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Antioxidant and antimicrobial activities of flower and bark extract of *Swietenia mahagoni* (L.) Jacq.

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ABSTRACT

Different Kupchan partitionates of flower and bark extracts of *Swietenia mahagoni* (L.) Jacq. were subjected to assays for determination of antioxidant activities by evaluating free radical scavenging activity and total phenolic content and also antimicrobial activity by using bacteria and fungi. In the first finding, aqueous soluble fraction of both extracts showed the highest free radical scavenging activity having IC_{50} values of 1.18±0.31 µg/ml and 7.60±0.54 µg/ml, respectively while reference standards tert-butyl-1-hydroxytoluene and ascorbic acid gave 27.50±0.95 µg/ml and 5.80±1.03 µg/ml, respectively. In total phenolic content assay, the highest amount of phenolic compounds was found in aqueous soluble fraction in both extracts (1.80±0.56 and 1.07±0.45 mg of gallic acid equivalent/gm of extractives, respectively). In antimicrobial screening, different fractions were applied to bacteria and fungi, among which, both the methanol extracts showed moderate activity with maximum activity against *Pseudomonas aeruginosa* having zone of inhibition 18±0.44 mm and 25±0.29 mm, respectively.

Keywords: *Swietenia mahagoni*, antioxidant, free radical scavenging, total phenolic content, antimicrobial.

1. Introduction

Swietenia mahagoni (L.) Jacq. (Meliaceae) is a medium to large evergreen tree native to Southern Florida, Cuba, The Bahamas, Hispaniola, and Jamaica [1]. The species are widely distributed in South Asian countries (India, Sri Lanka and Bangladesh). It is a large, deciduous, and economically important timber tree. Mahogany can reach 75 feet in height with a 50-foot-spread but is more often seen at 40 to 50 feet tall and wide [2]. The flowers are produced on panicles found in the axils of the leaves. The panicles emerge in the spring. The flowers are small and less than 0.3 inches wide. The flowers are yellow-green with fragrant. *S. mahagoni* has separate male and female flowers on the same plant (monoecism). Monoecism is frequent in the family Meliaceae, but not obvious. The bark of a young tree is relatively smooth and grayish compared to the older trees having stout trunk with rough, dark brown or gray bark that is coarsely fissured. The trunk ranges from 3 to 4.5 feet in diameter. The decoction of the bark of these mahoganies is extensively used as febrifuge, which could be associated with its use as an antimalarial drug [3]. Traditionally, different parts of this plant have been used in the treatment of fever, diabetes, malaria, hypertension and tuberculosis [4]. *S. mahagoni* seeds have been applied as a folk medicine for the treatment of hypertension, diabetes, and malaria [5].

In order to find out new bioactivities, the systematic screenings of various medicinal plants have become routine task in many phytochemical research laboratories. Therefore, *S. mahagoni* crude flower and bark extract and its different partitionates were used for the screening of antioxidant and antimicrobial activities.

2. Materials and methods

2.1 Plant materials

The flowers and bark of *S. mahagoni* were collected from Brahmanbaria, Bangladesh. The plant was identified at the Bangladesh National Herbarium, where a voucher specimen has been deposited for this collection (Accession number: 38501). The flower and bark were dried under sun light for several days and then oven dried for 24 hours at considerably low temperature (not exceeding 40 ^{0}C) for better grinding. The dried plants parts were then ground to a coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Department of Pharmacy, State University of Bangladesh.

2.2 Extraction
The powdered materials of flowers and bark (1200 gm each) were taken in two separate cleaned, amber color reagent bottles (5 liters) and soaked in methanol (1.6 L each). The containers with their contents were sealed by bottle cap and kept for a period of 2 weeks accompanying occasional shaking and stirring. The whole mixtures were then filtered separately through a fresh cotton plug and finally with a Whatman No.1 filter paper. The volume of the two filtrates was then individually allowed to evaporate at ambient temperature until approximately 70% solvent was evaporated. In both cases, an aliquot (5 gm) of the concentrated methanol extract was fractionated by modified Kupchan (Van Wagenen *et al.*, 1993) [6]. partition protocol and the resultant partitionates were evaporated to dryness with rotary evaporator to yield individual methanol, petroleum ether, carbon tetrachloride and aqueous soluble materials. The residues were then stored in a refrigerator until further use.

