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Quantification of total tannin, flavonoid contents and pharmacognostic study of the seeds of *Swietenia mahagoni* (Linn.)

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ABSTRACT

Objective: To justify the folkloric use of *Swietenia mahagoni* (*S. mahagoni*) seeds, 90% ethanolic extract and their aqueous and organic partitioning substances were evaluated for their possible antidiarrhoeal and antimicrobial potentials *in vivo*.
Methods: Crude ethanolic extract of *S. mahagoni* seeds were subjected and partitioned into fractions using solvents at different polarity. Antimicrobial and antidiarrheal activities were evaluated and subsequently outcomes were corresponded with the conventional standard drugs.
Results: The antidiarrheal activity was assessed using mouse model, where unfractionated ethanolic extract significantly reduced, the number, onset, rate and weight of diarrheal episodes. This fraction showed the limited number of defecation episodes of 27.0% and 40.9% at dose of 200 mg/kg and 400 mg/kg body weight respectively and reference drug, loperamide, showed 53% at a dose of 50 mg/kg. All extract fractions exhibited the significant potential to kill or subside the growth of known Gram-positive and Gram-negative bacteria.
Conclusions: Ethanolic extract and their aqueous and organic fractious revealed the seeds of *S. mahagoni* (Linn.) have the potential to be used as a remedy for diarrhea and known pathogenic microbes which ensured the folkloric use of the seeds of *S. mahagoni*.

1. Introduction

Medicinal plants are often used as traditional medicines. This exercise has been practiced since primitive times. There are many methods in which plants have been very useful especially in medicinal purposes. First, they may be used directly as infusions or in other extracted forms for their natural chemical ingredients. Second, they may be used effectively for synthesis and finally the organic molecules found in plants may be used as models for synthetic drugs. Traditionally, potential of medicinal plants were determined by trial and error. Modern methods were used to determine the medicinal efficacy of plants involved cooperative efforts of experts such as pharmacists, ethno botanists, anthropologists and physicians. Various modern medicines, which we see nowadays, had their origin from the medicinal plants. Examples include aspirin from willow bark (*Salix* spp.), digitalis from foxglove (*Digitalis purpurea*), and vinblastine from Madagascar periwinkle (*Vinca rosea*) for the management of childhood leukemia. *Swietenia mahagoni* (*S. mahagoni*) is a large, deciduous and economically important woody tree, locally known as mahogany which has a numerous genera and species[1,2]. In Bangladesh, mahogany trees are cultivated at warm zones[3]. *S. mahagoni* seeds have been used as a folk medicine for the treatment of malaria, hypertension, diabetes and diarrhea. It also possesses antibacterial and antifungal potential[4,5]. The seed extracts of *S. mahagoni* have also been reported to inhibit the aggregation properties of platelet[6]. On the basis of presence of bioactive compounds and also of folkloric use, we are interested in analyzing these evidences in order to justify the potentials of *Swietenia* seeds in case of infectious and diarrheal disorders.

2. Materials and methods

2.1. Sources of seeds

Seeds of *S. mahagoni* were accumulated from Keraniganj, Bangladesh in May 2015, and verified by a taxonomist of Bangladesh National Herbarium, Mirpur, Dhaka, who provided us with the voucher specimens (DACB accession No. 41103) for these seeds.

2.2. Preparation of extract

The fruits were unwrapped to get the seeds, and the seeds were dehydrated at 37 ˚C overnight for easily being crushed to find particle size powder by an electrical grinder. An amount of 416 g of the powder was dipped in 2000 mL of 90% ethyl alcohol. After 14

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All experimental procedures involving animals were conducted in accordance to United State Health Guidelines of the humane and ethical treatment of experimental animals and approved by the ethic committee of world university Bangladesh.

