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Sesquiterpenoids from Stem Bark of *Chisocheton lasiocarpus* and Their Cytotoxic Activity against MCF-7 Breast Cancer Cell

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ABSTRACT. Sesquiterpenoids are derivatives of terpenoids that have a diverse skeleton and broad spectrum of biological activities, particularly for anticancer activity. The existence of sesquiterpenoids can be found in higher plants, especially in *Chisocheton* genera of the Meliaceae family. This research reported the isolation and elucidation structure of sesquiterpenoid from *Chisocheton lasiocarpus* stem bark, as well as their cytotoxic activity. The *n*-hexane extract was separated and purified by various chromatography techniques to obtained four sesquiterpenoids **1**-**4**. The chemical structure of all compounds was identified by spectroscopic analysis, including IR, MS, ^{1}H-NMR, ^{13}C-NMR and DEPT, and compared with previous reported spectral data. These sesquiterpenoids were identified as eudesm-4(15)-ene-1β,6α-diol (**1**), *allo*-aromadendrane-10α,14-diol (**2**), *allo*-aromadendrane-10β,14-diol (**3**) and guaianediol (**4**), which led to the first report of sesquiterpenoid from *Chisocheton lasiocarpus*. The cytotoxic activity of the isolated compounds against MCF-7 breast cancer line were examined by using the resazurin method. The results showed eudesm-4(15)-ene-1β,6α-diol (**1**) shown the highest cytotoxic activity with IC_{50} 108.08 ± 0.58 μM.

Keyword: *Chisocheton lasiocarpus*, cytotoxic activity, MCF-7, sesquiterpenoid

INTRODUCTION

Sesquiterpenoids are class of natural product derived from terpenoid that formed by three isoprene units. Sesquiterpenoid exists in various form, including acyclic, monocyclic, bicyclic, tricyclic skeleton. Isomerization in sesquiterpenoids is very common, and several pairs of these isomers can be found within the same species or in different species. There are five basic forms of terpenoid isomers, namely structural, positional, geometrical, conformational, and stereochemical isomers. Sesquiterpenoid has properties as an oil but not infrequently as a crystalline (Ludwiczuk et al., 2017). Sesquiterpenoids have wide range of biological activities such as cytotoxic activities (Naini et al., 2022), anti-inflammatory (Wang et al., 2021), antibacterial and antifungal (Perveen et al., 2020), and antimalarial (Shen et al., 2020). The existence of sesquiterpenoid can be found in plants, especially in higher plants (Connolly & Hill, 1991).

Chisocheton (Meliaceae) is a genus of higher plant which consists of 53 species, distributed in tropical and subtropical region. This genus widely distributed in Asian lowland rainforest, from eastern India to southern China, Thailand, Philippines, Brunei, Malaysia, and Indonesia, Papua New Guinea, and found in the northern part of Australia and Vanuatu (Shilpi et al., 2016; Mebberley, 2010; Stevens, 1975). Plants from this genus, traditionally is used for medication of stomach, kidney, and back complaints, as well as treatment on fever, malaria, and rheumatism (Chan et al., 2012; Zhang et al., 2012; Chong et al., 2019). Furthermore, bioactivities that have been reported from this genus include antimalarial against *Plasmodium falciparum*, cytotoxic

against breast cancer cell line MCF-7, P-388 (murine leukemia cell), NCI-H187 (human small cell lung cancer), KB (oral human epidermal carcinoma), and B16-F10 (melanoma cell), anti-inflammation, anti-adipocyte differentiation activity, antimycobacterial activity, insecticidal, antifeedant, and antifungal (Chan et al., 2012; Hilmayanti et al., 2022; Hoai et al., 2018; Iijima et al., 2016; Katja, 2021; Katja et al., 2017, 2022; Maneerat et al., 2008; Nugroho et al., 2018, 2021; Nurlelasari et al., 2017; Salam et al., 2021; Shilpi et al., 2016; Supratman et al., 2019; Wong et al., 2014). In the previous study, the *Chisocheton* genus contains several sesquiterpenoids, there were aromadendrane, eudesmane, and guaiane-type sesquiterpenoid (Phongmaykin et al., 2008; Yang et al., 2012).

Chisocheton lasiocarpus is one of the species found as an endemic plant of Maluku and Solomon Islands (Stevens, 1975). The discovery of sesquiterpenoid in this species has not been reported. This study featured the isolation and elucidation structure of sesquiterpenoid from *C. lasiocarpus* alongside their cytotoxic activity against MCF-7 breast cancer cells. The compounds were isolated by using various chromatographic techniques and characterized with spectroscopic data (IR, MS, ^{1}H-NMR, ^{13}C-NMR, and DEPT). In addition, the resazurin method was used to measure the cytotoxic activity.

EXPERIMENTAL SECTION

General Experiment Procedure

The IR spectra were measured on a Perkin–Elmer spectrum-100 FT-IR in KBr (Waltham, Massachusetts, USA). NMR spectra were measured by JEOL JNM-ECZ500R/S1 (Tokyo, Japan) and TMS as an internal standard. Mass Spectra were measured by Waters QTOF-HRTOFMS-XEV^{otm} mass spectrometer (Waters, Milford, MA, USA). Chromatographic separation was carried out on silica gel 60 (70-230 mesh and 230-400 mesh, Merck, Darmstadt, Germany) and octa decyl silane (Fuji Sylisia Chemical LTD., Chromatorex® C_{18} DM1020 M, 100–200 mesh, Tokyo, Japan), while thin-layer chromatography (TLC) was performed on precoated silica gel 60 F_{254} (Merck, Darmstadt, Germany) and RP-18 F_{254}s plates (Merck, Darmstadt, Germany), and spot detection was achieved by spraying with 10% H_2SO_4 in ethanol, then heating.

