-Short communication

Bioassay of two Leguminous Plants: *Enterolobium saman* Prain and *Albizzia lebbek* Benth.

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The biological activities of *Enterolobium saman* Prain and *Albizzia lebbek* Benth, two species of Leguminosae family were investigated. The fruits of *E. saman* were extracted with methanol and the extract was subjected to acid-base treatment for the isolation of alkaloid. The stem bark of *A. lebbek* was also extracted with methanol and the extract was fractionated by using standard chromatographic techniques. The crude methanol extract and corresponding fractions were investigated for their antimicrobial and cytotoxic activities. The crude methanol extract of *E. saman* and caffeine (**1**) isolated from it were found to produce zone of inhibition of 8 to 25 mm and 10 to 27 mm, respectively. The crude methanol extract and mixed fractions (F-34 and 35) of *A. lebbek* produced zone of inhibition between 8 to 14 mm and 8 to 10 mm against the bacterial strains and 10 to 14 mm and 09 mm against fungal strains at 500 μg/disc and 250 μg/disc respectively. All samples were also screened for cytotoxic activities by using brine shrimp lethality bioassay. LC_{50} (lethal dose concentration at 50% mortality) for crude methanol extract and residue of *E. saman* were found to be 1.56 and 0.781 μg/mL, while *A. lebbek* showed LC_{50} of 3.125 and 1.563 μg/mL for crude methanol extract and mixed fractions (F-34 and 35), respectively. LC_{90} values were also observed to get an idea about the toxic concentration of extractives.

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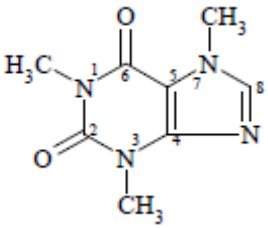
E. saman (Jacq.) Prain (Family-Leguminosae, sub-family- Mimosoideae) is a large tree available in Bangladesh with the Bengali name of "Koroi".[1] The fresh leaves of the plants are used in diarrhea.[1] Previous investigations with *E. saman* have revealed the presence of a number of secondary metabolites including triterpene, enterolosaponins A and B[2], and albizzine.[3-5] *A. lebbeck* is a moderate to large tree that reaches 30 m in height in rain forests. It is a fodder tree in the tropic and sub-tropic region.[6] The bark is used locally in India for tanning fishing nets, treating boils, as soap, anthelmintic, anti-inflammatory and in treatment of bronchitis, toothache and leprosy,[7,8] while the leaves and seeds were used for eye problem.[9] Previous phytochemical studies with *A. lebbek* led to the isolation of glycosides,[10] terpenoids, steroids, saponins,[11] anthraquinones and other phenolics,[12] volatile oils,[13] tannins,[14] gums,[15] and lipoidal matter.[16]

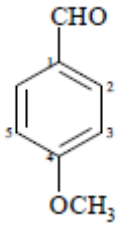
The fruit sample of *E. saman* and stem bark of *A. lebbek* were collected from the campus of the University of Dhaka, Dhaka-1000, Bangladesh in December 2005. The plants were properly identified and a voucher specimen has been deposited in the Bangladesh National Herbarium (BNH) under the accession number, DACB-32081 and DACB-32758, respectively.

The fruits of *E. saman* and stem bark of *A. lebbek* were cut into pieces, sun-dried for about 7 days and then ground to coarse powder. The coarse powdered samples were then stored in airtight container. The powdered fruits of *E. saman* and stem

bark of *A. lebbek* were cold extracted with methanol[17] at room temperature. The extract of fruits of *E. saman* was subjected to acid-base treatment for separation of the alkaloids. The xanthine alkaloid caffeine (**1**)[18] and also *p*-anisaldehyde (**2**)[19-21] were isolated from the column fractions of the 'alkaloid containing residue' by elution with ethyl acetate-methanol (90:10) and ethyl acetate-methanol (50:50), respectively.



