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Bioassay-guided Isolation and Identification of Antifungal Compounds from Seeds of *Swietenia Macrophylla* King

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ABSTRACT

Swietenia macrophylla King, a plant used extensively in many countries for medicinal purposes. The objective of the present study was to investigate in vitro antifungal activity of the extract as well as bioassay-guided isolation and identification of compounds from most active fraction of *S. macrophylla* seeds. In the antifungal assay, three clinical fungal strains; namely, *Aspergillus flavus*, *Candida albicans* and *Aspergillus niger* were tested by disc diffusion method. Solvent partitioning followed by Preparative High Performance Liquid Chromatography (prep-HPLC) of the most active fraction afforded four known limonoids and identified by spectroscopic techniques including Fourier Transform Infrared Spectroscopy (FTIR), Mass Spectrometry (MS) and Nuclear Magnetic Resonance Spectroscopy (NMR) analyses. The compounds were: swietenolide (1), proceranolide (2), 3-O-tigloyl-6-O-acetylswietenolide (3) and swietenine acetate (4). Among the fractions, ethyl acetate was the most dominant and effective fraction. Moreover, three compounds (1-3) showed moderate to weak activity, whereas all tested organisms completely resistant to compound 4 and no inhibition zone was observed. On overall, among the three active compounds; compound 1 showed more potent activity than other compounds against all tested organisms.

INTRODUCTION

Switenia macrophylla king is a genus of three species of plants in the family Meliaceae, which is natively distributed throughout tropical regions of the Americas, mainly in Brazil, Bolivia, Mexico and Central America (Eid *et al*., 2013; Li-Chai *et al*., 2015). *S. macrophylla* is a lofty, beautiful, evergreen shrub and large tree which grows to a height of 30-40 m and girth of 3-4 m, depending on the variety (Moghadamtousi *et al*., 2013). It has been included in traditional medicinal formulations for the treatment of diabetes, hypertension and to relieve pain (Dutta *et al*., 2013). The extracts of *S. macrophylla* showed different biological activities such as, antimicrobial, anti-inflammatory, antioxidant, anti-mutagenic, anticancer, antitumor and antidiabetic activities (Goh and Kadir, 2011). Limonoids and their derivatives isolated from *S. macrophylla* exhibited effective anti-inflammatory (Chen *et al*., 2010; Li-Chai *et al*., 2015) and antibacterial activities (Shahidur Rahman *et al*., 2009).

Infectious diseases caused by fungal represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide (Rahul *et al*., 2012). *Candida* and *Aspergillus* species are responsible for the majority (80 to 90%) of fungal infections (Espinel-Ingroff *et al*., 2005). *Candida albicans* is

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the third- or fourth-most-common isolate in nosocomial bloodstream infections in the USA. It is mainly responsible of gastrointestinal tract and candidiasis infections (Eggimann *et al*., 2003; Zadik *et al*., 2010). *Aspergillus niger* is a ubiquitous filamentous fungus found in grains, fruits, forage, mouldy vegetables and dairy products. *A. niger* is a toxinogenic specie that can produce ochratoxin A (Latifa *et al*., 2012). It is also a pathogenic mold that causes otomycosis and aspergillosis (Araiza *et al*., 2006; Bulpa *et al*., 2007). In spite of the introduction of new antifungal drugs, they are limited in number. The increase of fungal resistance to classical drugs and the treatment costs, justify the search for new antifungal drugs from different sources. However, the synthesis of a new drug is too expensive, wasting products in the process; the drugs unstable and may be creating toxic as by products. Furthermore, the environmental problems associated with synthetic of new drugs have to be considered. Plants are rich sources for a wide variety of phytochemicals. The presence of these phytochemicals in plants makes it to be a possible source of raw materials for many pharmaceutical industries as therapeutics sources. By this safer way and cheaper in price, natural drug can be originated. Nevertheless, several medicinal plants for different reasons have not received sufficient scientific studies and sometimes are classified as 'forgotten plants'. The growing interest in medicinal plants or herbs as a source of new pharmaceuticals and the increasing demand for herbal products in the world encourage us to revise this plant (*Swietenia macrophylla*) by evaluating its applicability and benefits using advanced scientific approaches to increase our information about its biological effects and the responsible phytochemicals from them. Thus, this study aimed to isolate and identify bioactive compounds (limonoids) from *S. macrophylla* seed, that might act as antifungal agents and to determine their antifungal potential against *Aspergillus flavus*, *Candida albicans* and *Aspergillus niger*, through a guided antifungal bioassay (Bioassay-guided isolation and identification).

