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

Hee Jung Yang, Mina Choi, Dong Young Lee, Sang Hyun Sung

College of Pharmacy, Kangwon National University, Chuncheon, College of Pharmacy and Research Institute of Pharmaceutical Science, Seoul National University, Seoul, Republic of Korea

Submitted: 20-01-2016 Revised: 10-02-2016 Published: 18-04-2017

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.

INTRODUCTION

Obesity is a major cause of the risk factors for various metabolic and cardiovascular diseases such as fatty liver, diabetes, hypertension, and hyperlipidemia. Adipogenesis is a process where fibroelastic preadipocytes become mature adipocytes. It brings the abnormal accumulation by an increase in the sizes or numbers of adipocytes from precursor cells. Thus, the control of the differentiation of preadipocytes into 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. 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. 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 malarial Indonesian folk medicine. Decoction of its bark has been used as febrifuge or antimalarial drug. Furthermore, some studies reported that it acts as a blood platelet aggregation inhibitor. The well known constituents are mainly limonoids, especially B, D-seco limonoids, which are derivatives of limonoids oxidized rings B and D of limonoid. 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.

This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-Share Alike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

Cite this article as: Yang H, Choi M, Lee DY, Sung SH. Anti-differentiation effect of B, D-seco limonoids of Swietenia mahogani. Phcog Mag 2017;13:293-9.
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 H₂O and successively partitioned into n-hexane fraction (1.2 g), CHCl₃ fraction (110.9 g), EtOAc fraction (0.4 g), and n-BuOH fraction (6.7 g), respectively. Among these fractions, CHCl₃ fraction showing significant anti-differentiation effect on 3T3-L1 cells was subjected to repeated column chromatography and HPLC to give compounds 1-9.

The CHCl₃ fraction was subjected to silica gel column chromatography (CC) and eluted with mixtures of n-hexane-EtOAc = 10:1, 3:1, 1:1, CHCl₃-MeOH = 10:1, to yield twelve fractions (C1 ~ C12) and compound 1 (1401 mg). C6 was subjected to ODS silica gel HPLC with MeCN-H₂O= 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 ODS silica gel HPLC with MeCN-CHCl₃ (CC) and eluted with mixtures of MeCN-CHCl₃ = 7:3, 6 ml/min) to give compound 4 (81 mg). C8 was subjected to ODS silica gel HPLC with MeCN-H₂O= 10:1, 1:1 to give compound 5 (22 mg) and 9 (209 mg).

**Swietenolide (1):** white amorphous powder; C₃₂H₅₀O₃ ×α [α]D: -140.1000 (c=0.1, chloroform); IR v max (KBr) (cm⁻¹): 3496, 1728, 876; HRMS (positive mode): m/z 487.2332 [M+H]⁺ (calc. mass: 487.2336); ¹³C NMR (CDCl₃, 500 MHz): see [Table 1]; ¹³C NMR (CDCl₃, 125 MHz): see [Table 2].

3-O-acetylswietenolide (2): white amorphous powder; C₃₀H₅₀O₃ ×α [α]D: -90.1000 (c=0.1, chloroform); IR v max (KBr) (cm⁻¹): 3495, 1732, 876; HRMS (positive mode): m/z 529.2438 [M+H]⁺ (calc. mass: 529.2438); ¹³C NMR (CDCl₃, 400 MHz): see [Table 1]; ¹³C NMR (CDCl₃, 100 MHz): see [Table 2].

3,6-Diacetylswietenolide (3): white amorphous powder; C₃₆H₆₀O₅ ×α [α]D: -206.7000 (c=0.1, chloroform); IR v max (KBr) (cm⁻¹): 1737, 876; HRMS (positive mode): m/z 571.2543 [M+H]⁺ (calc. mass: 571.2543); ¹H NMR (CDCl₃, 500 MHz): see [Table 1]; ¹³C NMR (CDCl₃, 125 MHz): see [Table 2].

