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Antioxidant, Antimicrobial and Cytotoxic Activities of *Samanea saman* (Jacq.) Merr.

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ABSTRACT

In the present investigation the n-hexane, carbon tetrachloride and choloroform soluble fractions of crude methanolic extract of *Samanea saman* bark were tested for antioxidant, antimicrobial and cytotoxic potential. Antioxidant activity of the extracts was evaluated by DPPH radical scavenging assay and total antioxidant activity test. Antimicrobial activity was tested using disc diffusion method against thirteen bacteria and three fungi and cytotoxicity was tested by brine shrimp lethality bioassay. Chloroform and hexane soluble fraction showed IC_{50} value of 12µg/ml and 14µg/ml respectively in scavenging DPPH radical while the reference Butylated hydroxytoluene showed an IC_{50} value of 10µg/ml. The carbon tetrachloride fraction showed the highest total antioxidant capacity. The carbon tetrachloride fraction was also found to possess mild to moderate microbial growth inhibitory capacity. In the brine shrimp lethality bioassay, the n-hexane, carbon tetrachloride, chloroform soluble fractions showed LC_{50} value of 14.94µg/ml, 0.831µg/ml and 3.288µg/ml respectively. The results suggest good antioxidant and cytotoxic potential of chloroform and hexane soluble fractions and antimicrobial activity of carbon tetrachloride fraction of *Samanea saman* bark extract.

Key Words: *Samanea saman*, Leguminoseae, Cytotoxicity, Antimicrobial, Antioxidant, Total antioxidant capacity.

INTRODUCTION

Plants have been the traditional source of medicine for long time to treat different types of ailments of human being. A rich heritage of knowledge to preventive and curative medicines was available in ancient scholastic works including the Atharva veda, Charaka, Sushruta, etc. An estimate suggests that about 13,000 plant species worldwide are known to have use as drugs. The trend of using natural products has increased and the active plant extracts are frequently explored for new drug discoveries. In recent years one of the areas which attracted a great deal of attention is the possible therapeutic potential of antioxidants in controlling degenerative diseases associated with marked oxidative damage. Several plant extracts and different classes of phytochemicals have been found to have quite prominent antioxidant activity (Larson, 1998; Sreejayan et al., 1997; Tripathi et al., 1996; Vani et al., 1997). Plants are also explored to identify the presence of effective antimicrobial agents (Das et al., 1999) due to the emergence of multi-drug resistant bacterial strains.

Samanea saman (Jacq.) Merr. belongs to the second largest family of flowering plants, Leguminoseae. It is locally known as 'Rendi' or 'Fulkoroi'. *Samanea saman* is easily recognized by its characteristic umbrella-shaped canopy, reaching a height of 20 to 25 meters, with the branches wide spreading. The tiny flowers (12–25 per head) are massed in pinkish heads 5–6 cm (2–2.4 in) across and about 4 cm (1.6 in) in height. Pods are fleshy, straight, more or less flat and black when ripe. Seeds 16 to 20, rounded, truncate at one end, pointed at the other, dark reddish brown with a paler ring at each side (Adams, 1972). In the Philippines, a decoction of the inner bark (fresh cambium) and the fresh leaves is traditionally used in diarrhoea (Staples and Elevitch, 2006). The plant is used traditionally in diarrhea, intestinal diseases, stomach ache, colds and headache (Prasad et al., 2008). Leafs are used as laxative and seeds in sore throat. The leaves were reported to contain tannins, flavonoids, steroids, saponins, cardiac glycosides are terpenoids (Prasad et al., 2008). Bark is reported to contain alkaloids called pithecolobine and a saponin called samarin (Anon, 1948-1876). The leaves possess antimicrobial activity and the plant is also

reported to have anticancer property (Raghavendra et al., 2008). The present investigation was carried out to explore the antioxidant, antimicrobial and cytotoxic potential of *Samanea saman*.

MATERIALS AND METHODS

Chemicals

DPPH (1,1-diphenyl, 2-dipicrylhydrazyl) was purchased from Sigma Chemicals Co., USA. Ammonium molybdate were obtained from Merck, Germany. Sodium phosphate was obtained from BDH, England. Ascorbic acid was purchased from SD Fine Chem. Ltd., Biosar, India.