Plant Material

The stem bark of *C. lasiocarpus* (Miq.) Valeton was collected in August 2019, from Bogor Botanical Garden, Bogor, West Java, Indonesia. The identification of the plant was carried out by Mr. Harto, the staff of Bogoriense Herbarium, Bogor, Indonesia and voucher specimen (VII. G. 168) was deposited at the herbarium.

Extraction and Isolation

The dried stem bark of *C. lasiocarpus* (1.5 kg) was macerated with methanol for 6 days (6 × 3L) and evaporated the solvent to conduce methanol extract (120 g). Furthermore, methanol extract was dissolved in water and fractionated with *n*-hexane, ethyl acetate and *n*-butanol followed by evaporated the solvent. The evaporated extract yield concentrated *n*-hexane (13.3 g), ethyl acetate (11.7 g) and *n*-butanol (5.2 g), respectively. The *n*-hexane extract was sapareted by vacuum liquid chromatography on silica gel G60 using an eluent gradient system of *n*-hexane-ethyl acetate-methanol, stepwise 10%, to give nine fractions (A-I). The E fraction (1.92 g) was separated with column chromatography on silica gel (70-230 mesh) using 5% eluent gradient system of *n*-hexane and ethyl acetate, to obtained eleven fractions (E1-E11). Moreover, E7 (343.0 mg) was column chromatographed on silica gel (230-400 mesh) with isocratic eluent system of dichloromethane:ethyl acetate (97:3) to yield three fractions (E7a-E7c). Subfraction E7b (22.2 mg) was purified with ODS (C_{18}, 100–200 mesh) with methanol:water (7:3) to afford compound **1** (4.9 mg).

Fraction F (1.40 g), was chromatographed on silica gel (70-230 mesh) with a gradient elution of dichloromethane:ethyl acetate, 2% stepwise, to obtained nine fraction (F1-F9). Subsequently, fraction F6 (149.2 mg) was chromatographed on ODS (C_{18}, 100–200 mesh) with acetonitrile:water (55:45) to conduce compound **2** (4.1 mg) and **3** (6.9 mg). Meanwhile, fraction F7 (164.0 mg) was then separated by column chromatography on silica gel (230-400 mesh) with isocratic eluent system of dichloromethane:ethyl acetate (7:3) to yield five fractions (F7a-F7e). F7b fraction (9.6 mg) was then purified by column chromatography on ODS (C_{18}, 100–200 mesh) and using a solvent of acetonitrile:water (55:45) to yield compound **4** (4.8 mg).

Eudesm-4(15)-ene-1β,6α-diol (**1**). Colorless oily; IR (KBr plate) ν_{max} cm^{-1}: 3353, 2959, 2875, 1706, 1465, 1377, 1052; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 3.43 (1H, dd, *J*= 6.5, 13.5 Hz, H-1), 1.85 (1H, m, H-2a), 1.55 (1H, m, H-2b), 2.33 (1H, ddd, *J*= 2.0, 4.5, 13.0 Hz, H-3a), 2.07 (1H, m, H-3b), 1.74 (1H, brd, *J*= 10 Hz, H-5), 3.71 (1H, t, *J*= 10 Hz, H-6), 1.29 (1H, m, H-7), 1.51 (1H, m, H-8a), 1.20 (1H, qd, *J*= 10.5, 3.5 Hz, H-8b), 1.91 (1H, m, H-9a), 1.16 (1H, m, H-9b), 2.23 (1H, d, *J*= 7.5, 3.0 Hz, H-11), 0.95 (3H, d, *J*= 7.5 Hz, H-12), 0.86 (3H, d, *J*= 7.5 Hz, H-13), 0.70 (3H, s, H-14), 5.01 (1H, brs, H-15a), 4.74 (1H, brs, H-15b); ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 1; HR-TOF MS *m/z* 239.2013 $[M+H]^+$ (calcd. *m/z* 239.2011 for $C_{15}H_{27}O_2$).

***Allo*-aromadendrane-10α,14-diol** (**2**). White needles; IR (KBr plate) ν_{max} cm^{-1}: 3293, 2928, 2868, 1453, 1375, 1029; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 2.06 (1H, m, H-1), 1.91 (2H, m, H-2), 1.31 (1H, dq, *J*= 5.5, 11,0 Hz, H-3a), 1.86 (1H, m, H-3b), 2.00 (1H, m, H-4), 1.74 (1H, m, H-5), 0.34 (1H, t, *J*= 9.6 Hz, H-6), 0.69 (1H, dd, *J*= 15.9, 9.2 Hz, H-7), 1.66

(1H, m, H-8a), 1.77 (1H, m, H-8b), 1.71 (1H, m, H-9a), 1.96 (1H, m, H-9b), 0.97 (3H, s, H-12), 1.04 (3H, s, H-13), 3.35 (1H, d, *J*= 11.0 Hz, H-14a), 3.46 (1H, d, *J*= 11.0, H-14b), 0.96 (3H, d, *J*= 6.7, H-15), ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 1; HR-TOF MS *m/z* 239.2013 $[M+H]^+$ (calcd. *m/z* 239.2011 for $C_{15}H_{27}O_2$).

