Chemical and Biological Investigations of *Samanea saman* (Jacq.) Merr.

Faisol Ferdous, Md. Khalid Hossain, Mohammad S. Rahman, Md. Aslam Hossain, Shaila Kabir and Mohammad A. Rashid

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

ABSTRACT: Two compounds namely lupeol (**1**) and epilupeol (**2**) were isolated from *n*-hexane soluble fraction of crude methanol extract of the powdered whole plant of *Samanea saman* Merr. for the first time. The structures of the isolated compounds were established by extensive analyses of their high resolution NMR spectral data as well as co-TLC with authentic samples. In our preliminary screening, the *n*-hexane, carbon tetrachloride, and dichloromethane soluble fractions of the crude methanolic extract of *S. saman* were subjected to antimicrobial activity and brine shrimp lethality bioassay. The carbon tetrachloride soluble partitionate of the methanol extract exhibited mild to moderate antimicrobial activity and strong cytotoxicity having LC_{50} of 0.831μg/ml.

Key words: *Samanea saman*, Leguminosae, lupeol, epilupeol, antimicrobial activity, brine shrimp lethality bioassay.

INTRODUCTION

Bangladesh is a good repository of medicinal plants comprising of various families, including Leguminosae.[1,2] The leguminous plants contain a wide range of pharmacologically active compounds including anti-inflammatory, antirheumatic, anti-diarrhea and anti-emetic activities. Throughout the world about 600 genera and 12,000 species are members of the family Leguminosae.[3] Investigations of a large number of liguminous plants have shown to contain a wide range of secondary metabolites including cytotoxic compounds. Therefore an attempt has been taken to study the chemical constituents as well as antimicrobial and cytotoxic activities of *S. saman*.

Correspondence to:
Mohammad A. Rashid
Tel: 880-2-8612069, 9661900-73, Extn. 4363, 4364, 8137
Fax: 880-2-8615583
E-mail: rashidma@univdhaka.edu

Samanea saman (Jacq.) Merr. (Bengali name- Rendi Koroi; Family- Leguminosae) is distributed in the tropics and generally known as rain tree, that grows all over Bangladesh. It is cultivated as an ornamental shade tree and its pods and leaves are valued as cattle fodder.[4] Rain tree is a folk remedy for cold, diarrhoea, headache, intestinal ailments and stomachache.[5] The antibacterial activity of an alkaloidal fraction of the leaves was reported to inhibit *Mycobacterium tuberculosis*.[6] Rain tree is also known to have anticancer property; the root decoction is used in hot bath for stomach cancer in Venezuela.[7] Previous phytochemical studies with *S. saman* revealed the occurrences of alkaloids, pithecolobin, samarin and steroidal compounds.[8] We, herein, report the isolation of lupeol **(1)** and epilupeol **(2)** as well as preliminary antimicrobial and cytotoxic activities of the extractives.

MATERIALS AND METHODS

General experimental procedures. The ^{1}H NMR spectra were recorded using a Bruker AMX-400 (400 MHz) instrument and the spectra were referenced to the residual nondeuterated solvent signal. PTLC and TLC were carried out using Merck Si gel 60 F_{254} on glass plates at a thickness of 0.5 mm. Spots on TLC and PTLC plates were visualized by spraying the developed plates with vanillin-sulfuric acid followed by heating for 5 minutes at 110 ^{o}C. All solvents used in this study were of reagent grade.

Plant material. Whole plant of *S. saman* was collected from Dhaka in September, 2008. A voucher specimen (DACB - 34204) for this collection has been deposited in Bangladesh National Herbarium for future reference.

Extraction and isolation. The powdered whole plant (550 g) of *S. saman* was extracted with 2.5 L of methanol for 7 days and filtered through a cotton plug followed by Whatman filter paper number 1. The extract was then concentrated with a rotary evaporator. An aliquot (5.0 g) of the concentrated aqueous methanol extract was fractionated by the modified Kupchan partioning protocol[9] into *n*-hexane, carbon tetrachloride, and dichloromethane. Subsequent evaporation of solvents afforded *n*-hexane (1.5 g), carbon tetrachloride (0.25 g), dichloromethane (0.15 g) and aqueous soluble (3.0 g) materials.

