Cytotoxic Triterpenoids from the Stem Barks of Dysoxylum arborescens and Dysoxylum excelsum against MCF-7 Breast Cancer Cell

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ABSTRACT

Three triterpenoids, taraxerone (1) and 18-epi-taraxerol (2), and masticadienolic acid (3) were isolated for the compounds was achieved by various extraction and chromatography column techniques, while their chemical structures were elucidated based on spectroscopic data and literature comparison. Compounds 1, 2, and 3 were found to show inhibitory activity against MCF-7 breast cancer cells with IC50 values 792.9, 59.6, and 3.5 µM correspondingly.

Keywords: Dysoxylum arborescens; Dysoxylum excelsum; MCF-7; triterpenoids

INTRODUCTION

Dysoxylum arborescens and Dysoxylum excelsum are members family of Meliaceae which are naturally members distributed in Asia - China, Taiwan, Andaman and Nicobar Islands, Malaysia, Indonesia to New Guinea, Northeast Australia, Solomon Islands, Vanuatu, India, and Southeast Asia (Mabberley 2008). Various types of compounds have been isolated from this genus, such as chromone alkaloids (Morita et al. 2014) sesquiterpenoids (Nugroho et al. 2015), diterpenoids (Duh et al. 2000) limonoids, and triterpenoids (Han et al. 2014). Some of them were reported to have interesting biological activities (Laksmi et al. 2009) such as cytotoxic sesquiterpenoids (Nugroho et al. 2015), antitumor diterpenoids (Fujioka et al. 1998), cytotoxic tirucallane triterpene (Kurimoto et al. 2011; Mohamad et al. 1999), cytotoxic triterpenoids and limonoids (Han et al. 2015; Zhou et al. 2015), antibacterial triterpenoids (Hu et al. 2014; Liu et al. 2011) and antibacterial steroids (Wah et al. 2013). As the tetracyclic and pentacyclic terpenoids typical compounds in the family of Meliaceae showed interesting bioactivities, herein we reported for the first time, the isolation and structural elucidation of two cytotoxic triterpenoids from the stem barks of D. arborescens and one from D. excelsum against MCF-7 breast cancer cells.

MATERIALS AND METHODS

GENERAL

Melting points were measured on Mettler Toledo MP50 apparatus and were uncorrected. Mass spectra were obtained from Water HR-QToF-MS, The IR spectra were recorded on Perkin-Elmer spectrum-100 FT-IR in KBr pellets. The 1H and 13C-NMR spectra were measured on JEOL JNM ECA-500 spectrometer using TMS as internal standard. Column chromatography was conducted on silica gel 60 (Merck, Germany) and RP-18 silica gel (Merck, Germany). TLC plates were precoated with silica gel GF254 (Merck, Germany, 0.25 mm) and detection was achieved by spraying with H2SO4 in ethanol (10%, v/v), followed by heating.

PLANT MATERIALS

The stem barks of D. arborescens were collected in Bogor Botanical Garden (Collection Number: III.E.64), Bogor, West Java Province, Indonesia in July 2014.
while the stem barks of *D. excelsum* were in June 2016 (Collection Number: III.F.67). The plants were identified by staff of Bogor Botanical Garden, Indonesian Science Institute.

**EXTRACTION AND ISOLATION**

The dried stem barks of *D. arborescens* (1.4 kg) were extracted with methanol exhaustively (5 L) at room temperature for 3 days. After removal of the solvent under reduced pressure, the viscous methanolic extract (61.1 g) was first suspended in H₂O and then partitioned with n-hexane and EtOAc, successively. The ethyl acetate crude (12.4 g) was subjected to chromatography column over silica gel using a gradient elution of n-hexane, EtOAc, and MeOH to afford 16 fractions (A-P). Fraction C (230 mg) was further purified on silica gel chromatography column, with mobile phase of n-hexane: dichloromethane (10:0–2:8, v/v) to afford 4 fractions (C1-C4). The fraction of C2 gave compound I (12 mg) and C3 gave compound 2 (11 mg).

