Journal of Faculty of Sciences

A Refereed Scientific Journal
Faculty of Pure and Applied Sciences
International University of Africa

Volume No. 6 , December , 2019

ISSN: 1858 – 51

Bioassay-Guided Isolation and Identification of Antibacterial Compounds from *Swietenia Macrophylla* Seed Extract

Mohammed B. Sulieman*[1], Azhari H. Nour[2], Abdurahman H. Nour[3], and Awatif A. Mohammed[1]

[1]*Medicinal, Aromatic Plants, Research Institute, National Centre for Research, 2404, Khartoum, Sudan*

[2]*Faculty of Pure and Applied Sciences, International University of Africa, 12223, Khartoum, Sudan*

[2]*Faculty of Chemical & Natural Resources Engineering, University of Malaysia Pahang, 26300, Malaysia*

*Corresponding author E-mail:*mohamedbabiiker@Gmail.com*; H/P: 00249910040080

Abstract

The medicinal plant *Swietenia macrophylla* (Family: Meliaceae) which is used by indigenous healers to treat various diseases was selected for the current work. Bioassay-guided fractionation process was applied to isolate compounds from the most active fraction of the seed extracts. Dried seeds were subjected to maceration and later fractionated by liquid-liquid extraction technique into different classes according to the polarity with various solvents. The isolation of the compounds has been carried out using preparative HPLC and the structures elucidation of the isolated compounds based on spectra including, MS, IR and NMR. The antibacterial activity of the fractions and isolated compounds was assessed against six multiple-drug resistant bacterial strains, namely; *Staphylococcus aureus* (ATCC1026), *Bacillus subtilis* (ATCC19659), *Enterococcus facalis* (ATCC29212), *Salmonella typhi* (ATCC14038), *Pseudomonas aeruginos* (ATCC15442) and *Escherichia coli* (ATCC14038) by the conventional disc diffusion

method. The results showed marked susceptibility of fractions against tested organisms. Among them, ethyl acetate fraction was the most dominant and effective fraction against all tested bacteria. Compounds isolation and structural elucidation of the most active fraction yielded four compounds, namely: swietenolide (**1**), proceranolide (**2**), 3-O-tigloyl-6-O-acetylswitenolide (**3**) and swietenine acetate (**4**). Among them, swietenolide showed highest activity against all of the tested bacteria. The MIC values of the compounds range from 4 to 256 µg/mL. The current results suggested the three compounds present in *S. macrophylla* are potential were antibacterial agents.

Keywords: Swietenia macrophylla; Limonoids; Antibacterial; Preparative LC.

1. Introduction

The plant genus *Swietenia* of which *S. macrophylla* (Family: Meliaceae) belongs to is a valuable timber tree closely related to the African genus *Khaya* which is one of the most popular traditional medicines in Africa (Degen,et al., 2013). The seeds extract of this plant has been included in traditional medicinal formulations for the treatment of diabetes, hypertension and to relieve pain, and as an abortifacient, antiseptic, antibacterial drug, astringent, depurative, purgative, and tonic (Dutta et al., 2013). The decoction of the bark reported as an anti-malarial drug (Moghadamtousi et al., 2013). A considerable number of limonoids have been isolated from the plant *S. macrophylla* and their structures determined on the basis of spectral data. Most of these have been isolated by classical column chromatography, with different work-up procedures, eluents and

adsorbents (Lin et al., 2011; Liu et al., 2012). The objective of the current work was isolation and identification of antibacterial compounds from *S. macrophylla* crude seed extract using bioassay guided fractionation.

2. Materials and Methods

2.1 General

The ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra in CD_3OD were performed on a Bruker NMR Spectrometer (Bruker Corp., Germany) with TMS as an internal standard. IR was achieved on a Perkin Elmer 100 FT-IR Spectrophotometer (Perkin Elmer, Inc., USA) system. The mass spectra were conducted using Agilent 1290 Infinity LC system (Waters Corp., USA) coupled to Agilent 6520 Accurate-Mass Q-TOF mass spectrometer (Agilent Tech., USA) with dual ESI source. The column used was Agilent Zorbax Eclipse plus C18, Rapid Resolution HD (2.1 x 50mm, 1.8um) Part no: 959757-902. HPLC carried out on Waters HT XDB- C18 column using 30-70% H_2O/ACN as solvent.

2.2 Plant materials

Seeds of *S. macrophylla* King were collected on 16 December, 2010 from Bukit Mertajam, Penang, Malaysia. The taxonomy and identification of the plant was done by the botanist in the School of Environment Science and Natural Resources, University of Malaya (UM) Malaysia.

2.3 Extraction and isolation

Dried and ground seeds of *S. macrophylla* (900 g) were macerated in methanol for five days at room temperature with occasional shaking and stirring. The resulting extracts were filtered through a sieve and finally with Whatman no. 1, filter papers. The volume of filtrate was concentrated using a rotary evaporator to obtain the crude extract (63.5 g). A portion of the crude extract (20 g) was re-dissolved in 5% methanol solution (200 mL), fractionated successfully with hexane, chloroform and ethyl acetate to give hexane (3.8 g), chloroform (5.7 g), ethyl acetate (4.4 g) and aqueous (4.5 g) fractions. The isolation was performed on the seed ethyl acetate fraction, which was the most active antimicrobial fraction. The ethyl acetate fraction (4.4 g) was purified through column chromatography packed with silica gel (70-230 mesh), and elution was conducted in an increasing polarity solvent system using (mobile phase: dichloromethane and methanol of increasing polarity), yielding two major fractions. The combination of these two fractions yielded 2.8 g sample. Exactly, 500 mg of this sample was dissolved in 50 mL methanol (HPLC grade) and filtered through a 0.2 mm membrane filter. The final solution was used for analytical and preparative HPLC analysis.

