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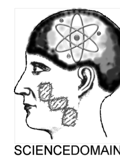

Cytotoxic, Thrombolytic and Membrane Stabilizing Activities of *Swietenia mahagoni* (L.) Jacq. Flower Extract

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Authors' contributions

This work was carried out in collaboration among all authors. Authors SMAR and MJN designed the study, performed the statistical analysis. Authors MA, TEH and TS wrote the protocol and wrote the first draft of the manuscript. Authors SMAR, MA and TEH managed the analyses of the study. Authors MJN and TS managed the literature searches. Author SMAR revised manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To investigate cytotoxic, thrombolytic and membrane stabilizing activities of methanol extract and its different Kupchan partitionates of flowers of *Swietenia mahagoni*.
Study Design: Evaluation of cytotoxic activity using brine shrimp nauplii, thrombolytic and membrane stabilizing activities on human RBCs.
Place and Duration of Study: Phytochemical Research Laboratory, Department of Pharmacy, School of Health Science, State University of Bangladesh, from April to September, 2013.
Methodology: The eggs of brine shrimp nauplii were hatched in artificial sea water for 24 hours. Cytotoxic activity was determined by measuring the percentage of their mortality after application of different partitionates to them. Human RBCs were obtained from 3

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healthy volunteers. Thrombolytic activity was calculated by weighing the clot before and after addition of different partitionates while membrane stabilizing activity was evaluated in terms of inhibition of percentage of haemolysis of RBCs by measuring optical density in both hypotonic and heat induced conditions.
Results: The highest cytotoxic activity was achieved with the crude methanol extract (LC_{50} = 0.10±0.01 μg/ml) among the partitionates while vincristine sulfate, the positive control, achieved an LC_{50} value of 0.40±0.02 μg/ml. While investigating thrombolytic activity, the petroleum ether soluble fraction achieved the highest clot lysis activity (34.30±0.78%) compared to the standard streptokinase (70.27±1.26%). While determining the membrane stabilizing activity, in hypotonic solution induced condition, the crude methanol extract inhibited 84.71±3.25% haemolysis of RBCs whereas in heat induced condition, the aqueous soluble fraction inhibited 86.30±4.98% haemolysis of human RBCs. Here, acetyl salicylic acid (0.01mg/ml) used as reference standard showed 71.91±2.29% and 45.45±4.87% inhibition of haemolysis of human RBCs in hypotonic solution and heat induced conditions, respectively.
Conclusion: From our investigation, it can be suggested that, the flower extractives can further be studied extensively to find out their efficacy.

Keywords: Swietenia mahagoni; flowers; brine shrimp lethality; thrombolytic; membrane stabilizing; hypotonic solution.

1. INTRODUCTION

Swietenia mahagoni L. Jacq. (Synonyms: *Swietenia mahogany* C. DC., *Swietenia acutifolia* Stokes, *Swietenia mahogani* JACQ; Bengali name: Mahagoni) is a large deciduous and economically important timber tree native to West Indies (Family: Meliaceae). It can reach 75 feet in height with a 50 feet spread [1]. It is a medium to large evergreen tree native to Southern Florida, Cuba, Bahamas, Hispaniola and Jamaica [2]. The species is widely distributed in South Asian countries like India, Sri Lanka and Bangladesh. *S. mahagoni* flowers bloom on panicles found in the axils of the leaves. The panicles emerge in the spring. Flowers are small and less than 0.3 inches wide. Flowers are yellow or green in color with characteristic fragrance. *S. mahagoni* has separate male and female flowers on the same plant (monoecism). It is an important medicinal plant and has various types of medicinal values like antimalarial and antidiarrhoeal effects [3-4]. The decoction of the bark is extensively used as febrifuge, which can be associated with its use as an anti-malarial drug [5]. Various parts of the plant have been used as by traditional healers for the treatment of hypertension, malaria, cancer, amoebiasis, chest pains, fever, anemia diarrhea, dysentery, depurative and intestinal parasitism [6-7].

In order to find out new bioactivities, the systematic screenings of different medicinal plants have become a regular task in many phytochemical research laboratories [8-9]. Therefore, the crude methanol extract of flowers of *S. mahagoni* and its aqueous and organic soluble partitionates were investigated for cytotoxic, thrombolytic and membrane stabilizing activity assays.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials and Extraction

The flowers of *S. mahagoni* were collected from Brahmanbaria, Bangladesh. The plant was identified at the Bangladesh National Herbarium. A voucher specimen has been deposited for this collection (Accession number DACB: 38501).