The dried stem barks of *D. excelsum* (2.7 kg) were treated in a manner similar to that of *D. arborescens* to produce 300 g of crude extract. The ethyl acetate-soluble fraction (30.0 g) was subjected to chromatography column over silica gel giving a gradient elution of n-hexane and EtOAc to give 8 fractions (F1-F8). Fraction F7 (5.8 g) was then separated on silica gel chromatography column and eluted with the mixtures of n-hexane/EtOAc (10:0–8:2, v/v) to give 3 fractions (F7a-F7c). Further purification was applied to the fraction of F7c (silica gel, n-hexane: acetone (10.0-7.3, v/v) to yield 4 fractions (F7c1-F7c4). The fraction of F7c2 gave compound 3 (20 mg).

Taraxerone (1) White needle-like crystals; m.p °C: 239.8-241.3; IR (KBr) νmax cm⁻¹: 3048, 2930, 1709, 1640, 1376; 1H-NMR (CDCl₃, 500 MHz), (Table 1); 13C-NMR (CDCl₃, 125 MHz), (Table 1); HR-ToF-MS (ESI) m/z 447.3675 (calc. m/z for C₃₀H₄₈O₅ 447.3597). Supported by its 1H and 13C NMR data (Table 1), the MS showed the molecular formula of C₃₀H₄₈O₅ with 7 degrees of unsaturation. The IR spectrum showed absorption peaks at 3048 (C-H sp²), 2930 (C-H), 1709 (C=O), 1640 (C=C), and 1376 cm⁻¹ (gem dimethyl). The 1H-NMR spectrum exhibited the presence of eight tertiary methyl groups which resonated at δH: 0.91, 0.83, 0.91, 0.95, 1.06, 1.08, 1.08 and 1.14 ppm assigned to H-26, H-28, H-30, H-29, H-24, H-23, H-25 and H-27, respectively. The presence of all methyl groups as singlet supported the fact that all methyl groups in compound I are attached to quaternary carbons. Double doublet signal appeared at δH 1.02 (J = 4.5 Hz) showed the presence of methine sp³ while another double doublet signal at 5.56 (J = 3.2 Hz) confirmed the presence of one olefinic proton at H-15 coupled with the protons of H-16. The ¹H-NMR, ¹³C-NMR and DEPT (SI.5) spectra of I showed 30 resonances, assigned to eight methyl groups, ten methylene sp³ groups, three methines sp³, one methine sp², six quaternary sp³ carbons and two quaternary sp². A carbonyl group was observed at δC 171.8. The double bond was represented by two singlets at δC 157.8 and 117.4 for carbons C-14 and C-15, respectively. The ¹H and ¹³C-NMR together with HR-ToF-MS data, suggested the presence of a typical pentacyclic triterpene skeleton. The structure elucidation of compound I was supported by HM.RC, COSY, and HMBC (SI.6-8) spectra as well as comparing with those reported previously (Ragasa et al. 2014) (SI.22 and 23). Thus compound I was identified as taraxerone.