The sample solution was screened for the presence of compounds via analytical method before conducting the preparative HPLC. In the analytical method, the separation was achieved on a prep-HPLC 25455 Binary Gradient Model System with analytical column C18-Zorbax Eclipse

XDB (4.6 x 150 mm i.d; particle size 5 µm) by using two chromatographic pumps. The elution was performed by gradient solvent systems with a flow rate of 1 mL/min at ambient temperature (25-28 °C). The mobile phase consisted of 70% water (solvent A) and 30% acetonitrile (solvent B) changed gradually to 90% acetonitrile within 15 minutes and held for another five minutes. The mobile phase was prepared freshly, filtered through a 0.2 mm membrane filter and degassed via sonication before use. Total running time was 20 minutes. The sample injection volume was 10 µL of the solution, while the wavelength of the UV-Vis detector was set at 230 nm. In the preparative method, the sample was injected into the same instrument and further separation and collection of the targeted compounds were done through the preparative column of C18-Zorbax Eclipse XDB (21.2 x 150 mm i.d; particle size 7 µm) and fractions collector. The solvent system was the same as the analytical analysis. The injection volume was increased to 210 µL at flow rate of 19 mL/min.

2.4 Antibacterial susceptibility test

All fractions and isolated compounds were screened against six bacterial strains including, three *Gram*-positive bacteria: *Staphylococcus aureus* (ATCC1026), *Bacillus subtilis* (ATCC19659) and *Enterococcus faecalis* (ATCC29212); three *Gram*-negative bacteria: *Salmonella typhi* (ATCC14038), *Pseudomonas aeruginosa* (ATCC15442) and *Escherichia coli* (ATCC14038) by the conventional disc diffusion method (Demetrio et al., 2015). Stock solutions 10 mg/mL were prepared from the 100 mg of

each fraction in 10 mL of solvent (9.5 mL H_2O + 0.5 mL DMSO), and sterilized discs were impregnated with 5 and 10 mg/mL of each sample, and dried. Standard discs of streptomycin (10 µg/disc) and 5% DMSO were used as the positive and negative controls, respectively. Each sample and control discs were carefully placed onto the previously marked zones on the agar plates pre-inoculated with test organisms. The plates were incubated at 37 °C for 24 h in upright positions. Whereas, a serial tube dilution technique was used to determine minimum inhibitory concentration (MIC) of the isolated compounds (Kiessoun et al., 2015).

2.5 Statistical analysis

The results obtained in the current work were analysed using SPSS software (SPSS Statistical Version 22). All values presented are mean values ± standard deviation of triplicates (n = 3), obtained from three separate experiments. The one-way ANOVA and post hoc multiple comparisons test (Scheffe) were performed to examine the differences among the groups. A P value of < 0.05 was considered to be statistically significant.

3. Results and Discussion

3.1 Antibacterial screening of the seed fractions

The result of the antibacterial activity of the four fractions (hexane, chloroform, ethyl acetate and aqueous) of S. macrophylla seed extract, against the six pathogenic bacteria is given in Table 1. Ethyl acetate is

potent than other fractions, at a concentration of 10 mg/mL, the inhibitions zones (>20 mm) were more against *P. aeruginosa* and *E. coli*, but inhibited less against *E. faecalis*, *B. subtilis*, *S. aureus* and *S. typhi*; the zones of inhibition were 15.5, 15.7,16.4 and 17.8 mm, respectively. Aqueous fraction also demonstrated significant antibacterial activity, but slightly less than ethyl acetate. The inhibitions zones with the same above concentration were 12.2, 14.7, 15.1, 16.3, 17.9 and 19.1 mm for the organisms, *B. subtilis*, *E. faecalis*, *S. aureus*, *S. typhi*, *P. aeruginosa* and *E. coli*, respectively. Hexane fraction showed activity against *E. coli*, *S. aureus*, *E. faecalis*, *B. subtilis*, *S. typhi* and *P. aeruginosa* with the inhibition zones of 16.3, 15.5, 14.9, 14.5, 12.6, and 1.6 mm, respectively.

Chloroform fraction showed moderate effect against S. typhi, P. aeruginosa, E. faecalis, E. coli and B. subtilis with inhibition zones of 13.4, 12.9, 12.8, 12.3 and 10.2 mm, respectively, whereas for S. aureus, it showed a higher degree of inhibition of 17.3 mm. The ANOVA results for the effect of the four different fractions at concentration of 10 mg/mL on growth inhibition for the six bacteria strains are given in Table 4.4. There is no significant differences among all fractions on the growth of S. aureus, but significant differences ($P<0.05$) were observed among all fractions on the growth of the all other tested bacteria strains.

