Sesquiterpenoids from the Stem Bark of *Lansium domesticum* Corr. Cv. Kokossan and Their Cytotoxic Activity against MCF-7 Breast Cancer Cell Lines

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Abstract: *The* n*-hexane fraction from the stem bark of* Lansium domesticum *Corr. Cv. Kokosan afforded 3 sesquiterpenoids, namely eudesm-4(15),7-dien-1β-ol (**1**), eudesm-4(15)-ene-1β,6α-diol (**2**), and octahydro-4-hydroxy-3a-methyl-7-methylene-a-(1-methylethyl)-1*H*-indene-1-methanol (**3**). These three compounds were discovered in the* Lansium *genus for the first time. Their chemical structures were determined based on data generated from various spectroscopic methods, including one- and two-dimensional NMR, as well as mass spectroscopy. Sesquiterpenoid compounds (**1**-**3**) have also been evaluated on MCF-7 breast cancer cell lines and the compound **1** showed the strongest activity with an IC_{50} value of 17.97 μg/mL while compounds **2** and **3** showed moderate and weak activity with IC_{50} values of 121.65 and 201.57 μg/mL, respectively. The implication of the findings of these compounds is as an illustration that one of these sesquiterpenoids has potential as an anticancer with the presence of double bond which played important role in the cytotoxic activity that can be studied for new drug discovery.*

Keywords: *cytotoxic activity;* Lansium domesticum *Corr. Cv. Kokosan; MCF-7 cell lines; sesquiterpenoid*

■ INTRODUCTION

Sesquiterpenoids are secondary metabolites of the terpenoid class that have important roles in the biological systems used by humans [1]. Sesquiterpenoids, generally found in higher plants as a volatile oil, are derived from the acyclic derivative of the initial compound, namely farnesyl pyrophosphate (FPP) derived from three isoprene units as the precursors. The FPP has a long chain structure and three double bonds, so it can form various cyclic formations with more flexibility such as monocyclic, tricyclic and tetracyclic [2]. Sesquiterpenoids have a C-15 framework and diverse structures due to the presence of functional groups and substituents [3]. In addition, sesquiterpenoids have also various biological activities such as anti-inflammatory, antibacterial, antioxidant, antitumor, and antimalarial [4]. The previous studies reported that aromadendrane-type sesquiterpenoids displayed antifeedant, antifouling, antimicrobial, antiviral, insect repellent, and cytotoxic activities [5]. Sesquiterpenoids are widely found in the Meliaceae family. The genera of the Meliaceae family which contain sesquiterpenoids are Trichilia [6-7], Capadessa [8], *Dysoxylum* [9], *Aglaia* [10], and *Lansium* [11-13]. Several sesquiterpenoids were isolated from Meliaceae family with good activity against MCF-7 breast cancer cells such as 10β-hydroxy-4α,4β-dimethyl-5α*H*,7α*H*-

eudesm-3-one and spatunelol. These two compounds were isolated from the stem bark of *D. parasiticum* with IC_{50} values for MCF-7 of 12.17 and 23.79 μM, respectively [14]. The other sesquiterpenoids isolated from the stem bark of plants from the Meliaceae family (*Aglaia harmsiana*) have good cytotoxic against MCF-7, namely; β-caryophyllene oxide, senecrassidiol; 4β,10α-dihydroxy aromadendrane; 4α,10α-dihydroxyaromadendrane with the IC_{50} values of 0.62, 1.32, 8.41, and 2.8 μM, respectively [15-16].

Lansium domesticum Corr. is a species and seasonal plant that belongs to the *Lansium* genus. *L. domesticum* Corr., which grows in tropical regions, especially in Southeast Asia, such as Philippines, Malaysia, Thailand, and Indonesia [17]. Previous research on this species reported that this species has compounds that have been isolated as follows: triterpenoids, onoceranoid-type onoceradiendione, limonoids, glycoside [18], and sesquiterpenoids. In Indonesia, this species has three cultivars and *Lansium domesticum* Corr. Cv. Kokosan is one of its cultivars which was observed to study the cytotoxicity of the Indonesian *Lansium* plant against MCF-7 breast cancer cell lines. We revealed the isolation, structure elucidation, and cytotoxic evaluation of an oppositane sesquiterpene and two eudesmane-type sesquiterpenoids **1**-**3**.