An aliquot of the carbon tetrachloride soluble fraction was fractionated by column chromatography (CC) over lipophilic Sephadex (LH-20) using *n*-hexane-dichloromethane-methanol (2:5:1) to provide 30 fractions, each 25 ml. Preparative thin layer chromatography [stationary phase: silica gel F_{254}, mobile phase: toluene - ethyl acetate (95 : 5) of column fractions 10-12 (mixed together due to their identical characteristics) yielded two compounds **1** (8 mg) and **2** (6 mg) as white amorphous mass.

Lupeol (**1**). Colorless mass; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.69 & 4.57 (each 1H, br. s, H_2-29), 3.21 (1H, dd, *J*=11.5, 5.03 Hz, H_α-3), 2.38 (1H, m, H-19), 1.67 (3H, s, H_3-30), 1.04 (3H, s, H_3-26), 0.98 (3H, s, H_3-23), 0.95 (3H, s, H_3-27), 0.84 (3H, s, H_3-25), 0.77 (3H, s, H_3-28), 0.77 (3H, s, H_3-24).

Epilupeol (**2**). White powder; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.68 & 4.59 (each 1H, brs, H_2-29), 3.40 (1H, t, *J*=3.0 Hz, H_β -3), 2.39 (1H, m, H-19), 1.69 (3H, s, H_3-30), 1.04 (3H, s, H_3-26), 0.97 (3H, s, H_3-23), 0.94 (3H, s, H_3-27), 0.83 (3H, s, H_3-25), 0.83 (3H, s, H_3-28), 0.80 (3H, s, H_3-24).

Antimicrobial screening. The antimicrobial activity of the extractives was determined by the disc diffusion method.[10] The bacterial and fungal strains used for the experiment (Table 1) were collected as pure cultures from the Institute of Nutrition and Food Sciences (INFS), University of Dhaka. The extractives were dissolved separately in chloroform and methanol as required and applied to sterile filter paper discs at 300 μg/disc and carefully dried to evaporate the residual solvent. Standard kanamycin (30μg/disc) discs were used as positive control.

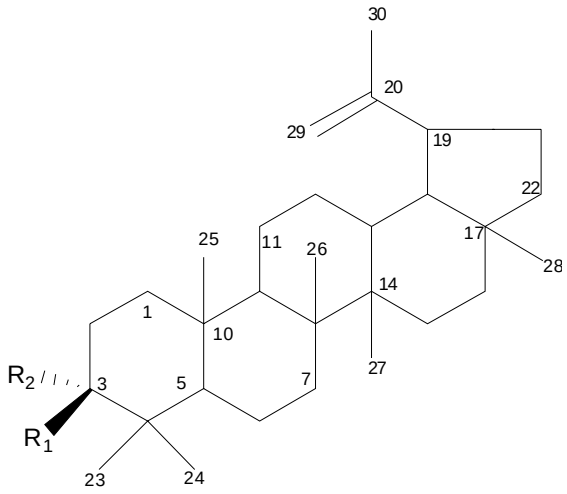
Cytotoxicity evaluation. For cytotoxicity screening, the *n*-hexane, carbon tetrachloride, and dichloromethane soluble materials of crude methanol extract were separately dissolved in DMSO. The test samples were then applied against *Artemia salina* in a 1-day in *vitro* assay.[11-12] Artificial sea water was prepared as described by Culkin[13] with slight modification of chemical composition. Four mg of each of the extractives was dissolved in DMSO and solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.78125 μg/mL were obtained by serial dilution technique. Vincristine sulfate and DMSO were used as the positive control and negative control respectively. The median lethal concentration (LC_{50}) of the test samples after 24 hrs of exposure were determined from a plot of % of the dead shrimps against the logarithm of the sample concentration.

Statistical analysis. 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.

RESULTS AND DISCUSSION

Repeated chromatographic separation and purification of the *n*-hexane soluble fraction of a methanol extract of the powdered whole plant of *S. saman* yielded two compounds (**1** and **2**), the structures of which were solved by extensive analyses of NMR data as well as by comparing with published values and co-TLC with authentic samples.