Table 1. Antibacterial activity of *S. macrophylla* seed fractions

		Inhibition zone (mm) [a]					
		Bacterial specie					
Fractions	Con.mg/mL	*S. aureus*	*B. subtilis*	*E. faecalis*	*S. typhi*	*P. aeruginosa*	*E. coli*
SF1	10	15.5 ± 0.4[a]	14.5 ± 0.7[a]	14.9 ± 0.3[a]	12.6 ± 0.1[a]	11.9 ± 0.4[a]	16.3 ± 0.4[a]
	5	11.0 ± 0.2	13.7 ± 0.8	13.3 ± 0.9	16.5 ± 0.3	10.7 ± 0.3	14.9 ± 0.4
SF2	10	17.3 ± 0.5[a]	10.2 ± 0.2[b]	12.8 ± 0.9[a]	13.4 ± 0.3[a]	12.9 ± 0.1[a]	12.3 ± 0.5[b]
	5	13.7 ± 0.2	9.8 ± 0.9	12.2 ± 0.7	11.8 ± 0.4	11.7 ± 0.4	12.1 ± 0.3
SF3	10	16.4 ± 0.4[a]	15.7 ± 0.9[a]	15.5 ± 0.2[b]	17.8 ± 0.5[b]	20.1 ± 0.4[b]	25.3 ± 0.2[c]
	5	12.0 ± 0.2	15.1 ± 0.8	14.1 ± 0.2	15.3 ± 0.3	16.3 ± 0.3	24.1 ± 0.1
SF4	10	15.1 ± 0.5[a]	12.2 ± 0.9[b]	14.7 ± 0.5a	16.3 ± 0.5[b]	17.9 ± 0.5[b]	19.1 ± 0.3[d]
	5	12.5 ± 0.3	12.1 ± 0.6	13.9 ± 0.9	12.6 ± 0.2	13.5 ± 0.3	14.4 ± 0.2
Sm	10 µg/mL	21.4 ± 0.1	18.1 ± 0.3	16.4 ± 0.2	22.5± 0.1	22.9 ± 0.2	27.6 ± 0.1
DMSO	5%	–	–	–	–	–	–

[a] Inhibition zone diameter including the diameter of the paper disc (6 mm); SF1: Hexane fraction, SF2: Chloroform fraction, SF3: Ethyl acetate fraction, SF4: Aqueous fraction; –: no growth inhibition; values were means of three replicates (± standard error mean); means with the different letter in the same column are significantly different at ($p < 0.05$).

3.2 Isolation of compounds

Bioassay-guided fractionation from most active fraction (ethyl acetate) of *S. macrophylla* seed extract, afforded four compounds. In spite of the big volume of the injected sample (210 µL), it can be seen in the chromatogram (Figure 1).that the column was not overloaded and all compounds were well separated. Yielding compounds (**1)** (at 2.15 min; 14.4 mg), compound (**2)** (at 3.69 min; 12.0 mg), compound (**3)** (at 4.51 min; 10.16 mg) and compound (**4)** (at 7.81 min; 10.18 mg). The pooled compounds were re-analysed by analytical HPLC to check the purity prior identification as indicated in Figure 2.

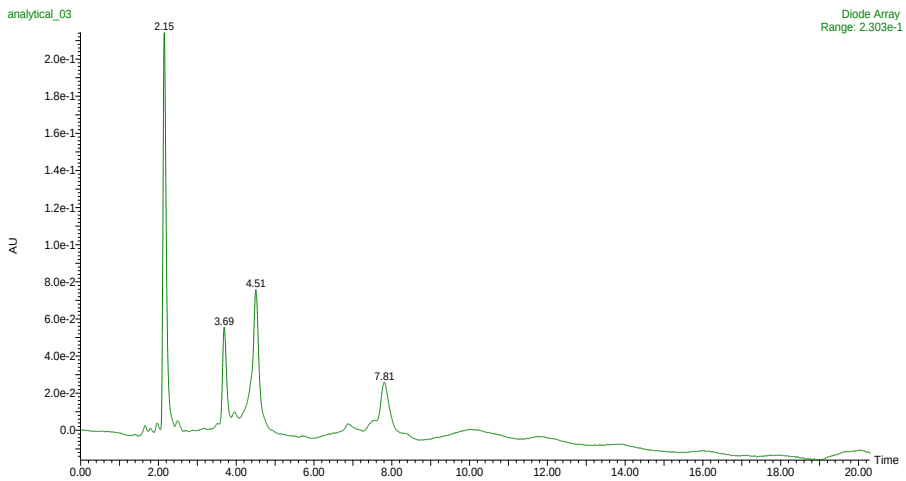

Figure 1 Chromatographic separation of the ethyl acetate fraction of *S. macrophylla* seed.

