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ORIGINAL ARTICLE

Anti-Differentiation Effect of B, D-*Seco* limonoids of *Swietenia mahogani*

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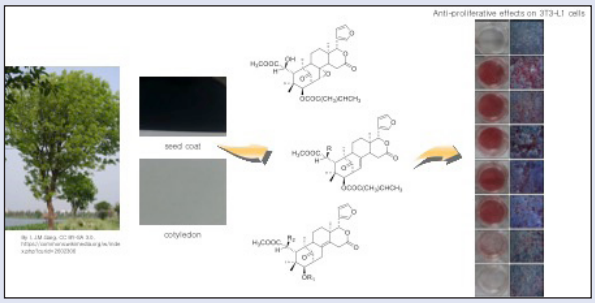
ABSTRACT

Background: Obesity is a pathological state caused by abnormal or excessive accumulation of fat. *Swietenia mahogani* JACQ., known as West Indian mahogany, is a medium-sized semi-evergreen tree belonging to Meliaceae. Their seeds are used in Indonesian folk medicine as a treatment for hypertension, diabetes, malaria, and it also has anti-feedant activities. The major components of *S. mahogani* are B, D-seco limonoids, a type of irregular triterpenes are well known. **Objective:** We tried to find the bioactive components, which have the inhibitory activity on adipocyte differentiation from the seeds of *S. mahogani*. **Material and Methods:** 3T3-L1 cells, derived from mouse preadipocyte, are widely used in studying adipogenesis process. In this study, we used 3T3-L1 cells to find natural products with the inhibitory activity on adipocyte differentiation. *S. mahogani* seeds were dried and extracted with 100% MeOH. **Results:** The methanolic extract was fractionated by bioassay-guided method to give nine B, D-seco limonoids (1-9) with slight structural modifications. Among nine compounds, compounds 4, 6 and 8 exhibited significant inhibitory effects of cell differentiation on 3T3-L1 cells. Those compounds have tigloyl residue at C-3 in common. Besides, compounds with no tigloyl residue at C-3 showed insignificant effect. Nevertheless, not all compounds with tigloyl residue at C-3 exerted significant inhibitory effect. **Conclusion:** These results suggested that tigloyl residue at C-3 may play a role in the anti-proliferative activity on a dipogenesis and the refined extract of *S. mahogani* may have a potential to be developed as a therapeutic agent to treat obesity.

Key words: 3T3-L1, adipogenesis, obesity, seco-limonoid, structure and activity relationship, *Swietenia mahogani*

SUMMARY

- Nine irregular seco-limonoids were isolated from *Swietenia mahogani*.
- Total extract and CHCl3 fraction of *S. mahogani* showed the significant inhibitory activities on 3T3-L1 cell differentiation.
- A tigloyl residue at C-3 in an aglycone may play a role in the anti-proliferative activity on adipogenesis.



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INTRODUCTION

Obesity is a major cause of the risk factors for various metabolic and cardiovascular diseases such as fatty liver, diabetes, hypertension, and hyperlipidemia.[1] Adipogenesis is a process where fibroblastic preadipocytes become mature adipocytes.[2] It brings the abnormal accumulation by an increase in the sizes or numbers of adipocytes from precursor cells.[3] Thus, the control of the differentiation of preadipocytesinto adipocytes is the crucial point for the prevention of obesity. However, many medicines available in the market for losing or controlling of weight are not successful in the long-term maintenance of over-weight patients.[4] To find the anti-adipogenetic agents from natural products, 3T3-L1 cells were selected as *in vitro* screening tool. These cells are preadipocytes derived from mouse and sub-clones of swiss mouse embryo 3T3, and differentiate into adipocyte-like phenotype under the exposure of adipogenic inducers, such as fetal bovine serum, dexamethasone, isobutylmethylxanthine, and insulin.[5] In the present study, we used 3T3-L1 cells to find natural products with the inhibitory activity on adipocyte differentiation, which are expected to lose or control body's over-weight.

Swietenia mahogani JACQ., known as West Indian mahogany, is a medium-sized semi-evergreen tree belonging to Meliaceae. It is native to West Indies and cultivated in tropical countries. Its seeds are used as a treatment for hypertension, diabetes and malariain Indonesian folk medicine.[6] Decoction of its bark has been used as febrifuge or anti-malarial drug.[7] Furthermore, some studies reported that it acts as a blood platelet aggregation inhibitor.[8] The well known constituents are mainly limonoids, especially B, D-*seco* limonoids, which are derivatives of limonoids oxidized rings B and D of limonoid.[9] To the best of our knowledge, the study of the extract or compounds of *S. mahogani* on anti-adipogenesis has not been reported. As a result, we isolated B, D-*seco* limonoids (1-9) from the seeds of *S. mahogani* and elucidated their anti-adipogenesis against 3T3-L1 cells.