■ EXPERIMENTAL SECTION

Materials

The stem bark of Kokosan was gathered from Pangandaran Botanical Garden, Pangandaran, West Java Province, Indonesia, in March 2021. The plant was determined at Laboratory Plant Taxonomy, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor, West Java and the voucher specimen has been deposited at the Laboratory. Organic solvents including ethanol, *n*-hexane, ethyl acetate, methanol, *n*-butanol, methylene chloride, and acetone were purchased from Kristata Gemilang Company, Bandung in technical quality and distilled.

Instrumentation

The IR spectra were obtained by Perkin Elmer Spectrum 100 FTIR spectrometer (Shelton, Connecticut, USA) using a NaCl plate. The mass spectra were recorded with Waters Q-TOF Xevo mass spectrometer instrument (Waters, Milford, Massachusetts, USA). The NMR spectra of all compounds were recorded on the JEOL JNM-ECX500R/S1 spectrometer (Tokyo, Japan) at 500 MHz for ^{1}H and 125 MHz for ^{13}C with TMS as an internal standard. The column chromatography was conducted on silica gel 60 (70–230 and 230–400 mesh, Merck, Darmstadt, Germany). The TLC analyses were implemented with silica GF_{254} (Merck, 0.25 mm) using various solvent systems and spot detection was attained by spraying with 10% of H_2SO_4 in EtOH, followed by heating and irradiating under ultraviolet-visible light (254 and 365 nm).

Procedure

Extraction and isolation

The dried bark Kokosan (3.18 kg) was macerated with ethanol (20 L) at room temperature (25 °C). The extract was evaporated by a rotary evaporator at 40 °C under reduced pressure to acquire the concentrated ethanol extract (300 g). Then, this residue was dissolved in water and partitioned successively with *n*-hexane (8 L) to yield 124 g, ethyl acetate (7 L) 54 g, and *n*-butanol (1.5 L) 15 g. This separation was monitored by thin layer chromatography and compound detection was carried out using 10% H_2SO_4 stain in ethanol and heated. Then, the *n*-hexane extract (124 g) was fractionated by vacuum liquid chromatography on silica gel G60 using 10% gradient elution of *n*-hexane:EtOAc:MeOH to acquire 12 (A-L) subfractions. Fraction B (15.2 g) was further fractionated by vacuum liquid chromatography using 2% gradient elution of *n*-hexane:EtOAc to afford eight fractions. Fraction B5a (4.37 g) was separated using column chromatography on silica gel (230–400 mesh) eluted with *n*-hexane:DCM:EtOAc (7.5:0.5:0.1) to afford compound **1** (10.5 mg). The B6 fraction (0.69 g) was separated using column chromatography on silica gel (230–400 mesh) eluted with using 5% gradient elution of *n*-hexane:EtOAc to afford 3 fractions (B.6a-B.6c). Fraction B6a (98.5 mg) was separated using column chromatography on octadecyl silane (ODS) eluted with methanol:water (5:5) to obtain compound **2** (10.2 mg) and **3** (9.3 mg).

Eudesm-4(15),7-dien-1β-ol (1). Pale yellow oil, IR ν_{max} 3364; 2955; 1645; 1464; 1379;1282; 1041 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz) data are shown in Table 1. HR-TOFMS *m/z* found 221.1904 $[M+H]^+$ (calculated for $C_{15}H_{24}O$, *m/z* 220.1905).

Eudesm-4(15)-ene-1β,6α-diol (2). White powder, IR ν_{max} 3397; 2933; 1456; 1386;1061 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz) data are shown in Table 1. HR-TOFMS *m/z* found 239.2007 $[M+H]^+$ (calculated for $C_{15}H_{26}O_2$, *m/z* 239.2011).

5-Octahydro-4-hydroxy-3r-methyl-7-methylene-r-(1-methylethyl)-1H-indene-1-methanol (3). Colorless oil, IR ν_{max} 3387; 2973;1650; 772 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz) data are shown in Table 1. HR-TOFMS *m/z* found 261.1816 $[M+Na]^+$, (calculated for $C_{15}H_{26}O_2Na$, *m/z* 261.1831).