khayasin T (4): white amorphous powder; C₃₂H₄₆O₃ ×α [α]D: -121.7000 (c=0.1, chloroform); IR v max (KBr) (cm⁻¹): 1728, 876; HRMS (positive mode): m/z 553.2801 [M+H]⁺ (calc. mass: 553.2801); ¹H NMR (CDCl₃, 400 MHz): see [Table 1]; ¹³C NMR (CDCl₃, 100 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 µg/mL 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% CO₂. 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 isopropl 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 isopropl alcohol for 5 minutes. The absorbance was measured at 544 nm. Lipid contents (% of control) = A₄₅₄nm (sample-undifferentiated control)/A₄₅₄nm (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        | 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 |
| 5        | 4.50 s | 4.52 s | 5.44 s | 2.36 m 2.38 m | 4.53 s | 4.52 s | 5.54 s | 4.44 s |     |
| 6        |     |     |     |     |     |     |     |     |     |
| 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 1.38 m 2.08 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 |     |
| 13       |     |     |     |     |     |     |     |     |     |
| 14       |     |     |     |     |     |     |     |     |     |
| 15       | 3.42 dt(21.1, 2.4) | 3.46 m 3.69 m | 3.43 dt(20.8, 2.45) | 3.19 m 3.59 m (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 |
| 18       |     |     |     |     |     |     |     |     |     |
| 19       |     |     |     |     |     |     |     |     |     |
| 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 |
| 24       |     |     |     |     |     |     |     |     |     |
| 25       |     |     |     |     |     |     |     |     |     |
| 26       |     |     |     |     |     |     |     |     |     |
| 27       |     |     |     |     |     |     |     |     |     |
| 28       |     |     |     |     |     |     |     |     |     |
| 29       |     |     |     |     |     |     |     |     |     |
| 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) | 3.70 s | 3.83 s | 3.69 s | 3.73 s | 3.70 s | 3.93 s |
| COOCH$_3$ | 3.78 s | 3.81 s | 3.73 s |     |     |     |     |     |     |
| 6-OCOCH$_3$ |     |     |     |     |     |     |     |     |     |
| 6-OCOCH$_3$ | 2.15 s | 2.15 s |     |     |     |     |     |     |     |
| 1'       |     |     |     |     |     |     |     |     |     |
| 2'       | 2.15 s | 2.12 s | 2.15 s |     |     |     |     |     |     |
| 3'       |     |     |     |     |     |     |     |     |     |
| 2'-CH$_3$ |     |     |     |     |     |     |     |     |     |
| 3'-CH$_3$ |     |     |     |     |     |     |     |     |     |
**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  |
| 18       | 120.8 | 120.7 | 120.5 | 120.8 | 120.8 | 120.7 | 121.2 | 120.9 | 120.8 |
| 19       | 141.0 | 141.1 | 141.5 | 141.7 | 141.0 | 141.9 | 140.5 | 141.2 | 140.6 |
| 20       | 109.8 | 109.7 | 109.8 | 110.0 | 109.7 | 109.7 | 109.1 | 109.4 | 109.8 |
| 21       | 142.9 | 143.1 | 143.0 | 142.8 | 143.1 | 143.0 | 143.1 | 143.1 | 143.5 |
| 22       | 17.9  | 18.4  | 18.0  | 17.3  | 17.7  | 21.6  | 21.2  | 21.4  | 27.0  |
| 23       | 17.9  | 17.5  | 16.7  | 16.7  | 17.6  | 15.8  | 16.4  | 15.6  | 17.0  |
| 24       | 23.2  | 23.3  | 23.3  | 20.2  | 23.0  | 22.6  | 22.7  | 22.7  | 23.2  |
| 25       | 23.6  | 23.1  | 23.0  | 23.8  | 23.7  | 20.2  | 22.9  | 22.8  | 23.1  |
| 26       | 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-OOCOCH$_3$ | 169.7 | 169.7  | 21.0  | 21.0  | 21.0  | 21.0  | 21.0  | 21.0  | 21.0  |
| 1’       | 170.3 | 170.3 | 167.4 | 167.2 | 167.1 | 166.9 | 166.9 | 166.9 | 166.8 |
| 2’       | 21.1  | 21.2  | 128.9 | 129.0 | 127.5 | 127.6 | 127.5 | 127.8 | 127.8 |
| 3’       | 139.3 | 139.0 | 139.5 | 139.0 | 139.0 | 139.5 | 139.5 | 139.6 | 139.6 |
| 2’-CH$_3$ | 12.3  | 12.2  | 14.6  | 11.6  | 11.7  | 11.7  | 11.7  | 12.3  | 12.3  |
| 3’-CH$_3$ | 14.6  | 14.5  | 11.8  | 14.5  | 14.6  | 14.6  | 14.6  | 14.6  | 14.6  |
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-O-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 C25H24O6 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 (876 cm⁻¹) as the functional groups. The 1H, 13C NMR and HMQC spectral data demonstrated the presence of one ketone at δ170.3 (C-1) and two benzyl groups at δ170.7 (C-2') and 171.3 (C-3') as the functional groups. The HMBC correlations from δ170.3 (C-1) and 5.44 (1H, s, H-17) to 170.28 (C-2') and 170.38 (C-3') by the 1D and 2D NMR spectroscopic techniques showed the similar structure with compounds 1 and 2 by the HMBC correlations from δ170.3 (C-1) and 5.44 (1H, s, H-17) to 170.28 (C-2') and 170.38 (C-3') by the 1D and 2D NMR spectroscopic techniques. The HMBC correlations from δ170.3 (C-1) and 5.44 (1H, s, H-17) to 170.28 (C-2') and 170.38 (C-3') by the 1D and 2D NMR spectroscopic techniques showed the similar structure with compounds 1 and 2 by the HMBC correlations from δ170.3 (C-1) and 5.44 (1H, s, H-17) to 170.28 (C-2') and 170.38 (C-3') by the 1D and 2D NMR spectroscopic techniques.