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days, extract was filtered and the filtrate was condensed by a vacuity revolving evaporator at 40 ˚C to recover solvent. Concentrated aqueous ethanolic extract was fractionated by Kupchan method[7]. Obtained fractions were ethyl acetate soluble fraction (EASF), petroleum ether soluble fraction (PSF), carbon tetrachloride soluble fraction (CTSF) and chloroform soluble fraction (CSF).

2.3. Phytochemical screening

The presence of biochemical of different classes was ensured by color variations using the technique of Sofowora[8].

2.4. Determination of total flavonoid contents

Kumaran and Karunakaran technique[9] was used to estimate the total flavonoid contented utilizing quercetin as a mentioned standard. For the purpose of whole flavonoid contents, 20 mg of crude ethanolic extract (CEE), EASF, PSF and CSF were dissolved in 1 mL of methanol to prepare 250 μg/mL of extract solutions, and were mixed with 1 mL of aluminum chloride in ethanol (20 mg/mL). A drip of acetic acid followed by up to 25 mL of ethanol was added for diluting into four different tubes. After 40 min, absorbance for test and standard solutions were estimated against the blank at 415 nm with an UV-vis. Series of quercetin solutions were prepared without extract as a reference standard. Total flavonoid contents were estimated from extrapolation of quercetin standardization curve.

2.5. Estimation of total tannin contents

The total tannin content of extracts of *S. mahagoni* seed was assessed by Folin-Coicalteu technique[10]. Concisely, 0.3 mL (300 μL) of the CEE and its extractives, 2.7 mL of Folin-Coicalteu (1:10) phenol mixture were taken into a 10 mL measuring cylinder. Five minutes later, 2 mL of 7.5% Na_2CO_3 solution was poured into every test tube, shaken followed by stored at room temperature for 30 min in shady area after warming at 45 ˚C. Series of tannic acid solutions were prepared without extract as a reference standard. Absorbance for test and standard solutions were estimated against the blank at 725 nm with an UV-vis spectrophotometer. Total tannin contents were estimated from extrapolation of tannic acid standardization curve.

2.6. Evaluation of the antidiarrheal activity

2.6.1. Experimental animals and drugs

Healthy Swiss-Wistar albino mice (body weight: 25–30 g) were selected to be used for the evaluation of *in-vivo* diarrheal episode. The diarrheal test was conducted by direct supervision of an animal specialist and a laboratory supervisor. The methods of the antidiarrheal tests were followed in the same way as was written in united state health Guidelines of the humane and ethical treatment of experimental animals (1985). This study was approved by the ethic committee of world university Bangladesh with the approval No. of WUB # ERW34. Loperamide (Opsonin Pharma) was used as the reference antidiarrheal drug.

2.6.2. Castor oil-induced diarrhea

The CEE of *S. mahagoni* was found effective in castor oil of prompted defecation in experimental animal model[11,12]. Before actual study, primarily all mice were given orally 0.5 mL of castor oil, and animals were selected for this test on the basis of animals diarrheal mode, then mice were divided into four groups (n = 5) and kept in clean spacious cages. At the study period, one group was given only distilled water, whereas another group was given loperamide, while the test groups received the extract at a dose of 200 mg and other groups at a dose of 400 mg/kg body weight. Extracts and loperamide were given orally for 60 min before given castor oil. We observed an aliquot of 0.5 mL of castor oil at 10 mL/kg body weight (mixed with few drops of 1% Tween® 80 in water) excreted the liquid stool and monitored for the diarrheal mode every 60 min for 4 h after the given of castor oil. Inhibition of defecation was calculated.

Percent inhibition = 100 × (C – EC)

where C means the number of defecation episodes in the control group and E means the number of defecation episodes in experimental group.