The chromatogram obtained using preparative column C-18 at 230 nm

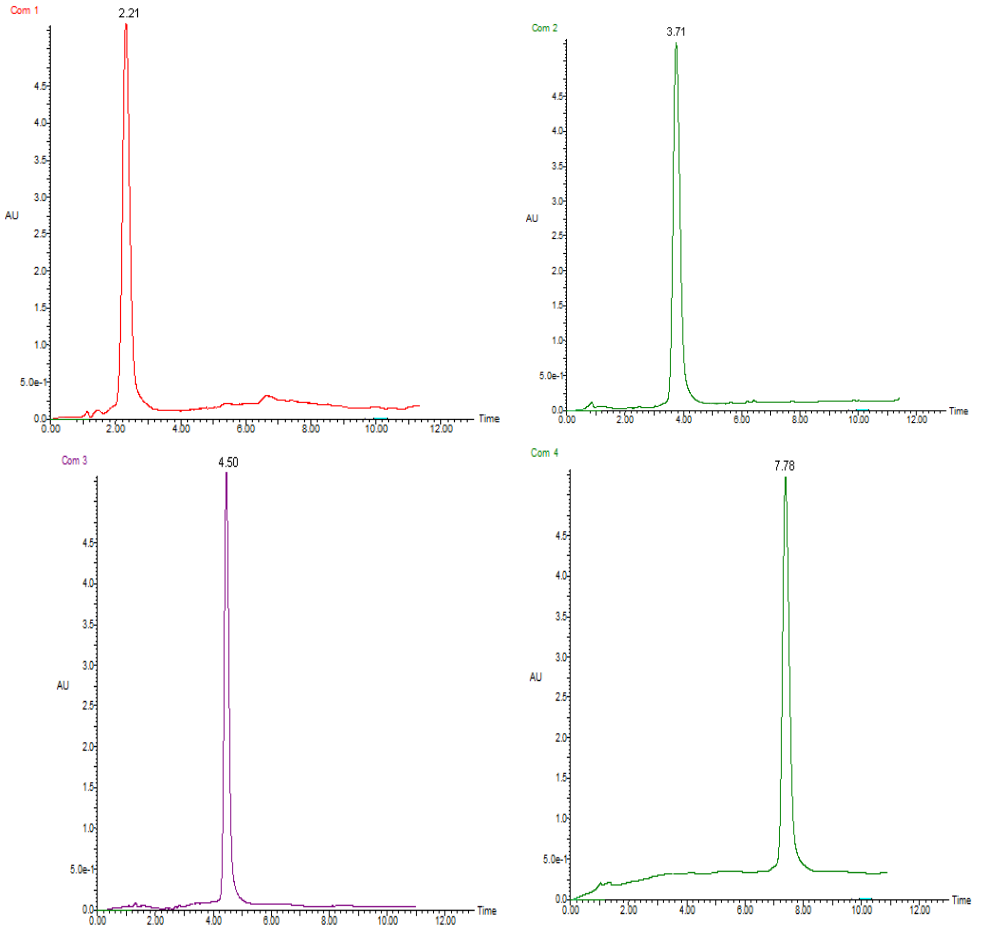

Figure 2. chromatographic separations of compounds 1-4, using analytical column. The chromatogram obtained using preparative column C-18 at 230 nm

3.3 Structure elucidation

Compound (**1)** was isolated as white crystals. The molecular formula was determined to be $C_{27}H_{34}O_8$ with 11 degrees of unsaturation in the molecule by positive FABMS (*m/z* 487 $[M + H]^+$); mp: 175-178 ºC; and NMR data (Tables 2 and 3). The IR spectra showed the absorptions for OH group (3473 cm^{-1}), carbonyl group (1718 cm^{-1}) and furan ring (880 cm^{-1}). The ^{13}C-NMR spectra (CD_3OD, 125 MHz) displayed 27 carbon signals due to three carbonyls (ketone, ester and lactone), six sp^3 methines, five methyl, four sp^3 methylenes, three sp^2 methines, three sp^3 quaternary carbons and three sp^2 quaternary carbons. Among them, three sp^3 methines δ_C 77.88 (C-3), 72.90 (C-6) and 80.50 (C-17), two sp^2 methines (δ_C 142.82 (C-23) and 109.63 (C-22) and one methyl 53.41 were ascribed to those bearing an oxygen atom. Comparison with information from literature, Schefer et al. (2006) suggested that the swietenolide limonoid for compound (**1)** (Figure 3).

Compound (2) was isolated as amorphous. The molecular formula was determined to be C27H34O7 with 11 degrees of unsaturation in the molecule by positive FABMS (m/z 471 [M + H]+); mp: 186-189 ºC; and NMR data (Tables 2 and 3). The IR spectra showed the absorptions for OH group (3413 cm-1), carbonyl group (1745 cm-1) and furan ring (871 cm-1). The 13C-NMR spectra (CD3OD, 125 MHz) confirmed that compound 2 has 27 carbon signals due to three carbonyls (one ketone, one ester and one lactone), five sp3 methines rather than six in comparison with compound

(1), five sp3 methlenes, three sp2 quaternary carbons, three sp3 quaternary carbons, three sp2 methines and five methyls. Among them, two sp3 methines at δC 142.82 (C-23) and 141.35 (C-21), one sp3 methyl 54.14 and two sp3 methines 80.49 (C-17) and 77.88 (C-3) were ascribed to those bearing an oxygen atom. By comparing the spectral data with those reported in the literature by Philip et al. (2005), the compound (2) was determined to be the proceranolide (Figure 3).