Compound 2 was obtained as a white needle-like crystals; m.p °C: 282.3-283.0. The HR-ToF-MS spectrum of 2 exhibited a quasi molecular ion with sodium at m/z 449.3658 (calc. m/z for C₃₀H₅₀ONa 449.3764). Supported by its ¹H and ¹³C-NMR data (Table 1), the MS showed the molecular formula of C₃₀H₅₀O with 6 degrees of unsaturation. The IR spectrum showed absorption peaks at 3486 (O-H), 3054 (C=C), 2934 (C-H), 1641 (C=C), while another...
and 1382 cm⁻¹ (gem dimethyl). The ¹H-NMR spectrum exhibited the presences of eight methyl groups which resonated at δ_H 0.82, 0.83, 0.91, 0.92, 0.94, 0.96, 0.99, and 1.10 ppm assigned to H-24, H-28, H-27, H-30, H-25, H-29, H-23 and H-26 respectively. The presence of all methyl groups as singlet supported the fact that all methyl groups in compound 2 are attached to quaternary carbons. Double doublet signal appeared at δ_H 0.97 (J = 5 Hz) showed the presence of methine sp³, while another double doublet signal appeared at 5.55 (J = 3.0 Hz) coupled with the protons of H-16. The ¹H, and ¹³C-NMR spectra of 2 showed 30 resonances, assigned to eight methyl groups, ten methylene sp³ groups, four methines sp³, one methine sp², six quaternary sp³ carbons and one quaternary sp². All of these spectra were similar with 1, other than the substituent at position 3. The position of the carbonyl group in compound 1 is replaced by the hydroxy group in compound 2 which is showed in ¹H-NMR of compound 2 at 3.21 ppm (1H, d, J = 7 Hz) and ¹³C-NMR at 218.8 and 79.1 ppm (each for compound 1 and 2, respectively). The HMBC crosspeak (SI.14) from methylene protons H-2 (δ_H 1.60 and 1.57) and H-1 (δ_H 1.61 and 0.96) to the oxymethine at δ_C 79.1, indicated the presence of a hydroxyl group at C-3. Methine proton at H-18 (δ_H 0.97 ppm, 1H, dd, J = 5) was indicated H-18 equatorial and thus the conformation was α-oriented. Supporting data of HSQC (SI.13) and HMBC Spectra (SI.14) as well as comparison of the NMR data of 2 with 18-epi-taraxerol (Mejin 2009) (SI.22 and 23) showed that the structures of the two compounds were very similar. Therefore compound 2 was identified as 18-epi-taraxerol.

Compound 3 was obtained as a white needle-like crystals, m.p °C 128.4-131.0. The HR-ToF-MS spectrum showed [M-H]⁻ at m/z 455.3831 (calc. 455.3531). Supported by its ¹H and ¹³C-NMR data (Table 1), the MS showed the molecular formula of C₃₀H₄₈O₃ and thus required seven degrees of unsaturation. The IR spectrum showed absorption peaks at 3420 (O-H), 2955 (C-H), 1680 (C=O), 1620 (C=C) and 1360 cm⁻¹ (gem dimethyl). The ¹H-NMR spectrum exhibited the presences of seven methyl groups, five singlets which resonated at δ_H: 0.76, 0.83, 0.91, 0.93 and 0.97 ppm for H-19, H-29, H-18, H-28 and H-30, respectively. A doublet and a singlet signal was observed at δ_H 0.89 and 1.91 ppm, respectively, which were assigned to H-21 and H-27 that attached to a quaternary sp² carbon. The ¹H, ¹³C-NMR and HSQC (SI.19) spectra of 3 showed 30 resonances, assigned to seven methyl groups, nine methylene sp³ groups, four methines sp³, four quaternary sp³ carbons, two quaternary sp² carbons, two methylene sp² groups, one oxygenated methine sp² and one carbonyl carbon. The position of carbons and protons was confirmed by HMBC (SI.20) and ¹H-¹H COSY spectra (SI.21). The structure of the euphane/tirucallane tetracyclic system (A, B, C and D) was determined by HMBC crosspeaks from H-18 (δ_H 0.91) to the C-20 (δ_C 36.2) required that the side chain was connected to C-20. Based on the literature data reported previously by Makino et al. (2003) and Unang et al. (2019) (SI.22 and 23), compound 3 was identified as masticadienolic acid.
TABLE 1. NMR Data (500 MHz for $^1$H and 125 MHz for $^{13}$C, in CDCl$_3$) for 1, taraxerone, 2, 18-epi-taraxerol, and 3, masticadienolic acid

