CYTOTOXIC ACTIVITY OF TWO LIMONOIDS ISOLATED FROM *SWIETENIA MAHAGONI B*Y USING BRINE SHRIMP LETHALITY BIOASSAY

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ABSTRACT

Solvent partitioning followed by column chromatography of the MeOH extract of the seeds of Swietenia mahagoni *afforded two limonoids, swietenolide (compound 1) and 2-hydroxy-3-*O*-tigloylswietenolide (compound 2), later one is new compound. The compounds were identified by spectroscopic means. The cytotoxic activity of these compounds was assessed by using the conventional brine shrimp lethality bioassay. While both compounds were found to have moderate cytotoxic activity, compound 2 displayed overall more potent activity than compound 1.*

Key words: *Cytotoxic, Limonoid,* Swietenia mahagoni, *Brine shrimp lethality bioassay*

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INTRODUCTION

Swietenia mahagoni (L.) Jacq., a deciduous and economically important timber tree commonly known as 'Mahogani', belongs to the family Meliaceae, which has 146 genera and about 1500 species[1]. *S. mahagoni* is native to a number of North and South American countries including USA, Cuba, Haiti, Jamaica, San Domingo and the Bahamas, and has been distributed elsewhere. Nowadays, it is widely cultivated in both plane land and hill tracts of Bangladesh. Traditionally, various parts of this plant have been used in the treatment of fever, diabetes, malaria, hypertension and tuberculosis, and as an abortifacient, antiseptic, astringent, depurative, purgative and tonic[2-4]. The antifeedant activity of the limonoids from this plant has been reported recently[5]. The extract of this plant showed ameliorative effects on diabetic mice[6], antimicrobial properties[7,8], platelet aggregation inhibitory[9] and anti-HIV activities[10]. Chlorogenic acid from the methanol extract of this plant displayed human immunodeficiency virus protease inhibitory activity[11]. Previous phytochemical investigations on this species led to isolation and identification of more than 45 limonoids belonging to the structural classes andirobin, gendunin, mexicanolide and phragmalin, triterpens, tetranortriterpenes and chlorogenic acid[4,5,9,11,12]. Two limonoids swietenolide (compound **1**) and 2-hydroxy-3-*O*-tigloylswietenolide (compound **2**) previously isolated from from the seeds of *Swietenia mahagonii* were found to have promising antibacterial against a number of multiple drug resistant (MDR) bacterial strains[13]. As a part of our on-going phytochemical and bioactivity studies on Bangladeshi plants[14-19], we now report on the isolation, identification and cytotoxic properties of two limonoids, swietenolide (compound **1**) and 2-hydroxy-3-*O*-tigloylswietenolide (compound **2**), the latter being a new natural product, from *Swietenia mahagoni.* The cytotoxic properties were determined by using brine shrimp lethality

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MATERIALS AND METHODS

Bioassay. This bioassay is indicative cytotoxicity and a wide range of pharmacological activity of the compounds

General procedures

NMR spectra were recorded in CD_3OD on a Bruker DRX 500 MHz NMR Spectrometer (500 MHz for ^1H and 125 MHz for ^{13}C) using residual solvent peak as internal standard. FABMS analyses were performed, on a Finnigan MAT95 spectrometer.

Plant material

The seeds of *Swietenia mahagoni* (L.) Jacq. were collected from Sirajgonj, Bangladesh in December, 2004. The plant was taxonomically identified by the Department of Botany, University of Dhaka, where a voucher specimen (no.18) has been retained.