Cytotoxic activity test by the MTT assay

Cell viability was assessed by MTT reagent (Thermo Fisher Scientific, Uppsala, Sweden) based on the reduction of resazurin (blue), which works as a function of redox potential. Actively respiring cells convert the water-soluble MTT to an insoluble purple formazan. The formazan is then solubilized and its concentration is determined by optical density. In the initial step, MCF-7 cell cultures that were 80% confluent were washed with 1 mL 1X PBS twice. Cells were added with 1 mL of trypsin EDTA and then incubated for 3 min until the cells were released. Then, the cells were transferred into a Falcon tube containing 5 mL of culture medium and centrifuged at 1,200 rpm for 4 min. The supernatant was discarded and cells were resuspended with 1 mL of culture medium. then, cells were counted using a haemocytometer and planted in 96 well plates with a series number for the standard curve (6 times replications) and 3 times repetitions for the treatment then added 100 µL of the medium, incubated at 37 °C for 24 h, 5% CO_2.

The medium was replaced with 180 µL of new medium and added 20 µL of compound **1**-**3** (1, 10, 100, 250, and 500 ppm) with co-solvent with various percentages of DMSO (0.5–2.5%) in PBS (Uppsala, Sweden). incubated at 37 °C for 24 h, 5% CO_2. After that, cells were added with MTT 20 µL, incubated for at 37 °C for 3 h, 5% CO_2. The absorbance was determined at 570 nm and the IC_{50} value was appointed with the equation given below based on the comparison of the percentage of cytotoxicity to untreated cells. In this trial, the used control positive was Doxorubicin. Based on the literature [14], all assays and analyses were respectively run in duplicate and all averaged so that a plot of % cytotoxicity versus sample concentration was used to calculate the concentration indicating 50% cytotoxicity (IC_{50}).

■ RESULTS AND DISCUSSION

The *n*-hexane extract from the stem bark of the Kokosan was separated and purified using the column chromatography method repeatedly, to produce three sesquiterpene compounds **1**-**3**.

Compound **1**, pale yellow oil, with the yield of 0.2% (10.5 mg) from B5a fraction (4.37 g), was elucidated by interpreting peaks of the IR spectrum (Fig. S1) which appeared at 3364 (hydroxyl group), 2955 (C-H sp^3) 1645 (olefinic bond), 1645 (C=C double bonds), 1464 and 1379 (*gem*-dimethyl), and 1041 cm^{-1} (C-O stretching). The structural formula was established with HR-TOF-MS (Fig. S2) which showed the molecular ion peak of 221.1904 $[M+H]^+$ and the calculated mass of $C_{15}H_{24}O$ *m/z* 220.1827. The prediction was supported by NMR data. The ^{1}H-NMR spectrum (Fig. S3) showed the presence of one tertiary methyl at δ_H 0.64 ppm (CH_3-14), *gem*-dimethyl at δ_H 0.99 ppm (CH_3-12 and CH_3-13) as well as two olefinic protons at 4.63 and 4.82 ppm (CH_2-15). The ^{13}C-NMR spectrum (Fig. S4) showed 15 signals of carbons and the classification of these signals based on their chemical shifts and DEPT 135° (Fig. S5) as three methyls, four methylenes, three methines (one oxygenated methine), one quaternary carbon, and four olefinic carbons (including two quaternary carbons, one methylene and one methine). Based on the structure elucidation of compound **1**, there were 4 degrees of

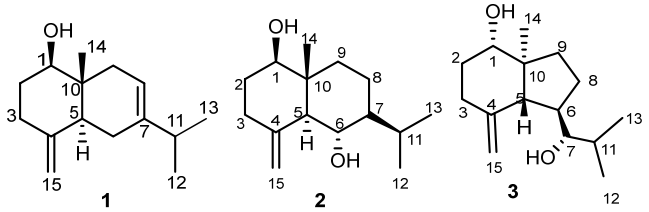

Fig 1. Structures of sesquiterpenoids **1**-**3**

unsaturation consisting of 2 double bonds and bicyclic skeleton, each consisting of six carbons. This compound showed the presence of an isopropyl group at C-7 and a methyl group at C-10 which was a characteristic of eudesmane-type sesquiterpene compounds [1] and was supported by previous report [19]. Compound **1** was determined as the known compound eudesm-4(15),7-dien-1β-ol (**1**) and the structure of this compound can be seen in Fig. 1 number 1. This compound was isolated from the genus *Lansium* for the first time.