***Allo*-aromadendrane-10β,14-diol** (**3**) White needles; IR (KBr plate) ν_{max} cm^{-1}: 3376, 2948, 2868, 1462, 1375, 1065; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 1.86 (1H, m, H-1), 1.57 (2H, m, H-2), 1.28 (1H, q, *J*= 11.5 Hz H-3a), 1.80 (1H, m, H-3b), 1.99 (1H, m, H-4), 1.86 (1H, m, H-5), 0.12 (1H, t, *J*= 9.5 Hz, H-6), 0.64 (1H, dd, *J*= 9.5, 6.5 Hz, H-7), 1.40 (1H, q, *J*= 13.0 Hz H-8a), 1.64 (1H, m, H-8b), 1.51 (1H, m, H-9a), 1.68 (1H, m, H-9b), 1.01 (3H, s, H-12), 1.04 (3H, s, H-13), 3.28 (1H, d, *J*= 10.5 Hz, H-14a), 3.42 (1H, d, *J*= 10.5 Hz, H-14a), 0.93 (3H, d, *J*= 6.5, H-15), ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 2; HR-TOF MS *m/z* 239.2012 $[M+H]^+$ (calcd. *m/z* 239.2011 for $C_{15}H_{27}O_2$).

Guaianediol (**4**) Colorless oily; IR (KBr plate) ν_{max} cm^{-1}: 3376, 2955, 1730, 1373, 1059; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 1.87 (1H, m, H-1), 1.64 (1H, m, H-2a), 1.77 (1H, m, H-2b), 1.71 (1H, m, H-3a), 1.61 (1H, m, H-3b), 2.17 (1H, m, H-5), 5.50 (1H, brs, H-6), 2.20 (1H, m, H-8a), 1.92 (1H, m, H-8b), 1.82 (1H, m, H-9a), 1.47 (1H, m, H-9b), 2.25 (1H, sep, *J*= 5.5 Hz, H-11), 0.98 (3H, d, *J*= 5.5 Hz, H-12), 0.97 (3H, d, *J*= 5.5 Hz, H-13), 1.27 (3H, s, H-14), 1.21 (3H, s, H-15); ^{13}C-NMR ($CDCl_3$, 125 MHz), see Table 2; HR-TOF MS *m/z* 239.2001 $[M+H]^+$ (calcd. *m/z* 239.2011 for $C_{15}H_{27}O_2$).

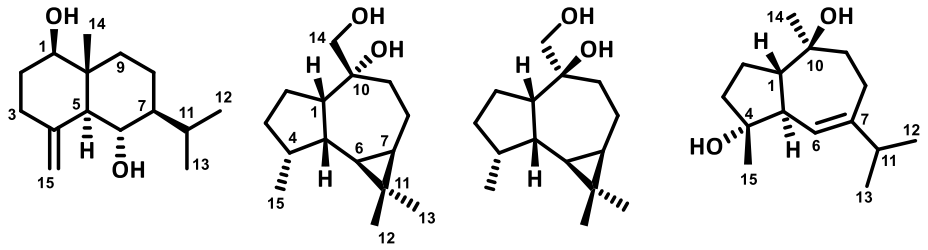
Determination of Cytotoxic Activity

The method used to determine cytotoxic activity of compound **1**-**4** was measured with a cell viability with resazurin base by PrestoBlue® assay. The cells were maintained in RPMI (Rosewell Park Memorial Institute) medium, 10% (v/v) FBS (Fetal Bovine Serum), as well as 1 μL/mL antibiotic. The cells were cultured into 96 well plates at 37 ºC in a humidified atmosphere of 5% CO_2 incubation for 24 h or until the cells reach a density of 1.7×10^4 cell/well. The medium was discarded, then added the fresh medium containing sample with different concentration (1,000.00, 500.00, 250.00, 125.00, 62.50, 31.25, 15.63, and 7.81 μg/mL) and also control cisplatin. The cells with sample then incubated for 48 h, after that PrestoBlue® reagent was added and the result was read using a multimode reader at 570 nm and converted the absorption into cell viability so that the IC_{50} of each compound were obtained.

RESULTS AND DISCUSSION

The methanol extract of *C. lasiocarpus* stem bark was dissolved in water and partitioned with *n*-hexane, ethyl acetate and *n*-butanol, successively. The *n*-hexane extract was separated by column chromatography on silica gel 60 and C_{18}-ODS, the separation process was observed by TLC using silica GF_{254} led to isolation of four sesquiterpenoids **1**-**4** (**Figure 1**).