Compound (3) was isolated as colourless needles crystal. The molecular formula was determined to be C34H42O10 with 14 degrees of unsaturation in the molecule by positive FABMS (m/z 611 [M + H]+); mp: 154-156 ºC ; and NMR data (Tables 2 and 3). The IR spectra showed the absorptions for OH group (3401 cm-1), carbonyl group (1641 cm-1) and furan ring (877 cm-1). The 13C-NMR spectra confirmed that compound (3) has 34 carbon resonances due to five carbonyls rather than three or four in comparison with previous compounds (one ketone, three ester and lactone), six sp3 methines, eight methyls, four sp3 methylenes, three sp2 methines, three sp3 quaternary carbons and five sp2 quaternary carbons. Among them, three sp3 methines δC 77.44 (C-3), 72.64 (C-6) and 78.33 (C-17), two sp2 methines (δC 143.23 (C-23) and 109.21 (C-22) and one methyl 50.01, were ascribed to those bearing an oxygen atom. It was consistent with the skeleton of 3-O-tigloyl-6-O-acetylswietenolide (Jih-Jung et al., 2010). Therefore, compound (3) was determined to be 3-O-tigloyl-6-O-acetylswietenolide (Figure 3).

Compound (4) was isolated as colourless needles. The molecular formula was determined to be C34H42O10 with 14 degrees of unsaturation in the molecule by positive FABMS (m/z 611 [M + H]+); mp: 130-133 ºC; and NMR data (Tables 2 and 3). The IR spectra showed the absorptions for OH group (3461 cm-1), carbonyl group (1639 cm-1) and furan ring (875 cm-1). The NMR spectra confirmed that compound (4) is sharing similar NMR data as found for compound (3) indicating that the two structures are closely related, differing in only one aspect. While compound (3) has a double bond between C8-C14 carbons, compound (4) has a double bond between C8-C30 carbons. A relevant and interesting point is the presence of a signal at δH 5.32 ppm, instead of a signal at δH 2.26 ppm characteristic of the hydrogen H-30 when there is a double bond between C8-C30 carbons, which was noted in the 1H-NMR spectrum. Another prove lies in the presence of a signal at δC 122.15 instead of a signal at δC 36.53 characteristic of the carbon (C-30) when there is a double bond between C8-C30 carbons, which was noted in the 13C-NMR spectrum. By comparing the spectral data with those reported in the literature by Dewanjee et al. (2009), the compound (4) was confirmed to be the swietenine acetate (Figure 3).

Table 2. ^{1}H NMR data for compounds **1-4** (at 500 MHz in CD_3OD, □ in ppm, *J* in Hz).

Position (H)	1	2	3	4
2	2.95 (1H, *m*)	2.95 (1H, *m*)	3.47 (1H, *m*)	3.48 (1H, *m*)
3	3.31 (1H, *d*, *J* = 0.5)	3.31 (1H, *d*, *J* = 0.5)	4.89 (1H, *d*, *J* = 0.5)	4.65 (1H, *d*, *J* = 0.5)
5	3.37 (1H, *s*)	3.51 (1H, *dd*, *J* = 7.5, 1.5)	4.67 (1H, *s*)	3.37 (1H, *s*)
6	4.57 (1H, *s*)	2.59 (2H, *m*)	5.65 (1H, *s*)	5.65 (1H, *s*)
9	1.89 (1H, *m*)	2.05 (1H, *m*)	1.78 (1H, *m*)	2.23 (1H, *m*)
11	1.13 (2H, *m*)	1.81 (2H, *m*)	2.30 (2H, *m*)	1.88 (2H, *m*)
	1.75 (2H, *m*)	1.88 (2H, *m*)	1.95 (2H, *m*)	1.99 (2H, *m)*
12	1.96 (2H, *m*)	1.12 (2H, *m*)	1.55 (2H, *m*)	1.85 (2H, *m*)
	1.76 (2H, *m*)	1.69 (2H, *m*)	1.72 (2H, *m*)	1.16 (2H, *m*
14	–	–	–	2.40 (1H, *m*)
15	3.26 (2H, *d*, *J* = 2.5)	3.31 (2H, *d*, *J* = 2.5)	2.99 (2H, *d*, *J* = 2.5)	2.26 (2H, *d*, *J* = 2.5)
17	5.67 (1H, *s*)	5.67 (1H, *s*)	5.63 (1H, *s*)	5.63 (1H, *s*)
18	0.97 (3H, *s*)	1.01 (3H, *s*)	1.05 (3H, *s*)	1.05 (3H, *s*)
19	1.39 (3H, *s*)	1.37 (3H, *s*)	1.22 (3H, *s*)	1.22 (3H, *s*)
21	7.61 (1H, *t*, *J* = 0.5)	7.61 (1H, *t*, *J* = 0.5)	7.80 (1H, *t*, *J* = 0.5)	7.80 (1H, *t*, *J* = 0.5)
22	6.52 (1H, *dd*, *J* = 2, 0.5)	6.52 (1H, *dd*, *J* = 2, 0.5)	6.56 (1H, *dd*, *J* = 1.5, 05)	6.56 (1H, *dd*, *J* = 1.5, 0.5)
23	7.53 (1H, *t*, *J* = 2)	7.53 (1H, *t*, *J* = 2)	7.58 (1H, *t*, *J* = 1.5)	7.58 (1H, *t*, *J* = 1.5)
28	1.01 (3H, *s*)	0.92 (3H, *s*)	1.15 (3H, *s*)	1.15 (3H, *s*)
29	0.92 (1H, *s*)	0.97 (3H, *s*)	1.01 (3H, *s*)	1.01 (3H, *s*)
30	2.04 (2H, *m*)	3.14 (2H, *m*)	2.38 (2H, *m*)	5.31 (1H, *d*, *J* = 7.5)
OCH_3	3.97 (3H, *s*)	3.79 (3H, *s*)	3.77 (3H, *s*)	3.77 (3H, *s*)
32	–	–	2.21 (1H, *s*)	2.21 (3H, *s*)
35	–	–	6.94 (1H, *qq*, *J* = 7, 1.5)	6.94 (1H, *qq*, *J* = 7, 1.5)
36	–	–	1.73 (3H, *d*, *J* = 1)	1.73 (3H, *d*, *J* = 1)
37	–	–	1.71 (3H, *d*, *J* = 1)	1.72 (3H, *d*, *J* = 1