The HMBC correlations from δ170.3 (C-1) and 5.44 (1H, s, H-17) to 170.28 (C-2') and 170.38 (C-3') by the 1D and 2D NMR spectroscopic techniques showed the similar structure with compounds 1 and 2 by the HMBC correlations from δ170.3 (C-1) and 5.44 (1H, s, H-17) to 170.28 (C-2') and 170.38 (C-3') by the 1D and 2D NMR spectroscopic techniques.

Compound 2 exhibiting the molecular ion peak at m/z 529.2438 [M+H]+ has the molecular formula for C25H22O6. The 1H, 13C NMR and HMQC spectrum demonstrated similar patterns with compound 1 except for the presence of an acetyl group at δ2.12 (3H, s, H-2') and 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 δ6.93 (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 established as a B, D-seco limonoid found in Meliaceae plants. The HMBC correlations from δ5.44 (1H, s, H-17) to 170.28 (C-2') and 170.38 (C-3') by the 1D and 2D NMR spectroscopic techniques showed the similar structure with compounds 1 and 2 by the HMBC correlations from δ170.3 (C-1) and 5.44 (1H, s, H-17) to 170.28 (C-2') and 170.38 (C-3') by the 1D and 2D NMR spectroscopic techniques.

Compound 3 exhibiting the molecular ion peak at m/z 571.2543 [M+H]+ showed the similar structure with compounds 1 and 2 by the HMBC correlations from δ5.44 (1H, s, H-17) to 170.28 (C-2') and 170.38 (C-3') by the 1D and 2D NMR spectroscopic techniques. The HMBC analysis indicated that two acetoxyl groups were located in C-ring by the HMBC signals from δ3.15 (1H, dd, J = 14.4 and 2.3 Hz, H-30a) and 1.99 (1H, m, H-30b) to δ129.0 (C-8) and 130.8 (C-14). With above data, the structure of compound 3 was determined as 3-O-acetylswietenolide. The HMBC correlations from δ5.44 (1H, s, H-17) to 170.28 (C-2') and 170.38 (C-3') by the 1D and 2D NMR spectroscopic techniques showed the similar structure with compounds 1 and 2 by the HMBC correlations from δ5.44 (1H, s, H-17) to 170.28 (C-2') and 170.38 (C-3') by the 1D and 2D NMR spectroscopic techniques.