Compound **1** was isolated as a colorless oil, with a molecular formula of $C_{15}H_{26}O_2$ based on HR-TOFMS of the positive ion peak at *m/z* 239.2013 $[M+H]^+$ (calcd. 239.2011), requiring three degrees of unsaturation. The IR spectra showed absorption bands which indicates the presence of hydoxyl (3353 cm^{-1}), aliphatic (C-H sp^3) (2959 and 2875 cm^{-1}), olefinic (C=C) (1706 cm^{-1}), *gem*-dimethyl (1465 and 1377 cm^{-1}), and ether group (1052 cm^{-1}). The ^{1}H-NMR spectra showed proton resonance related to one tertiary methyl at δ_H 0.70 (3H, s, CH_3-14), and two secondary methyls at δ_H 0.95 (3H, d, *J*= 7.5 Hz, CH_3-12) and 0.86 (3H, d, *J*= 7.5 Hz, CH_3-13), and one olefinic methylene group at δ_H 5.01 (1H, brs, H-15a), 4.74 (1H, brs, H-15b). In addition, two oxygenated proton signals were observed at δ_H 3.43 (1H, dd, *J*= 6.5, 13.5 Hz, H-1) and 3.71 (1H, t, *J*= 10 Hz, H-6). The ^{13}C-NMR spectra indicated the presence of 15 carbons, assigned by DEPT 135º spectra into three methyls at δ_C 21.1 (C-12), 16.2 (C-13), and 11.6 (C-14), five methylenes (one olefinic) at δ_C 31.9 (C-2), 35.1 (C-3), 18.2 (C-8), 36.3 (C-9) and 107.8 (C-15), five methines including two oxygenated at δ_C 55.9 (C-5), 49.3 (C-7), 26.0 (C-11), 79.0 (C-1), 67.0 (C-6), and two quaternary carbons at δ_C 41.7 (C-10) and 146.2 (C-4) as quaternary olefinic. The ^{13}C-NMR data above suggested that the olefinic moiety was take the disubstituted structure or terminal olefinic and accounted for one degree of unsaturation, so the two unassigned hidrogen deficiency indexes has coresponded to the bicyclic sesquiterpenoid structure. Based on analysis of ^{1}H-NMR, ^{13}C-NMR, and DEPT spectra, compound **1** indicited the characteristic of eudesmane-type sesquiterpenoid. It is supported by the evidence of one tertiary methyl at δ_H 0.70 (3H, s, CH_3-14) and one quaternary carbons at δ_C 41.7 (C-10), as well as the existence of two secondary methyls with the same value of *J* coupling constant [δ_H 0.95 (3H, d, *J*= 7.5 Hz, CH_3-12), 0.86 (3H, d, *J*= 7.5 Hz, CH_3-13)] which proved the isopropyl in eudesmane skeleton. The stereochemistry of compound **1** established by several approach. The biosynthesis of eudesmane led *β*-orientation of CH_3-10 (Christianson & Blank, 2020). Proton of oxygenated methine H-1 has a *dd* split pattern with *J* coupling constant 6.5 and 13.5 Hz indicated the axial conformation, implied β-orientation of 1-OH. The *J* coupling constant of H-5 (10.0 Hz) indicated the axial-axial conformation to H-6 provided *α*-orientation of H-5 and 6-OH. The isopropyl configuration can be established by the chemical shift of H-7 (δ_H 1.29), showed α-orientation (δ_H 1.26) of H-7 than β-orientation (δ_H1.71) (Zhang et al., 2003), led β-orientation of isopropyl.



1 2 3 4

Figure 1. Structure of compound **1-4**

The comparison of the NMR data of compound **1** with the data for eudesm-4(15)-ene-1β,6*α*-diol (Zhang et al., 2003), involved with its stereochemistry, indicated the structure of both were similar. Therefore, the structure of **1** was indentified as an eudesm-4(15)-ene-1β,6*α*-diol.

Compound **2** was isolated as a white neddles, with a molecular formula of $C_{15}H_{26}O_2$, established by HR-TOFMS spectra of the positive ion peak at *m/z* 239.2013 $[M+H]^+$ (calcd. 239.2011), requiring the three degrees of unsaturation. The IR spectra gave absorption bands indicates the presence of hydroxyl (3293 cm^{-1}), aliphatic (C-H sp^3) (2928 and 2868 cm^{-1}), *gem*-dimethyl (1453 and 1375 cm^{-1}), and ether group (1029 cm^{-1}). The ^{1}H-NMR spectra showed proton resonances related to two tertiary methyls at δ_H 0.97 (3H, s, CH_3-12) and 1.04 (3H, s, CH_3-13), one secondary methyl at δ_H 0.96 (3H, d, *J*= 6.7, CH_3-15), two oxygenated protons with the geminal *J* coupling constant also appeared at δ_H 3.35 (1H, d, *J*= 11.0 Hz, H-14a) and 3.46 (1H, d, *J*= 11.0, H-14b). Furthermore, the ^{1}H-NMR displayed the upfield protons at δ_H 0.34 (1H, t, *J*= 9.6 Hz, H-6) and 0.69 (1H, dd, *J*= 15.9, 9.2 Hz, H-7) which suggested the presence of cyclopropane moiety on a sesquiterpenoid aromadendrane-type framework. The ^{13}C-NMR spectra revealed the presence of 15 carbons, detailed with DEPT (**Table 1**) showed three methyls, four methylenes involving one oxygenated methylene, five methines, and two quaternary carbons (including one oxygenated quaternary carbon). The ^{13}C-NMR evidenced that no other olefinic resonances were present, so three degrees of unsaturation originated from tricyclic sesquiterpenoid skeleton. The quaternary carbon at δ_C 19.2 and the upfield proton at H6/H7 verified the cyclopropane on aromadendrane-type (Feliciano et al., 1989). Determination of the structure through the approach of aromadendrane-type sesquiterpenoids that have been isolated from *Chisocheton* genera. Aromadendranes that have been isolated from *Chisocheton* genera were *allo*-aromadendrane-10*α*,14-diol, *allo*-aromadendrane-10β,14-diol, and *allo*-aromadendrane-10β,13,14-triol. *Allo*-aromadendrane-10β,13,14-triol is impossible to be compound **2**, because of triol. The distinction of *allo*-aromadendrane-10*α*,14-diol and *allo*-aromadendrane-10β,14-diol is the stereochemistry of hydroxymethyl and hydroxyl moiety attached to C-10. The chemical shift of C-14 (hydroxymethyl) of *allo*-aromadendrane-10*α*,14-diol was at 69.9 and *allo*-aromadendrane-10β,14-diol was at 70.7 (Phongmaykin et al., 2008), while compound **2** was at 69.9. That evidence led to *allo*-aromadendrane-10*α*,14-diol. The NMR data of compound **2** showed resembled signals to those of *allo*-aromadendrane-10*α*,14-diol (Phongmaykin et al., 2008). Therefore, the structure of compound **2** was identified as *allo*-aromadendrane-10*α*,14-diol.