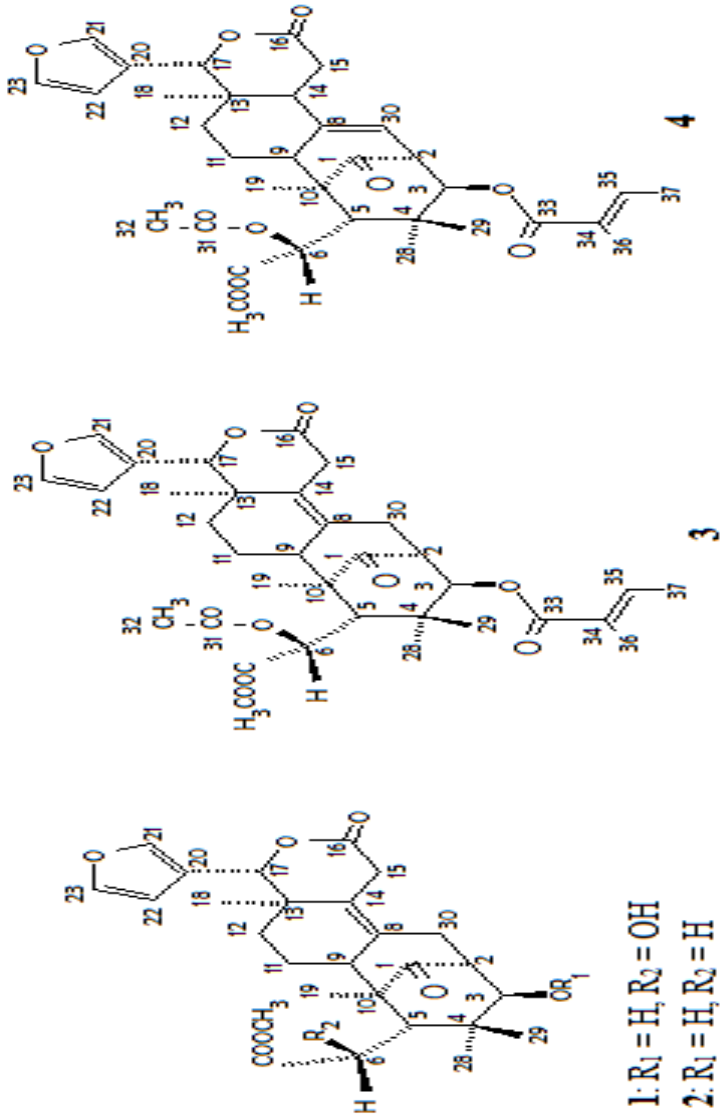

Figure 3. Structures of compounds **1-4**.

Table 3 ^{13}C NMR data for compounds **1-4** (at 125 MHz in CD_3OD □ in ppm)

Position (C)	**1**	**2**	**3**	**4**
1	217.2 (C)	215.8 (C)	216.26 (C)	216.65 (C)
2	50.99 (CH)	51.58 (CH)	48.86 (CH)	48.87 (CH)
3	77.88 (CH)	77.88 (CH)	77.44 (CH)	78.33 (CH)
4	44.02 (C)	50.99 (C)	44.44 (C)	38.72 (C)
5	39.50 (CH)	44.01 (CH)	44.59 (CH)	44.45 (CH)
6	72.90 (CH)	37.98 (CH2)	72.64 (CH)	72.64 (CH)
7	176.57 (C)	176.58 (C)	171.48 (C)	171.48 (C)
8	129.03 (C)	129.02 (C)	122.15 (C)	139.03 (C)
9	51.59 (CH)	53.40 (CH)	52.39 (CH)	57.32 (CH)
10	54.14 (C)	72.88 (C)	57.32 (C)	50.01 (C)
11	28.94 (CH2)	18.39 (CH2)	20.31 (CH2)	19.45 (CH2)
12	17.00 (CH2)	28.93 (CH2)	29.02 (CH2)	33.96 (CH2)
13	37.70 (C)	39.58 (C)	38.72 (C)	36.54 (C)
14	130.35 (C)	130.35 (C)	139.30 (C)	44.59 (CH)
15	32.63 (CH2)	33.83 (CH2)	33.96 (CH2)	29.01 (CH2)
16	172.69 (C)	172.70 (C)	169.92 (C)	169.92
17	80.50 (CH)	80.49 (CH)	78.33 (CH)	77.24 (CH)
18	18.40 (CH3)	16.99 (CH3)	21.71 (CH3)	20.30 (CH3)
19	16.90 (CH3)	16.88 (CH3)	14.77 (CH3)	14.77 (CH3)
20	120.99 (C)	120.98 (C)	121.06 (C)	121.05 (C)
21	141.35 (CH)	141.35 (CH)	141.18 (CH)	141.18 (CH)
22	109.63 (CH)	109.63 (CH)	109.21 (CH)	109.21 (CH)
23	142.82 (CH)	142.82 (CH)	143.23 (CH)	143.23 (CH)
28	22.61 (CH3)	22.20 (CH3)	21.71 (CH3)	21.71 (CH3)
29	23.07 (CH3)	22.60 (CH3)	22.09 (CH3)	22.09 (CH3)
30	33.83 (CH2)	32.62 (CH2)	36.53 (CH2)	122.15 (CH)
$COOCH_3$	53.41 (CH3)	54.14 (CH3)	50.01 (CH3)	52.39 (CH3)
31	–	–	170.28 (C)	170.28 (C)
32	–	–	20.96 (CH3)	20.97 (CH3)
33	–	–	166.88 (C)	166.88 (C)
34	–	–	127.38 (C)	127.38 (C)
35	–	–	139.47 (CH)	139.47 (CH)
36	–	–	10.93 (CH3)	10.63 (CH3)
37	–	–	13.36 (CH3)	13.37 (CH3)