Compound 4 has C25H20O6 as its molecular formula determined by the molecular ion peak at m/z 535.2801 [M+H]+. Similar to compound 1 and 2, it had the tigloylmoiety at C-3 by the signals at δ167.4 (C-1'), 128.9 (C-2') and δ5.44 (1H, s, H-17)/170.3 (C-2') and 5.44 (1H, s, H-17)/169.7 (C-2'). Consequently, compound 3 was established as 3, 6-O-diacetylswietenolide. Compound 4 has C25H20O6 as its molecular formula determined by the molecular ion peak at m/z 535.2801 [M+H]+. Similar to compound 1 and 2, it had the tigloylmoiety at C-3 by the signals at δ167.4 (C-1'), 128.9 (C-2') and δ5.44 (1H, s, H-17)/170.3 (C-2') and 5.44 (1H, s, H-17)/169.7 (C-2'). Consequently, compound 3 was established as 3, 6-O-diacetylswietenolide.
Figure 2: The chromatogram of CHCl₃ 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.
HEEJUNG YANG, et al.: Anti-Differentiation Effect of B, D-Seco limonoids

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, CHCl₃ and BuOH fraction, and aqueous residue [Figure 3A]. After inducing of the differentiation of 3T3-L1 cells, total extract and CHCl₃ 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 CHCl₃ 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 CHCl₃ 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 hydroxyl, acetyl or tigloyl moieties and showed different inhibitory activities from CHCl₃ total extract, sub-fractions, and B, D-seco limonoids derivatives with slight modifications, 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.

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.

Acknowledgement

This research was supported by a grant of the Korea Health Technology R and D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health and Welfare, Republic of Korea (No. HI15C0075) and 2015 Research Grant from Kangwon National University (No. 520150296)

Financial support and sponsorship

Nil

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Park HJ, Cho JY, Kim MK. Anti-obesity effect of Schisandra chinensis in 3T3-L1 cells and high fat diet-induced obese rats. Food Chem 2012;134:227-34.
2. Kim CK, Kim M, Oh SD. Effects of Atractylodes macrocephala Koidzumi rhizome on 3T3-L1 adipogenesis and an animal model of obesity. J Ethnopharmacol 2011;137:396-402.
3. Lee M, Lee HH, Lee JK, Ye SK, Kim SH, Sung SH. Anti-adipogenic activity of compounds isolated from Idesia polycarpa on 3T3-L1 cells. Bioorg Med Chem Lett 2013;23:3170-4.
4. Wadden TA. Treatment of Obesity by Moderate and Severe Caloric Restriction-Results of Clinical Research Trials. Ann Intern Med 1993;119:688-93.
5. Green H, Kehinde O. Established Pre-Adipose Cell Line and Its Differentiation in Culture 2. Factors Affecting Adipose Conversion. Cell 1975;5:19-27.
6. Kadota S, Marpaung L, Kikuchi T, Ekimoto H. Constituents of the Seeds of Swietenia-Mahagoni Jacq.1. Isolation, Structures, and H-1-Nuclear and C-13-Nuclear Magnetic-Resonance Signal Assignments of New Tetranortriterpenoids Related to Swietenine and Swietenolide. Chem Pharm Bull 1990;38:639-51.
7. Nagalakshmi MAH, Thangadurai D, Rao DM, Puliaiah T. Phytochemical and antimicrobial study of Chukrasia tabularis leaves. Fitoterapia 2001;72:82-4.
8. Kadota S, Marpaung L, Kikuchi T, Ekimoto H. Antagonists of Platelet Activating Factor from Swietenia-Mahagoni (L) Jacq. Tetrahedron Lett 1989;30:1111-14.
9. Nakatani M, Abdelgaleli SIAM, Saadi MMG, Huang RC, Doe M, Iwagawa T. Pragmalin limonoids from Chukrasia tabularis. Phytochemistry 2004;65:2833-41.
10. Narendran T, Khaliq T, Shweta. 13 C NMR spectroscopy of D and B, D-rings seco-limonoids of Mielicaee family. Nat Prod Res 2008;22:763-800.
11. Leite AC, Fernandes JB, da Silva MFDF, Vieira PC. Limonoids from Cipadessa fruticosa. Z Naturforsch B 2005;60:351-55.
12. Kadota S, Marpaung L, Kikuchi T, Ekimoto H. Constituents of the Seeds of Swietenia-Mahagoni Jacq. 2. Structures of Swietenmahonin-a, Swietenmahonin-b, Swietenmahonin-c, Swietenmahonin-d, Swietenmahonin-e, Swietenmahonin-f, and Swietenmahonin-g and Swietenolmahonide. Chem Pharm Bull 1990;38:949-901.