Compound **2** was acquired as a white powder with melting point of 118–120 °C in 10.36% yield (10.2 mg) from B6a fraction (98.5 mg). The IR spectrum (Fig. S6) showed the absorption band of the hydroxyl group (3397 cm^{-1}), C-H sp^3 (2933 cm^{-1}), *gem*-dimethyl (1456 and 1386 cm^{-1}), and C-O stretching (1061 cm^{-1}). The structural formula was established with HR-TOFMS (Fig. S7) which showed molecular ion peak of *m/z* 239.2007 $[M+H]^+$ and the calculated mass of $C_{15}H_{26}O_2$ *m/z* 238.1933. The ^{1}H-NMR spectrum (Fig. S8) showed the presence of one tertiary methyl at δ_H 0.65 ppm (CH_3-14), *gem*-dimethyl at 0.89 and 0.80 ppm (CH_3-12 and CH_3-13) and also two olefinic protons at δ_H 4.68 and 4.95 ppm (-CH_2-15). The ^{13}C-NMR spectrum (Fig. S9) showed 15 signals of carbons and the classification of these signals based on their chemical shifts and DEPT 135° (Fig. S10) as three methyls, four methylenes, five methines (two oxygenated methine), one quaternary carbon, and two olefinic carbons (including one quaternary carbons, and one methylene).

The structure elucidation of compound **2** showed that there were 3 degrees of unsaturation consisting of 1 double bond and bicyclic moiety, each consisting of six carbons, similar to compound **1**. This compound showed the presence of an isopropyl group at C-7 and a methyl group at C-10 which was a characteristic of eudesmane-type sesquiterpenoid [1] and was supported by the previous report [20]. The position of the functional group was proved by the correlation of HSQC (Fig. S9). This compound was isolated from the genus *Lansium* for the first time and its structure can be seen in Fig. 1 number 2.

Compound **3** was acquired as a colorless oil in 9.44% yield (9.3 mg) from B6a fraction (98.5 mg). The IR spectrum (Fig. S11) showed the absorption band of the hydroxyl group (3387 cm^{-1}), C-H sp^3 (2973 cm^{-1}), and C=C double bond (1650 cm^{-1}). The structural formula was established with HR-TOFMS (Fig. S12) which showed molecular ion peak of 261.1816 $[M+Na]^+$ and the calculated mass of $C_{15}H_{26}O_2Na$ *m/z* 261.1933. This prediction was supported by NMR data. The ^{1}H-NMR spectrum (Fig. S13) showed the presence of one tertiary methyl at δ_H 0.60 ppm (CH_3-14), *gem*-dimethyl at 0.92 and 0.84 ppm (CH_3-12 and CH_3-13) as well as two olefinic protons at 4.74 and 4.88 ppm (CH_2-15). The ^{13}C-NMR spectrum (Fig. S14) showed 15 signals of carbons and the classification of these signals based on their chemical shifts and DEPT 135° (Fig. S15) as three methyls, four methylenes, five methines (two oxygenated methines), one quaternary carbon, and two olefinic carbons (including one quaternary carbon and one methylene). This compound had 3 degrees of unsaturation consisting of 1 double bond and bicyclic skeleton.

The ^{1}H-NMR, ^{13}C-NMR and DEPT spectra were supported by spectral data from HSQC (Fig. S16) where the spectral matching pattern was appropriate and the chemical shift can be seen in Table 1. Apart from being compared to the literature, compound **3** was reviewed through the ^{1}H-^{1}H COSY correlation (Fig. S17) where there was a relationship between H_1-H_2-H_3 and the continuous relationship between H_5-H_6-H_8-H_9 and H_6-H_7-H_{11}-$H_{12,13}$ (Fig. 2). These correlations showed the presence of rings A and B (bicyclic bonds) with the presence of an isobutanol group attached to the B ring. This compound had a different skeleton from compound **2**, where the skeleton of compound **2** had bicyclic each six carbons whereas compound **3** had six carbon (ring A) and five-carbon (ring B) showed by the relationship of spectrum ^{1}H-^{1}H COSY (H_5-H_6-H_8-H_9 and H_6-H_7-H_{11}-$H_{12,13}$) (Fig. 2). This data supported that this compound is an opposite sesquiterpenoid- type and was proved by the HMBC correlation (Fig. S18).