3.4 Antibacterial activity of the isolated compounds (1-4)

The results of the antibacterial activity of the four isolated compounds at a concentration of 100 µg/mL; against the tested bacterial strains were represented in Table 4. Compound (**1)** showed maximum zone of inhibition (34.5 mm) against *P. aeruginosa*, followed by *S. typhi* (28.1 mm), *E. coli* (24.3 mm), *S. aureus* (23.2 mm), *B. subtilis* (15.5 mm) and *E. faecalis* (13.1 mm). Compound (**2)** showed maximum zone of inhibition (20.6 mm) against *P. aeruginosa*, followed by *S. typhi* (19.4 mm), *E. coli* (18.2 mm), *S. aureus* (15.3 mm), *E. faecalis* (11.0 mm) and *B. subtilis* (8.5 mm). Compound (**3)** showed maximum zone of inhibition (18.3 mm) against *S. typhi*, followed by *E. coli* (13.4 mm), *P. aeruginosa* (10.2 mm), *E. faecalis* (8.2 mm), *S. aureus* (7.5 mm) and *B. subtilis* (7.1 mm), whereas, compound (**4)** was inactive against all the tested bacterial strains.

Since compounds (1), (2) and (3) showed good antibacterial activity against the tested bacteria strains, their inhibitory activity was evaluated by determining MIC values, which are indicated in Table 5. As can be seen, the MIC values varied to each compound, from 4 to 128 µg/mL for compound 1, from 32 to 256 µg/mL for compound (2), from 64 to 256 µg/mL for compound (3) and from 2 to 32 µg/mL for the control (streptomycin). The results obtained in these assays indicated that P. aeruginosa strain was the most sensitive bacteria to compound (1), with a MIC value of 4 µg/mL; which was equal with the reference antibiotic streptomycin (4 µg/mL). Low

MIC value (32 µg/mL) for this strain was also obtained for compound (2), compared to other strains.

Table 4. Inhibition zone (mm) of the tested compounds 1-4 at 100 µg/mL

Bacterial species	**Inhibition zone (mm) [a]**				
	Streptomycin	**1**	**2**	**3**	**4**
S. aureus	21.4 ± 0.1	23.4 ± 0.1	15.3 ± 0.1	7.5 ± 0.1	–
B. subtilis	18.1 ± 0.3	15.5 ± 0.3	8.5 ± 0.1	7.1 ± 0.2	–
E. faecalis	16.4 ± 0.2	13.1 ± 0.2	11.0 ± 0.2	8.2 ± 0.4	–
S. typhi	22.5± 0.1	28.1 ± 0.2	19.4 ± 0.1	18.3 ± 0.1	–
P. aeruginosa	22.9 ± 0.2	34.5 ± 0.2	20.6 ± 0.2	10.2 ± 0.1	–
E. coli	27.6 ± 0.1	24.3 ± 0.1	18.2 ± 0.1	13.4 ± 0.1	–

[a] Inhibition zone diameter including the diameter of the paper disc (6 mm); –: no growth inhibition; values were means of three replicates (± standard error mean). 1-4: isolated compounds.

Table 5. Minimum inhibitory concentration (MIC) of the tested compounds 1-3

Compounds	**Minimum inhibitory concentration MIC (µg/mL)**					
	Bacterial specie					
	S. aureus	*B. subtilis*	*E. faecalis*	*S. typhi*	*P. aeruginosa*	*E. coli*
1	16	128	128	8	4	16
2	128	256	256	64	32	64
3	256	256	256	64	256	128
Streptomycin	4	32	32	4	4	2

On comparing the antimicrobial activity of the three isolated active compounds in term of MIC values, it was found that compound (**1)** showed the highest MIC values (4 to 128 µg/mL) against all the tested bacteria. This compound, previously reported as a new natural antibacterial product from *S. mahagoni* against eight of bacterial strains (Shahidur Rahman et al., 2009). Compounds (**2)** and (**3)** exhibited considerable MIC values in the

range of 32-256 µg/mL and 64-256 µg/mL against all tested multiple drug-resistant bacteria strains, respectively. The observed antimicrobial activity of these two compounds against tested bacteria is novel and important because there are no treatments available for infections caused by many of the antibiotic-resistant bacteria strains. They developed mechanisms of resistance to common antibacterial agents (Shahidur Rahman et al., 2009; Sibhghatulla et al., 2015).