2.6.3. Acute toxicity test

The acute toxicity of CEE was measured in mice according to the system of El Hilaly *et al.*[13] with modifications. Purpose of studies was to create the therapeutic guide, *i.e.*, the relation between the pharmacologically active and the lethal dose on the experimental mice. Twenty mice were taken and starved for 16 h, and then divided into four groups consisting of five mice each group. Administered doses of the CEE (200, 400, 800, 1 600 and 3 200 mg/kg) were given to every group of mice orally. The animals were then permitted for regular food and their behaviors/activities were closely and continuously monitored for the first 3 h then every 4 h for the next 48 h. The extract did not result in mortality during the 48 h of observation but some changes were observed such as ataxia, diarrhea and weight loss. Thus, at least, the CEE was acutely proved to be safe at all tested doses.

2.7. Antimicrobial activity

The extracts were evaluated for antibacterial action by the standard disc diffusion technique. A total of nine bacterial strains were used in the present study, including five Gram-negative strains and four Gram-positive strains. The five Gram-negative strains included *Escherichia coli* (*E. coli*), *Proteus mirabilis, Pseudomonas fluorescens, Pseudomonas aeruginosa* and *Salmonella typhi* (*S. typhi*). The four Gram-positive bacteria species included *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*), *Sarcina lutea* (*S. lutea*) and *Bacillus cereus* (*B. cereus*). Bacteria were obtained from the Department of Microbiology, University of Dhaka. The bacterial strains were maintained on nutrient agar slants tubes at 4 ˚C overnight.

2.8. Determination of minimum inhibitory concentration (MIC)

The MIC was determined by successive dilution process using nine sterile tubes[14,15] for *E. coli, Shigella dysenteriae* (*S. dysenteriae*), *S. aureus, B. subtilis, S. typhi, B. cereus* and *S. lutea*, remaining two tubes for controls only. Serial dilutions were made from 250 mg/mL of the CEE with nutrient agar to make 250.000, 125.000, 62.500, 31.250, and 15.625 mg/mL, and all the tubes were specifically inoculated with 0.1 mL of inoculum (10^9 CFU/mL) followed by aerobically incubation at 37 ˚C for 18–24 h. Cephalosporin (0.1 mg/mL) and gentamycin (0.1 mg/mL) were used as controls. The minimum concentration (maximum dilution) of the CEE exhibited no growth of detectible bacterial (no turbidity) when compared to the controls and hence was regarded as the MIC.

2.9. Antibacterial activity

The CEE and extractives were screened for their antimicrobial

actions and compared to the conventional drug kanamycin (30 µg/disc) by the *in-vitro* disc diffusion technique[16] by known bacterial strains. Broth medium was sterilized at 121 ˚C for 15 min and cooled to 45–50 ˚C followed by the inoculation with the 0.1 mL test microorganisms in test tubes, and organisms (10^9 CFU/mL) were spread on sterile agar plates[17]. One mL of plant extract was added in the paper discs (6 mm diameter, Whatman No. 1 filter paper, containing 100 µg/mL plant extract), then the dried separate paper disc was placed on the agar containing petri dish surface by keeping at 4 ˚C overnight and incubating at 37 ˚C for 16 h to promote bacterial growth. The inhibition zone formed was measured and the test was conducted in triplicate for every sample.

3. Results

3.1. Flavonoid and tannin contents

The qualitative screening of ethanolic extract was conducted to expose the existence of alkaloids, flavonoids and tannins in all extract fractions (9 g, Table 1). It was the same for carbohydrates with the exception of PSF. Reducing sugar was detected only in EASF and CSF, and steroid only in PSF. The EASF (47.38 ± 0.15) exhibited the highest flavonoid content while the PSF (33.34 ± 0.11) was found to possess the lowest flavonoid content. The total tannin contents were the highest in the EASF (48.08 ± 0.22) as well and the lowest in the CEE (20.98 ± 0.59) (Table 2).

Table 1
Fractionated 9 g of CEE of *S. mahagoni*.

Fraction	WSF	PSF	EASF	CSF	CTSF
Amount (%)	26.6 ± 2.4	25.5 ± 2.3	22.2 ± 2.0	17.7 ± 1.6	7.7 ± 0.7

Values were expressed by mean ± SE. WSF: Water soluble fraction.