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MATERIAL AND METHODS

Reagents

First grade solvent for extraction, fractionation and isolation were purchased from Dae Jung Pure chemical Eng. Co. Ltd. (Ansan, Korea), and HPLC grade solvents from Fisher Scientific (Pittsburgh, PA, USA). Dulbecco's modified Eagle's media (DMEM), penicillin/streptomycin, trypsin, PBS for cultures of 3T3-L1 cells were purchased from Sigma Chemical Co. (St. Louis, MO, USA). MTT, IBMX, dexamethasone, insulin, EGCG, Nonidet P-40, isopropyl alcohol, and reagents for ORO were also obtained from Sigma Chemical Co. (St. Louis, MO, USA). FBS, CS were obtained from Hyclone Co. (Logan, UT, USA), Gibco Co. (Grand Island, NY, USA), respectively. Multi-well culture plate and cell culture dishes were purchased from Corning (New York, NY, USA).

Plant materials

The Seeds of *S. mahogani* were collected at Jakarta, Indonesia in August 2012 and air-dried. These seeds were identified by Prof. Sang Hyun Sung, one of the authors in the present study; (SNUPH-0830) has been stored in the Herbarium of the Medicinal Plant Garden, Seoul National University.

Isolation of compounds 1-9 from the seeds of *S. mahogani*

Dried seeds (1.2 kg) of *S. mahogani* were extracted with 100% MeOH (4L × 3) in an ultrasonic apparatus. After removal of the solvent *in vacuo*, the 100% MeOH extract (444.6 g) was suspended in H_2O and successively partitioned into n-hexane fraction (1.2 g), $CHCl_3$ fraction (110.9 g), EtOAc fraction (0.4 g), and n-BuOH fraction (6.7 g), respectively. Among these fractions, $CHCl_3$ fraction showing significant anti-differentiation effect on 3T3-L1 cells was subjected to repeated column chromatography and HPLC to give compounds 1-9.

The $CHCl_3$ fraction was subjected to silica gel column chromatography (CC) and eluted with mixtures of *n*-hexane-EtOAc = 10:1, 3:1, 1:1, $CHCl_3$-MeOH= 10:1, to yield twelve fractions (C1 ~ C12) and compound 1 (1401 mg). C6 was subjected to ODS silica gel HPLC with MeCN-H_2O (6:4, 6 ml/min) to give compound 4 (81 mg). C8 was subjected to silica gel CC and eluted with mixtures of $CHCl_3$-MeOH = 100:1, 50:1, 10:1, 1:1 to give compounds 6 (143 mg) and 8 (247 mg). Compound 3 (895 mg) was isolated from C10 by recrystallization (MeOH). C11 was subjected to silica gel MPLC gradient eluting with mixtures of *n*-hexane-EtOAc (62:38, 0:100) to give thirteen fractions (C11-1 ~ C11-13) and compound 7 (117 mg). C11-12 was further separated into six fractions (C11-12-1 ~ C11-12-6) by ODS silica gel HPLC with MeCN-H_2O (5:5, 6 ml/min). C11-12-2 was subjected to ODS silica gel HPLC with MeCN-H_2O (7:3, 6 ml/min) again to give compound 2 (42mg). C11-12-6 was subjected to ODS silica gel HPLC in same condition to give compounds 5 (22 mg) and 9 (209 mg).

Swietenolide (1): white amorphous powder; $C_{27}H_{34}O_8$;$[\alpha]^{25}_D$: -140.1000 (*c*=0.1, chloroform); IR ν_{max} (KBr) (cm^{-1}): 3496, 1721, 876; HRMS (positive mode): *m/z* 487.2332 $[M+H]^+$(calc. mass: 487.2336); ^1H NMR ($CDCl_3$, 500 MHz): see [Table 1]; ^{13}C NMR ($CDCl_3$, 125 MHz): see [Table 2].

3-*O*-acetylswietenolide (2): white amorphous powder; $C_{29}H_{36}O_9$; $[\alpha]^{25}_D$: -90.1000 (*c*=0.1, chloroform); IR ν_{max} (KBr) (cm^{-1}): 3495, 1732, 876; HRMS (positive mode): *m/z* 529.2438 $[M+H]^+$(calc. mass: 529.2438); ^1H NMR ($CDCl_3$, 400 MHz): see [Table 1]; ^{13}C NMR ($CDCl_3$, 100 MHz): see [Table 2].

3,6-*O,O*-diacetylswietenolide (3): white amorphous powder; $C_{31}H_{38}O_{10}$; $[\alpha]^{25}_D$: -206.7000 (*c*=0.1, chloroform); IR ν_{max} (KBr) (cm^{-1}): 1737, 876; HRMS (positive mode): *m/z* 571.2543 $[M+H]^+$(calc. mass: 571.2543); ^1H NMR ($CDCl_3$, 500 MHz): see [Table 1]; ^{13}C NMR ($CDCl_3$, 125 MHz): see [Table 2].

khayasin T (4): white amorphous powder; $C_{32}H_{40}O_8$; $[\alpha]^{25}_D$: -121.7000 (*c*=0.1, chloroform); IR ν_{max} (KBr) (cm^{-1}): 1728, 876; HRMS (positive mode): *m/z* 553.2801 $[M+H]^+$(calc. mass: 553.2801); ^1H NMR ($CDCl_3$, 400 MHz): see [Table 1]; ^{13}C NMR ($CDCl_3$, 100 MHz): see [Table 2].

