

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

M. Kaisarul Islam¹, Asma Rahman², Md. S. Hossain³ and A. Jabbar¹

¹Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh

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

³Bioassay Section, Analytical Research Division, BCSIR, Dhaka-1205, Bangladesh

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. LC₅₀ (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 LC₅₀ of 3.125 and 1.563 µg/mL for crude methanol extract and mixed fractions (F-34 and 35), respectively. LC₉₀ values were also observed to get an idea about the toxic concentration of extractives.

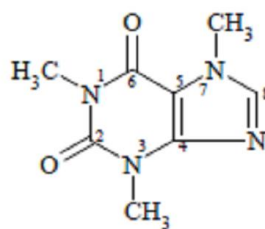
Correspondence to: M. Kaisarul Islam
Email: m_kislam@yahoo.com

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 B², and albizzine.³⁻⁵ *A. lebbek* 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

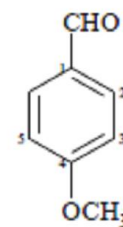
The fruit sample of *E. saman* and stem bark of *A. lebbek* were collected from the campus of the University of Dhaka, Dhaka-1000, Bangladesh in December 2005. The plants were properly identified and a voucher specimen has been deposited in the Bangladesh National Herbarium (BNH) under the accession 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 methanol¹⁷ 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			
<i>Bacillus cereus</i> (BTCC-19)	20	25	50
<i>B. megaterium</i> (BTCC-18)	22	27	46
<i>B. subtilis</i>	22	25	30
<i>Staphylococcus aureus</i> (BTCC-43)	12	10	46
Gram Negative			
<i>Escherichia coli</i> (BTCC-172)	25	-	35
<i>Salmonella typhi</i>	08	21	38
<i>Sa. paratyphi</i>	09	20	35
Fungi			
<i>Saccharomyces cevevaceae</i>	-	-	38
<i>Candida albicans</i>	22	20	40
<i>Aspergillus niger</i>	-	-	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			
<i>Bacillus cereus</i> (BTCC-19)	10	09	50
<i>B. megaterium</i> (BTCC-18)	10	10	46
<i>B. subtilis</i>	09	08	30
<i>Staphylococcus aureus</i> (BTCC-43)	10	10	46
<i>Sarcina lutea</i>	09	10	35
Gram Negative			
<i>Pseudomonas aeruginosa</i>	08	10	40
<i>Salmonella paratyphi</i>	09	09	35
<i>Salmonella typhi</i>	14	10	38
<i>Shigella boydii</i>	10	10	40
<i>Sh. dysenteriae</i>	-	10	35
<i>Vibrio mimicus</i>	10	-	40
<i>V. parahemolyticus</i>	14	09	40
Fungi			
<i>Saccharomyces cevevaceae</i>	14	09	38
<i>Aspergillus niger</i>	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 method²²⁻²⁴ 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 °C 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 *Saccharomyces 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 LC₅₀ were found to be 0.625 and 1.56 µg/mL for vincristine sulfate and crude methanol

extract of *E. saman*, respectively. The LC₉₀ 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 LC₅₀ 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 LC₉₀ value were found to be 170.0 and 50.0 µg/mL, respectively to determine the toxic level of the extracts.

ACKNOWLEDGEMENTS

The authors are thankful to Analytical Research Division, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh; Bangladesh National Herbarium (BNH) for identifying the plant and Institute of Nutrition and Food Science (INFS), University of Dhaka for supplying microorganisms as pure culture.

REFERENCES

1. Staples, G.W. and Elevitch, C.R. 2006. *Species Profiles for Pacific Island Agroforestry*. Permanent Agriculture Resources (PAR), Holualoa, Hawaii. pp. 1-15.
2. Mimaki, Y., Harada, H., Sakuma, C., Haraguchi, M., Yui, S., Kudo, T., Yamazaki, M. and Sashida. 2003. Enterolosaponins A and B, novel triterpene bisdesmosides from *Enterolobium contortisiliquum*, and evaluation for their macrophage-oriented cytotoxic activity. *Bioorg. Med. Chem. Lett.* **13**, 623-627.
3. Kjaer, A. and Vesterager, E. 1960. Amino Acid Studies. Part III, Synthesis and properties of some isomerides of albizziine. *Acta Chemica Scandinavica* **14**, 961-964.
4. Inouye, S., Shomura, T., Tsuruoka, T., Ogawa, Y., Watanabe, H., Yoshida, J. and Niida, T. 1975. L-beta-(5-Hydroxy-2-pyridyl)-alanine and L-beta-(3-Hydroxyureido)-alanine from *Streptomyces*. *Chem. Pharm. Bull.* **23**, 2669-2677.
5. Buckingham, J., 1996. *Dictionary of Natural Products*, Chapman and Hall, London, UK, p. 545.
6. Prinsen, J.H. 1986. Potential of *Albizia lebbek* as a tropical fodder tree - a review of literature. *Tropical Grassland* **29**, 78-83.
7. Rashid, R.B., R. Chowdhury, Hasan, C.M. and Rashid, M.A. 2003. Constituents of *Albizia lebbek* and antibacterial activity of an isolated flavone derivative. *Saudi Pharm. J.* **11**, 52-56.