Plant material

The bark of *Samanea saman* was collected from Dhaka in January 2008. The plant was identified by the experts of Bangladesh National Herbarium and a voucher specimen has been deposited there. The bark of *Samanea saman* was collected, washed properly, cut into small pieces and then sun and air dried for several days. The pieces were then dried for 24 hours in oven at considerably low temperature to effect grinding. The dried plant was then ground in coarse powder using high capacity grinding machine.

Extraction

About 550 gm of the powdered material was taken in a clean, round bottomed flask (5 liters) and soaked in 2.5 liter of methanol. The container with its content was sealed by foil and kept for a period of 15 days accompanying occasional shaking and stirring. The whole mixture was then filtered through filter paper and the filtrate thus obtained was concentrated at 50°C with a rotary evaporation. 5 gm of methanol extract was triturated with 100ml of methanol containing 10ml of distilled water. The crude extract went to the solution completely. The mother solution was taken in a separating funnel. 100ml of the *n*-hexane was added to it and the funnel was shaken and then kept undisturbed. The organic portion was collected. The process was repeated thrice; *n*-hexane fractions were collected together (350.0 mg) and evaporated. To the mother solution left after washing with *n*-hexane, 12.5ml of distilled water was added and mixed. The mother solution was then taken in a separating funnel and extracted with CCl_4 (100ml X 3). The CCl_4 fractions were collected together and evaporated. The aqueous fraction was preserved for the next step. To the mother solution that left after washing with *n*-hexane and CCl_4, 16ml of distilled water was added and mixed uniformly. The mother solution was then taken in a separating funnel and extracted with $CHCl_3$ (100ml X 3). The $CHCl_3$ soluble fractions were collected together and evaporated (250 mg).

Antioxidant activity

DPPH radical scavenging activity

Antioxidant activity of the different fractions of methanolic extract was determined on the basis of their scavenging potential of the stable DPPH free radical. The assay was performed on the basis of the modified method described by Gupta et al. (2003). Stock solution (10mg/ml) of the plant extracts were prepared in ethanol from which serial dilutions were carried out to obtain concentrations of 1, 5, 10, 50, 100, 500µg/ml. Diluted solutions (2ml) were added to 2ml of a 0.004% ethanol solutions of DPPH, mixed and allowed to stand at 25^0C for 30 min for reaction to occur. The absorbance was determined as 517nm and from these values corresponding percentage of inhibitions were calculated from the formula $[(A_0-A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract/standard. Then % inhibitions were plotted against log concentration and from the graph IC_{50} was calculated. The experiment was performed in triplicate and average absorption was noted for each concentration. Ascorbic acid was used as positive control.

Determination of total antioxidant capacity

The antioxidant activity of the extract was evaluated by the phosphomolybdenum method according to the procedure of Prieto et al. (1999). The assay is based on the reduction of Mo (VI)–Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. 0.3ml extract was combined with 3ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95^0C for 90 min. Then the absorbance of the solution was measured at 695nm using a spectrophotometer (HACH 4000 DU UV–visible spectrophotometer) against blank after cooling

to room temperature. Methanol (0.3ml) in the place of extract was used as the blank. The antioxidant breaks the free radical chain by donating a hydrogen atom. The antioxidant activity is expressed as the number of equivalents of ascorbic acid.

Antimicrobial assay

The disc diffusion method describe by Bauer et al. (1966) was used to test antimicrobial activity against thirteen bacteria and three fungi (Table 3). Solutions of known concentration (mg/ml) of the test samples were made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper disc (6mm diameter) were then impregnated with known amounts of the test substances using micropipette. Discs containing the test material were placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic discs (kanamycin 30 µg/disc) and blank discs (impregnated with solvents) were used as a positive and negative control. These plates were then kept at low temperature (4^0C) for 24 h to allow maximum diffusion. There is a gradual change in concentration in the media surrounding discs. The plates were then incubated at 37^0C for 24 h to allow maximum growth of the organisms. The test materials having antimicrobial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. The antimicrobial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter.