3-*O*-tigloylswietenolide (4): white amorphous powder; $C_{32}H_{40}O_9$; $[\alpha]^{25}_D$: -120.4000 (*c*=0.1, chloroform); IR ν_{max} (KBr) (cm^{-1}): 3489, 1719, 876; HRMS (positive mode): *m/z* 569.2751 $[M+H]^+$(calc. mass: 569.2744); ^1H NMR ($CDCl_3$, 500 MHz): see [Table 1]; ^{13}C NMR ($CDCl_3$, 125 MHz): see [Table 2].

Febrifugin (6): white amorphous powder; $C_{32}H_{40}O_8$; $[\alpha]^{25}_D$: -100.6000 (*c*=0.1, chloroform); IR ν_{max} (KBr) (cm^{-1}): 1727, 876; HRMS (positive mode): *m/z* 553.2808 $[M+H]^+$(calc. mass: 553.2808); ^1H NMR ($CDCl_3$, 500 MHz): see [Table 1]; ^{13}C NMR ($CDCl_3$, 125 MHz): see [Table 2].

Swietenine (7): white amorphous powder; $C_{32}H_{40}O_9$; $[\alpha]^{25}_D$: -76.0000 (*c*=0.1, chloroform); IR ν_{max} (KBr) (cm^{-1}): 3470, 1725, 876; HRMS (positive mode): *m/z* 569.2751 $[M+H]^+$(calc. mass: 569.2748); ^1H NMR ($CDCl_3$, 600 MHz): see [Table 1]; ^{13}C NMR ($CDCl_3$, 150 MHz): see [Table 2].

Swietenine acetate (8): white amorphous powder; $C_{34}H_{42}O_{10}$; $[\alpha]^{25}_D$: -139.3000 (*c*=0.1, chloroform); IR ν_{max} (KBr) (cm^{-1}): 1728, 876; HRMS (positive mode): *m/z* 611.2857 $[M+H]^+$(calc. mass: 611.2856); ^1H NMR ($CDCl_3$, 500 MHz): see [Table 1]; ^{13}C NMR ($CDCl_3$, 125 MHz): see [Table 2].

Swietemahonin E (9): white amorphous powder; $C_{32}H_{40}O_{10}$; $[\alpha]^{25}_D$: -100.7000 (*c*=0.1, chloroform); IR ν_{max} (KBr) (cm^{-1}): 3495, 1729, 876; HRMS (positive mode): *m/z* 585.2717 $[M+H]^+$(calc. mass: 585.2700); ^1H NMR ($CDCl_3$, 500 MHz): see [Table 1]; ^{13}C NMR ($CDCl_3$, 125 MHz): see [Table 2].

Cell culture and adipocyte differentiation

Mouse embryo fibroblasts 3T3-L1 cells were obtained from the American Type Culture Collection (Manassas, VA, U.S.A.) and incubated in DMEM supplemented with 10% bovine calf serum until confluence. Two days after confluence (designated day 0), preadipocytes were stimulated to differentiate with differentiation medium (DM, DMEM with 10% FBS, 0.5 mM 3-isobutyl-1-methyl-xanthine, 10 µg/mL insulin and 1 µM dexamethasone) for 2 days (day 2). Cells were then maintained in DM (DMEM 10% FBS and 10 µg/mL insulin) for other 2 days (day 4), followed by culturing with DM (DMEM with 10% FBS) for additional 4 days (day 8). All media contained 100 IU/mL penicillin and 100 mg/mL streptomycin. The cells were maintained at 37°C in a humidified atmosphere of 95% air - 5% CO_2. Test compounds were dissolved in DMSO (final concentration of 0.1% in media). The cultures were treated with test samples for the whole culture period (day 0-8).

Oil red o staining

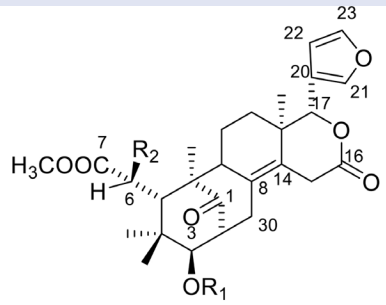
Lipid droplets in cells were stained with Oil Red O (ORO) on day 8. Briefly, culture dishes were washed three times with PBS and fixed with 10% formalin for 1 hour at room temperature. After fixation, cells were washed once with PBS and stained with a filtered ORO solution (6 parts of saturated 0.6% ORO in isopropyl alcohol and 4 parts of water) for 15 minutes at room temperature. Cells were washed twice with water for 15 minutes and visualized. To quantify the intracellular lipids, spectrophotometrical quantification of the stain was performed by dissolving the stained lipid droplets with 4% Nonidet P-40 in isopropyl alcohol for 5 minutes. The absorbance was measured at 544 nm. Lipid contents (% of control) $=A_{540nm}$ (sample-undifferentiated control)/A_{540nm} (differentiated control-undifferentiated control) × 100.

Table 1: ^{1}H NMR spectral data of compounds 1-9.