The flowers were subjected to shade drying under sun light for several days and then oven drying for 24 hours at considerably low temperature (not exceeding 40ºC) for better grinding. The dried flowers were then ground to a coarse powder. The powdered material (1200g) was taken in a properly cleaned, amber color reagent bottle (5 liters) and soaked in 2.5L methanol. After 2 weeks, the bottle content was filtered through a fresh cotton plug and finally with a Whatman filter paper No.1 and concentrated with a rotary evaporator at reduced temperature and pressure. An aliquot (5g) of the concentrated methanol extract was fractionated by modified Kupchan partitioning protocol [10] and the resultant partitionates were evaporated to dryness with rotary evaporator to yield petroleum ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF) and aqueous soluble materials (AQSF). The residues were then stored in a refrigerator until further use.

2.2 Brine Shrimp Lethality Bioassay

The cytotoxic activity was determined by using brine shrimp nauplii following the method of Meyer et al. [11]. According to the method, all partitionates were taken separately in vials and dissolved in pure dimethyl sulfoxide (DMSO) to get stock solution of 400µg/ml. From the stock solution, different concentrations such as 200µg/ml, 100µg/ml, 50µg/ml, 25µg/ml, 12.50µg/ml, 6.25µg/ml, 3.125µg/ml, 1.5625µg/ml and 0.78125µg/ml were obtained by serial dilution technique. In this study vincristine sulphate was used as the positive control. Measured amount of the vincristine sulphate was dissolved in DMSO to obtain an initial concentration of 20µg/ml from which serial dilution was done by using DMSO to get 10µg/ml, 5 µg/ml, 2.5µg/ml, 1.25µg/ml, 0.625µg/ml, 0.3125µg/ml, 0.15625µg/ml, 0.078125µg/ml and 0.0390 µg/ml. After that, for each partitionate and standard, the obtained solutions were added to the properly labeled vials containing ten live brine shrimp nauplii in 5 ml simulated sea water. After 24 hours, the vials were inspected using a magnifying glass and the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration. The lethal concentrations (LC_{50}) of the test samples were obtained by a plot of percentage of lethality against the logarithm of the sample concentration. Besides, 100µl of DMSO was added to three properly labeled glass vials containing 5 ml of simulated sea water and 10 brine shrimp nauplii to use as control groups in order to check validity of the method.

2.3 Thrombolytic Activity

The thrombolytic activity was evaluated by the method developed by Prasad et al. [12] by using streptokinase (SK) as positive control. According to the method, all partitionates (100 mg) were suspended in 10 ml of distilled water in test tubes and kept overnight. Then the soluble supernatant was decanted and filtered from each test tube. Again, from commercially available lyophilized altepase (streptokinase) vial (Beacon Pharmaceutical Ltd.) of 1,500,000 I.U., a stock of standard streptokinase of 100µl (30,000 I.U) was prepared for *in vitro* thrombolysis. Using sterile syringe, venous blood was drawn from healthy human volunteers having no history of oral contraceptive or anticoagulant therapy and then 1 ml of blood was

transferred to the previously weighed sterile 7 microcentrifuge tubes each and the tubes were allowed to incubate at 37ºC for 45 minutes for clot formation. After that, the serum was completely removed from all tubes without disturbing the clots and each tube having clot was again weighed to determine the individual clot weight (clot weight = weight of clot containing tube – weight of tube alone).To each microcentrifuge tube containing pre-weighed clot, 100 µl aqueous solutions of 5 different partitionates along with 100 µl of streptokinase, the positive control, and 100 µl of distilled water, the negative control, were added separately. All the tubes were then incubated at 37ºC for 90 minutes and observed for clot lysis. After incubation, the released fluid was removed from all tubes and tubes were again weighed to observe the differences in weight after clot disruption. Differences obtained in weight taken before and after clot lysis were expressed as percentage of clot lysis as shown below:

% of clot lysis = (wt of released clot /clot wt) × 100%.

2.4 Membrane Stabilizing Activity

The membrane stabilizing activity of the extractives was determined by their ability to inhibit heat and hypotonic solution induced haemolysis of human erythrocytes following the method adopted by Kawsar et al. [13].

