 25% ethyl acetate in petroleum ether provided β-sitosterol.

***p*-Anisaldehyde** (**1**) (4 mg, yield: 0.08 %) : White powder ; ^{1}H NMR spectrum (400 MHz, $CDCl_3$): δ 9.63 (1H, s, CHO-5), 7.58 (2H, d, *J* = 8.8 Hz, H-3 & H-5), 6.75 (2H, d, *J* = 8.8 Hz, H-2 & H-6), 3.69 (3H, s, OCH_3 -1).

The antimicrobial activity of the extractives was determined by the disc diffusion method.[8,9] The samples were dissolved separately in specific volume of chloroform and applied to sterile discs at a concentration of 400 µg/disc and carefully dried to evaporate the residual solvent. For cytotoxicity screening, DMSO solutions of fractions obtained from the crude extract were applied against *Artemia salina* in a 1-day *in vivo* assay.[10-12] For the experiment, 4 mg of each of the Kupchan fractions was dissolved in DMSO and 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 hours of exposure was obtained by plotting percentage of the shrimps killed against the logarithm of the sample concentration (toxicant concentration).

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

The ^{1}H NMR spectrum of compound **1** showed a sharp downfield singlet at δ 9.63 characteristic for an aldehyde group proton. It also showed two doublets (*J* = 8.8 Hz) centered at δ 7.58 and δ 6.75 (each 2H) which could be assigned to the *ortho* coupled aromatic protons at C-3 & C-5 and C-2 & C-6, respectively. The singlet of three proton intensity at δ 3.69 was demonstrative of a methoxyl group proton. These ^{1}H NMR data suggested that the compound must be a *para*-disubstituted benzene, where one of the substitutions was an aldehydic group and the other substituent was a methoxyl function. By comparing these values with published data,[13] compound **1** was characterized as *p*-anisaldehyde.



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β-sitosterol was identified by comparing its ^{1}H NMR data with published values[14] and thorough Co-TLC with an authentic sample.

The extractives of the *A. carambola* when subjected to antimicrobial screening at 400 µg/disc demonstrated mild inhibition of microbial growth (Table 1). The average zone of inhibition produced by the petroleum ether, carbon tetrachloride and chloroform soluble fractions of the methanol extract were 8-12 mm, 8-12 mm, and 8-15 mm, respectively. The petroleum ether extract moderately inhibited the growth of *E. coli* and *S. dysenteriae* having the zone of inhibition of 12 mm each. On the other hand, the carbon tetrachloride soluble fraction moderately inhibited the growth of *E. coli* only. In the same time, the chloroform soluble material strongly inhibited the growth of *E. coli* with zone of inhibition 15 mm. In case of fungi, mild inhibitory activity was exhibited by all extractives.

Following the procedure of Meyer,[12] the lethality of petroleum ether (PE), carbon tetrachloride (CT), chloroform (CF) and aqueous soluble (AQ) partitionates of the methanolic extract to brine shrimps was determined (Table 2). The degree of lethality was directly proportional to the concentration of the extract ranging from the lowest concentration (0.78125µg/ml) to the highest concentration (400 µg/ml). The LC_{50} obtained from the best-fit line slope were 0.32, 0.70, 0.06 and 3.14 µg/ml for standard vincristine sulfate (VS), petroleum ether, carbon tetrachloride and chloroform soluble

materials, respectively and the carbon tetrachloride (CT) soluble partitionate of methanolic extract exhibited promising activity.

Although the extractives showed strong cytotoxicity against brine shrimp nauplii, none of them demonstrated significant antimicrobial activity.

Table 1. Antimicrobial activity of *A. carambola* extractives.

Test microorganisms	Diameter of zone of inhibition (mm)			
	PE (400 µg/disc)	CT (400 µg/disc)	CF (400 µg/disc)	KAN (30 µg/disc)
Gram positive bacteria				
Bacillus cereus	09.20 ± 1.10	11.27 ± 0.36	08.27 ± 0.36	25.33 ± 0.25
B. megaterium	10.11 ± 2.10	08.26 ± 0.94	-	24.15 ± 1.25
B. subtilis	10.10 ± 2.42	10.47 ± 0.37	-	22.03 ± 0.68
Staphylococcus aureus	10.33 ± 0.86	10.33 ± 0.67	08.21 ± 0.69	26.41 ± 1.21
Sarcina lutea	10.36 ± 1.25	08.24 ± 1.36	10.45 ± 1.25	28.34 ± 1.98
Gram negative bacteria				
Escherichia coli	12.25 ± 1.29	12.22 ± 0.25	15.11 ± 2.14	28.37 ± 0.29
Pseudomonas aeruginosa	08.00 ± 1.11	-	-	27.24 ± 0.69
Salmonella typhi	10.11 ± 1.87	10.11 ± 1.34	-	27.24 ± 1.37
S. paratyphi	10.41 ± 1.34	10.12 ± 2.10	-	26.29 ± 0.98
Shigella dysenteriae	12.27 ± 1.36	10.31 ± 1.31	-	25.34 ± 1.29
Vibrio mimicus	10.16 ± 0.89	09.00 ± 1.17	08.22 ± 0.23	24.37 ± 0.49
V. parahemolyticus	10.19 ± 0.47	10.03 ± 0.59	08.25 ± 0.16	29.14 ± 1.11
Fungi				
Candida albicans	10.18 ± 1.15	08.21 ± 1.30	08.34 ± 0.28	27.46 ± 1.40
Aspergillus niger	08.42 ± 1.37	10.14 ± 1.89	08.37 ± 0.27	29.44 ± 1.20
Sacharomyces cerevaceae	10.24 ± 1.67	-	-	26.34 ± 1.27

The diameters of zones of inhibition are expressed as mean ± SD (n=3); a diameter less than 8 mm was considered inactive; PE: petroleum ether soluble fraction of the methanolic extract; CT: carbon tetrachloride soluble fraction of the methanolic extract; CF: chloroform soluble fraction of the methanolic extract; Kan: kanamycin.

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

Samples	LC_{50} (µg/ml)
Vincristine sulphate	0.32 ± 0.15
PE	0.70 ± 0.33
CT	0.06 ± 0.25
CF	3.14 ± 1.33

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

ACKNOWLEDGEMENT

Authors wish to thank the Ministry of Science and Information & Communication Technology, Government of the People's Republic of Bangladesh for partial financial support for carrying out the research.

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