Brine Shrimp lethality bioassay

Brine shrimp lethality bioassay was carried out according to the method described by Persoone (1980) for the determination of cytotoxic property of the hexane soluble fraction, carbontetrachloride soluble fraction and chloroform soluble fraction of the crude bark extract. The eggs of Brine Shrimp (*Artemia salina* Leach) were hatched in a tank with 24 hour oxygen supply facility at 37℃ temperature. Stock solutions of plant extract samples were prepared by dissolving the appropriate amount of extracts in calculated volume of dimethyl sulfoxide (DMSO). Samples of different concentrations (0.781, 1.563, 3.125, 6.25, 12.5, 25, 50, 100, 200, 400µg/ml) were prepared. 10 living nauplii were taken to each of the vial containing different concentrations of test sample with Pasteur pipette. Vincristine sulphate was used as the positive control. Measured amount of the vincristine sulphate was dissolved in DMSO to get an initial concentration of 20µg/ml from which serial dilutions are made using DMSO to get 10 µg/ml, 5 µg/ml, 2.5µg/ml, 1.25µg/ml, 0.625µg/ml, 0.3125µg/ml, 0.15625µg/ml, 0.078125µg/ml and 0.0390µg/ml. Then the positive control solutions are added to the premarked vials containing ten living brine shrimp nauplii in 5ml simulated sea water. 30µl of DMSO was added to each of three premarked glass vials containing 5ml of simulated seawater and 10 shrimp nauplii to use as negative control groups. After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial were counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration.

RESULTS & DISCUSSION

DPPH radical scavenging activity

Free radical scavenging activities of different fractions of methanolic extract and standard tested by DPPH method are shown in Figure 1. Chloroform soluble fraction of the methanolic extract of *Samanea saman* exhibited significant radical scavenging activity having IC_{50} value of 12µg/ml. IC_{50} value of the standard (BHT) obtained 10µg/ml. Hexane soluble fraction also showed high antioxidant potential (IC_{50}=14µg/ml). The carbontetrachloride fraction of the methanolic extract showed moderate antioxidant property having IC_{50} value of 65µg/ml (Table 1).

Total antioxidant activity

Phosphomolybdenum method was used to determine the total antioxidant capacity of the extracts. Total antioxidant activity of the extracts is expressed as ascorbic acid equivalent (AAE) and is given in Table 2. The carbon tetrachloride fraction showed highest activity followed by chloroform and n-hexane soluble fractions. Tannins, flavanoids and phenolic compounds present in the plant may be responsible for antioxidant properties of the extracts (Larson, 1988).

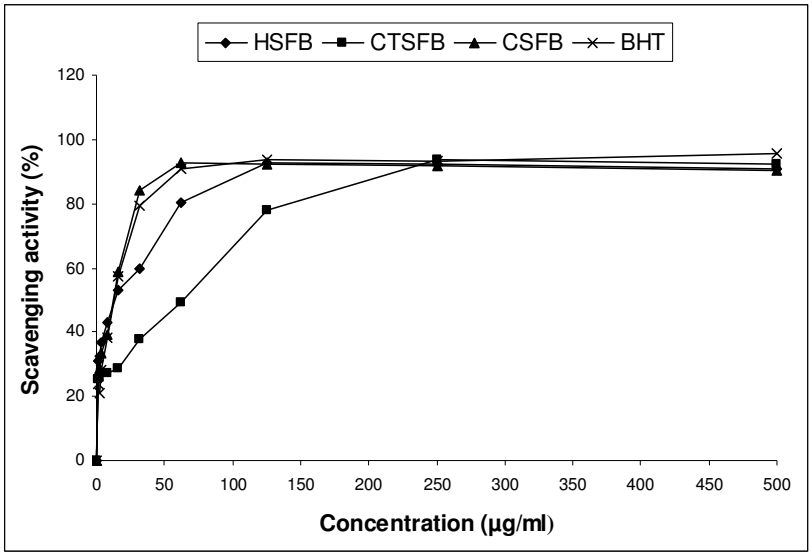

Figure 1: DPPH radical scavenging activity of different fractions of *Samanea saman*. (Values are the mean of duplicate experiments and represented as mean ±SD).
HSFB = Hexane soluble fraction of bark; CTSFB = Carbon tetrachloride soluble fraction of bark; CSFB = Chloroform soluble fraction.