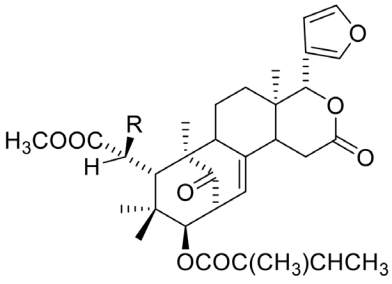
Position	1	2	3	4	5	6	7	8	9
1									
2	3.01 m	3.12 m	3.13 m	3.25 m	3.18 m	3.49 dd(8.5, 7.9)	3.47 m	3.49 m	3.59 dd(9.5, 2.6)
3	3.54 dd(9.7, 3.4)	4.79 d (9.9)	4.85 d (10.0)	4.82 d (9.7)	4.67 d (9.8)	4.80 d (9.4)	4.59 dd(9.7, 2.8)	4.66 d (9.5)	4.82 d (9.5)
4									
5	3.21 s	3.19 s	3.39 s	3.34 dd(9.6, 3.3)	3.34 s	3.44 dd(9.1, 2.5)	3.46 s	3.68 s	3.34 s
6	4.50 s	4.52 s	5.44 s	2.36 m 2.38 m	4.53 s	2.34 m	4.52 s	5.54 s	4.44 s
7									
8									
9	2.04 br s	2.15 m	2.07 m	2.05 m	2.10 m	2.19 m	2.26 m	2.23 m	1.98 dd(14.3, 4.1)
10									
11	1.10 m 1.72 m	1.16 m 1.73 m	1.17 m 1.84 m	1.80 m 1.69 m	1.75 m 1.84 m	1.61 m 2.06 m	1.78 m 1.97 m	1.78 s 2.16 s	1.75 m 1.91 m
12	1.75 m 1.82 m	1.77 m 1.84 m	1.76 m 1.89 m	1.14 m 1.74 m	1.15 m 1.74 m	1.37 m 1.61 m	1.44 m 1.74 m	1.39 m 1.69 m	1.38 m 2.08 m
13									
14						2.17 m	2.19 m	2.18 m	
15	3.42 dt(21.1, 2.4) 3.99 d (21.1)	3.46 m 3.69 m	3.43 dt(20.8, 2.45) 3.66 d (20.9)	3.19 m 3.59 d (20.0)	3.24 t (2.9) 3.52 s	2.74 s	2.78 m 2.75 m	2.78 d (6.3)	2.70 m, 3.14 m
16									
17	5.44 s	5.53 s	5.58 s	5.52 s	5.40 s	5.59 s	5.51 s	5.52 s	5.06 s
20									
21	7.44 s	7.45 m	7.51 s	7.54 s	7.45 s	7.80 s	7.52 s	7.67 s	7.40 m
22	6.37 s	6.38 m	6.44 s	6.45 d (1.2)	6.36 m	6.44 s	6.35 s	6.41 m	6.32 m
23	7.38 s	7.40 m	7.41 s	7.38 t (1.6)	7.40 s	7.39 t (1.5)	7.41 m	7.41 s	7.42 m
18	0.95 s	1.00 s	1.03 s	1.00 s	0.95 s	1.04 s	0.93 s	0.99 s	1.01 s
19	1.37 s	1.39 s	1.14 s	1.14 s	1.40 s	1.11 s	1.41 s	1.16 s	1.32 s
28	0.96 s	1.06 s	1.05 s	0.79 s	1.06 s	0.78 s	1.08 s	0.93 s	1.09 s
29	0.83 s	0.77 s	0.83 s	0.75 s	0.83 s	0.80 s	0.85 s	1.07 s	0.90 s
30	1.99 m 3.15 dd(14.4, 2.3)	2.15 m 2.80 m	2.13 s, 2.78 dd(15.3, 1.9)	2.05 dd(15.1, 5.5) 2.64 dd(15.2, 2.0)	2.10 m	5.30 d (7.1)	5.29 m	5.30 d (7.1)	3.08 d (2.6)
$COOCH_3$	3.78 s	3.81 s	3.73 s	3.70 s	3.83 s	3.69 s	3.73 s	3.70 s	3.93 s
6-$OCOCH_3$									
6-$OCOCH_3$	2.15 s		2.15 s					2.16 m	
1’									
2’	2.15 s	2.12 s	2.15 s						
3’				6.93 m	6.89 m	6.90 m	6.82 m	6.86 m	6.98 m
2’-CH_3				1.86 s	1.88 m	1.71 d (7.0)	1.77 s	1.78 m	1.91 s
3’-CH_3				1.81 m	1.80 m	1.79 s	1.70 m	1.70 m	1.89 m

Table 2: ^{13}C NMR spectral data of compounds 1-9.

