Two Limonoids from The Seeds of Chisocheton Macrophyllus and Their Cytotoxic Activity Against MCF-7 Breast Cancer Cells

Intan Rahmayanti1, Nurlelasari1*, Desi Harneti1, Rani Maharani1,2, Darwati1, Yoshihito Shiono2, Unang Supratman1,2

1Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor 45363, Indonesia
2Central Laboratory, Universitas Padjadjaran, Jatinangor 45363, Indonesia
3Department of Food, Life, and Environmental Science, Faculty of Agriculture, Yamagata University, Tsuruoka, Yamagata 997-8555, Japan.

*Corresponding author email: nurlelasari@unpad.ac.id

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ABSTRACT. Limonoids (tetrnnortriterpenoids) are triterpenoid compounds that lose four terminals in their structural framework. These compounds have a wide variety of structures and interesting activities including anti-inflammatory, anticancer, anti-tumor and anti-malarial properties. The purpose of this study was to find limonoid compounds from the Indonesian Chisocheton plant, and one of which is Chisocheton macrophyllus. The dried and powdered seeds of C. macrophyllus (4.5 kg) were extracted with methanol and partitioned successively with n-hexane, ethyl acetate and n-butanol. Evaporation of each extract resulted in the crude extracts of n-hexane (346.6 g), ethyl acetate (60.8 g) and n-butanol (14.6 g). The n-hexane fraction was subjected to a silica gel vacuum-liquid chromatography (VLC) column packed with silica gel 60 using gradient of n-hexane, ethyl acetate and methanol (10% stepwise) to afford thirteen fractions (A–M). Fraction F (4.4 g) was subjected to silica gel column chromatography using gradient of n-hexane and ethyl acetate (5% stepwise). Subfraction F5 (1.2 g) was chromatographed on a column of silica gel eluted with n-hexane: CH2Cl2: EtOAc (2:7.5:0.5) to give compound 1 (19.7 mg) and fraction H (1.8 g) was subjected to silica gel column chromatography using gradient of n-hexane and ethyl acetate (5% stepwise) as eluting solvent to give 2 (12.0 mg). Chemical structures of 1 and 2 were elucidated by spectroscopic methods and determined as 6α-acetoxyepoxyazadiradione (1) and Dysobinin(2). Dysobinin (2) showed weak cytotoxic activity against MCF-7 breast cancer cells with an IC50 value of 228.15 μM.

Keywords: 6α-acetoxyepoxyazadiradione, C. macrophyllus, dysobinin, limonoid, MCF-7

INTRODUCTION

Limonoids are a class of tetranortriterpenoids that are formed through the loss of four terminal carbons of side chain of euphane (20-S) or iricallane (20-R) skeleton that are followed by a cyclization to form a 17β-furan ring (Tan, & Luo, 2011; Shi et al., 2020). Limonoids are classified into ten classes based on the differences on A, B, C, D, and furan ring of the limonoid skeleton and can be identified by their biosynthetic relationships (Fang, Di, & Hao, 2011; Tan & Luo, 2011; Shi et al., 2020). Ten classes of limonoids include protolimonoids, apoeuphol skeleton, D-ring seco, B, D-ring seco, A-ring seco, AB-ring seco, C-ring seco, AD-ring seco and B-ring seco.