Compound **3** was a white neddles, with a molecular formula of $C_{15}H_{26}O_2$, established by HR-TOFMS spectra of the positive ion peak at *m/z* 239.2012 $[M+H]^+$ (calcd. 239.2011), requiring the three degrees of unsaturation. The IR spectrum showed absorption bands which implies the presence of hydroxyl (3376 cm^{-1}), aliphatic (C-H sp^3) (2948 and 2868 cm^{-1}), *gem*-dimethyl (1462 and 1375 cm^{-1}), and ether group (1065 cm^{-1}). The ^{1}H-NMR and ^{13}C-NMR of **3** have similarity to those of **2**, expect for several chemical shift of carbon which were slightly shifted. Thus compound **3** was predicted to have the same planar structure as compound **2**. Two methyls appeared as singlet at δ_H [1.01 (3H, s, CH_3-12) and 1.04 (3H, s, CH_3-13)] implying a *gem*-dimethyl and one secondary methyl at δ_H 0.93 (3H, d, *J*= 6.5, CH_3-15). The upfield protons at δ_H 0.12 (1H, t, *J*= 9.5 Hz, H-6) and 0.69 (1H, dd, *J*= 9.5, 6.5 Hz, H-7) as well as quaternary carbon at δ_C 19.2 (C-11) provided the existence cyclopropane on aromadenrane-type in **2**. In addition, two oxygenated protons at δ_H 3.28 (1H, d, *J*= 10.5 Hz, H-14a) and 3.42 (1H, d, *J*= 10.5, H-14b) was assigned to one oxygenated methylene at δ_C 70.7 (C-14). The oxygenated quaternary carbon at δ_C 76.4 (C-10) also appeared. Since the NMR data of compound **2** and **3** have similarity and was predicted to have the same planar structure, the chemical structure and its stereochemistry of compound **3** was identified by the same approach and the comparison NMR data of compound **3** and *allo*-aromadendrane-10*β*,14-diol (Phongmaykin et al., 2008), revealed that both compounds were very similar, consequently **3** was identified as *allo*-aromadendrane-10*β*,14-diol, the stereoisomer of compound **2**.

Table 1. Comparison of ^{13}C-NMR data of compound **1**-**2** and literatures ($CDCl_3$, 125 MHz)

Carbon Position	Compounds			
	1 δ_C (mult.)	eudesm-4(15)-ene-1β,6α-diol (Zhang et al., 2003) δ_C (mult.)	**2** δ_C (mult.)	*allo*-aromadendrane-10α,14-diol (Phongmaykin et al., 2008) δ_C (mult.)
1	79.0 (d)	78.9 (d)	50.0 (d)	50.0 (d)
2	31.9 (t)	31.8 (t)	23.8 (t)	23.8 (t)
3	35.1 (t)	35.0 (t)	30.3 (t)	30.2 (t)
4	146.2 (s)	146.2 (s)	38.3 (d)	38.3 (d)
5	55.9 (d)	55.8 (d)	40.7 (d)	40.7 (d)
6	67.0 (d)	67.0 (d)	23.4 (d)	23.3 (d)
7	49.3 (d)	49.2 (d)	25.0 (d)	25.1 (d)
8	18.2 (t)	18.0 (t)	19.3 (t)	19.2 (t)
9	36.3 (t)	36.2 (t)	33.6 (t)	33.5 (t)
10	41.7 (s)	41.6 (s)	76.3 (s)	76.3 (s)
11	26.0 (d)	25.9 (d)	19.2 (s)	19.1 (s)
12	21.1 (q)	21.1 (q)	16.0 (q)	16.0 (q)
13	16.2 (q)	16.1 (q)	28.6 (q)	28.6 (q)
14	11.6 (q)	11.5 (q)	69.9 (q)	69.9 (q)
15	107.8 (t)	107.8 (t)	15.6 (q)	15.6 (q)

Table 2 Comparison of ^{13}C-NMR data of compound **3**-**4** and literatures ($CDCl_3$, 125 MHz)

Carbon Position	Compounds			
	3 δ_C (mult.)	*allo*-aromadendrane-10α,14-diol (Phongmaykin et al., 2008) δ_C (mult.)	**4** δ_C (mult.)	guaianediol (El Sayed & Hamann, 1996) δ_C (mult.)
1	53.5 (d)	53.5 (d)	50.6 (d)	50.7 (d)
2	24.6 (t)	24.5 (t)	21.5 (t)	21.5 (t)
3	29.0 (t)	29.0 (t)	40.5 (t)	40.5 (t)
4	38.2 (d)	38.2 (d)	80.2 (s)	80.2 (s)
5	40.0 (d)	40.0 (d)	50.3 (d)	50.3 (d)
6	22.4 (d)	22.4 (d)	121.3 (d)	121.3 (d)
7	28.7 (d)	28.7 (d)	149.7 (s)	149.6 (s)
8	18.3 (t)	18.3 (t)	25.1 (t)	25.1 (t)
9	32.1 (t)	32.0 (t)	42.6 (t)	42.6 (t)
10	76.4 (s)	76.5 (s)	75.3 (s)	75.3 (s)
11	19.2 (s)	19.1 (s)	37.3 (d)	37.3 (d)
12	16.2 (q)	16.2 (q)	21.4 (q)	21.4 (q)
13	29.1 (q)	29.0 (q)	21.3 (q)	21.3 (q)
14	70.7 (q)	70.7 (q)	21.1 (q)	21.1 (q)
15	16.1 (q)	16.1 (q)	22.5 (q)	22.5 (q)