| Position | $^{13}$C NMR $\delta_c$ | $^1$H NMR $\delta$ (Int., mult, $J$=Hz) | $^{13}$C NMR $\delta_c$ | $^1$H NMR $\delta$ (Int., mult, $J$=Hz) | $^{13}$C NMR $\delta_c$ | $^1$H NMR $\delta$ (Int., mult, $J$=Hz) |
|----------|----------------|----------------------------------|----------------|----------------------------------|----------------|----------------------------------|
| 1        | 38.5           | 1.87;1.36 (2H, m)                | 37.7           | 1.61;0.96 (2H, m)                | 31.4           | 1.47;1.39 (2H, m)                |
| 2        | 34.3           | 2.57;2.33 (2H, m)                | 27.1           | 1.60;1.57 (2H, m)                | 25.5           | 1.95;1.63 (2H, m)                |
| 3        | 217.8          | -                                | 79.0           | 3.21 (1H, dd, 7)                 | 76.5           | 3.46 (1H, s)                     |
| 4        | 47.7           | -                                | 38.7           | -                                | 37.5           | -                                |
| 5        | 55.9           | 1.34 (1H, m)                     | 55.5           | 0.79 (1H, dd, 11)                | 44.7           | 1.76 (1H, m)                     |
| 6        | 20.1           | 1.56;1.57 (2H, m)                | 18.7           | 1.61;1.48 (2H, m)                | 24.0           | 2.13;1.91 (2H, m)                |
| 7        | 35.2           | 1.35;1.34 (2H, m)                | 41.3           | 1.36;2.05 (2H, dd, 13)           | 118.0          | 5.25 (1H, s)                     |
| 8        | 39.0           | -                                | 38.9           | -                                | 146.2          | -                                |
| 9        | 48.8           | 1.50 (1H, m)                     | 49.2           | 1.43 (1H, m)                     | 49.0           | 2.30 (1H, bd, 11)               |
| 10       | 35.9           | -                                | 37.9           | -                                | 34.8           | -                                |
| 11       | 17.6           | 1.54;1.67 (2H, m)                | 17.4           | 1.65;1.48 (2H, m)                | 18.1           | 1.52;1.47 (2H, m)                |
| 12       | 37.8           | 1.64;1.92 (2H, dd, 11.7)         | 33.6           | 1.60;1.54 (2H, m)                | 33.9           | 1.78;1.60 (2H, m)                |
| 13       | 37.9           | -                                | 37.5           | -                                | 43.6           | -                                |
| 14       | 157.7          | -                                | 158.0          | -                                | 51.3           | -                                |
| 15       | 117.3          | 5.56 (1H, d, 3.25)               | 116.8          | 5.55 (1H, dd, 3)                 | 34.1           | 1.43;1.45 (2H, m)                |
| 16       | 36.8           | 1.32;1.26 (2H, m, 3.25)          | 37.7           | 1.63;1.93 (2H, dd,3)             | 27.0           | 2.56;2.44 (2H, m)                |
| 17       | 37.7           | -                                | 35.7           | -                                | 52.9           | 1.47 (1H, m)                     |
| 18       | 48.9           | 1.02 (1H, dd, 4.5)               | 48.7           | 0.97 (1H, m, 5)                  | 21.9           | 0.91 (3H, s)                     |
| 19       | 40.7           | 2.07;1.31 (2H, m, 4.5)           | 36.6           | 1.33;0.97 (2H, m)                | 13.1           | 0.76 (3H, s)                     |
| 20       | 28.9           | -                                | 28.7           | -                                | 36.2           | 1.39 (1H, m)                     |
| 21       | 33.7           | 1.67;1.57 (2H, m)                | 33.0           | 1.27;1.35 (2H, m)                | 18.4           | 0.89 (3H, d, 5.5)                |
| 22       | 33.2           | 1.38;1.24 (2H, m)                | 35.1           | 1.37;1.01 (2H, m)                | 35.8           | 1.52;1.14 (2H, m)                |
| 23       | 26.2           | 1.08 (3H, s)                     | 27.9           | 0.99 (3H, s)                     | 28.3           | 1.95;1.25 (2H, m)                |
| 24       | 21.6           | 1.06 (3H, s)                     | 15.4           | 0.82 (3H, s)                     | 147.3          | 6.08 (1H, s)                     |
| 25       | 14.9           | 1.08 (3H, s)                     | 15.4           | 0.94 (3H, s)                     | 125.8          | -                                |
| 26       | 30.0           | 0.83 (3H, s)                     | 25.9           | 1.10 (3H, s)                     | 172.7          | -                                |
| 27       | 25.7           | 1.14 (3H, s)                     | 21.3           | 0.92 (3H, s)                     | 20.7           | 1.91 (3H, s)                     |
| 28       | 30.1           | 0.91 (3H, s)                     | 29.8           | 0.83 (3H, s)                     | 27.9           | 0.93 (3H, s)                     |
| 29       | 33.5           | 0.95 (3H, s)                     | 33.3           | 0.96 (3H, s)                     | 22.0           | 0.83 (3H, s)                     |
| 30       | 21.5           | 0.91 (3H, s)                     | 29.9           | 0.92 (3H, s)                     | 27.4           | 0.97 (3H, s)                     |