Limonoids occur mainly in the plant order of Rutales and most of them are found in Meliaceae and Rutaceae families (Li, Peng, & Zheng, 2016). Limonoids isolated from species of the family of Meliaceae have been of interest due to their diverse structures and their biological activities, including antifeedant, anticaner, antimicrobial, antimalarial, and antiviral properties (Tan, & Luo, 2011; Wong et al., 2011; Gualdani, Cavalluzzi, Lentini, & Habtemariam, 2016; Shilpi et al., 2016; Chong et al., 2019; Supratman et al., 2020). Nimbolide is a major limonoid isolated from the leaves of Azadirachta indica A. Juss or known as neem tree. Nimbolide as a neem limonoid is widely used for anti-malaria, antibacterial activity against S. aureus and S. coagulase, anti-feedant and insectcidal activity (Kumar & Navaratnam, 2013; Bodduluru, Kasala, Thota, Barua & Sistla, 2014; Wang et al., 2016; Sophia et al., 2018). Nimbolide was presumed to be a more potent anticancer. Nimbolide shows anticancer activity throughout selective modulation of signaling pathways linked to inflammation, survival, growth, invasion, angiogenesis and metastasis. Nimbolide was reported to induce apoptosis by disruption of Mitochondrial Outer Membrane Potential (MOMP) and inhibits tumor cell proliferation through alterations of cyclins, cdks, PCNA and p53 levels. In addition, nimbolide also reducing the nuclear alterations of cyclins, cdks, PCNA and p53 levels. In addition, nimbolide also reducing the nuclear
translocation and DNA-binding activity of NF-xB in cancer cells (Kumar & Navaratnam, 2013; Bodduluru, Kasala, Thota, Barua, & Sistla, 2014; Wang et al., 2016; Sophia et al., 2018). Beside nimboide, other limonoids, such azadirachtin, salannin, nimbin and nimbinic acid, have been isolated from A. indica (Wang et al., 2016). Azadirachtin-A (AzaA) is a prominent limonoid known as strong antifeedant and has been exploited commercially. AzaA is present in seed, leaves and other parts of A. indica. Natural pesticide like AzaA is widely used to control the insect. AzaA can keep the insect engaged in defensive while reducing food consumption. In silico studies suggests that AzaA accommodated in the hydrophobic pocket of juvenile hormone esterase and interact with active site residues. AzaA generally targets more than one protein and was presumed to be a poten biopesticide (Dawkar et al., 2019). Other limonoids from Meliaceae family also have potential applications in the food and pharmaceutical industries and have been used as food additives and pesticides (Gualdani, Cavalluzzi, Lentini, & Habtemariam, 2016; Shi et al., 2020).

Chisocheton plant is a genus from Meliaceae that consists of more than 50 species. The genus is distributed mainly in India, Thailand, Malaysia, Indonesia and becomes the second largest genus of family Meliaceae (Katja et al., 2016; Supriatno et al., 2018). Previous phytochemical studies on Chisocheton have discovered several limonoid compounds, such as malayanines A and B, two novel limonoids, that were isolated from the bark of Malaysian C. erythrocarpus (Hiern (Chong et al., 2012), chisomicines D and E, two new limonoids, that have been isolated from the bark of Malaysian C. ceramicus (Miq.) (Najmuldeen et al., 2012), chisotrijugin, a trijugin-type limonoid, from the bark of C. cumingianus (Katja et al., 2016), and pentandricine, a new vilacinine-type limonoid, that was isolated from the stembark of C. pentandrus, together with ceramicine B, 6-de(acetyloxy)-23-oxochisocheton, and 6-de(acetyloxy)-23-oxo-7-O-deacetylchisocheton that have been (Supriatno et al., 2018). Chisocheton genus has also been known as the producers of limonoid compounds with interesting biological activities, for example, ceramicine G and I from C. ceramicus, which have cytotoxic activities against MCF-7 breast cancer cells (Wong et al., 2011) and erythrophorpine E from C. erythrocarpus, which has anticancer properties against HSC-4 human oral cancer cells (Nagoor et al., 2011).

In order to investigate cytotoxic limonoids from Indonesian Chisocheton plants (Katja et al., 2016; Nurlelasari et al., 2017), we continue to carry out a phytochemical investigation on Chisocheton macrophyllus seeds. C. macrophyllus species are distributed in Nicobar Islands, peninsular Thailand, Peninsular Malaysia, Singapore, Sumatra, Anambas Islands, Java and also Borneo. C. macrophyllus is a higher plant with the tree up to 35 m tall. The oil isolated from C. macrophyllus seed has been used for lighting in Indonesia. The wood of C. macrophyllus are used as timber because it is not durable and splits easily (Vossen, & Umali, 2002; Nurlelasari et al., 2017). Previous investigation on limonoids from C. macrophyllus seeds has showed that the plant resulted in bioactive limonoids including dysobinol, 7α-hydroxyneotricilenone, dysobinin and nimonol with cytotoxic activity against P-388 murine leukemia cells (Nurlelasari et al., 2017). In this paper, we describe the isolation, structure elucidation and cytotoxic properties against MCF-7 breast cancer cells of 6α-acetoxyoxazadirodine (1) and dysobinin (2), isolated from C. macrophyllus seeds.