8. Kirtikar, K.R. and B.D. Basu, 1980. *Indian Medicinal Plants*, Bishen Singh Mahendra Pal Singh, Dehra Dun, India, p. 2793.
9. Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. and Anthony, S. 2009. *Agroforestry Database: A Tree Reference and Selection Guide*. International Centre for Research in Agroforestry, Nairobi, Kenya, p. 6.
10. Varshney, I.P. 1976. Glycosides and carbohydrates from the members of the family Leguminosae. *Univ. Indore Res. J. Sci.* **4**, 13-22.
11. Pal, B.C., Achari, B., Yoshikawa, K. and Arihara, S. 1995. Saponins from *Albizia lebbek*. *Phytochemistry* **38**, 1287-1291.
12. Deshpande, V.H. and Shastri, R.K. 1977. Phenolics of *Albizia lebbek*, *A. amara* and *A. procera*. *Indian J. Chem.* **15**, 201-204.
13. Jain, A.S. and Mishra, K.L. 1963. Assay the volatile oil of the flowers of *Albizia lebbek* L. *Tetrahedron Lett.* **1**, 19-20.
14. Rayudu, K.H. and Rajadurai, G.F. 1965. Investigation of D catechin in the bark of *Albizia lebbek* L. *Phytochemistry* **113**, 258-263.
15. Farooqi, M.I.H. and Kaul, K.N. 1962. Investigation of the chemical composition of the gum of *Albizia lebbek* L. trees. *J. Sci. Ind. Res.* **21**, 454-455.
16. Kufuku, U.H. and Huta, L.K. 1934. Analysis the oil of *Albizia lebbek* L. *Tetrahedron Lett.* **1**, 15-16.
17. Trease, G.E. and Evans, W.C. 1989. *A Text-Book of Pharmacognosy*. Baillière Tindall, Alden Press, Oxford, p. 53.
18. Kan, L.S., Borer, P.N., Cheng, D.M. and Ts'o, P.O.P. 1980. ^1H and ^{13}C -NMR studies on Caffeine and its interaction with nucleic acids. *Biopolymers* **19**, 1641-1654.
19. Dhami, K.S. and Stothers, J.B. 1966. ^{13}C NMR studies: VIII. ^{13}C spectra of some substituted anisoles. *Canadian J. Chem.* **44**, 2855-2866.
20. Birkinshaw, J.H. and Chaplen, P. 1995. Biochemistry of the wood-rotting fungi. 8. Volatile metabolic products of *Daedalea juniperina* Murr. *Biochem. J.* **60**, 255-261.
21. Sylvie, R., Sophie, B., Thierry, T., Yves, P. and Bessière, J.M. 2002. The anise-like odor of *Clitocybe odora*, *Lentinellus cochleatus* and *Agaricus essetiei*. *Mycologia* **94**, 373-376.
22. Zavala, S.M.A., Perez, G.S. and Perez, G.M. 1997. Antimicrobial screening of some medicinal plants. *Phytotherapy Res.* **11**, 368-371.
23. Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C. and Tenover, R.H. 1995. *Manual of Clinical Microbiology*, Washington DC, USA, pp. 214-15.
24. Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Truck, M. 1966. Antibiotic susceptibility testing by standard single disc diffusion method. *Am. J. Clin. Pathol.* **45**, 426-493.
25. Meyer, B.N., Ferringni, N.R., Puam, J.E., Lacobsen, L.B., Nichols, D.E. and McLaughlin, J.L. 1982. Brine shrimp: a convenient general bioassay for active constituents. *Planta Med.* **45**, 31-32.