Caffeine (**1**)



p-Anisaldehyde (**2**)

Table 1. Antimicrobial activity of *E. saman* extractives

Test microorganism	Diameter of zone of inhibition (mm)		
	ME	C-1	KAN
Gram Positive			
Bacillus cereus (BTCC-19)	20	25	50
B. megaterium (BTCC-18)	22	27	46
B. subtilis	22	25	30
Staphylococcus aureus (BTCC-43)	12	10	46
Gram Negative			
Escherichia coli (BTCC-172)	25	-	35
Salmonella typhi	08	21	38
Sa. paratyphi	09	20	35
Fungi			
Saccharromyces cevevaceae	-	-	38
Candida albicans	22	20	40
Aspergillus niger	-	-	38

ME: methanol extract; C-1: caffeine; KAN: standard kanamycin disc; diameter of zone of inhibition less than 7 mm was considered inactive (-).

Table 2. Antimicrobial activity study *A. lebbek* extractives

Test microorganism	Diameter of zone of inhibition (mm)		
	ME (500 µg/disc)	F-34, 35 (250 µg/disc)	KAN (30 µg/disc)
Gram Positive			
Bacillus cereus (BTCC-19)	10	09	50
B. megaterium (BTCC-18)	10	10	46
B. subtilis	09	08	30
Staphylococcus aureus (BTCC-43)	10	10	46
Sarcina lutea	09	10	35
Gram Negative			
Pseudomonas aeruginosa	08	10	40
Salmonella paratyphi	09	09	35
Salmonella typhi	14	10	38
Shigella boydii	10	10	40
Sh. dysenteriae	–	10	35
Vibrio mimicus	10	–	40
V. parahemolyticus	14	09	40
Fungi			
Saccharromyces cevevaceae	14	09	38
Aspergillus niger	10	09	38

ME: methanol extract; F-34, 35: Mixed Fraction

The preliminary antimicrobial activity of the crude methanol extract and caffeine (**1)** from *E. saman* was determined at a concentration of 250 µg/disc by the disc diffusion assay method[22-24] against a number of gram positive and gram negative bacteria and fungi (Table 1), by using kanamycin (30µg/disc) as reference. Nutrient agar medium (DIFCO) was used in the present study to prepare fresh culture. The discs were then incubated on the plate aerobically at 37 ºC for 24 hours. The zones of inhibition were measured and recorded at the end of the incubation period. Similarly antimicrobial activity of the crude methanol extract and mixed fractions (F-34 and 35) from *A. lebbek* were also determined by the same process (Table 2). The crude methanol extract and caffeine (**1)** of *E. saman* were found to produce zone of inhibition of 8 to 25 mm and 10 to 27 mm, respectively, with prominent activity against *Candida albicans* with zone of inhibition of 22 and 20 mm, respectively. From Table 1, it is evident that the zone of inhibition for the crude methanol extract was prominent against *E. coli* (25 mm), *B. megaterium* (22 mm) and *B. subtilis* (22 mm). From Table 2, the zone of inhibition produced by the crude methanol extract and mixed fractions (F-34 and 35) of *A. lebbek* were found to be 08 to 14 mm and 08 to 10 mm at the concentrations of 500 µg/disc and 250 µg/disc, respectively. The crude methanol extract showed good activity against *Salmonella typhi* (14 mm), *Vibrio parahemolyticus* (14 mm) and *Saccharromyces cevevaceae* (14 mm).

Following the procedure of Meyer,[25] the lethality of crude methanol extract to brine shrimp was determined on *Artemia salina*. For the experiment 2.0 mg of each of the extract was dissolved in DMSO and serially diluted to get solutions of varying concentrations such as 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.781 and 0.3905 µg/mL. In the cytotoxicity screening, vincristine sulfate was used as standard. The results of the brine shrimp lethality testing were obtained after 24 hours of exposure to the samples and the positive control, vincristine sulphate. The LC_{50} were found to be 0.625 and 1.56 µg/mL for vincristine sulfate and crude methanol extract of *E. saman*, respectively. The LC_{90} value was also determined 39 µg/mL for crude methanol extract of *E. saman* to find the toxicity level of the fractions. In comparison with the positive control (vincristine sulfate) the cytotoxic activity of the crude extract of *E. saman* was significant. The LC_{50} of crude methanol extract and mixed fractions (F-34 and 35) from *A. lebbek* were 3.125 and 1.56 µg/mL, respectively. While the LC_{90} value were found to be 170.0 and 50.0 µg/mL, respectively to determine the toxic level of the extracts.

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