2.4.1 Hypotonic solution-induced haemolysis

0.5ml of each partitionates (1.0mg/ml of methanol) and acetyl salicylic acid (0.1 mg/ml of methanol), the standard, were added to 4.5ml hypotonic solution (50mM NaCl) in 10 mM sodium phosphate saline (pH 7.4). 4.5ml of the hypotonic solution was also taken separately that was considered as control. To the partitionates, standard and control, stock erythrocyte suspension (0.5ml) was added. Then, the mixtures were incubated for 10 min at room temperature, centrifuged for 10 min at 3000 g and the absorbances of the supernatant were measured at 540nm. The percentage of inhibition of haemolysis i.e. membrane stabilization was calculated using the following equation:

% inhibition of haemolysis = 100 x (OD_1-OD_2/OD_1) %, where, OD_1 = optical density of hypotonic-buffered saline solution alone (control) and OD_2 = optical density of test sample in hypotonic solution.

2.4.2 Heat-induced haemolysis

5 ml of aliquots of different partitionates (1.0 mg/ml of isotonic buffer) as well as acetyl salicylic acid (0.1mg/ml of isotonic buffer), the standard, were put into two duplicate sets of centrifuge tubes. The vehicle, in the same amount, was added to another tube as control. Stock erythrocyte suspension (30 µL) was added to all tubes and mixed gently by inversion. Total 6 tubes (one tube from each pair) were incubated at 54ºC for 20 min in a water bath while the other 6 tubes were maintained at 0-5ºC in an ice bath. The reaction mixtures were centrifuged for 3min at 1300g and the absorbances of the supernatants were measured at 540nm. The percentage of inhibition of haemolysis i.e. membrane stabilization was calculated using the following equation:

% Inhibition of haemolysis = 100 x [1- (OD_2-OD_1/OD_3-OD_1)] %, where, OD_1 = optical density of unheated test sample, OD_2 = optical density of heated test sample and OD_3 = optical density of heated control sample.

2.5 Statistical Analysis

Three replicates of each sample were used for statistical analysis and the values have been reported as Mean ± Standard Deviation (n=3). Subsequently, all the values were subjected to One-way Analysis of Variance (ANOVA) followed by Bonferroni Multiple Comparison Test at 95% Confidence Interval.

3. RESULTS AND DISCUSSION

3.1 Results

The present study was undertaken to evaluate the cytotoxic, thrombolytic and membrane stabilizing activities of different organic and aqueous soluble materials of the crude methanol extract of flowers of *S. mahagoni* (L.) Jacq. The yields of resultant Kupchan partitionates such as petroleum ether (PESF), carbon tetrachloride (CTCSF), chloroform (CSF) and aqueous (AQSF) soluble fraction were 1.5mg, 0.95mg, 0.4mg and 0.5mg, respectively.

In brine shrimp lethality bioassay, it was found that all the partitionates but aqueous soluble fraction possessed cytotoxic activity. The highest cytotoxic activity was revealed by crude methanol extract (LC_{50} = 0.10±0.01µg/ml) followed by petroleum ether soluble fraction (LC_{50} = 0.45±0.10 µg/ml) while vincristine sulfate used as positive control demonstrated an LC_{50} value of 0.40±0.02µg/ml (Table 1).

In thrombolytic activity assay, all partitionates were evaluated using streptokinase as standard. The standard demonstrated 70.27±1.26% lysis of clot after addition to the clot and subsequent 90 minutes of incubation. Among the partitionates, the petroleum ether soluble fraction exhibited the highest thrombolytic activity (34.30±0.78%) (Table 1).

Table 1. Cytotoxic, thrombolytic and membrane stabilizing activities of different partitionates of methanol extract of flowers of *S. mahagoni*

Sample	Brine shrimp lethality bioassay LC_{50} (µg/ml)	% of lysis	% inhibition of haemolysis	
			Hypotonic solution induced	Heat induced
CME[a]	0.10±0.01	19.57±2.15[#,***]	84.71±3.25**	15.14±3.04***
PESF[b]	0.45±0.10	34.30±0.78***	17.77±1.16***	10.01±2.21***
CTCSF[c]	1.29±0.31	27.14±3.94***	64.72±1.54	5.44±1.01***
CSF[d]	2.61±0.75	22.39±2.72***	39.24±3.68***	24.78±2.28***
AQSF[e]	19.80±2.27***	29.83±4.09***	47.62±4.35***	86.30±4.98***
VS[f]	0.40±0.02	-	-	-
Blank	-	28.03±2.18***	-	-
SK[g]	-	70.27±1.26[###]	-	-
ASA[h]	-	-	71.91±2.29	45.45±4.87