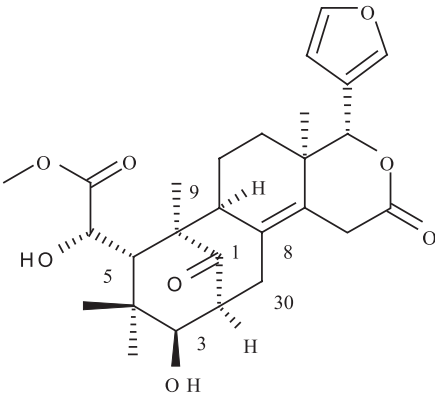
Extraction and isolation of compounds

The dried and ground seeds of *S. mahagoni* (850 g) were percolated in methanol (2 x 1.5 L) for 3 days at room temperature with occasional shaking and stirring. The resulting extract was filtered through a fresh cotton plug and finally with a Whatman no.1 filters paper. The volume of filtrate was concentrated using a rotary evaporator at low temperature and reduced pressure to obtain a reddish-brown gummy extract (58.74 g). A portion of the extract (10 g) was suspended in 5% MeOH in water (100 mL), and partioned against *n*-hexane (3 x 100 mL) to yield *n*-hexane fraction (1.9 g) and aqueous fraction (6.7 g). The aqueous fraction was further partioned against chloroform (3 x 100 mL) and then ethyl acetate (EtOAc) (3 x 100 mL) to produce 3.0 g of ethyl acetate fraction. A portion of the EtOAc fraction (1.5 g) was subjected to column chromatography on Kieselgel 60, mesh 70- 230 (mobile phase = dichloromethane (DCM) and MeOH of increasing polarity), which resulted in ten fractions. Compounds **1** (22 mg, R_f = 5.0) and **2** (R_f = 2.5, 17.5 mg) were obtained from the column fractions 3 and 5, respectively, on evaporation of solvent, and after being washed with petroleum ether. The purity of the compounds were assessed by TLC using solvent systems, DCM: MeOH = 4:1, and DCM: MeOH = 3:2, respectively, and vanillin-sulphuric acid as the spray reagent.

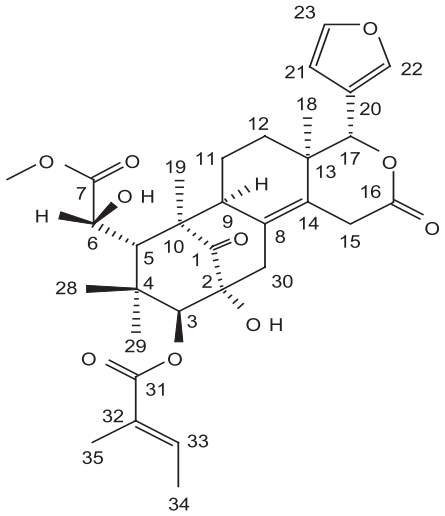
Swietenolide (compound 1)

Reddish-brown amorphous solid (yield 0.00517%); 22.0 mg; m.p. 175-178 ^{o}C; FABMS *m/z* 509 $[M + Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$ and ^{13}C NMR (125 MHz, $CDCl_3$) as published data [20], Table-I and Figure-1.

2-Hydroxy-3-*O*-tigloylswietenolide (compound **2**)



Compound 1 : Swietenolide



Compound 2 : 2-Hydroxy-3-O-tigloylswietenolide

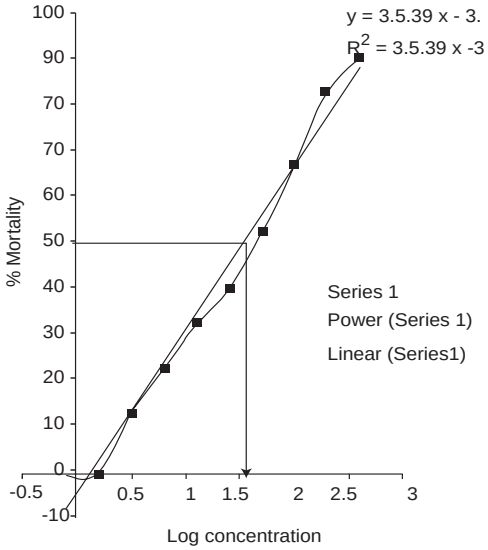


Fig.-1: *Structure of the compound 1 and 2*

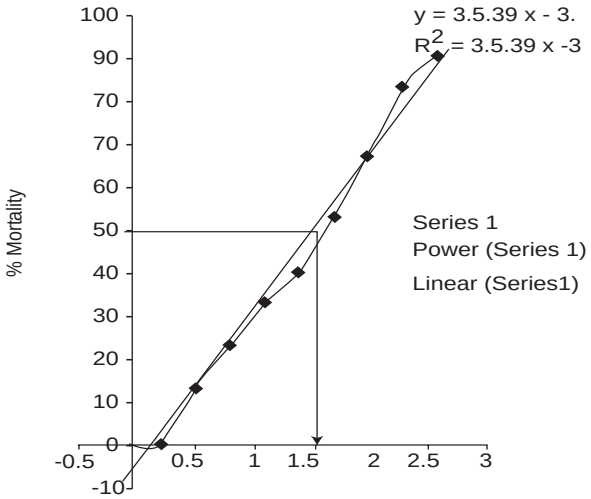


Table-I

*^{1}H NMR (500 MHz, coupling constant J in Hz in parentheses) and ^{13}C NMR (125 MHz) data of compounds **1** and **2**, and ^{1}H-^{13}C long-range (^{2}J and ^{3}J) HMBC key correlation of **2***