MATERIALS AND METHODS

General:

The proton nuclear magnetic resonance (^{1}H NMR, 500 MHz) and carbon nuclear magnetic resonance (^{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.

Plant Materials:

Seed 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).

Extraction and Isolation:

Dried and ground seed of *S. macrophylla* (900 g) were macerated in methanol for five days at room temperature with occasional shaking and stirring. The result extract was 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. About, 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 mins. 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.

Antifungal Susceptibility Test:

All fractions and isolated compounds were screened against three funguses namely: *A. flavus* (MTCC 1973), *C. albicans* (MTCC 183) and *A. niger* (MTCC 16404) by the conventional disc diffusion method (Majid *et al*., 2004). 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 fraction, and dried. Standard discs of Fluconazole (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 five days in upright positions. After incubation, the antifungal effect of tested materials was determined by measuring the diameter of the inhibition zones in mm with a transparent scale. Figure 1 shows the flow chart process for the bioassay-guided isolation and identification of limonoids from *S. macrophylla* King

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.

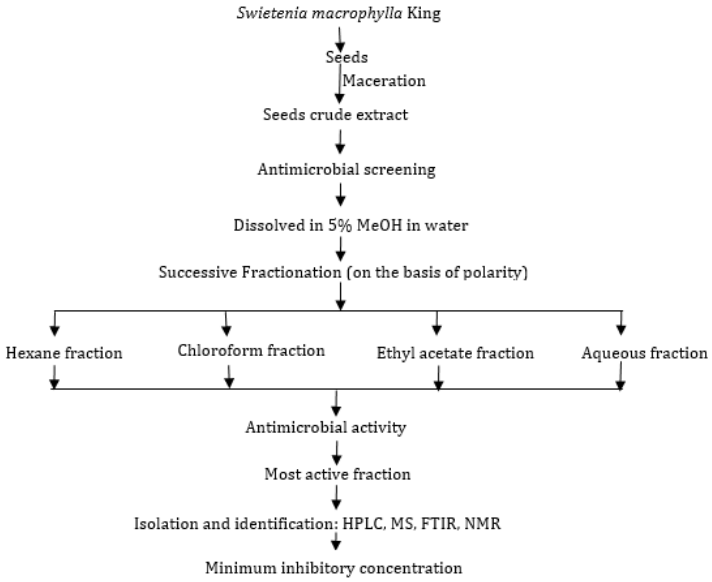


Fig. 1: Flow chart for the bioassay-guided isolation and identification of limonoids from *S. macrophylla* King

RESULTS AND DISCUSSION

Antifungal Screening of the Seed Fractions:

The antifungal effect of the seed fractions has shown good levels of activity when screened against *C. albicans*, but some were found to have less effect on *A. niger* and *A. flavus* (Table 1). Assay showed that among fractionated extracts, ethyl acetate is more active than other fractions; the inhibition zones against all funguses ranging from 9.1 to 16.5 mm at a concentration of 10 mg/mL. Aqueous fraction showed good inhibition but slightly less than ethyl acetate against *C. albicans* (16.2 mm), *A. niger* (13.1 mm) and *A. flavus* (9.0 mm). Both hexane and chloroform fractions showed moderate to weak inhibitions against *C. albicans* (13.4 and 13.9 mm), against *A. niger* (12.4 and 12.1 mm) and against *A. flavus* (8.9 and 8.5 mm), respectively, at the same concentration.

Table 1: Antifungal activity of *S. macrophylla* seed fractions

Inhibition zone (mm) [a]				
		Bacterial specie		
Fractions	Con.mg/mL	*A. niger*	*C. albicans*	*A. flavus*
Hexane	10	12.4 ± 0.4a	13.4 ± 0.4a	8.9 ± 0.5a
	5	11.3 ± 0.3	12.1 ± 0.3	8.2 ± 0.3
Chloroform	10	12.1 ± 0.3a	13.9 ± 0.2a	8.7 ± 0.4a
	5	12.3 ± 0.2	11.6 ± 0.5	8.5 ± 0.3
Ethyl acetate	10	13.3 ± 0.5a	16.5 ± 0.1b	9.1 ± 0.2a
	5	10.3 ± 0.2	13.8 ± 0.3	8.3 ± 0.4
Aqueous	10	13.1 ± 0.2a	16.2 ± 0.4b	9.0 ± 0.2a
	5	12.4 ± 0.4	15.8 ± 0.2	8.6 ± 0.1
Fluconazole	10 µg/mL	23.6 ± 0.2	26.1 ± 0.1	21.0 ± 0.3
DMSO	5%	–	–	–

[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); means with the different letter in the same column are significantly different at ($p < 0.05$).