Characterization of 1 as lupeol. The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **1** showed a double doublet (J = 11.5, 5.03 Hz) of one proton intensity centered at δ 3.21 typical for an oxymethine proton at C-3 of a triterpene skeleton. The splitting pattern of this proton confirmed the β (beta) orientation of the C-3 oxygenated substituent. The spectrum displayed two broad singlets at δ 4.69 and δ 4.57 (1H each) assignable to the vinylic protons at C-29. It also showed seven singlets at δ 0.98, 0.77, 1.04, 0.84, 0.95, 0.77 and 1.67 (3H each) assignable to the methyl group protons at C-4 (H_3-23, H_3-24), C-8 (H_3-26), C-10 (H_3-25), C-14 (H_3-27), C-17 (H_3-28) and C-20 (H_3-30), respectively. On this basis, compound **1** was characterized as lupeol. The identity of **1** was further confirmed by comparing its spectral data with previously reported values as well as Co-TLC with an authentic sample of lupeol, previously isolated in our laboratory.[14]



1: R_1 = OH, R_2 = H
2: R_1 = H, R_2 = OH

Characterization of 2 as epilupeol. The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **2** was almost identical to that recorded for lupeol (**1**). Thus it exhibited a triplet (J=3.0 Hz) of one proton intensity at δ 3.40 characteristic for H-3 of a terpenoid type carbon skeleton. The absence of a double doublet and the appearance of a triplet suggested that the hydroxy group on C-3 was at the α (alpha)- position, thus confirming the β-orientation of the C-3 proton. The spectrum also displayed two broad singlets at δ 4.68 and δ 4.59 (1H each) attributable to the vinylic protons at C-29. A multiplet at δ 2.39 could be ascribed to proton at C-19. The spectrum further showed seven singlets at δ 0.97, 0.80, 1.04, 0.83, 0.94, 0.83 and 1.69 (3H each) for methyl protons at C-4 (H_3-23, H_3-24), C-8 (H_3-26), C-10 (H_3-25), C-14 (H_3-27), C-17 (H_3-28) and C-20 (H_3-30), respectively. On the basis of above spectral data and by comparing these with published values, the structure of **2** was elucidated as epilupeol.[15] Again, the identity of **2** as epilupeol was confirmed by Co-TLC with an authentic sample.

Antibacterial and antifungal activities of *S. saman*. Different partitionates of methanol extract of *S. saman* were tested for antibacterial and antifungal activities against a number of gram positive and gram negative bacteria as well as some fungi. Among all the partitionates, the carbon tetrachloride soluble fraction of the methanol extract exhibited mild to moderate antibacterial and antifungal activity (Table-1). The carbon tetrachloride soluble fraction demonstrated moderate antibacterial activity against *Shigella dysenteriae* and *Sarcina lutea* having the diameter of zone of inhibition of 12 mm each. This fraction also showed mild antibacterial activity against *Bacillus cereus* and *B. subtilis* with zone of inhibition of 11 mm and 10 mm respectively.

Brine shrimp lethality bioassay of *S. saman*. Table 2 shows the results of the brine shrimp lethality assay after 24 hr exposure to the samples and the positive control vincristine sulfate. The positive control, compared with the negative control (sea water) was lethal, depicting significant mortality to the shrimp.

Table 1. Antibacterial activity of carbon tetrachloride soluble partitionate (CTSF) of methanol extract of *S. saman* at 300 µg/disc

Test microorganisms	Diameter of zone of inhibition (mm)	
	CTSF	Kanamycin
Gram positive bacteria		
Bacillus cereus	10	40
Bacillus megaterium	8	38
Bacillus subtilis	11	40
Staphylococcus aureus	7	50
Sarcina lutea	12	35
Gram negative bacteria		
Escherichia coli	8	45
Pseudomonas aeruginosa	10	47
Salmonella paratyphi	8	38
Salmonella typhi	8	38
Shigella boydii	9	34
Shigella dysenteriae	12	48
Vibrio mimicus	7	50
Vibrio parahemolyticus	7	38
Fungi		
Candida albicans	7	35
Aspergillus niger	9	35
Sacharomyces cerevacae	9	35

Table 2. Results of cytotoxicity screening of *S. saman*.