position	1	2	3	4	5	6	7	8	9
1	219.7	217.6	216.9	218.3	218.0	217.3	216.6	216.0	213.8
2	50.0	47.8	47.8	48.2	48.1	48.8	48.9	48.7	49.0
3	78.5	79.9	79.5	79.3	80.5	76.6	78.3	77.8	79.1
4	39.6	38.7	38.8	38.7	39.1	38.5	38.9	38.9	40.0
5	44.0	45.2	44.4	40.5	45.0	41.2	45.3	44.5	46.1
6	73.6	73.3	73.0	33.4	73.3	32.8	72.7	72.7	72.3
7	175.8	175.4	171.2	174.2	175.6	174.0	175.9	171.1	175.8
8	129.0	128.5	127.6	127.8	128.4	138.5	138.3	138.4	60.3
9	53.0	53.6	53.1	52.2	53.5	56.7	57.5	57.4	55.0
10	54.0	53.2	53.3	53.2	53.4	49.8	50.3	50.1	48.6
11	29.1	29.7	29.4	18.7	18.9	20.6	21.2	21.1	20.5
12	18.7	18.9	18.7	29.1	29.7	34.4	34.5	34.4	32.5
13	37.8	38.1	38.1	38.1	38.1	36.9	36.6	36.7	35.6
14	130.8	131.4	132.3	131.9	131.5	45.1	44.9	45.1	43.4
15	33.1	33.6	33.4	32.9	33.1	29.6	29.5	29.5	32.4
16	171.3	169.5	169.6	169.7	164.3	168.9	168.5	168.6	170.8
17	80.5	80.9	80.8	80.9	81.1	77.0	76.7	76.6	80.8
20	120.8	120.7	120.5	120.8	120.8	120.7	121.2	120.9	120.8
21	141.0	141.1	141.5	141.7	141.0	141.9	140.5	141.2	140.6
22	109.8	109.7	109.8	110.0	109.7	109.7	109.1	109.4	109.8
23	142.9	143.1	143.0	142.8	143.1	143.0	143.1	143.1	143.5
18	17.9	18.4	18.0	17.3	17.7	21.6	21.2	21.4	27.0
19	17.9	17.5	16.7	16.7	17.6	15.8	16.4	15.6	17.0
28	23.2	23.3	23.3	20.2	23.0	22.6	22.7	22.7	23.2
29	23.6	23.1	23.0	23.8	23.7	20.2	22.9	22.8	23.1
30	33.8	33.9	33.7	33.6	34.0	123.0	123.5	123.1	63.0
$COOCH_3$	53.2	53.2	53.1	52.0	53.1	52.1	53.2	53.1	53.4
6-$OCOCH_3$			169.7					169.7	
6-$OCOCH_3$			21.0					21.0	
1’		170.3	170.3	167.4	167.2	167.1	166.9	166.9	166.8
2’		21.1	21.2	128.9	129.0	127.5	127.6	127.5	127.8
3’				139.3	139.0	139.5	139.0	139.5	139.6
2’-CH_3				12.3	12.2	14.6	11.6	11.7	12.3
3’-CH_3				14.6	14.5	11.8	14.5	14.6	14.6



	R_1	R_2
1	H	OH
2	$COCH_3$	OH
3	$COCH_3$	$OCOCH_3$
4	$COCCH_3$=$CHCH_3$	H
5	$COCCH_3$=$CHCH_3$	OH



	R
6	H
7	OH
8	$OCOCH_3$

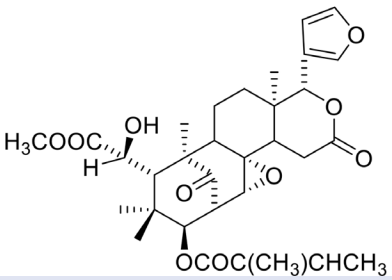


Figure 1: The structures of compounds**1**-**9**

Statistical analysis

Statistical analysis was performed using Student's *t* test. Data were expressed as the means ± standard deviation (SD). Statistical significance was represented with an asterisk for *p* values < 0.05, two asterisks for *p* values < 0.01 and three asterisks for *p* values < 0.001.

RESULTS

Identification of chemical structure of compounds from the seeds of *S. mahogani*

Nine B, D-*seco* limonoids (1-9) were isolated from the seeds of *S. mahogani* using series of column chromatographies and elucidated as swietenolide (1), 3-O-acetylswietenolide (2), 3, 6-*OO*-diacetylswietenolide (3), khayasin T (4), 3-*O*-tigloylswietenolide (5), febrifugin (6), swietenine (7), swietenine acetate (8) and swietemahonin E (9) by the various spectroscopic techniques [Figure 1].