Table 2
Total flavonoid and tannin contents of CEE of *S. mahagoni* and their various fractions.

Extract fractions	Flavonoid[a]	Tannin[b]
CEE	None	20.98 ± 0.59
EASF	47.38 ± 0.15	48.08 ± 0.22
CSF	41.66 ± 1.65	27.09 ± 0.13
PSF	33.34 ± 0.11	27.41 ± 0.01

[a]: Total flavonoid contents were directed in terms of mg of quercetin equivalents per gram of dry extract. [b]: Total tannin contents were directed in terms of mg of tannic acid equivalents per gram of dry extract.

3.2. Antidiarrheal activity

The evaluation of antidiarrhoeal activity used the mouse model. The ethanolic extract limited the mean number of defecation episode in a dose-dependent manner (Table 3). The unfractionated CEE limited the number of defecation episodes by 27.0% at a dose of 200 mg/kg and by 40.9% at dose 400 mg/kg body weight, which was matched by castor oil-provoked diarrhoea and standard loperamide.

Table 3
Antidiarrheal potential of the CEE compared to positive and negative control groups in mice model.

Groups	Latent period	Number of defecation episodes	Inhibition (%)
Distilled water (0.5 mL)	0.79 ± 0.06	9.90 ± 0.86	0.0
Loperamide (50 mg/kg)	2.21 ± 0.16**	4.33 ± 0.45**	53.6
CEE (TG1) (200 mg/kg)	1.05 ± 0.07*	7.00 ± 0.86*	27.0
CEE (TG2) (400 mg/kg)	1.59 ± 0.19**	5.50 ± 0.63**	40.9

All agents were given orally. Values were expressed by mean ± SE. *: $P < 0.05$ and **: $P < 0.001$ both indicating statistical difference with respect to the effect of distilled water.

3.3. Determination of MICs

The MICs against *E. coli, S. dysenteriae, S. aureus, B. subtilis, S. typhi, B. cereus* and *S. lutea* were determined only for the CEE of the seeds of *S. mahagoni*, and results were corresponded with the control. The MICs ranged between 12 and 56 µg/mL. The highest MIC was (56 ± 0.81) against *B. subtilis* and the lowest was (12 ± 0.22) against controls (Table 4).

Table 4
MIC of the CEE of *S. mahagoni* ($n = 3$).

Strains	MIC (µg/mL)
E. coli	13.00 ± 0.81
S. aureus	12.00 ± 0.22
B. subtilis	56.00 ± 0.81
S. dysenteriae	25.00 ± 0.01
S. typhi	35.00 ± 0.29
B. cereus	50.00 ± 0.11
S. lutea	38.00 ± 0.61

Values were expressed by mean ± SD.

3.4. Antimicrobial activity

The antibacterial activity of the CEE and extractives of seeds of *S. mahagoni* were studied against both Gram-positive and Gram-negative species and antibacterial activity compared with the standard kanamycin (35 µg/mL)[18]. The results of antibacterial screening of petroleum ether, chloroform, carbon tetrachloride, ethanol extract and water soluble fractions of *S. mahagoni* were presented in Table 5. Growth of inhibition of each extract against the known bacteria was not the same. Concentration of 100 µg/mL of CEE and extractives of seeds of *S. mahagoni* with the PSF showed the maximum sensitivity (17.0 ± 0.35) against *B. cereus* among the various extract fractions, whereas the lowest sensitivity was observed with CSF against Gram-positive species *B. subtilis* (7.0 ± 0.1). PSF appeared to be the most effective extract against *B. cereus*. No inhibitory effect was observed by CSF against Gram-positive bacteria *S. lutea* and Gram-negative bacteria *E. coli*. The Gram-positive species *B. cereus* and *B. subtilis* were sensitive to all extracts. No antibacterial activity was shown by the aqueous soluble fraction.

Table 5
The antibacterial potential of the CEE various fractions and their extracts of the seeds of *S. mahagoni* ($n = 3$).