Table 1: IC_{50} values of standard and different fractions of *Samanea saman.*

Extract	IC_{50} values
Hexane soluble fraction	14
Carbon tetrachloride soluble fraction	65
Chloroform soluble fraction	12
Butylated hydroxytoluene	10

Table 2: Total antioxidant capacity of different fractions of *Samanea saman* (Values are the mean of duplicate experiments and represented as mean ± SD).

Sample	Total antioxidant capacity, mg/g plant extract in AAE
Hexane soluble fraction	118.15±3.14
Carbon tetrachloride soluble fraction	173.70±2.62
Chloroform soluble fraction	133.70±2.10

Antimicrobial assay
Different partitionate of Methanolic extract of *Samanea saman* was tested for antibacterial and antifungal activity against a number (13) of gram positive and gram negative bacteria as well as some (3) fungi (Table 3) by disc diffusion method. Standard disc of Kanamycin (30µg/disc) was used for comparison purpose. Among all the partitionate only the carbon tetra chloride soluble fraction of the methanolic extract exhibited mild to moderate antibacterial and antifungal activity, listed in Table 3. The carbon tetrachloride soluble fraction showed highest antibacterial activity against *Shigella dysenteriae and Sarcina lutea* having the diameter of zone of inhibition of 12mm each. This fraction also showed moderate antibacterial activity against *Bacillus cereus* and *Bacillus subtilis* having the diameter of zone of inhibition of 11mm and 10mm respectively. None of the crude extracts demonstrated significant inhibition of growth of the test microorganisms.

Table 3: Antibacterial activity of the different fractions of methanolic bark extract of *Samanea saman*.

Test microorganisms	Diameter of zone of inhibition (mm)			
	HSFB	CTSFB	CSFB	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

HSFB = Hexane soluble fraction of bark; CTSFB = Carbon tetrachloride soluble fraction of bark; CSFB = Chloroform soluble fraction of bark.

Brine Shrimp lethality bioassay

The lethality of all the crude extracts to brine shrimp was determined on *A. salina* (Meyer et al., 1982). Table 4 shows the results of the brine shrimp lethality testing after 24 hours of exposure to the samples and the positive control, vincristine sulphate. The LC_{50} obtained from the best-fit line slope were found to be 0.812µg/ml, 14.94µg/ml, 0.831µg/ml and 3.288µg/ml for Vincristine sulphate (positive control), Hexane soluble fraction, Carbontetrachloride soluble fraction and chloroform soluble fraction of methenolic crude extract respectively. The chloroform soluble fraction was found very effective compared to the standard. The results of this study indicate the presence of potent bioactive principles in this crude extract which might be very useful as antiproliferative, antitumor, pesticidal and other bioactive agents.

Table 4: Effects of HSFB, CTSFB and CSFB on brine shrimp nauplii.

Conc. (C) (µg/ml)	Log C	% Mortality			LC_{50} (µg/ml)			Vincristine Sulfate			
		HSFB	CTSFB	CSFB	HSFB	CTSFB	CSFB	Conc (C) (µg/ml)	Log C	% Mortality	LC_{50} (µg/ml)
400	2.602	100	100	100				40	1.602	100	
200	2.301	90	100	100				20	1.301	100	
100	2	90	90	100				10	1.000	90	
50	1.699	80	80	100				5	0.698	80	
25	1.398	70	90	80	14.94	0.831	3.288	2.5	0.397	70	0.812
12.5	1.097	50	60	70				1.25	0.096	50	
6.25	0.796	50	50	70				0.625	-0.204	40	
3.125	0.495	40	40	60				0.3125	-0.505	30	
1.563	0.194	30	50	50				0.15625	-0.806	30	
0.781	-0.107	20	40	40				0.078125	-1.107	20	

HSFB = Hexane soluble fraction of bark; CTSFB = Carbon tetrachloride soluble fraction of bark; CSFB = Chloroform soluble fraction of bark.

CONCLUSION

Based on the results of the present study, it can be commented that different fractions of the plant extract possess remarkable antioxidant and cytotoxic property while only the carbontetrachloride soluble fraction possess antimicrobial property. However, further studies are needed to understand the exact mechanisms of such bioactivity and to isolate and purify the compound(s) responsible for such activity.

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