Multiple drug-resistant bacteria strains used in this study are common causes of infections in long-term care units in hospitals. S. aureus is the most important bacteria that cause disease in humans, E. coli is mainly responsible for urinary tract infections and P. Aeruginosa and S. tyhi are the main strains responsible for 16% of nosocomial pneumonia cases. They are the leading cause of skin and soft tissue infection (Aqumas et al., 2014; Truong et al., 2011). The significant antibacterial activity caused by compounds (1), (2) and (3) against S. aureus, S. typhi, E. coli and P. aeruginosa are particularly interesting due to its importance as a nocosomial infectious agent. They can possibly be used as antibacterial agents in new drugs for the therapy of infectious diseases caused by multiple-drug-resistant (MDR) bacterial strains.

References

Aqumas S., A. Tamrat and M. Adane, 2014. Antimicrobial susceptibility pattern of nasal *Staphylococcus aureus* among Dessie Referral Hospital health care workers, Dessie, Northeast *Ethiopia. Int. J. Infect. Dis*, **25**, 22-25.

Degen B., S. Ward, M. Lemes, C. Navarro, S. Cavers and A. Sebbenn, 2013. Verifying the geographic origin of mahogany (*Swietenia macrophylla* King) with DNA-fingerprints, *Forensic. Sci. Int. Gen*. **7**, 55-62.

Demetrio L. V. J., I. A. Jeannie, J. M. P. Juliana, C. C. Esperanaza and L. R. Windell, 2015. Antibacterial activities of ethanol extracts of Philippine medicinal plants against multidrug-resistant bacteria, *Asian Pac J Trop Biomed*. **5**, 532-540.

Dewanjee S., A. Maiti, A. K. Das, S. C. Mandal and S. P. Dey, 2009. Swietenine: A potential oral hypoglycemic from *Swietenia macrophylla* seed. *Fitoterapia*, **80**, 49-251.

Dutta M., U. K. Biswas, R. Chakraborty, P. Banerjee, D. Maji, M. C. Mondal and U. Raychaudhuri, 2013. Antidiabetic and antioxidant effect of *Swietenia macrophylla* seeds in experimental type 2 diabetic rats, *Int. J. Diabetes Dev. Ctries.* **33***,* 60-65.

Jih-Jung C., H. Sheng-Shiou, L. Chang-Hui, W. Dau-Chang, S. Ping-Jyun and W. Tai-Chi, 2010. A new phragmalin-type limonoid and anti-inflammatory constituents from the fruits of *Swietenia macrophyllA. Food chemistry*. **120**, 379-384.

Kiessoun K., Y. Kassi, S. Oksana and B. Marian, 2015. Antidiarrheal and antimicrobial profiles extracts of the leaves from *Trichilia emetic* Vahl. (Meliaceae). Asia, *Pac J Trop Biomed*. **5**, 242-248.

Lin B-D., C-R. Zhang, S-P. Yang, S. Zhang, Y. Wu and J-M. Yue, 2011. D-ring-opened phragmalin-type limonoid orthoesters from the twigs of *Swietenia macrophylla*, *J. Nat. Prod*. **72**, 1305-1313.

Liu J-O., C-F. Wang, J-C. Chen and M-H. Qiu, M.-H, 2012. Limonoids from the leaves of *Swietenia macrophylla*, *Nat. Prod. Res*. **26,** 1887-1891.

Moghadamtousi S. Z., B. H. Goh, C. K. Chan, T. Sabah and H. A. Kadir, 2013. Biological activities and phytochemical of *Swietenia macrophylla* King, *Molecules.* **18**, 10465-10483.

Philip H. C., M. Dulcie and R. Milijaona, 2005. Mexicanolide limonoids from the Madagascan Meliaceae *Quivisia papinae. Phytochemistry*, **66**, 1100-1107.

Schefer A. B., U. Braumann, L.H. Tseng, M. Spraul, M. G. Soares, J. B. Fernandes, M. F. daSilva, P. C. Vieira and A. G. Ferreira, 2006. Application of high-performance liquid chromatography nuclearmagnetic resonance coupling to the identification of limonoids from *mahogany* tree (*Switenia macrophylla*, Meliaceae) by stopped-flow 1D and 2D NMR spectroscopy, *J. Chromatography. A*. **1128**, 152-163.

Shahidur Rahman A. K., A. K. Azad Chowdhury, H. Ali, S. Z. Raihan, M. S. Ali, L. Nahar and S. D. Sarker, 2009. Antibacterial activity of two limonoids from *swietenia mahogani* aganist multiple-drug-resistant (MDR) bacterial strains. *J. Nat. Med*, **63**, 41-45.

Sibhghatulla S., F. Jamale, S. Shazi, M. D. R. Syed and A. K. Mohammed, 2015. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment, *Saudi. J. Biol. Sci*. **22**, 90-.101.

Truong H., S. S. Shah, L. Ludmir, E. O. Twananana, M. Bafana and S. M. Wood, 2011. *Staphylococcus aureus* skin and soft tissue infections at a tertiary hospital in Botswana*. S. Afr. Med. J*. **101**, 413-416.

مجلة
كلية العلوم

مجلة علمية محكّمة
كلية العلوم البحتة والتطبيقية
جامعة إفريقيا العالمية

العدد السادس – ديسمبر – 2019م

ردمك: 513x – 1858