Table 3. Cytotoxic activity of compound **1**-**4** against MCF-7 breast cancer line

Compounds	IC_{50} (μM)
1	108.08 ± 0.58
2	258.70 ± 2.49
3	231.99 ± 5.40
4	1039.62 ± 2.02
Cisplatin	53.00

Meanwhile, compound **4** was isolated as a colorless oil, with a molecular formula of $C_{15}H_{26}O_2$, established by HR-TOFMS spectra of the positive ion peak at *m/z* 239.2001 $[M+H]^+$ (calcd. 239.2011), indicating three degrees of unsaturation. The IR spectra displayed the presence of hydroxyl (3376 cm^{-1}), aliphatic (C-H sp^2) (2955 cm^{-1}), olefinic (C=C) (1730 cm^{-1}) *gem*-dimethyl (1373 cm^{-1}), and ether group (1059 cm^{-1}). The ^{1}H-NMR gave proton resonances related to two doublet methyls as *gem*-

dimethyl and two singlet methyls relatively deshielded at δ_H 0.98 (3H, d, *J*= 5.5 Hz, CH_3-12), 0.97 (3H, d, *J*= 5.5 Hz, CH_3-13), 1.27 (3H, s, H-14), and 1.21 (3H, s, H-15), respectively. Moreover, the presence of one proton olefinic at δ_H 5.50 (1H, brs, H-6) implied the trisubstituted olefinic group. The ^{13}C-NMR spectra possessed the resonances for 15 carbons, with the aid of DEPT spectra (**Table 2**), corresponding to four methyls, four methylenes, four methines (one olefinic), and three quaternary carbons involving two oxygenated and one olefinic carbon. Taking into consideration the presence of one pair double bond and two degrees of unsaturation thus indicated that **4** possessed two additional rings from a bicyclic sesquiterpenoid framework. Furthermore, compound **4** showed the absence of quaternary aliphatic carbon (non-oxygenated) together with NMR data analysis revealed that **4** was a sesquiterpenoid guaiane-type and was suggested as guaianediol or (+)-alismoxide. The stereochemistry of compound **4**, determined by the oxygenated quaternary carbon chemical shift of guaianediol and alismoxide. Stereocenter of guaianediol is known that H-5 and CH_3-14 has *α*-orientation while H-1 and CH_3-15 has *β*-orientation (the δ_C see **Table 2**) (El Sayed & Hamann, 1996). Whereas, (+)-alismoxide has opposite orientation of guaianediol. The chemical shift of C-1, C-4, C-5, C-10, C-14, and C-15 on (+)-alismoxide were at δ_C 50.9, 80.4, 50.5, 75.2, 21.4, and 22.8, respectively (Blay et al., 2006). Despite the NMR data nearly same, it can be determined that the stereochemistry of compound **4** is highly similar to that of guaianediol than that of alismoxide. Based on the NMR data spectra and agreed with the literature (El Sayed & Hamann, 1996), compound **4** was established as guaianediol.

Sesquiterpenoids that isolated from *C. lasiocarpus* (**1-4**) were tested against MCF-7 breast cancer cell line based on the method described previously (Izdihar et al., 2021) and used Cisplatin (IC_{50} 53 *μ*M) as positive control (Lukyanova et al., 2009) and the results shown in **Table 3**. Compound **1** shown the best cytotoxic activity than all of the compound, even though **1-3** were weak activity and **4** was not active according to Wibowo et al. (2011). Compound **3** exhibited more active than **2**, it indicated that β alcohol and α hydroxymethyl orientation on C-10 responsible for the effect of activity compere to the opposite.

CONCLUSIONS

Four sesquiterpenoids namely, eudesm-4(15)-ene-1β,6β-diol (**1**), *allo*-aromadendrane-10α,14-diol (**2**), *allo*-aromadendrane-10β,14-diol (**3**) and guaianediol (**4**) has been isolated from the stem bark of *C. lasiocarpus* for the first time. Compound **1** showed the strongest cytotoxic activity against MCF-7 breast cancer line (IC_{50} 108.08 ± 0.58 *μ*M). Alcohol and methyl alcohol orientation at C-10 on compound **2** and **3** slightly provide different activity, which β alcohol orientation responsible for higher activity.