The tertiary methyl (CH_3-14) (δ_H 0.60) was correlated to C-9 (δ_C 37.3), C-10 (δ_C 49.5), C-5 (δ_C 56.4), and C-1 (δ_C 79.0). Then, the correlation of CH_3-13 (δ_H 0.84) to C-12 (δ_C 20.6), C-11 (δ_C 31.3), C-7 (δ_C 82.7), and

the correlation of CH_3-12 (δ_H 0.92) to C-13 (δ_C 14.8), C-11 (δ_C 31.3), C-7 (δ_C 82.7) showed that C-13 and C-12 are *gem*-dimethyl group attached to C-11 and indicated that the partial structure of **3** contains isobutanol group which bound to C-6, cyclopentane ring and C-7 has a hydroxyl group. The correlation of methylene protons at δ_H 1.24 and 1.84 (H-8) to C-9 (δ_C 37.3), C-10 (δ_C 49.5), C-7 (δ_C 82.7), and C-6 (δ_C 39.4) and methylene protons at δ_H 1.31 and 1.69 (H-9) to C-8 (δ_C 37.3), C-6 (δ_C 39.4), C-10 (δ_C 49.5), C-5 (δ_C 56.4), C-1 (δ_C 79.0 showed the oxygenated C with hydroxyl group) indicated that the compound **3** contained cyclopentane ring at C-5, C-6, C-8, C-9 and C-10 in the presence of isobutanol group located at C-6.

Another partial skeleton was determined by the strong correlation of methine proton at δ_H 1.77 (H-5) to C-6 (δ_C 39.4), C-4 (δ_C 148.9) C-10 (δ_C 49.5), C-7 (δ_C 82.7), C-3 (δ_C 34.9), C-1 (δ_C 79.0), C-14 (δ_C 12.3), and C-15 (δ_C 107.6), indicated that H-5 was between cyclopentane and another part of the skeleton. This correlation showed that this skeleton indicated a double bond at C-4 (δ_C 148.9) and C-15 (δ_C 107.6). This conjecture was confirmed by the proton H-15 at δ_H 4.88 and 4.74 (H-15) to C-4 (δ_C 148.9), C-3 (δ_C 34.9), and C-5 (δ_C 56.4) which showed the double bond attached to C-4, which was adjacent to C-3 and C-5.

The other correlation of methylene protons were at δ_H 2.04 and 2.23 ppm (H-3) to C-4, C-5, C-15, C-2, and C-1 and at δ_H 1.41 and 1.78 ppm (H-2) to C-1, C-3, C-10, C-4, and C-1. The data correlations proved that methylene CH_2 (2) and CH_2 (3) were next to each other and in the same cyclic as evidenced by the HMBC correlation, that this cyclic was composed of six carbons (C-1, C-2, C-3, C-4, C5 and C-10), cyclohexane (ring A), wherein C-5 and C-10 were bridges to ring B (cyclopentane). In ring A, there was a carbon that was oxygenated by a hydroxyl group, located in C-1 (δ_C 79.0) and there was a double bond between carbon C-4 and C-15. The relative stereochemistry of compound **3** was

Table 1. NMR data of compounds **1**-**3** ($CDCl_3$ at 500 MHz for ^1H and 125 MHz for ^{13}C)

Position Carbon	Compounds					
	1		**2**		**3**	
	δ_C (mult.)	δ_H (∑H, mult., *J* = Hz)	δ_C (mult.)	δ_H (∑H, mult., *J* = Hz)	δ_C (mult.)	δ_H (∑H, mult., *J* = Hz)
1	79.6 (d)	3.62 (1H, t, 11.7)	79.0 (d)	3.38 (1H, dd, 11.6, 4.7)	79.0 (d)	3.53 (1H, dd, 11.0, 5.0)
2	31.5 (t)	1.83 (2H, m)	31.9 (t)	1.82 (2H, m)	31.9 (t)	1.41 (1H, m)
						1.78 (1H, m)
3	34.3 (t)	2.35 (2H, m)	35.1 (t)	2.3 (2H, m)	34.9 (t)	2.04 (1H, m)
						2.23 (1H, m)
4	148.4 (s)	-	146.2 (s)	-	148.9 (s)	-
5	43.0 (d)	1.72 (1H, m)	55.9 (d)	1.7 (1H, m)	56.4 (d)	1.77 (1H, m)
6	25.6 (t)	1.95 (2H, m)	67.0 (d)	3.68 (1H, t, 9.8)	39.4 (d)	2.24 (1H, m)
7	141.7 (s)	-	49.3 (d)	1.26 (1H, m)	82.7 (d)	3.17 (1H, dd, 2.5, 9.5)
8	115.8 (d)	5.31 (1H, dd, 5.4, 2.1)	18.2 (t)	1.5 (2H, m)	26.1 (t)	1.25; 1.84 (m)
9	38.4 (t)	1.90 (2H, m)	36.3 (t)	1.88 (2H, dt, 12.4, 2.9)	37.3 (t)	1.31 (1H, m)
						1.69 (1H, m)
10	38.9 (s)	-	41.7 (s)	-	49.5 (s)	-
11	35.1 (d)	2.16 (1H, m)	26.0 (d)	2.2 (1H, m)	31.3 (d)	1.77 (1H, m)
12	21.7 (q)	0.99 (3H, d, 6.8)	21.1 (q)	0.89 (3H, d, 7.0)	20.6 (q)	0.92 (3H, d, 7.0)
13	21.3 (q)	0.99 (3H, d, 6.8)	16.2 (q)	0.80 (3H, d, 7.0)	14.9 (q)	0.84 (3H, d, 7.0)
14	10.4 (q)	0.63 (3H, s)	11.5 (q)	0,65 (3H, s)	12.4 (q)	0.60 (3H, s)
15	107.8 (t)	4.62 (1H, d, 1.5)	107.8 (d)	4.68 (1H, d, 1.1)	107.7 (t)	4.88 (1H, d, 1.5)
		4.83 (1H, d, 1.5)		4.95 (1H, d, 1.1)		4.74 (1H, d, .5)