EXPERIMENTAL SECTION

Material and instrumentation

Seeds of C. macrophyllus were collected from Bogor Botanical Garden, Bogor, West Java Province, Indonesia with voucher specimen (No. Bo-1295453). IR spectra and mass spectra were recorded on an One PerkinElmer spectrum-100 FT-IR in KBr and Waters Xevo QTOF MS respectively. NMR spectra were obtained with JEOL JNM-ECZ300R/51 at 500 MHz for 1H and 125 MHz for 13C. Chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. Chromatographic separations were carried out on the silica gel 60 (70–230 and 230–400 mesh, Merck). TLC analysis was carried out on 60 GF254 (Merck, 0.25 mm) using various solvent systems, and detection by irradiating under ultraviolet-visible light Vilber Lourmat (λ 254 nm dan λ 365 nm) followed by heating of silica gel plates, sprayed with 10% H2SO4 in ethanol and Ehrlich’s reagent (p-dimethylaminobenzaldehyde).

Extraction and isolation

The dried and powdered seeds of C. macrophyllus (4.5 kg) was extracted with methanol at room temperature for 3 x 4 L x 24 hours. After the solvent removal under vacuum, a total 560 g of methanol extract was obtained and partitioned with n-hexane, ethyl acetate and n-butanol. Evaporation on each extract resulted the crude extracts of n-hexane (346.6 g), ethyl acetate (60.8 g) and n-butanol (14.6 g).

The n-hexane soluble fraction was subjected to a silica gel vacuum-liquid chromatography (VLC) column packed with silica gel 60 using gradient of n-hexane, ethyl acetate and methanol (10% stepwise) to afford thirteen fractions (A-M). Fraction F (4.4 g) was subjected to silica gel column chromatography using gradient of n-hexane and ethyl acetate (5% stepwise) as eluting solvent to afford twelve subfractions (F1-F12). Subfraction F5 (1.2 g) was chromatographed on a column of silica gel eluted with n-hexane: CH3Cl2:EtOAc (2:7.5:0.5) to give compound 1 (19.7 mg). Fraction H (1.8 g) was subjected to silica gel column chromatography using gradient of n-hexane and ethyl acetate (5% stepwise) as eluting solvent to give 2 (12.0 mg).
6a-acetoxypentadecanone (1)

Colorless crystals 220-221°C; IR (KBr) νmax 2922, 1741, 1668, 1503, 1365 and 1244 cm⁻¹; ¹H-NMR (CDCl₃, 500 MHz) and ¹³C-NMR (CDCl₃, 125 MHz) see Table 1; HR-TOFMS m/z 547.2302 [M+Na]⁺, (calcld. C₃₀H₃₂O₆Na m/z 547.2308).

Dysobin (2)

Colorless crystals 196-197°C; IR (KBr) νmax 2919, 1740, 1667, 1502, 1382; 1362, 1234 cm⁻¹; ¹H-NMR (CDCl₃, 500 MHz) and ¹³C-NMR (CDCl₃, 125 MHz) see Table 1; HR-TOFMS m/z 495.2741 [M+H]⁺, (calcld. C₂₀H₂₆O₆ m/z 495.2747).

Bioassays for cytotoxic activity

The cytotoxic assay was conducted according to the method previously described by Examinati, Wulandari, Harneti, & Poniah (2018) and Supriatno et al., (2018). MCF-7 cells plated in 96 multwell culture plates at a density of 1.7 × 10⁴ cells/well. After twenty-four hours, medium was discarded and fresh medium containing sample with different concentrations 7.81, 15.63, 31.25, 62.50, 125.00, 250.00, 500.00, 1000.00 μg/mL and control was added. After incubation with sample for 24h, prestoBlue® reagent (resazurin dye) was added. The PrestoBlue® assay results were read using multimode reader at 570 nm. The IC₅₀ values were determined by linear regression method using Microsoft Excel software. The IC₅₀ value corresponds to the concentration of compounds that decreases 50% number of viable cells and the absorbance in control corresponds to 100% viability.