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REFERENCES

Blay, G., Garcıa, B., Molina, E., & Pedro, J. R. (2006). Syntheses of (+)-alismoxide and (+)-4-epi-alismoxide. *Journal of Organic Chemistry*, *71*(12), 7866–7869. https://doi.org/10.1021/jo061278y

Chan, K. Y., Mohamad, K., Ooi, A. J. A., Imiyabir, Z., & Chung, L. Y. (2012). Bioactivity-guided fractionation of the lipoxygenase and cyclooxygenase inhibiting constituents from *Chisocheton polyandrus* Merr. *Fitoterapia*, *83*(5), 961–967. https://doi.org/10.1016/j.fitote.2012.04.018

Chong, S.L., Hematpoor, A., Hazni, H., Azirun, M.S., Litaudon, M., Supratman, U., Murata, M., Awang, K., 2019. Mosquito larvicidal limonoids from the fruits of Chisocheton erythrocarpus Hiern. Phytochem. Lett. 30, 69-73. https://doi.org/10.1016/j.phytol.2018.12.013

Christianson, D. W., & Blank, P. N. (2020). Structural biology of template-directed catalysis by terpene synthases. In *Comprehensive Natural Products III* (3rd ed.). Elsevier Inc. https://doi.org/10.1016/b978-0-12-409547-2.14563-9

Connolly, J. D., & Hill, R. . (1991). *Dictionary of Terpenoids*. Chapman & Hall.

El Sayed, K. A., & Hamann, M. T. (1996). A new norcembranoid dimer from the Red Sea soft coral *Sinularia gardineri*. *Journal of Natural Products*, *59*(7), 687–689. https://doi.org/10.1021/np960207z

Ellithey, M. S., Lall, N., Hussein, A. A., & Meyer, D. (2013). Cytotoxic, cytostatic and HIV-1 PR inhibitory activities of the soft coral *Litophyton arboreum*. *Marine Drugs*, *11*(12), 4917–4936. https://doi.org/10.3390/md11124917

Feliciano, A. S., Medarde, M., Gordaliza, M., Olmo, E. Del, & Corral, J. M. M. del. (1989). Sesquiterpenoids and phenolics of *Pulicaria paludosa*. *Phytochemistry*, *28*(10), 2717–2721. https://doi.org/10.1016/ S0031-9422(00)98074-9

Hilmayanti, E., Nurlelasari, Katja, D. G., Hidayat, A. T., Fajriah, S., Darmawan, A., Supratman, U., Azmi, M. N., & Shiono, Y. (2022). Azadirone-type limonoids from the fruit of *Chisocheton lasiocarpus* and their cytotoxic activity against MCF-7 breast cancer lines. *Molekul*, *17*(1), 60–67. https://doi.org/https://doi.org/ 10.20884/1.jm.2022.17.1.5593

Hoai, N. T., Duc, H. V., Raal, A., & Morita, H. (2018). A new limonoid from *Chisocheton paniculatus* fruit collected in Vietnam and its NO production inhibitory activity. *Natural Product Communications*, *13*(10), 1255–1257. https://doi.org/10.1177/1934578x1801301005

Iijima, C., Wong, C. P., Nugroho, A. E., Sotozono, Y., Someya, S., Hirasawa, Y., Kaneda, T., Hadi, A. H. A., & Morita, H. (2016). Anti-melanin deposition activity of ceramicines from *Chisocheton ceramicus*. *Journal of Natural Medicines*, *70*(4), 702–707. https://doi.org/10.1007/s11418-016-1016-y

Izdihar, G., Naini, A. A., Harneti, D., Maharani, R., Nurlelasari, N., Safari, A., Farabi, K., Supratman, U., & Azmi, M. N. (2021). Sesquiterpenoids from the stem bark of *aglaia simplicifolia* and their cytotoxic activity against B16-F10 melanoma skin cancer cell. *Indonesian Journal of Chemistry*, *21*(6), 1560–1567. https://doi.org/10.22146/ijc.68383

Katja, D. G. (2021). Senyawa stigmasterol (steroid) dari kulit batang *Chisocheton celebicus* Koord (Meliaceae). *Chemistry Progress*, *14*(1), 56–61. https://doi.org/10.35799/cp.14.1.2021.34154

Katja, D. G., Farabi, K., Harneti, D., Mayanti, T., & Supratman, U. (2017). Cytotoxic triterpenoid from the stembark of *Chisocheton celebicus* (Meliaceae). *Makara Journal of Science*, *21*(1), 8–12. https://doi.org/10.7454/ mss.v21i1. 7531

Katja, D. G., Hilmayanti, E., Nurlelasari, Mayanti, T., Harneti, D., Maharani, R., Farabi, K., Darwati, Lesmana, R., Fajriah, S., Supratman, U., Azmi, M. N., & Shiono, Y. (2022). Limonoids from the fruits of *Chisocheton lasiocarpus* (Meliaceae). *Journal of Asian Natural Products Research*, 1–8. https://doi.org/10.1080/10286020.2022.2032678

Ludwiczuk, A., Skalicka-Woźniak, K., & Georgiev, M. I. (2017). Terpenoids. In *Pharmacognosy: Fundamentals, Applications and Strategy* (pp. 233–266). https://doi.org/10.1016/B978-0-12-802104-0.00011-1

Lukyanova, N. Y., Rusetskya, N. V., Tregubova, N. A., & Chekhun, V. F. (2009). Molecular profile and cell cycle in MCF-7 cells resistant to cisplatin and doxorubicin. *Experimental Oncology*, *31*(2), 87–91.