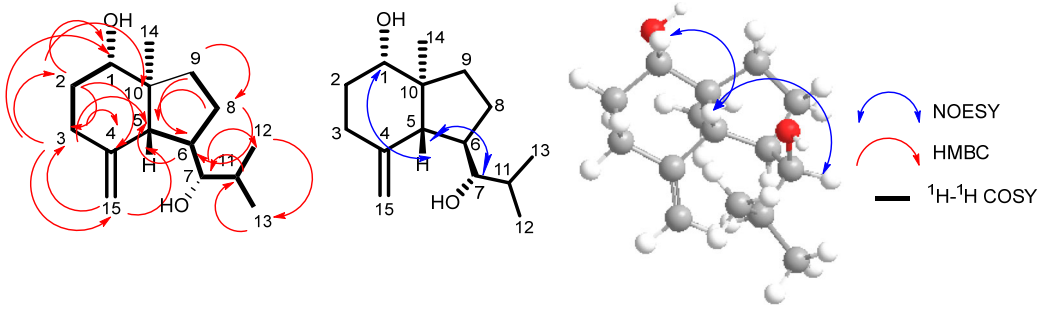

Fig 2. Selected HMBC, ^{1}H-^{1}H-COSY and NOESY correlations of compound **3**

Table 2. The cytotoxic activity of compounds **1**-**3** against MCF-7 breast cancer cell lines

Compounds	IC_{50} (μg/mL)
Eudesm-4(15),7-dien-1β-ol (**1**)	17.97
Eudesm-4(15)-ene-1β, 6α-diol (**2**)	121.65
Octahydro-4-hydroxy-3r-methyl-7-methylene-r-(1-methylethyl)-1H-indene-1methanol (**3**)	201.57
Doxorubicin (+)	0.17

supported by NOESY correlations (Fig. S19). The results displayed that the correlation of H-5 (β-oriented) with H-1 and H-7, and hydroxyl group at C-1 and C-7 was α-oriented. Whereas H-14 and H-6 have no correlation with H β-oriented, so they have α-configuration. Compound **3** has a different cyclicity from compounds **1** and **2** (Fig. 1), detailed examination of the NMR spectral data and comparison with those reported [20] for compound **3** identified that the structure of this compound is octahydro-4-hydroxy-3a-methyl-7-methylene-a-(1-methyl ethyl)-1*H*-indene-1methanol (**3**), similar to compounds eudesm-4(15),7-dien-1β-ol (**1**), and eudesm-4(15)-ene-1β, 6α-diol (**2**). The three compounds have many similarities in the proton shift NMR and C-NMR data which can be seen in Table 2, this compound is also the first to be isolated from the genus *Lansium*. The structure of compound **3** can be seen in Fig. 1 and the correlation between HMBC, ^{1}H-^{1}H COSY and the determination of its configuration through NOESY can be seen in Fig. 2.