Bacteria		CTSF	PSF	CSF	CEE	Kanamycin (30 µg/disc)
G(+)	*B. cereus*	13.00 ± 0.13	17.00 ± 0.35	10.00 ± 0.12	8.00 ± 0.12	29.50 ± 0.10
	B. subtilis	10.00 ± 0.51	10.00 ± 0.90	7.00 ± 0.10	10.00 ± 0.24	28.00 ± 0.91
	S. aureus	9.80 ± 0.20	-	8.00 ± 0.91	-	30.00 ± 0.38
	S. lutea	10.00 ± 0.84	8.10 ± 0.10	-	10.00 ± 0.12	26.00 ± 0.51
G(-)	*S. typhi*	9.00 ± 0.16	13.00 ± 0.12	9.00 ± 0.16	-	35.00 ± 0.31
	VP	11.00 ± 0.80	10.00 ± 0.91	11.00 ± 0.10	-	37.00 ± 0.10
	E. coli	11.00 ± 0.90	12.10 ± 0.58	-	10.00 ± 0.31	23.00 ± 0.18
	Vibrio mimicus	11.00 ± 0.66	-	16.00 ± 0.80	12.00 ± 0.10	28.00 ± 12.0
	S. dysenteriae	10.00 ± 0.29	-	10.00 ± 0.11	12.00 ± 0.20	23.00 ± 0.19

The values were expressed by mean ± SD. The amount of extract fraction used in each case was 100 µg/mL. G(+): Gram-positive; G(-) : Gram-negative; VP: *vibrio parahaemolyticus.*

4. Discussion

In this experiment, we investigated to justify the traditional use of *S. mahagoni* seeds for aforesaid disorders. In the initial screening, all the extract fractions were ensured for flavonoid

and tannin and the contents of these biochemical were also ensured using the aluminum chloride colorimetric method[19]. We decided to investigate pharmacological potential of the seeds, the outcomes of the study directed that the seeds of *S. mahagoni* (Linn.) have the potential to be used as a remedy for diarrhea and against known pathogenic microbes.

The experiment revealed that the ethanolic extracts limited the mean number of defecation episodes in a dose dependent mode. It may be due to the presence of biologically active compounds found in the extracts such as tannins and flavonoids, which counteracted the irritant actions of ricinoleic acid on the epithelium of the intestine[20]. In acute toxicity test, normal behavior was found, and no toxic effect was also observed. Hence, there was no lethal effect in any of the groups.

Seeds of *S. mahagoni* were investigated against known bacteria and the growth of inhibition was compared with the conventional standard drug kanamycin[17]. Kanamycin is a conventional bactericidal drug (inhibit the synthesis of bacterial cell wall). It acts on 30S ribosome by anticodon recognition, so synthesis of defective protein occurs. A variability was observed in growth of inhibition of each extract against the given bacteria. Among the various extract fractions, the PSF showed the highest activity against Gram-positive species *B. cereus* in comparison to the standard. The lowest activity was observed with CSF against Gram-positive species *B. subtilis*. No antibacterial activity was shown by the aqueous soluble fractions. We can claim that the seeds of *S. mahagoni* could be used against both Gram-positive and Gram-negative pathogens fruitfully. According to the previous investigations and review literature, a number of medicinal plants have shown anti-infective or bactericidal and bacteriostatic properties[21]. Seeds of mahagoni perhaps possess the same properties, which we already found in our study. So we can state that the presence of antibacterial active ingredients in the seeds of *S. mahagoni* are sensitive to both Gram-positive and Gram-negative pathogens.

Phytochemical study of the various extract fractions of *S. mahagoni* seeds showed the existence of a significant amount of phenolic compounds, which ensured that the plant possesses aforesaid pharmacological potentials. On the basis of investigations, it is justified the folkloric use of *S. mahagoni* seeds has the potential remedy of microbial infection and diarrheal disorders.

Conflict of interest statement

We declare that we have no conflict of interest.

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