Compound 1 exhibited molecular ion peak at m/z 487.2332 $[M+H]^+$ indicating $C_{27}H_{34}O_8$ as its molecular formula in the positive HRMS analysis. The IR spectrum showed the presence of hydroxyl (3496 cm^{-1}), carbonyl (1721 cm^{-1}) and furan ring (876cm^{-1}) as the functional groups. The ^1H, ^{13}C NMR and HMQC spectral data demonstrated the presence of one ketone at δ_C 219.7 (C-1), two carboxyl groups at δ_C 175.8 (C-7) and 171.3 (C-16), two hydroxyl groups at δ_H/δ_C3.54 (1H, J = 9.7 and 3.4 Hz, H-3)/78.5 (C-3) and 4.50 (1H, s, H-6)/73.6 (C-6), and four methyl groups at δ_H/δ_C0.95 (3H, s, H-18)/17.9 (C-18), 1.37 (3H, s, H-19)/17.9 (C-19) and 0.96 (3H, s, H-28)/23.2 (C-28), 0.83 (3H, s, H-29)/23.6 (C-29). In addition, furan ring was present at δ_H/δ_C 120.8 (C-20) and 7.44 (1H, s, H-21)/141.0 (C-21), and 6.37 (1H, s, H-22)/109.8 (C-22) and 7.38 (1H, s, H-23)/142.9 (C-23). On the basis of above information, compound 1 was estimated to be a B, D-*seco* limonoid found in Meliaceae plants.[10] The HMBC correlations from δ_H5.44 (1H, s, H-17) to δ_C120.8 (C-20), 141.0 (C-21) and 109.8 (C-22) indicated the existence of the furan ring at C-17. A double bond existing between C-8 (δ_C 129.03) and C-14 (δ_C 130.78) was located in C-ring by the HMBC signals from δ_H3.15 (1H, dd, J = 14.4 and 2.3 Hz, H-30a) and 1.99 (1H, m, H-30b) to δ_C 129.0 (C-8) and 130.8 (C-14). With above data, the structure of compound 1 was established as swietenolide.[6] Compound 2 exhibiting the molecular ion peak at m/z 529.2438 $[M+H]^+$ has the molecular formula for $C_{29}H_{36}O_9$. The ^1H, ^{13}C NMR and HMQC spectrum demonstrated similar patterns with compound 1 except for the presence of an acetyl group at δ_C170.28 (C-1') and δ_H/δ_C 2.12 (3H, s, H-2')/21.1 (C-2') instead of the hydroxyl group at C-3 in compound 1. The location of the acetyl group was determined by the HMBC correlations from δ_H4.79 (1H, d, J = 9.9 Hz, H-3) to δ_C170.3 (C-1').On the basis of the above data, the structure of compound **2** was established as 3-*O*-acetylswietenolide.[6] Compound 3 with $C_{31}H_{38}O_{10}$ determined by the molecular ion peak at m/z 571.2543 $[M+H]^+$ showed the similar structure with compounds 1 and 2 by the ^1H, ^{13}C NMR and HMQC spectra. The HMBC analysis indicated that two acetyl groups were located at C-3 and C-6, respectively, by the signals at δ_H/δ_C4.85 (1H, d, J = 10.0 Hz, H-3)/170.3 (C-1') and 5.44 (1H, s, H-6)/169.7 (C-2"). Consequently, compound 3 was established as 3, 6-*O,O*-diacetyl swietenolide.[6] Compound 4 has $C_{32}H_{40}O_8$ as its molecular formula determined by the molecular ion peak at *m/z* 553.2801 $[M+H]^+$. Similar to compound 1 and 2, it had the tigloylmoiety at C-3 by the signals at δ_C167.4 (C-1'), 128.9 (C-2') and δ_H/δ_C 6.93 (1H, m, C-3')/139.3 (C-3'), 1.86 (3H, s, CH_3at C-2')/12.3 (CH_3 at C-2'), 1.81 (3H, m, CH_3 at H-3')/14.6 (CH_3 at C-3') by the 1D and 2D NMR spectroscopic data. Also the HMBC correlations of δ_H/δ_C4.82 (1H, d, *J*=9.7 Hz, H-3)/176.4 (C-1') indicated that the tigloyl group existed at C-3. With above data, the structure of compound 4 was determined as khayasin T.[6] Compound 5 with the molecular ion peak at m/z 569.2751