Isolation of Compounds:

Bioassay-guided fractionation using prep-HPLC of the ethyl acetate fraction led to isolation of four compounds, which were identified as limonoids. These compounds have been previously isolated from S. mahogany seed by column chromatography technique (Schefer *et al*., 2006; Philip *et al*., 2005). In this study, when the sample injected, four compounds were separated (Figure 2) and gave compounds **1** (14.4 mg at 2.15 min); compound **2** (12.0 mg at 3.69 min); compound **3** (10.16 mg at 4.51 min) and compound **4** (10.18 mg at 7.81 min). The pooled compounds were re-analysed by analytical HPLC to check the purity prior identification as indicated in Figure 3.

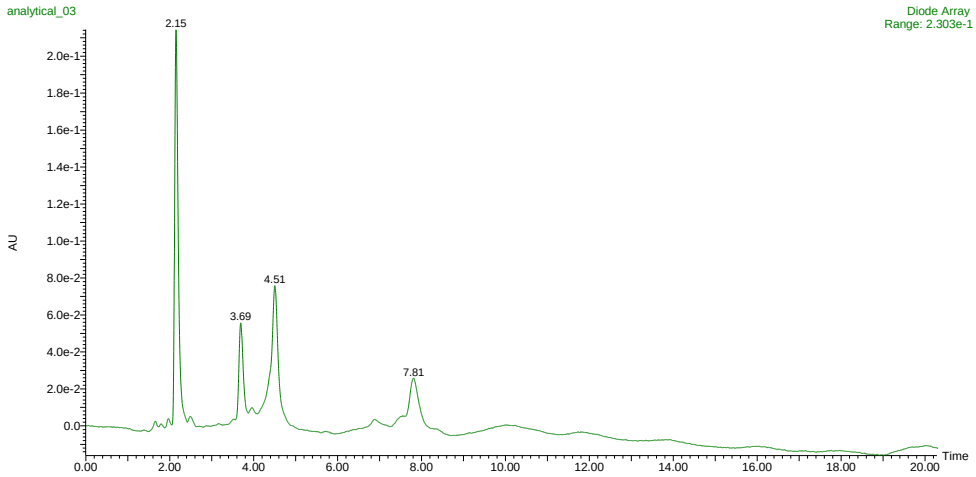
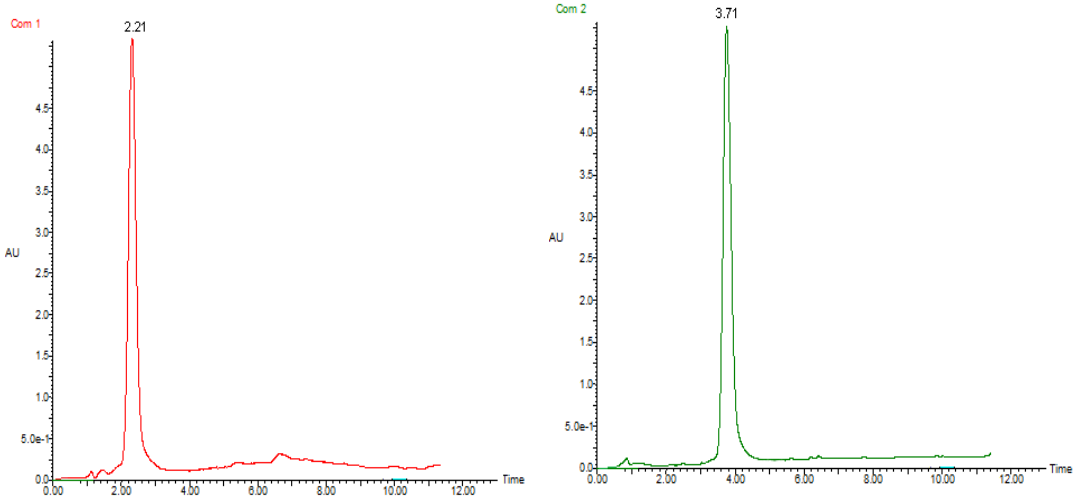


Fig. 2: Chromatographic separation of the ethyl acetate fraction of *S. macrophylla* seed.