RESULTS AND DISCUSSION

The n-hexane extract from the seeds of C. macrophyllus was subjected to a silica gel vacuum-liquid chromatography (VLC) column packed with silica gel 60 by gradient elution. The VLC fractions were repeatedly subjected to normal phase column chromatography on silica gel to yield compounds 1 and 2 (Figure 1).

Compound 1 was isolated as a colorless crystals with 220-221°C. The HR-TOFMS showed the presence of [M+Na]⁺ peak at m/z 547.2302 (calcld m/z for C₃₀H₃₂O₆Na 547.2308), indicating the molecular formula of C₃₀H₃₂O₆. UV spectrum in MeOH showed λmax 220 nm and IR absorptions suggested the presence of aliphatic (vmax 2922 cm⁻¹), carbonyl ester (vmax 1741 cm⁻¹), α,β-unsaturated carbonyl (vmax 1668 cm⁻¹), olefinic (vmax 1503 cm⁻¹), gem dimethyl, (vmax 1365 cm⁻¹), and ether groups (vmax 1244 cm⁻¹). The ¹H-NMR spectrum showed five tertiary methyls at δH 1.01 (3H, s, Me-29), 1.14 (3H, s, Me-18), 1.19 (3H, s, Me-30), 1.23 (3H, s, Me-19) and 1.30 (3H, s, Me-28). Two acetoxyl groups δH 2.06 (3H, s, H-1') and 2.00 (3H, s, H-1'). Three oxygenated protons at δH 5.34 (1H, dd, J = 2.6, 12.5 Hz, H-6), 5.01 (1H, d, J = 2.6 Hz, H-7) and 3.40 (1H, s, H-15), a β-furan moiety at δH 6.21 (1H, d, J = 1.45 Hz, H-22), 7.38 (1H, d, J = 1.45 Hz, H-23) and 7.54 (1H, s, H-21) and two olefinic protons at δH 5.93 (1H, d, J = 10.5 Hz, H-2), and 7.11 (1H, d, J = 10.5 Hz, H-1). The ¹³C-NMR and DEPT spectra showed thirty carbons consisting of an α,β-unsaturated carbonyl at δC 204.5 (C-3), carbonyl ketone at δC 208.1 (C-16), two acetyl oxyl groups at δC 21.2 (C-1'), 170.0 (C-2'), 21.3 (C-1') and 170.1 (C-2'), five methyls at δC 19.1 (Me-28), 20.3 (Me-18), 21.7 (Me-30), 24.8 (Me-29) and 31.7 (Me-19). The spectra also showed two methylene carbons at δC 16.2 (C-11) and 28.7 (C-12), three sp³ methine carbons at δC 38.5 (C-9), 48.5 (C-5) and 50.9 (C-17), three sp³ methine carbons at δC 111.0 (C-22), 126.6 (C-2) and 156.9 (C-1), three oxygenated sp³ methine carbons at δC 57.1 (C-15), 69.9 (C-6) and 73.1 (C-7), two oxygenated sp³ methine carbons at δC 141.7 (C-21) and 142.6 (C-23), four sp³ quaternary carbons at δC 40.5 (C-10), 42.5 (C-8), 43.3 (C-4), and 45.3 (C-13), an oxygenated sp³ quaternary carbons at δC 72.4 (C-14), and one sp³ quaternary carbons at δC 116.5 (C-20). These functionalities accounted for seven out of the total thirteen degrees of unsaturation (C₃₀H₃₂O₆), while the remaining six degrees of unsaturation corresponded to the pentacyclic limonoid structure (Wong et al., 2011; Najmuldeen et al., 2012; Nurlesari et al., 2017; Supriatno et