Overall, compounds 1 and 2 (Figure 1) were isolated for the first time from the genus *Dysoxylum*, while compound 3 was the first time from the corresponding species. The presence of euphan/tirucallane-type compounds like masticadienolic acid 3 have been widely reported from the genus *Dysoxylum*. Variations in tirucallane-type from the genus *Dysoxylum* generally occur through oxidation or epoxidation at side chain (Luo et al. 2000; Mohammad et al. 1999).

The cytotoxic effects of the three isolated compounds 1, 2, and 3 against MCF-7 breast cancer cell lines were investigated (SI.22). A 13 folds difference of
cytotoxic effect was observed between compound 1 and 2 with the IC$_{50}$ 792.9, 59.6 and 3.5 μM, respectively. According to the structure relationship, presumably due to the hydroxyl substituent (compound 2) which inhibits cancer cell lines much better than ketone group at C-3 (compound 1). In contrast, the most potent cytotoxic compound against the cancer cell was possessed by compound 3 with IC$_{50}$ 3.5 μM.

It was known that cytotoxic activity of an active compound against MCF7 cell lines is due to inhibition of aromatase targeting CyP19A1 (Shibahara et al. 2012). Based on molecular docking studies of cytotoxic tetracyclic triterpenoids typical compound such as, Oenotheranstrol-A (Lasnot-5-en-2β, 3β, 26, 27-tetraol-21-oic acid) and Oenotheranstrol-B (Lasnot-5-en-2β, 3β, 26, 30-tetrol-21-oic acid) against the aromatase CYP19A1, the carboxylic acid and olefinic moieties were most probably responsible for the inhibition activity (Prakash et al. 2014). As compound 3 contained similar moieties, it is implied that the inhibition mechanism of the compound against the aromatase targeting CYP19A1 of MCF7 cell lines behaves in the same manner with those reported by Prakash et al. (2014). Thus, compound 3 was the most potent against MCF7 cell lines. Nevertheless, further molecular docking studies of compound 3 against the aromatase targeting CYP19A1 of MCF7 cell lines must be carried out to clarify the hypothesis.

CONCLUSION
Three triterpenoid compounds, taraxerone (1) and 18-epi-taraxerol (2), and masticadienolic acid (3), have been isolated for the first time from the stem barks of D. arborescens, and D. excelsum correspondingly. The IC$_{50}$ values of compound 1, 2, and 3 against MCF-7 breast cancer cell lines in vitro were 792.9, 59.6, and 3.5 μM, respectively. Further studies on phytochemistry, bioactivity and structure and activity relationships through bioinformatics of the two species are expected to increase Meliaceae plants potential in medical application.

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