2.3 DPPH free radical scavenging assay
According to the method developed by Brand-Williams *et al*., [7] the free radical scavenging activity (antioxidant capacity) of the methanol extracts of flowers and bark along with their partitionates were assessed by using 1,1-diphenyl-2-picrylhydrazyl (DPPH). Thus, 2.0 ml of serially diluted different concentrations (500 µg/ml to 0.977 µg/ml) of each test samples were mixed with 3.0 ml of DPPH solution (20 µg/ml) in methanol. After reaction period of 30 minutes at room temperature in dark place, the absorbance was measured at 517 nm. Then, the concentration of sample required to scavenge 50% of free radicals i.e. IC_{50} values were calculated from the regression equation, produced by plotting concentration (µg/ml) of the samples versus percentage inhibition of free radicals. In this case, ascorbic acid and butylated hydroxytoluene (BHT), synthetic antioxidants were used as positive control.

2.4 Total phenolic content
Total phenolic content of flowers and bark of *S. mahagoni* extractives was measured employing the method as described by Škerget *et al*. [8] involving Folin-Ciocalteu reagent as oxidizing agent and gallic acid as standard [9]. 2.5 ml of 10 times diluted Folin-Ciocalteu reagent and 2.0 ml of sodium carbonate (7.5%, w/v) in water were added to the test samples (three replicates), and subsequently incubated for 15 min at 45 °C. The absorbance of the test samples was measured at 765 nm using UV-visible spectrophotometer. The total phenolic contents were expressed as milligrams of gallic acid equivalent per gram (mg GAE/g) of dry extract.

2.5 Antimicrobial assay
The antimicrobial activity of the partitionates was evaluated against bacteria and fungi according to the 'Disc diffusion method' [10, 11]. Dried and sterilized filter paper discs were impregnated by the test samples dissolved in methanol and the residual solvents were completely evaporated. Discs containing the test material (400 µg/disc) were placed on nutrient agar medium evenly seeded with the test microorganisms collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka. Standard discs of Ciprofloxacin (30 µg/disc) and blank discs (impregnated with only methanol followed by evaporation) were used as positive and negative control, respectively. These plates were then kept at low temperature (4 ^{0}C) for 24 hours for maximum diffusion of test samples followed by incubation at 37 ^{0}C for 24 hours for maximum growth of the organisms. The diameters of the clear, distinct zones of inhibition visualized surrounding the discs were determined (in millimeter).

2.6 Statistical analysis.
Three replicates of each sample were used for statistical analysis and the values are reported as mean ± SD (n=3).

3. Results and discussion
The crude methanol extracts of flowers and bark of *S. mahagoni* as well as different Kupchan partitionates obtained from these were subjected to assays for free radical scavenging activity, total phenolic content and antimicrobial activity.

In the DPPH free radical scavenging assay, aqueous soluble fraction of both flower and bark extracts showed the highest free radical scavenging activity (IC_{50} = 1.18±0.31 µg/ml and 7.60±0.54 µg/ml, respectively) followed by petroleum ether soluble fraction (IC_{50} = 11.87±1.33 µg/ml) among the partitionates derived from methanol extract of flowers and carbon tetrachloride soluble fraction (IC_{50} = 9.70±0.45 µg/ml) among the partitionates of bark extract (Table 1).

Table 1: Free radical scavenging activity and total phenolic content of different partitionates of flower and bark extract of *S. mahagoni*

Plant parts	Test samples	IC_{50} values (µg/ml)	Total phenolic content (mg of GAE/gm of sample)
Flowers of *S. mahagoni*	ME	23.63±1.25	1.27±0.32
	PSF	11.87±1.33	1.33±0.45
	CCSF	41.00±0.89	0.73±0.16
	CSF	65.91±2.51	0.15±0.28
	ASF	1.18±0.31	1.80±0.56
Bark of *S. mahagoni*	ME	47.60±0.85	0.90±0.20
	PSF	184.00±1.25	0.50±0.12
	CCSF	9.70±0.45	0.93±0.83
	CSF	98.20±1.11	0.87±0.69
	ASF	7.60±0.54	1.07±0.45
	BHT	27.50±0.95	-
	ASA	5.80±1.03	-

ME= Methanol crude extract; PSF= Petroleum ether soluble fraction; CCSF= Carbon tetrachloride soluble fraction; CSF= Chloroform soluble fraction; ASF= Aqueous soluble fraction; BHT= t-Butyl-1-hydroxytolune and ASA= Acetyl salicylic acid

On the other hand, the total phenolic content of different partitionates was found to be the maximum in aqueous soluble fraction of both flower and bark extracts (1.80±0.56 mg of GAE/gm of sample and 1.07±0.45 mg of GAE/gm of sample, respectively) and minimum in chloroform soluble fraction of flower extract (0.15±0.28 mg of GAE/gm of sample) and in petroleum ether soluble fraction (0.50±0.12 mg of GAE/gm of sample) among the partitionates derived from bark extract (Table 1).