*[a]Crude methanol extract; [b]Petroleum ether soluble fraction; [c]Carbon tetrachloride soluble fraction; [d]Chloroform soluble fraction; [e]Aqueous soluble fraction; [f]Vincristine sulfate; [g]Streptokinase; [h]Acetyl salicylic acid; [In cytotoxic activity, ***P<0.001 compared to the standard VS]; [In thrombolytic activity, [###]P < 0.001 and [#]P < 0.05 compared to the blank; ***P<0.001 compared to the standard SK]; [In membrane stabilizing activity, *** P<0.001 and ** P < 0.01 compared to the standard ASA]*

The flower extract and its different partitionates were evaluated to measure their potential to prevent lysis of human RBC membrane induced by hypotonic solution and heat. In hypotonic solution induced condition, the crude methanol extract inhibited 84.71±3.25% haemolysis of RBCs followed by the carbon tetrachloride soluble fraction (64.72±1.54%). On the other hand, under heat induced condition, the maximum membrane stabilizing activity was exhibited by the aqueous soluble fraction (86.30±4.98% inhibition of haemolysis of RBCs). Acetyl salicylic acid (0.1mg/ml), used as reference standard showed 71.91±2.29% and 45.45±4.87% inhibition of haemolysis of RBCs in hypotonic solution and heat induced conditions, respectively (Table 1).

3.2 Discussion

The objective of this study was to evaluate the cytotoxic, thrombolytic and membrane stabilizing potentials of *S. mahagoni* flower extract. While investigating cytotoxic activity using brine shrimp nauplii, all partitionates except aqueous soluble fraction gave non-significant differences compared to standard vincristine sulfate. So, all partitionates but aqueous soluble fraction are as active as the standard in cytotoxicity. In determining the thrombolytic activity of the partitionates, we observed that streptokinase and crude methanol extract showed significant differences compared to the blank. On the other hand, all the partitionates gave significant differences compared to the standard streptokinase. So, none of the partitionates is as active as the standard streptokinase in disrupting the blood clot. We investigated the membrane stabilizing activity in hypotonic solution induced and heat induced condition. All the partitionates but carbon tetrachloride soluble fraction exhibited significantly different activities than the standard acetyl salicylic acid i.e. the carbon tetrachloride soluble fraction is equally active, the crude methanol extract was more active whereas others were less active compared to the standard acetyl salicylic acid in stabilizing the membrane of RBCs in hypotonic solution induced condition. In heat induced condition, all the partitionates gave significantly different activity than the standard acetyl salicylic acid i.e. aqueous soluble fraction was more active and others were less active than the standard acetyl salicylic acid in stabilizing the membrane of RBCs. So, the above findings suggest that the test samples of flower extract of *S. mahagoni* possess significant cytotoxic and membrane stabilizing activities with insignificant thrombolytic activity. Previous studies on *S. mahagoni* extractives suggested that the species possesses other medicinal values like antibacterial [14] and antioxidant activities [15] as well as antidiabetic property [16]. The crude ethanol extract of *S. mahagoni* leaf was reported to have an anti-ulcer activity against ethanol-induced gastric ulcer in experimental animals [7]. Tetranortriterpenoids and fatty acids are the biologically active ingredients isolated from this species that are considered to be responsible for these therapeutic effects [17]. From *S. mahagoni*, eighteen tetranotriterpenoids were isolated [18] and the presence of known fatty acids and terpenoids was reported [19]. More than 45 limonoids belonging to the structural types of andirobin, gendunin, mexicanolide, phragmalin, triterpens, tetranortriterpenes, and chlorgenic acid have been isolated from *S. mahogani* in previous phytochemical investigations [20-21]. Therefore, we can say that the bioactivities demonstrated by the *S. mahagoni* flower extractives are due to the presence of different biologically active phytoconstituents (that have already been reported from other parts of the tree) in the flowers.

4. CONCLUSION

The flower extract of *S. mahagoni* possesses potential cytotoxic and membrane stabilizing activities with negligible thrombolytic activity. From our investigation, it can be suggested

that, the flower extractives should be further screened for bioactivities as well as isolation, characterization and evaluation of the bioactive compounds.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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