Position	Chemical shift in ppm				HMBC correlation in **2**	
	^{1}H		^{13}C			
	1	2	1	2	^{2}J	^{3}J
1	-	-	219.0	216.0	-	-
2	3.02 m	-	50.0	78.9	-	-
3	3.54 d (10.6)	4.54 s	78.5	80.2	C-2, C-4	C-1, C-28, C-29, C-30, C-31
4	-	-	39.6	39.0	-	-
5	3.25 br s	3.48 s	44.0	45.0	C-4, C-6	C-1, C-3, C-7, C-9, C-19, C-28, C-29,
6	4.50 bd	4.61 bd	73.5	72.9	-	C-4, C-10
7	-	-	175.8	176.0	-	-
8	-	-	129.0	123.6	-	-
9	2.09 m	2.10 m	53.0	57.5	-	C-1, C-12, C-14
10	-	-	54.0	50.4	-	-
11	1.14 m	1.14 m	29.1	29.7	C-9, C-12	-
12	1.78 m	1.77 m	17.9	19.1	C-11, C-13	-
13	-	-	38.2	39.0	-	-
14	-	-	130.8	138.3	-	-
15	3.45 d (20.3) 3.98 d (20.3)	3.50 m3.97 m	33.1	34.0	C-16	-
16	-	-	171.4	170.0	-	-
17	5.44 s	5.53 s	80.5	78.4	C-20	C-12, C-14, C-16
18	0.95 s	0.96 s	17.9	18.0	C-13	C-12, C-14, C-17
19	1.36 s	1.42 s	18.7	18.9	C-10	C-1, C-5, C-9
20	-	-	120.8	121.0	-	-
21	7.44 s	7.54 s	141.1	141.2	C-20, C-23	C-22, C-17
22	6.36 s	6.37 s	109.8	110.1	-	C-21, C-23
23	7.37 s	7.43 s	142.9	143.0	C-21	C-22
28	0.95 s	1.11 s	23.2	21.3	C-4	C-3, C-5, C-29
29	0.84 s	0.88 s	23.6	22.8	C-4	C-3, C-5, C-28
30	2.09 m3.14 m	2.60 bs	33.8	34.4	C-2, C-8	-
31	-	-	-	169.8	-	-
OMe	3.78 s	3.75 s	53.2	53.3	-	C-7
32	-	-	-	127.7	-	
33	-	6.85 q (7.4)	-	143.2	C-32	C-31, C-35
34	-	1.73 d (7.4)	-	14.6	C-33	C-32
35	-	1.80 s	-	11.7	C-32	C-31, C-33

Spectra obtained in CDC3

Table 2.1
Effects of compound 1 on brine shrimp nauplii

Concentration(C) (µg/ml)	Log C	% Mortality SMS1				LC_{50} (µg/ml) SMS1
		Group-A	Group-B	Group-C	Average	
400	2.60206	100	100	100	100	33.46
200	2.30103	100	90	90	93	
100	2.00000	80	70	80	77	
50	1.69897	60	70	50	60	
25	1.39794	40	40	50	43	
12.5	1.09691	20	20	20	20	
6.25	0.79588	10	20	10	13	
3.125	0.49485	00	10	00	3	
1.562	0.19382	00	00	00	00	
0.781	-0.10720	00	00	00	00	

Table 2.1
Effects of compound 2 on brine shrimp nauplii

Concentration(C) (µg/ml)	Log C	% Mortality SMS2				LC_{50} (µg/ml) SMS2
		Group-A	Group-B	Group-C	Average	
400	2.60206	100	100	100	100	29.13
200	2.30103	100	100	100	100	
100	2.00000	90	80	80	83	
50	1.69897	60	70	70	67	
25	1.39794	40	50	50	47	
12.5	1.09691	30	20	20	23	
6.25	0.79588	10	20	20	17	
3.125	0.49485	00	10	10	7	
1.562	0.19382	00	10	00	3	
0.781	-0.10720	00	00	00	00	

White crystals (yield 0.00411%); 17.5 mg; m.p. 214-216 ^{o}C; FABMS *m/z* 607 $[M + Na]^+$; HR-FABMS *m/z* 607.2518 calculated 607.2519 for $C_{32}H_{40}O_{10}Na$. ^{1}H NMR (500 MHz, $CDCl_3$ and ^{13}C NMR (125 MHz, $CDCl_3$) Table 1 and Figure 1.

Cytotoxic activity

Preparation of sea water- 38 gm salt (pure NaCl) was weighed, dissolved in 1 liter of distilled water and filtered off to get clear solution.

Hatching of brine shrimps- *Artemia salina* leach (brine shrimp eggs) collected from pet shop was used as the test organism. Sea water was taken in the small tank and shrimp eggs were added one side of the tank and then this side was covered. One day (24 hours) was allowed to hatch the shrimps and to be matured as nauplii. Constant oxygen supply was provided throughout the hatching time. The hatched shrimps were attracted to the lamp through the perforated dam and with the help of Pasteur pipette 10 living shrimps were added to each of the test tubes containing 5 ml of sea water.