Antimicrobial activity of the test samples was investigated *in vitro* against five gram positive bacteria (*Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, *Sarcina lutea* and *Staphylococcus aureus*) eight gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* Paratyphi, *Salmonella* Typhi, *Shigella boydii*, *Shigella dysenteriae*, *Vibrio mimicus* and *Vibrio parahaemolyticus)* and three fungi (*Saccharomyces cerevisiae*, *Candida albicans* and *Aspergillus liger*). Among all the partitionates of flower and bark extracts, only the methanol extract gave moderate antimicrobial activity against the microorganisms. Both methanol fractions of flower and bark extracts showed maximum activity against *Pseudomonas aeruginosa* having zone of inhibition 18±0.44 mm and 25±0.29 mm, respectively. Overall, the partitionates of bark extract exhibited more antimicrobial activities than partitionates obtained from flower extract (Table 2).

Table 2: Antimicrobial activity of different partitionates of flower and bark extract of *S. mahagoni*

Test microorganisms	Diameter of zone of inhibition (mm)										
	Flowers of *S. mahagoni*					Bark of *S. mahagoni*					CF
	ME	PSF	CCSF	CSF	ASF	ME	PSF	CCSF	CSF	ASF	
A) Gram positive bacteria											
B. cereus	10± 0.54	-	3± 0.80	-	5± 0.65	20± 0.41	-	10± 0.92	-	12± 0.77	37± 0.89
B. megaterium	12± 0.80	-	8± 0.38	2± 0.75	3± 0.22	22±	-	15± 0.65	-	15± 0.98	40± 0.41
B. subtilis	8± 0.77	3± 0.52	5± 0.25	-	-	25± 0.38	10± 0.51	8± 0.12	3± 0.80	5± 0.89	34± 0.57
S. lutea	5± 0.79	-	2± 0.54	8± 0.45	3± 0.58	18± 0.73	-	5± 0.45	-	15± 0.65	38± 0.12
S. aureus	12± 0.63	3± 0.33	-	-	5± 0.77	17± 0.89	8± 0.26	10± 0.38	-	3± 0.09	41± 0.28
B) Gram negative bacteria											
E. coli	12± 0.31	-	-	8± 0.44	-	18± 0.96	-	-	5± 0.82	15± 0.70	38± 0.54
***S.* Paratyphi**	15± 0.50	3± 0.56	6± 0.19	-	-	20± 1.07	-	15± 0.92	10± 0.54	-	42± 0.30
***S.* Typhi**	8± 0.40	3± 0.77	-	-	5± 0.55	15± 0.62	-	-	5± 0.65	3± 0.25	40± 0.80
S. boydii	10± 0.41	8± 0.27	-	5± 0.80	-	5± 0.54	-	5± 0.89	-	8± 0.60	37± 0.55
S. dysenteriae	12± 0.30	10± 0.77	3± 0.80	-	-	18± 0.63	12± 0.79	-	-	-	42± 0.71
P. aeruginosa	18± 0.44	10± 0.50	5± 0.21	3± 0.69	12± 0.80	25± 0.29	5± 0.05	10± 0.74	3± 0.92	12± 0.54	41± 0.93
V. mimicus	10± 0.79	-	-	3± 0.11	8± 0.30	20± 1.21	5± 0.39	12± 0.85	-	10± 0.41	39± 1.12
V. parahemolyticus	15± 0.73	-	-	3± 0.40	6± 0.87	15± 0.41	-	12± 0.54	-	10± 0.89	40± 0.38
C) Fungi											
S. cerevisiae	5± 0.88	-	-	3± 0.55	2± 0.21	20± 1.02	-	8± 0.33	5± 0.62	10± 0.72	37± 0.99
C. albicans	8± 0.67	5± 0.71	-	-	-	12± 0.80	5± 0.41	10± 0.92	3± 0.29	20± 0.68	35± 0.54
A. liger	10± 0.52	-	-	5± 0.35	-	20± 0.86	8± 0.38	-	5± 0.54	13± 0.42	38± 0.77

ME= Methanol crude extract; PSF= Petroleum ether soluble fraction; CCSF= Carbon tetrachloride soluble fraction; CSF= Chloroform soluble fraction; ASF= Aqueous soluble fraction and CF= Ciprofloxacin

4. Conclusion

Therefore, it can be concluded that, in the preliminary studies, some of the test samples obtained from the flower and bark of *S. mahagoni* revealed significant antioxidant activity and moderate antimicrobial activity.

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