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



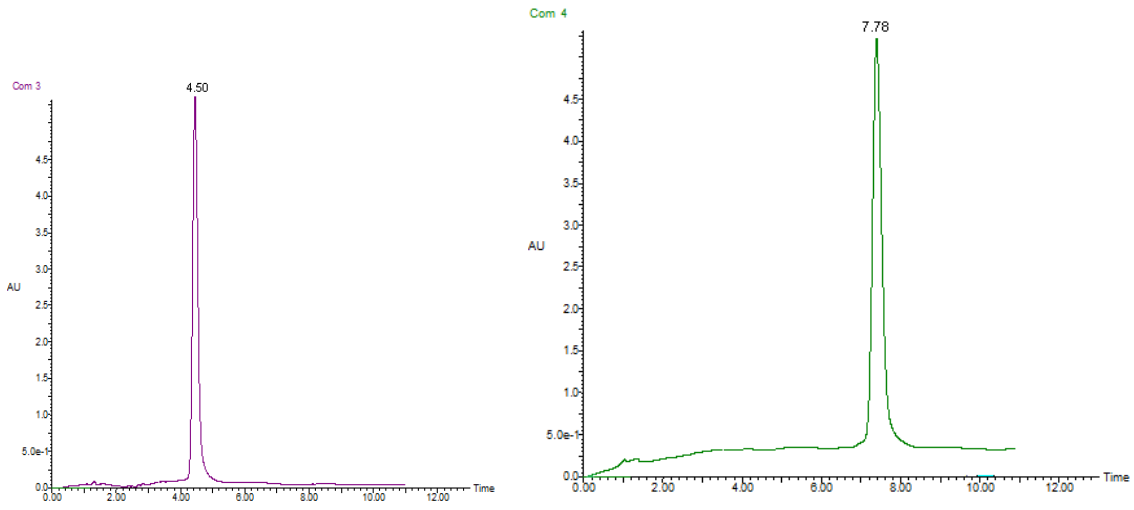


Fig. 3: Chromatographic separations of compounds 1-4, using analytical column.

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) suggests the swietenolide limonoid for compound 1 (Figure 4).

Compound 2 was isolated as amorphous. The molecular formula was determined to be $C_{27}H_{34}O_7$ with 11 degrees of unsaturation in the molecule by positive FABMS (m/z 471 $[M + H]^+$); mp: 186-189 °C; 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 ^{13}C-NMR spectra (CD_3OD, 125 MHz) confirmed that compound 2 has 27 carbon signals due to three carbonyls (one ketone, one ester and one lactone), five sp^3 methines rather than six in comparison with compound 1, five sp^3 methlenes, three sp^2 quaternary carbons, three sp^3 quaternary carbons, three sp^2 methines and five methyls. Among them, two sp^3 methines at δ_C 142.82 (C-23) and 141.35 (C-21), one sp^3 methyl 54.14 and two sp^3 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 (Philip *et al*., 2005) compound 2 was determined to be the proceranolide (Figure 4).

Compound 3 was isolated as colourless needles crystal. The molecular formula was determined to be $C_{34}H_{42}O_{10}$ with 14 degrees of unsaturation in the molecule by positive FABMS (m/z 611 $[M + H]^+$); mp: 154-156 °C ; 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 ^{13}C-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 sp^3 methines, eight methyls, four sp^3 methylenes, three sp^2 methines, three sp^3 quaternary carbons and five sp^2 quaternary carbons. Among them, three sp^3 methines δ_C 77.44 (C-3), 72.64 (C-6) and 78.33 (C-17), two sp^2 methines (δ_C 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 4).

Compound 4 was isolated as colourless needles. The molecular formula was determined to be $C_{34}H_{42}O_{10}$ with 14 degrees of unsaturation in the molecule by positive FABMS (m/z 611 $[M + H]^+$); mp: 130-133 °C; 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 δ_H 5.32 ppm, instead of a signal at δ_H 2.26 ppm characteristic of the hydrogen H-30 when there is a double bond between C8-C30 carbons, which was noted in the ^{1}H-NMR spectrum. Another prove lies in the presence of a signal at δ_C 122.15 instead of a signal at δ_C 36.53 characteristic of the carbon (C-30) when there is a double bond between C8-C30 carbons, which was

noted in the ^{13}C-NMR spectrum. By comparing the spectral data with those reported in the literature (Dewanjee *et al*., 2009) compound 4 was determined to be the swietenine acetate (Figure 4).