al., 2018) with an additional cyclic. The NMR spectra data of 1 were resembled to those of previously reported dysobin (Nurlesari et al., 2017), except for the absence of olefinic signals at C-14/C-15 and instead the appearance of oxygenated signals [δC 3.40 (1H, s), δC 57.1 and δC 72.4] and carbonyl at δC 208.1, thus suggesting the appearance epoxide between C-14 and C-15 and carbonyl at C-16 in structure 1. Position of these carbonyl at C-16 and epoxide between C-14 and C-15 was determined through the ¹H-¹H COSY and HMBC experiments (Figure 2). ¹H-¹H COSY spectrum of 1 in CDCl₃ (Figure 2), showed correlation in H-1-H₂, H₃-H₄, H₂-H₃, H₅-H₆ and H₂-H₃₂. Four partial structures a (from C-1 to C-2), b (from C-5 to C-7), c (C-11 to C-12), and d (from C-22 to C-23) were deduced from this ¹H-¹H COSY data and supporting the presence of a havanesin-type of limonoid structure in 1. Data from HMBC spectrum showed 3J correlations between sp² methine proton signal δC 7.11 (H-1) and δC 48.5 (C-5) and carbonyl at δC 204.5 (C-3) and a correlation between δC 5.93 (H-2) and δC 40.5 (C-10). Furthermore, olefinic protons at δC 7.11 (1H, d, J = 10.5 Hz, H-1) and δC 5.93 (1H, d, J = 10.5 Hz, H-2) are coupled each other indicating that an α,β-unsaturated carbonyl was locating at C-1, C-2 and C-3. Correlations from oxygenated sp³ methine protons at δC 5.34 (H-6) to δC 48.5 (C-5), δC 73.1 (C-7) and δC 170.0 (C-2'), δC 5.01 (H-7) to δC 48.5 (C-5), δC 69.9 (C-6), δC 42.5 (C-8), δC 38.5 (C-9) and δC 170.1 (C-2'), δC 2.06 (H-1') to δC 170.0 (C-2') and δC 2.00 (H-1') to δC 170.1 (C-2') indicating that acetyl group was attached at C-6 and C-7.
Correlations from oxygenated sp$^3$ methine protons at $\delta_H$ 3.40 (H-15) to $\delta_C$ 208.1 (C-16) and $\delta_C$ 50.9 (C-17) and correlations from $\delta_H$ 3.86 (H-17) to $\delta_C$ 28.7 (C-12), $\delta_C$ 45.3 (C-13), $\delta_C$ 208.1 (C-16), $\delta_C$ 116.5 (C-20), $\delta_C$ 141.7 (C-21) and $\delta_C$ 111.0 (C-22) were used to assign position of an epoxide between C-14 and C-15, a carbonyl located on C-16 and a furan ring attached at C-17. Chemical structure of 1 was presumed to be the same as 6$\alpha$-acetoxyepoxyazadiradione due to the high similarity of the NMR chemical shifts that was previously reported (Table 1) (Pereira et al., 2014). The indicating the relative stereochemistry of epoxide between C-14/C-15 of 1 are $\beta$-oriented and acetyl group at C-6 and C-7 is $\alpha$-oriented. Therefore, compound 1 was identified as a 6$\alpha$-acetoxyepoxyazadiradione and showed in this plant for the first time.

