- Short communication

Bioactivities of *Sesbania sesban* Extractives

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The *n*-hexane, carbon tetrachloride and chloroform soluble fractions of crude methanolic extract of the leaves of *Sesbania sesban* were subjected to microbiological investigation and brine shrimp lethality bioassay. In case of antimicrobial screening, the carbon tetrachloride soluble partitionate of the methanolic extract appeared to be very potent in terms of both zone of inhibition and spectrum of activity. In the brine shrimp lethality bioassay, the chloroform soluble fraction of methanolic extract revealed strongest cytotoxicity having LC_{50} of 0.13 μg/ml.

Sesbania sesban (Bengali name- Kathshola, Daincha or Joyanti; Family- Papilionaceae) is a small perennial tree with woody stems, yellow flowers and linear pods.[1] It is available throughout Bangladesh. Leaves are anthelmintic and also useful in diabetes, colic and skin diseases. Seeds are stimulant, emmenagogue and astringent and also useful in diarrhoea. Paste of seeds is used to cure itches and other skin eruptions.[2] Previous phytochemical investigations of the plant led to the isolation of oleanolic acid, stigmasta-5,24(28)-dien-3-ol-3-O-β-D galactopyranoside,[3] fatty acids and amino acids,[1,4] various types of lignins composed of guaiacyl, syringyl and *p*-hydroxyphenylpropane building units[5] and also an anti-tumour principle, kaempferol trisaccharide.[6]

The leaves of *S. sesban* was collected from BCSIR Laboratoriers campus, Chittagong in August 2005 and was identified at the Plant Taxonomy Division of BCSIR and at the Department of Botany, University of Dhaka. The leaves were cleaned, dried and ground into a coarse powder.

The powdered leaves (270 g) of *S. sesban* was soaked in 1.5 L of methanol for 7 days and then filtered through a cotton plug followed by Whatman filter paper number 1. The extract was concentrated with a rotary evaporator. A portion of the concentrated methanol extract (ME, 5 g) was fractionated by the modified Kupchan partitioning method[7] into *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions. Subsequent evaporation of solvents afforded *n*-hexane (HX, 2.50 g), carbon tetrachloride (CT, 0.54 g), chloroform (CF, 0.48 g) and aqueous soluble materials (AQ, 1.48 g).

The antimicrobial activity of the extractives was determined by the disc diffusion method.[8] The

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samples were dissolved separately in 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 the plant extracts were applied against *Artemia salina* in a 1-day *in vivo* assay [9,10] according to published protocol. For the experiment, 1 mg of each of the Kupchan fractions was dissolved in DMSO. Solutions of varying concentrations such as 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781, 0.40, 0.20 µg/ml were obtained by serial dilution technique. The lethal concentration LC_{50} of the test samples after 24 hrs was obtained from a plot of percentage of the shrimps killed against the logarithm of the sample concentration.

In the antimicrobial screening, the zones of inhibition produced by the *n*-hexane, carbon tetrachloride and chloroform soluble partitionates of the methanolic extract of *S. sesban* were ranged from 8-10 mm, 8-12 mm and 8-11 mm, respectively at a concentration of 400 µg/disc (Table 1). The carbon tetrachloride partitionate of the methanolic extract showed the strongest inhibitory activity against *E. coli* having the zone size 12 mm. The growth of *B. cereus*, *S. lutea*, *P. aeruginosa* and *S. dysenteriae* was also moderate inhibited. Mild activity was found against *B. megatenum* (9 mm), *S .typhi* (9 mm), and *V. parahemolyticus* (8 mm). At the same time, the chloroform fraction of the methanolic extract showed moderate inhibitory activity against *E. coli, P. aeruginosa* and *S. dysenteriae* having zone of inhibition 10 mm for each. *B. megatenum* (9 mm) and *S. lutea* (9 mm) were mildly inhibited. The *n*-hexane soluble material of the methanolic extract moderately inhibited the growth of *E.coli* and *A. niger* each having zone of inhibition 10 mm. It also showed mild activity against *B. cereus* (9 mm), *P. aeruginosa* (9 mm), *S. dysenteriae* (9 mm), and *S. cerevaceae* (9 mm). In case of fungal strains, all the extractives strongly inhibited the growth of *A. niger*.

Table 1. Antimicrobial activity of the *Sesbania sesban* extractives (400 µg/disc) and kanamycin (30 µg/disc).

Test microorganisms	Diameter of zone of inhibition (mm)			
	HX	CT	CF	KAN
Gram positive bacteria				
Bacillus cereus	09	10	-	38
B. megaterium	08	09	09	40
B. subtilis	-	-	08	40
Staphylococcus aureus	08		08	38
Sarcina lutea	08	10	09	32
Gram negative bacteria				
Escherichia coli	10	12	10	25
Pseudomonas aeruginosa	09	10	10	25
Salmonella paratyphi	-	-	08	25
S. typhi	08	09	08	25
Shigella dysenteriae	09	10	10	36
Vibrio mimicus	-	-	08	38
V. parahemolyticus	08	08	08	36
Fungi				
Candida albicans	08	10	09	38
Aspergillus niger	10	12	11	30
Sacharomyces cerevaceae	09	08	09	25

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; KAN: kanamycin (30 µg/disc); a diameter of zone of inhibition less than 8 mm was considered inactive.

Following the procedure of Meyer,[9] the lethality of the *n*-hexane (HX), carbon tetrachloride (CT) and chloroform (CF) partitionates to brine shrimp were investigated. The degree of lethality was directly proportional to the concentration of the extract ranging from the lowest concentration (0.20 µg/ml) to the highest concentration (100 µg/ml). Maximum mortalities took place at a concentration of 100

µg/ml, whereas least mortalities were observed at 0.20 µg/ml. The LC_{50} obtained from the best-fit line slope were 0.23, 3.38, 0.16 and 0.13 µg/ml for Standard (vincristine sulphate), HX, CT and CF, respectively. 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 various diseases.

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