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

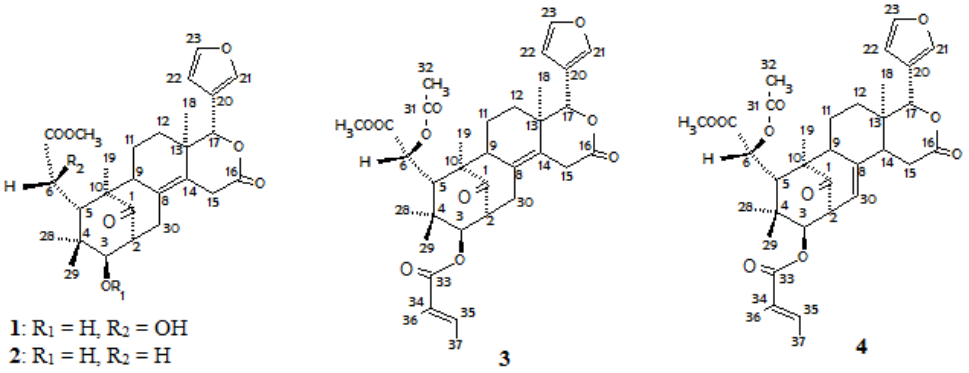


Fig. 4: Structures of compounds 1-4.

Table 3: ^{13}C NMR data for compounds 1-4 (at 125 MHz in CD_3OD, δ 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)

Antifungal Activity of the Isolated Compounds (1-4):

Antifungal activity of the four isolated compounds prepared in two different concentrations (50 and 100 µg/mL) was tested against three fungal strains which were studied. Of the four compounds evaluated, compounds 1, 2 and 3 showed inhibition zones in all the fungal strains tested. Whereas, all tested funguses completely resistance to compound 4 and not observed any inhibition zones (Table 4). The highly strong antifungal activity was observed in compound 1 at a concentration 10 µg/mL against *C. albacans* (13.1 m), *A. niger* (10.2 m) and *A. flavus* (9.1 m). In the same above concentration, compounds 2 and 3 showed similar activity to each other, both inhibited the growth of the fungal *A. flavus, C. albacans* and *A. niger* with the inhibition zones of 4.2-4.3, 5.1-5.4 and 4.0-4.4 mm, respectively. It can explain that *C. albacans* is most sensitive of the tested funguses to these isolated compounds.

Table 4: Inhibition zones (mm) of the isolated compounds 1-4

Compounds	Conc. µg/mL	Inhibition zone (mm)[a]		
		A. favus	*C. albacans*	*A. niger*
Compound (1)	50	8.6 ± 0.3	12.1 ± 0.2	9.0 ± 0.1
	100	9.1 ± 0.4	13.1 ± 0.1	10.2 ± 0.1
Compound (2)	50	4.0 ± 0.1	5.1± 0.1	3.9 ± 0.4
	100	4.3 ± 0.2	5.4 ± 0.1	4.4 ± 0.3
Compound (3)	50	3.9 ± 0.1	4.8 ± 0.3	3.7 ± 0.2
	100	4.2 ± 0.2	5.1 ± 0.3	4.0 ± 0.2
Compound (4)	50	–	–	–
	100	–	–	–
Fluconazole	10	21.0 ± 0.3	26.1 ± 0.1	23.6 ± 0.2

[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)

Compounds (1-3) had at least one free hydroxyl group as a common structural feature in carbon 3 or 6 positions. Other than the presence of the free hydroxyl group, the individual compounds differed in the double bond position. Compound 4 has a characteristic 8,30-double bond, while compounds 1, 2 and 3 have an 8,14-double bond. The result indicated that the double bond-position does not influence to the antifungal activity but suggested that hydroxyl group substitutions may be critical for the antifungal activity. No results concerning antifungal activity have been found in the literature for the isolated compounds. However, Maiti *et al*. (2007) reported the antifungal activity of *S. macrophylla* seed extract against Candida sp (*C. albicans* and *C. albidus*.) and *Aspergillus* sp (*A. flavus* and *A. niger*). Assay showed that the seed extract was more active against Candida sp. *C. albicans* strain in particular, used in this study is common causes of infections in long-term care units in hospitals. It is the leading cause of gastrointestinal tract, candidosis and otomycosis infections (Bulpa *et al*., 2007; Eggimann *et al*., 2003). The significant antifungal activity caused by compound 1 against *C. albicans*, suggesting that this compound could play an active role in the protection against fungi related to several diseases.

Conclusion:

In conclusion, among four compounds which isolated from *S. macrophylla* seed, compound 1 (swieteniolide) showed moderate antifungal activity against tested organisms. Its effect on tested organisms had the following order: *C. albacans* > *A. niger* > *A. flavus*. Compound 2 (proceranolide) and compound 3 (3-O-tigloyl-6-O-acetylswietenolide), both are showed weak and similar activity. Whereas, all tested organisms completely resistance to compound 4 (swietenine acetate) and not observed any inhibition zones.

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