Sample	LC_{50} (µg/mL)	Regression equation	R^2
Vincristine sulphate	0.812	y = 33.219x + 52.781	0.9717
HSF	14.94	y = 27.381x + 17.845	0.9404
CTSF	0.831	y = 20.334x + 51.635	0.9065
DCMSF	3.288	y = 27.381x + 35.845	0.9341

HSF = Hexane soluble fraction of methanol extract, CTSF = Carbon tetrachloride soluble fraction of methanol extract, DCMSF = Dichloromethane soluble fraction of methanol extract

The median lethal concentration (LC_{50}) of the test samples after 24 hr was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration (toxicant concentration) and the best-fit line was obtained from the graph by means of regression analysis.

The *n*-hexane soluble partitionate showed less cytotoxicity than other fractions. Comparison with positive control, vincristine sulfate indicated that cytotoxicity exhibited by CTSF and DCMSF were promising and further bioactivity guided investigation should be conducted to find out the antitumor and pesticidal compounds.

CONCLUSION

Successive chromatographic separation and purification of the *n*-hexane soluble fraction of a methanol extract of *S. saman* yielded two isomeric compounds. The structures of the compounds were elucidated as lupeol (**1**) and epilupeol (**2**). The carbon tetrachloride soluble fraction of crude methanol extract of *S. saman* showed moderate antimicrobial activity whereas the carbon tetrachloride and dichloromethane soluble fractions demonstrated potent cytotoxic activity. These support the traditional uses of this plant in various infectious diseases. The plant can be further screened against various diseases in order to find out its unexplored

efficacy and can be a potential source of chemically interesting and biologically important drug candidates.

REFERENCES

1. Rahman, S., Hasnat, A., Hasan, C.M., Rashid, M.A**.**, and Ilias M. 2001. Pharmacological evaluation of Bangladeshi medicinal plants- a review. *Pharmaceutical Biology*. **39**, 1-6.
2. Yusuf, M., Chowdhury, J.U., Yahab, M.A. and Begum J. 1994. Medicinal Plants of Bangladesh. BCSIR Laboratories, Bangladesh, p. 38.
3. Trease and Evans, *Pharmacognosy,* 12th edition, 1987, p. 188.
4. Anonymous, 1945. Pharmacopoeia of India. Government of India, New Delhi, Ministry of Health and Family Welfare.
5. Duke, J.A. and Warin, K.K. 1981. Medicinal plants of the world. Computer index with more than 85000 entries.
6. Perry, L.M. 1980. Medicinal plants of east and southeast Asia. MIT press, Cambridge.
7. Hartwell, J.L. 1967-1971. Plants used against cancer. A survey, Lloydia, pp. 30-34
8. Anonymous, 1948-1976, wealth of India, Raw Materials, Vol. I-XI, Publication and Information directorate, C.S.I.R., New Delhi.
9. Van Wagenen, B.C., Larsen R., Cardellina J.H., Ran dazzo D., Lidert Z.C. and Swithenbank, C. 1993. Ulosantoin, a potent insecticide from the sponge *Ulosa ruetzleri. J. Org. Chem.* **58**, 335-337.
10. Bauer, A.W., Kirby, W.M.M., Sherries, J.C. and Truck, M. 1966. Antibiotic susceptibility testing by standard single disc diffusion method. *Am J. Clini. Pathol.* **45**, 426-493.
11. Meyer, B.N., Ferrigni, 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 Medica*. **45**, 31-32.
12. McLaughlin, J.L. and Rogers, L.L. 1998. The use of biological assays to evaluate botanicals. *Drug Info. J*. **32**, 513-524.
13. Culkin F. 1965. The major constituents of seawater in "*Chemical Oceanography"* (J.P. Riley and G. Skirrow eds. Academic Press), pp 121-61.
14. Aratanechemuge, Y., Hibasami, H., Sanpin, K., Katsuzaki, H., Imai, K., and Komiya, T. 2004. Induction of apoptosis by lupeol isolated from mokumen (*Gossampinus malabarica* L. *Merr*) in human promyelotic leukemia HL-60 cells. *Oncol. Rep.* **11**, 289-292.
15. Rahman, M.Z., Sultana, S.J., Faruquee, C.F., Ferdous, F., Rahman, M.S., Islam, M.S. and Rashid, M.A. 2007. Phytochemical and Biological investigation of *Erythrina variegate*. *Saudi Pharm. J.* **15**, 140-145.