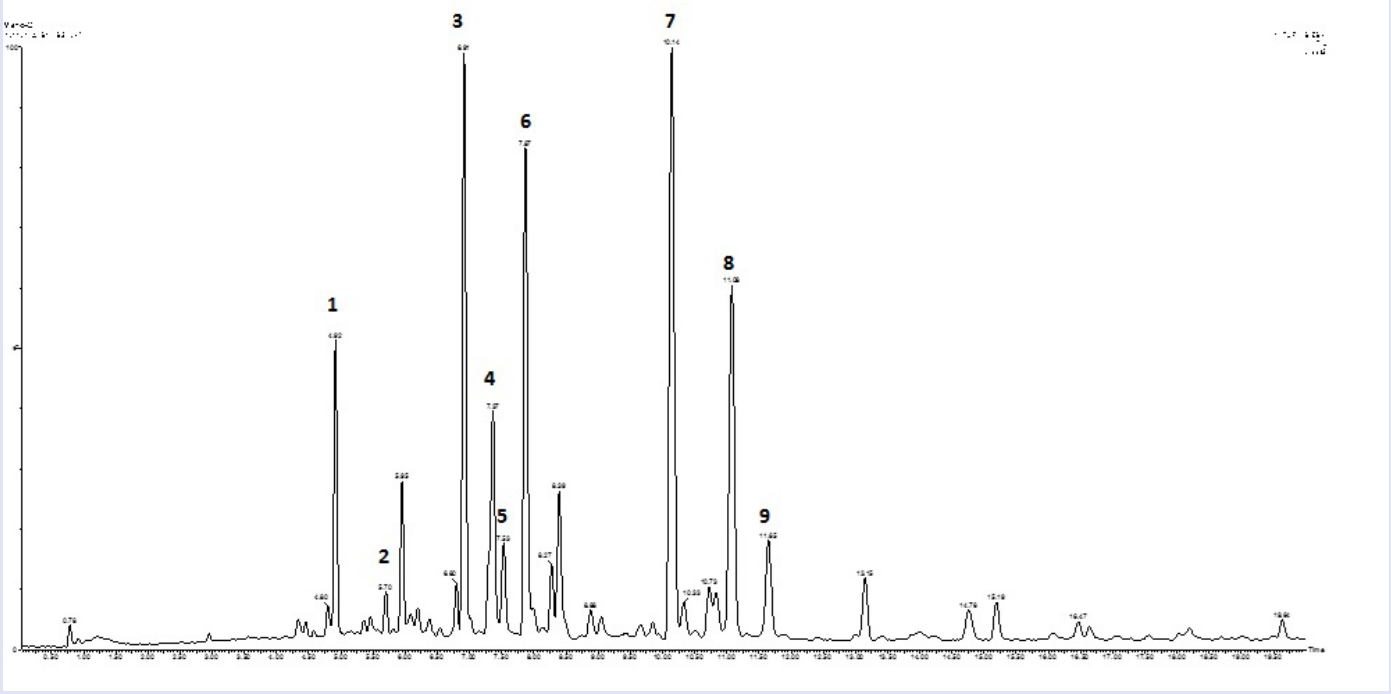


Figure 2: The chromatogram of $CHCl_3$ fraction from *S. mahogani* analyzed by UPLC-QTOF-MS.

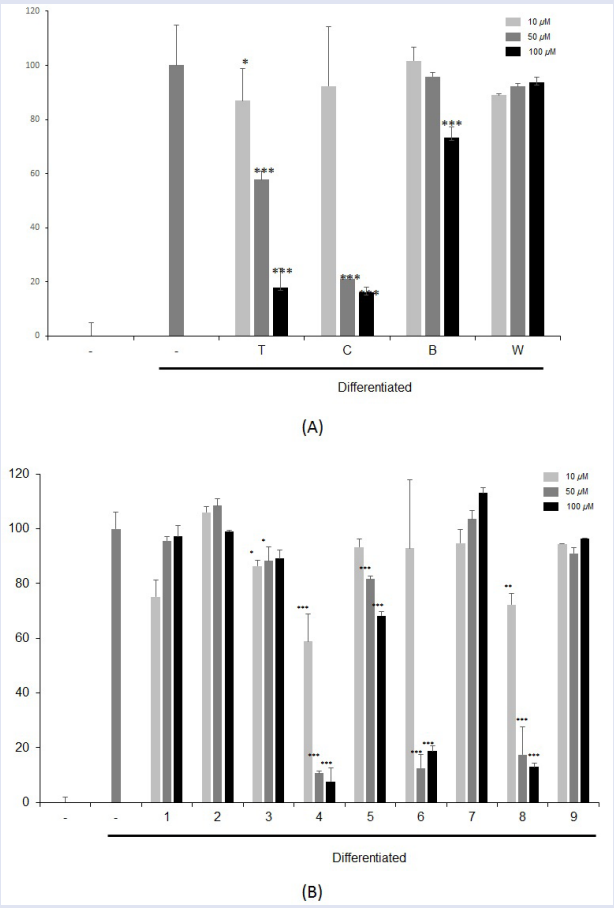


Figure 3: Inhibitory effect of total extract and fractions (A) and compounds **1-9** (B) of *S. mahogani* seeds on 3T3-L1 differentiation

$[M+H]^+$ with $C_{32}H_{40}O_9$ indicated the similar structure with compound 4. According to the HMBC correlations, compound **5** had a tigloyl moiety at C-3 by the characteristic values from δ_H4.67 (1H, d, J = 9.8 Hz, H-3) to δ_C167.2 (C-1'), from δ_H 1.88 (3H, m, CH_3at C-2') to δ_C 129.0 (C-2') and from δ_H 1.80 (3H, m, CH_3at C-3') to δ_C 139.0 (C-3'). A hydroxyl moiety was located at C-6 by the signals at δ_H/δ_C3.34 (1H, s, H-6)/73.3 (C-6). Therefore, compound 5 was identified as 3-*O*-tigloyl swietenolide.[6] Compound 6 with $C_{32}H_{40}O_8$ by the molecular ion peak at m/z 553.2808 $[M+H]^+$. Compound 6 was significantly similar to those of compound 4 by 1D and 2D NMR spectroscopic data except for the signals responding to the double bond at δ_H/δ_C138.5 (C-8) and 5.30 (1H, d, J = 7.1 Hz, H-30)/123.0 (C-30). Thus, compound 6 was established as febrifugin.[11] Compounds **7** ($C_{32}H_{40}O_9$, m/z 569.2751 $[M+H]^+$) and 8 (m/z 611.2857 $[M+H]^+$, $C_{34}H_{42}O_{10}$) had same backbones with compound 6 except for the derivatives attached at C-6 in 1D and 2D NMR spectra data. Compounds **7** and 8 has a hydroxyl moiety indicated by δ_C72.7 (C-6 in **7**) and acetyl moiety by δ_C169.7 ($COCH_3$ in 8) and 21.0 ($COCH_3$ in 8) instead of a hydrogen bond, respectively. Therefore, they were identified as swietenine (**7**) and swietenine acetate (8), respectively.[6] Compound 9 ($C_{32}H_{40}O_{10}$, *m/z* 585.2717 $[M+H]^+$) had one more oxygen molecule by the comparison to compound **7**. Two oxygenated signals at δ_C60.3 (C-8) and 63.0 (C-30) existed instead of those of a double bond. Their up-fielded signals indicated the presence of an epoxide moiety. Consequently, the structure of compound 9 was established as swietemahonin E.[12]