Maneerat, W., Laphookhieo, S., Koysomboon, S., & Chantrapromma, K. (2008). Antimalarial, antimycobacterial and cytotoxic limonoids from *Chisocheton siamensis*. *Phytomedicine*, *15*(12), 1130–1134. https://doi.org/10.1016/j.phymed.2008.05.004

Naini, A. A., Mayanti, T., Nurlelasari, Harneti, D., Maharani, R., Safari, A., Hidayat, A. T., Farabi, K., Lesmana, R., Supratman, U., & Shiono, Y. (2022). Cytotoxic sesquiterpenoids from *Dysoxylum parasiticum* (Osbeck) Kosterm. stem bark. *Phytochemistry Letters*, *47*(April 2021), 102–106. https://doi.org/10.1016/j.phytol.2021.11.010

Nugroho, A. E., Hashimoto, A., Wong, C. P., Yokoe, H., Tsubuki, M., Kaneda, T., Hadi, A. H. A., & Morita, H. (2018). Ceramicines M–P from *Chisocheton ceramicus*: isolation and structure–activity relationship study. *Journal of Natural Medicines*, *72*(1), 64–72. https://doi.org/10.1007/s11418-017-1109-2

Nugroho, A. E., Okabe, M., Hirasawa, Y., Wong, C. P., Kaneda, T., Tougan, T., Horii, T., & Morita, H. (2021). A novel trimeric triterpene from *Chisocheton ceramicus* Miq. *Natural Product Communications*, *16*(11). https://doi.org/10.1177/1934578X211053202

Nurlelasari, Katja DG, Harneti D, Wardayo MM, Supratman U, Awang K (2017) Limonoids from the seeds of *Chisocheton macrophyllus*. *Chemistry of Natural Compounds 53*, 83–87. https://doi.org/10.1007/s10600-017-1916-4.

Perveen, S., Alqahtani, J., Orfali, R., Aati, H. Y., Al-taweel, A. M., Ibrahim, T. A., Khan, A., Yusufoglu, H. S., Abdel-kader, M. S., & Taglialatela-scafati, O. (2020). Aerial parts of *Anvillea garcinii*. *Molecules*, *25*, 1730. https://doi.org/10.3390/molecules25071730

Phongmaykin, J., Kumamoto, T., Ishikawa, T., Suttisri, R., & Saifah, E. (2008). A new sesquiterpene and other terpenoid constituents of *Chisocheton penduliflorus*. *Archives of Pharmacal Research*, *31*(1), 21–27. https://doi.org/10.1007/s12272-008-1115-8

Salam, S., Harneti, D., Maharani, R., Safari, A., Tatang, A., Lesmana, R., Azlan, M., Supratman, U., Alexander, T., Prescott, K., & Shiono, Y. (2021). Cytotoxic triterpenoids from *Chisocheton pentandrus*. *Phytochemistry*, *187*(March), 112759. https://doi.org/10.1016/j.phytochem.2021.112759

Shen, Y., Cui, S. J., Chen, H., Shen, L., Wang, M., Dong, X., Xiao, C. J., & Jiang, B. (2020). Antimalarial eudesmane sesquiterpenoids from *Dobinea delavayi*. *Journal of Natural Products*, *83*(4), 927–936. https://doi.org/10.1021/acs.jnatprod.9b00761

Shilpi, J. A., Saha, S., Chong, S. L., Nahar, L., Sarker, S. D., & Awang, K. (2016). Advances in chemistry and bioactivity of the genus Chisocheton Blume. *Chemistry and Biodiversity*, *13*(5), 483–503. https://doi.org/10.1002/cbdv.201400373

Stevens, P. F. (1975). *Review of Chisocheton (Meliaceae) in Papuasia* (Issue 11).

Supratman, U., Naibaho, W., Salam, S., Maharani, R., Hidayat, A. T., Harneti, D., Nurlelasari, & Shiono, Y. (2019). Cytotoxic triterpenoids from the bark of *Chisocheton patens* Blume

(Meliaceae). *Phytochemistry Letters*, *30*(January), 81–87. https://doi.org/10.1016/j.phytol.2019.01.034

Wang, X., Peng, X., Tang, C., Zhou, S., Ke, C. Q., Liu, Y., Yao, S., Ai, J., & Ye, Y. (2021). Anti-inflammatory eudesmane sesquiterpenoids from *Artemisia hedinii*. *Journal of Natural Products*, *84*(5), 1626–1637. https://doi.org/10.1021/acs.jnatprod.1c00177

Wibowo, A., Ahmat, N., Hamzah, A. S., Sufian, A. S., Ismail, N. H., Ahmad, R., Jaafar, F. M., & Takayama, H. (2011). Malaysianol A, a new trimer resveratrol oligomer from the stem bark of *Dryobalanops aromatica*. *Fitoterapia*, *82*(4), 676–681. https://doi.org/10.1016/j.fitote.2011.02.006

Wong, C. P., Kaneda, T., Hadi, A. H. A., & Morita, H. (2014). Ceramicine B, a limonoid with anti-lipid droplets accumulation activity from *Chisocheton ceramicus*. *Journal of Natural Medicines*, *68*(1), 22–30. https://doi.org/10.1007/s11418-013-0755-2

Yang, M. H., Wang, J. S., & Kong, L. Y. (2012). Chemical constituents of *Chisocheton cumingianus*. *Chinese Journal of New Drugs*, *21*, 555–560.

Zhang, H. J., Tan, G. T., Santarsiero, B. D., Mesecar, A. D., Van Hung, N., Cuong, N. M., Soejarto, D. D., Pezzuto, J. M., & Fong, H. H. S. (2003). New sesquiterpenes from *Litsea verticillata*. *Journal of Natural Products*, *66*(5), 609–615. https://doi.org/10.1021/np020508a