Compound (2) was isolated as a colorless crystals with 196-197°C. The [M+H]$^+$ peak at m/z 495.2741 (calcd for C$_{36}$H$_{50}$O$_6$, 495.2747), HR-TOFMS indicated the molecular formula C$_{36}$H$_{50}$O$_6$, and NMR data (Table 2), thus requiring twelve degrees of unsaturation. IR absorptions suggested the presence of aliphatic (v$_{max}$ 2919 cm$^{-1}$), carbonyl ester (v$_{max}$ 1740 cm$^{-1}$), $\alpha$,$\beta$-unsaturated carbonyl (v$_{max}$ 1667 cm$^{-1}$), olefinic (v$_{max}$ 1502 cm$^{-1}$), gem dimethyl (v$_{max}$ 1382; 1362 cm$^{-1}$), and other groups (v$_{max}$ 1234 cm$^{-1}$). The $^1$H-NMR spectrum showed five tertiary methyls at $\delta_H$ 0.78 (3H, s, Me-28), 1.17 (3H, s, Me-19), 1.17 (3H, s, Me-30), 1.23 (3H, s, Me-29) and 1.31 (3H, s, Me-18). Two acetyl groups $\delta_H$ 1.99 (3H, s, H-1') and 2.03 (3H, s, H-11'). Two oxygenated protons at $\delta_H$ 5.36 (1H, dd, J = 4.5, 3.5 Hz, H-6) and 5.41 (1H, d, J = 2.6 Hz, H-7), a $\delta$-furan moiety at $\delta_H$ 6.25 (1H, d, J = 1.45 Hz, H-22), 7.36 (1H, d, J = 1.45 Hz, H-23) and 7.22 (1H, s, H-21) and two olefinic protons at $\delta_H$ 5.90 (1H, d, J = 10.5 Hz, H-2), and 7.12 (1H, d, J = 10.5 Hz, H-1). The $^{13}$C NMR and DEPT spectra showed thirty carbons consisting of an $\alpha$$\beta$-unsaturated carbonyl at $\delta_C$ 204.8 (C-3), two acetoxy groups at $\delta_C$ 21.0 (C-1'), 170.2 (C-2'), 21.4 (C-1") and 170.3 (C-2"), five methyls at $\delta_C$ 20.5 (Me-28), 20.8 (Me-29), 22.7 (Me-30), 26.8 (Me-18) and 31.7 (Me-19). The spectra also showed three methylene carbons at $\delta_C$ 16.5 (C-11), 34.4 (C-12) and 32.7 (C-16), three sp$^3$ methine carbons at $\delta_C$ 37.3 (C-9), 47.9 (C-5) and 51.6 (C-7), four sp$^2$ methine carbons at $\delta_C$ 111.0 (C-22), 119.8 (C-15), 126.2 (C-2) and 157.4 (C-1), two oxygenated sp$^3$ methine carbons at $\delta_C$ 70.0 (C-6) and 74.6 (C-7), two oxygenated sp$^2$ methine carbons at $\delta_C$ 139.8 (C-21) and 142.7 (C-23), four sp$^3$ quaternary carbons at $\delta_C$ 40.8 (C-10), 43.0 (C-8), 45.0 (C-4), and 47.1 (C-13) and two sp$^2$ quaternary carbons at $\delta_C$ 124.5 (C-20) and 158.2 (C-14). These functionalities accounted for seven out of the total twelve degrees of unsaturation, while the remaining five degrees of unsaturation corresponded to the pentacyclic limonoid structure (Wong et al., 2011; Najmuldeen et al., 2012; Nurlelasari et al., 2017; Supriatno et al., 2018). Structure of 2 was presumed to be the same as dysobinin because of the high similarity of the NMR chemical shifts of the backbone skeleton (Nurlelasari et al., 2017). Therefore, the structure of 2 was elucidated as havanensin-type of limonoid and namely as dysobinin.