Chemical fingerprint of *S. mahogani* seeds using ESI-QTOF-MS

The $CHCl_3$ fraction of the seeds of *S. mahogani* was analyzed to monitor the chemical profile by UPLC-ESI-QTOF-MS [Figure 2]. Nine compounds isolated from $CHCl_3$ fraction were detected and identified by the comparison of the retention times and *m/z* valuesin $CHCl_3$ fraction compared with those of compounds 1-9 [Table 3].

Table 3: Spectral and chromatographic data of compounds **1-9** detected in $CHCl_3$ fraction of ***S. mahogani*** seeds.

Peak	Compound name	RT (min)	Mol. formula	$[M+H]^+$
1	swietenolide	4.92	$C_{27}H_{34}O_8$	487.2336
2	3-*O*-acetylswietenolide	5.70	$C_{29}H_{36}O_9$	529.2438
3	swietenine	6.91	$C_{32}H_{40}O_9$	569.2748
4	3-*O*-tigloylswietenolide	7.37	$C_{32}H_{40}O_9$	569.2744
5	swietemahonin E	7.53	$C_{32}H_{40}O_{10}$	585.2700
6	3,6-*O*,*O*-diacetylswietenolide	7.89	$C_{31}H_{38}O_{10}$	571.2543
7	swietenine acetate	10.14	$C_{34}H_{42}O_{10}$	611.2856
8	febrifugin	11.08	$C_{32}H_{40}O_9$	553.2808
9	khayasin T	11.65	$C_{32}H_{40}O_9$	553.2801

Anti-differentiation activities of total extract, fractions and the compounds from the seeds of *S. mahogani* against 3T3-L1 cells

Inhibitory effect on adipocyte differentiation in 3T3-L1 cells was investigated with total extract, $CHCl_3$ and BuOH fraction, and aqueous residue [Figure 3A]. After inducing of the differentiation of 3T3-L1 cells, total extract and $CHCl_3$ fraction showed significant inhibitory activities on 3T3-L1 cell differentiation compared to the other fractions, BuOH fraction and aqueous residue.

Compounds 1-9 isolated from $CHCl_3$ fraction were treated on 3T3-L1 cells [Figure 3B]. Among them, compounds 4, 6 and 8 showed significant anti-differentiation activities in dose-dependent manner at the concentrations of 50 and 100 μM against 3T3-L1 cells.

DISCUSSION

S. mahogani produces active secondary metabolites, limonoids, which have been known to possess a wide range of biological activities. We isolated nine B, D-*seco* limonoids derivatives with slight modifications, such as hydroxylation, acetylation and tigloylation, from the seeds of *S. mahogani* and evaluated their anti-differentiation activities against 3T3-L1 cells. Blocking adipogenesis (adipocyte differentiation) has been considered as an effective prevention strategy for obesity. 3T3-L1 cells are being used as a useful tool to investigate biological factors affecting adipogenesis. We performed *in vitro* study for the evaluation of the inhibitory effect on adipocyte differentiation in 3T3-L1 cells by total extract, sub-fractions, and B, D-*seco* limonoids (1-9) isolated from $CHCl_3$ fraction. Compounds 1-9 were highly substituted with hydroxyl, acetyl or tigloyl moieties and showed different inhibitory activities against 3T3-L1 cells. Compounds 4, 6 and 8 showing the significant anti-differentiation activities had a tigloyl moiety at C-3 in common. But it is in appropriate to make an assumption that it is essential for leading to inhibitory effect on adipocyte differentiation because compounds 5, 7 and 9 having a tigloyl moiety showed weak inhibitory activities. It was estimated that a tigloyl residue may not be an essential factor for anti-differentiation of 3T3-L1 cells, but give an effect on anti-differentiation.

CONCLUSION

Limonoids are known to have a wide range of activities including anti-malarial, anti-cancer, anti-HIV, and anti-feedant on insects. We reported B, D-*seco* limonoids have an anti-differentiation against 3T3-L1 cells. It might need the structure and activity relationship study against the 3T3-L1 cells differentiation according to the types of backbone or functional groups in the further study.

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Nil

Conflicts of interest

There are no conflicts of interest.

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