Preparation of the test solution

2 mg of each sample (compound **1** and compound **2**) was dissolved in specific volume of DMSO

(Dimethylsulfoxide) to obtain the desired concentration of the prepared solution as 2000µgm/30µl. Then a series of solutions of lower concentrations were prepared from this solution serial dilution with DMSO. Thus the concentrations of the solutions were obtained as 2000µg/30µl, 1000µg/30µl, 500µg/30µl, 250µg/30µl, 125µg/30µl, 62.5µg/30µl, 31.25µg/30µl, 15.63µg/30µl, 7.81µg/30µl, 3.90µg/30µl. From each of these solutions 30µl were added to the pre-marked test tubes containing 5ml of sea water and 10 shrimp nauplii. So, the final concentration of samples in the test tubes were 400µg/30µl, 200µg/30µl, 100µg/30µl, 50µg/30µl, 25µg/30µl, 12.5µg/30µl, 6,25µg/30µl, 3.125µg/30µl, 1.56µg/30µl, 0.78µg/30µl respectively.

Preparation of the control group

Vincristine sulfate served as the positive control.

Preparation of the negative control

As for negative control, 30 µl of DMSO was added to each of three pre-marked glass vials containing 5ml of simulated sea water and 10 shrimp nauplii to use for negative control. If the brine shrimps in these vials show a rapid mortality rate, then the test tube is considered as the nauplii died due to some reason other than the cytotoxicity of the compounds.

Counting of nauplii and determination of LC_{50}- After 24 hours, the vials were observed using a magnifying glass and number of survived nauplii in each vial was counted. From this data, the percent (%) of the lethality of the brine shrimp nauplii was calculated for each concentration. Percentage (%) of the mortality of brine shrimp against log concentration were plotted on graph paper by using SPSS Software and Microsoft Office Excel to determine the LC_{50} of the compound **1** and compound **2**.

RESULTS AND DISCUSSION

Solvent partitioning followed by column chromatography of the MeOH extract of the seeds of Swietenia *mahagoni* afforded two limonoids, swietenolide (compound **1**) [20] and 2-hydroxy-3-*O*-tigloylswietenolide (compound **2**). The compounds were identified by spectroscopic means.

The FABMS spectrum of compound **1** revealed the *pseudo*molecular ion $[M+Na]^+$ at *m/z* 509 corresponding to the molecular formula $C_{27}H_{34}O_8$. The ^1H and ^{13}C NMR data (Table 1) of compound **1** were in total agreement with the data published for the known limonoid swietenolide (compound **1**)[20]. The HR-FABMS spectrum of compound **2** showed the *pseudo*molecular ion $[M+Na]^+$ at *m/z* 607.2518, and confirmed the molecular formula $C_{32}H_{40}O_{10}$ for this compound. The ^1H and ^{13}C NMR spectra (Table 1) of compound **2** were quite similar to those of compound **1**, with the notable exceptions that there were signals for a tigloyl moiety ($?_H$ 6.85, 1.73, 1.80; $?_C$ 169.8, 127.7, 143.2, 14.6, 11.7), the signal for the C-2 methine (as in compound **1**) was absent, and an additional oxygenated quarternary carbon signal ($?_C$ 78.9) appeared. These data indicated hydroxylation at C-2, and the presence of an *O*-tigloyl functionality, which could be placed at C-3, and confirmed from the ^3J ^1H-^{13}C HMBC correlation (Table 1) between H-3 ($?_H$ 4.54) and the tigloyl carbonyl (C-31, $?_C$ 169.8). The ^1H and ^{13}C NMR data (Table 1) of **2** were also comparable with the data published for the similar compound, 2-hydroxy-3-*O*-tigloyl-6-*O*-acetylswietenolide[21] with the exception of the acetyl signal. Thus, compound **2** was identified exclusively as 2-hydroxy-3-*O*-tigloylswietenolide, which is a new natural product.

In this bioassay, two pure compounds (Compound **1** & Compound **2**) isolated from the ethyl acetate fraction of seeds of *Swietenia mahagoni* were showed positive results indicating that the compounds are more or less biologically active. Each of the test samples were showed different mortality rates at different concentrations.

The mortality rate of brine shrimp was found increased with the increasing concentration of the samples. A plot of log of concentration versus percent mortality on the graph paper produced an approximately linear correlation between them. From the graph, the concentration at which fifty percent mortality (LC_{50}) of the brine shrimp nauplii was determined by extrapolation.

The LC_{50} values for the pure compounds (Compound **1** and Compound **2**) were found 33.46 and 29.13 µg/ml respectively, indicating that these compounds are moderately active against brine shrimp (Table 2.1 and 2.2 , Figure 2.1, 2.2 and 2.3).

CONCLUSION

Swietenolide (compound **1**) and 2-hydroxy-3-*O*-tigloylswietenolide (compound **2**) showed significant cytotoxic activity by using brine shrimp lethality bioassay, which is indicative cytotoxicity and a wide range of pharmacological activity of the compounds and this finding will certainly contribute to the on-going search for new anticancer agents to fight against bacterial infections caused by MDR bacterial strains.

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