Compounds 1 and 2 were evaluated for their cytotoxic activity against MCF-7 breast cancer cell and cisplatin as a positive control according to a method previously described (Examinati, Wulandari, Harneti, & Poniah, 2018; Supriatno et al., 2018). 6$\alpha$-Acetoxyepoxyazadiradione (1) was found to be inactive and dysobinin (2) demonstrated weak cytotoxic activity against MCF-7 breast cancer cell line with IC$_{50}$ values of 228.15 μM whereas cisplatin as the positive control has IC$_{50}$ value of 11.42 μM. The bioassay result, suggested that the carbonyl at C-16 and epoxide at C-14/C-15 in 6$\alpha$-acetoxyepoxyazadiradione (1) decreased the cytotoxic activity.

Figure 1. Chemical Structures of Compound 1 and 2.
**Figure 2.** Selected $^1$H-$^1$H COSY and HMBC Correlations for 1.

**Tabel 1.** NMR data for compounds 1 dan 6α-acetoxyepoxyazadiradione (Pereira et al., 2014) (CDCl$_3$, 500 MHz for $^1$H and 125 for $^{13}$C)

| Posisi C | $^1$H-NMR *F5 | $^{13}$C-NMR | $^1$H-NMR $^{6α}$-acetoxyepoxyazadiradione | $^{13}$C-NMR |
|---|---|---|---|---|
| | $\delta_H$ ppm (3H; mult; $J$=Hz) | $\delta_C$ ppm | $\delta_H$ ppm (3H; mult; $J$=Hz) | $\delta_C$ ppm |
| 1 | 7.11 (1H; d; 10.5) | 156.9 | 7.16 (1H; d; 10.1) | 156.7 |
| 2 | 5.93 (1H; d; 10.5) | 126.6 | 5.99 (1H; d; 10.1) | 126.5 |
| 3 | - | 204.5 | - | 204.4 |
| 4 | - | 43.3 | - | 45.2 |
| 5 | 2.48 (1H; d; 12.5) | 48.5 | 2.54 (1H; d; 12.5) | 48.5 |
| 6 | 5.34 (1H; dd; 2.6; 12.5) | 69.9 | 5.40 (1H; dd; 2.6; 12.5) | 69.8 |
| 7 | 5.01 (1H; d; 2.6) | 73.1 | 5.06 (1H; d; 2.6) | 73.0 |
| 8 | - | 42.5 | - | 43.2 |
| 9 | 2.64 (1H; dd; 2.6; 12.5) | 38.5 | 2.71 (1H; dd; 4.0; 12.5) | 38.4 |
| 10 | - | 40.5 | - | 40.5 |
| 11 | 1.90 (2H; m) | 16.2 | 2.19 (1H; m); 1.88 (1H; m) | 16.1 |
| 12 | 2.65 (2H; dd; 4.0; 12.5) | 28.7 | 2.03 (1H; m); 1.95 (1H; m) | 28.6 |
| 13 | - | 45.3 | - | 42.5 |
| 14 | - | 72.4 | - | 72.2 |
| 15 | 3.40 (1H; s) | 57.1 | 3.46 (1H; s) | 57.0 |
| 16 | - | 208.1 | - | 207.9 |
| 17 | 3.86 (1H; s) | 50.9 | 3.92 (1H; s) | 50.8 |
| 18 | 1.14 (3H; s) | 20.3 | 1.07 (3H; s) | 24.7 |
| 19 | 1.23 (3H; s) | 31.7 | 1.24 (3H; s) | 21.5 |
| 20 | - | 116.5 | - | 116.4 |
| 21 | 7.54 (1H; s) | 141.7 | 7.43 (1H; t; 1.6) | 142.5 |
| 22 | 6.21 (1H; d; 1.45) | 111.0 | 6.26 (1H; d; 1.6) | 110.9 |
| 23 | 7.38 (1H; d; 1.45) | 142.6 | 7.41 (1H; s) | 141.6 |
| 28 | 1.30 (3H; s) | 19.1 | 1.29 (3H; s) | 31.6 |
| 29 | 1.01 (3H; s) | 24.8 | 1.20 (3H; s) | 20.2 |
| 30 | 1.19 (3H; s) | 21.7 | 1.36 (3H; s) | 19.0 |
| 1' | 2.06 (3H; s) | 21.2 | 2.11 (3H; s) | 21.2 |
| 2' | - | 170.0 | - | 169.9 |
| 1'' | 2.00 (3H; s) | 21.3 | 2.05 (3H; s) | 21.1 |
| 2'' | - | 170.1 | - | 169.8 |

* (CDCl$_3$; $^1$H-NMR 500 MHz; $^{13}$C-NMR 125 MHz)

** (CDCl$_3$; $^1$H-NMR 500 MHz; $^{13}$C-NMR 125 MHz)
Table 2. NMR data for compounds 2 and dysobinin (Nurlelasari et al., 2016).

| Posisi C | $^1$H -NMR | $^{13}$C-NMR | $^1$H -NMR | $^{13}$C-NMR |
|----------|-------------|--------------|-------------|--------------|
|          | δH ppm (2H; mult; J=Hz) | δC ppm | δH ppm (2H; mult; J=Hz) | δC ppm |
| 1        | 7.12 (1H; d; 10.5) | 157.4 | 7.3 (1H; d; 10.3) | 158.2 |
| 2        | 5.90 (1H; d; 10.5) | 126.2 | 5.84 (1H; d; 10.3) | 126.6 |
| 3        | - | 204.8 | - | 204.1 |
| 4        | - | 45.0 | - | 45.6 |
| 5        | 2.48 (1H; d; 14.0) | 47.9 | 2.5 (1H; m) | 48.9 |
| 6        | 5.36 (1H; dd; 4.5; 3.5) | 70.0 | 5.40 (1H; m) | 70.0 |
| 7        | 5.41 (1H; d; 2.6) | 74.6 | 5.40 (1H; m) | 75.1 |
| 8        | - | 43.0 | - | 43.9 |
| 9        | 2.41 (1H; m) | 37.3 | 1.28 (1H; m) | 38.3 |
| 10       | - | 40.8 | - | 41.6 |
| 11       | 1.62 (2H; m) | 16.5 | 1.80 (2H; m) | 17.0 |
| 12       | 2.32 (2H; m) | 34.4 | 2.3 (1H; m); 2.5 (1H; m) | 35.3 |
| 13       | - | 47.1 | - | 47.9 |
| 14       | - | 158.2 | - | 159.7 |
| 15       | 2.24 (1H; m) | 119.8 | 2.26 (1H; m) | 120.2 |
| 16       | 1.73 (1H; m); 1.91 (1H; m) | 32.7 | 1.73 (1H; m); 1.93 (1H; m) | 33.6 |
| 17       | 2.79 (1H; dd; 11.0; 18.5) | 51.6 | 2.84 (1H; dd; 7.4; 11.3) | 52.7 |
| 18       | 1.31 (3H; s) | 26.8 | 1.35 (3H; s) | 27.1 |
| 19       | 1.17 (3H; s) | 31.7 | 1.22 (3H; s) | 32.1 |
| 20       | - | 124.5 | - | 125.5 |
| 21       | 7.22 (1H; s) | 139.8 | 7.40 (1H; s) | 140.9 |
| 22       | 6.25 (1H; d; 1.45) | 111.0 | 6.40 (1H; s) | 112.0 |
| 23       | 7.36 (1H; d; 1.45) | 142.7 | 7.50 (1H; s) | 143.7 |
| 24       | 0.78 (3H; s) | 20.5 | 1.15 (3H; s) | 20.7 |
| 25       | 1.23 (3H; s) | 20.8 | 1.22 (3H; s) | 20.9 |
| 26       | 1.17 (3H; s) | 22.7 | 1.22 (3H; s) | 21.2 |
| 1*       | 1.99 (3H; s) | 21.0 | 2.00 (3H; s) | 21.3 |
| 2*       | - | 170.2 | - | 170.6 |
| 1*       | 2.03 (3H; s) | 21.4 | 2.00 (3H; s) | 22.4 |
| 2*       | - | 170.3 | - | 170.6 |

* (CDCl$_3$; $^1$H-NMR 500 MHz; $^{13}$C-NMR 125 MHz)

** (CDCl$_3$; $^1$H-NMR 500 MHz; $^{13}$C-NMR 125 MHz)

CONCLUSIONS

6α-Acetoxyepoxyazadiradione (1) and dysobinin (2) have been isolated from the seeds of C. macrophyllus. The discovery of 1 and 2 supported the occurrence of limonoid in the Chisocheton genus. Compound 1 and 2 were evaluated for their cytotoxic activity against MCF-7 breast cancer cell line. Compound 1 was inactive and compound 2 demonstrated weak cytotoxic activity (228.15 μM) against MCF-7 breast cancer cell line. The bioassay data suggested that the carbonyl at C-16 and epoxide at C-14/C-15 in 6α-acetoxyepoxyazadiradione (1) decreased the cytotoxic properties.

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