The cytotoxic activity of the sesquiterpenoids **1**-**3** was carried out against the MCF-7 cancer cell according to a method described [21-22]. The used positive control was doxorubicin (0.17 μg/mL) and the results are shown in Table 2. Among all sesquiterpenoid compounds, eudesm-4(15),7-dien-1β-ol (**1**) showed the highest cytotoxic activity, while octahydro-4-hydroxy-3a-methyl-7-methylene-a-(1-methylethyl)-1*H*-indene-1-methanol (**3**) showed the lowest activity. The IC_{50} value indicated that the presence of an olefinic group at C-7 of compound **1** significantly increased cytotoxic activity compared to compound **2** which has lost one of the olefinic group at C-7 and underwent the addition of a hydroxyl group at C-6. This conversion reduced cytotoxic activity as well as compound **3** which had lower IC_{50} than that of compound **2** apart from the loss of olefinic group and addition of hydroxyl group. Compound **3** also underwenr a structural change from cyclohexane substituted with isopropyl group to cyclopentane substituted 2-methylpropanol at C-6. Therefore, changes in ring shape and substituents of this compound may reduce the IC_{50} value. These three compounds were also compared with the new eudesman 10β-hydroxy-4α,4β-dimethyl-5αH,7α*H*-eudesm-3-one compound which was successfully isolated by Naini et al. [14] from *Dysoxylum parasiticum* which had a higher IC_{50} value with the presence of a ketone group at C-3 and only one hydroxyl group was substituted at C-10 while compounds **1**-**3** have a methyl group at C-10. The significant probability of the presence of a π electron can increase the IC_{50} value. These results indicated that the hydroxyl and olefinic groups played several critical structural features in the cytotoxic activity of eudesmane-type sesquiterpenoids.

■ CONCLUSION

Three eudesmane-type sesquiterpenoid, eudesm-4(15),7-dien-1β-ol (**1**), eudesm-4(15)-ene-1β,6α-diol (**2**), and octahydro-4-hydroxy-3a-methyl-7methylene-a-(1-methylethyl)-1*H*-indene 1-methanol (**3**) were successfully isolated from the *n*-hexane extract of the stem bark of Kokosan. All compounds (**1**-**3**) were reported from the genus *Lansium* for the first time and were evaluated for their cytotoxic activity against MCF-7 breast cancer cell lines. Among the eudesm-type sesquiterpenoids, compound **1** showed the highest activity while compound **3** showed the lowest activity. The different values of IC_{50} were caused by the different functional groups of the skeleton eudesm-type. The presence of hydroxyl group decreased the IC_{50} value and the presence of olefinic group increased the IC_{50} value. The hydroxyl group and the olefinic group played some important structural features for cytotoxic activity in eudesmane-type sesquiterpenoid, one of these sesquiterpenoids had the potential as an anticancer breast that can be developed as a target molecule through a mechanism that can be studied for new drug discovery.

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■ REFERENCES

[1] Chappell, J., and Coates, R.M., 2010, "Sesquiterpenes" in *Comprehensive Natural Products II*, vol. 1. Eds. Mandler, L., and Liu, H.W., Elsevier, Kidlington, United Kingdom, 609–641.

[2] Talapatra, S.K., and Talapatra, B, 2015, *Chemistry of Plant Natural Products: Stereochemistry, Conformation, Synthesis, Biology, and Medicine*, Springer-Verlag, Berlin Heidelberg.

[3] Gong, D.Y., Chen, X.Y., Guo, S.X., Wang, B.C., and Li, B., 2021, Recent advances and new insights in biosynthesis of dendrobine and sesquiterpenes, *Appl. Microbiol. Biotechnol.*, 105 (18), 6597–6606.

[4] Zhao, W.Y., Yan, J., Liu, T.T., Gao, J., Huang, H.L., Sun, C.P., Huo, X.K., Deng, S., Zhang, B.J., and Ma, X.C., 2020, Natural sesquiterpenoid oligomers: A chemical perspective, *Eur. J. Med. Chem.*, 203, 112622.

[5] Jaeger, R., and Cuny, E., 2016, Terpenoids with special pharmacological significance: A review, *Nat. Prod. Commun.*, 11 (9), 1373–1390.

[6] Nangmo, K.P., Tsamo, T.A., Zhen, L., Mkounga, P., Akone, S.H., Tsabang, N., Müller, W.E.G., Marat, K., Proksch, P., and Mkengfack, A.E., 2018, Chemical constituents from leaves and root bark of *Trichilia monadelpha* (Meliaceae), *Phytochem. Lett.*, 23, 120–126.

[7] Hou, L., Tang, G.H., Zhang, Y., Hao, X.J., Zhao, Q., and He, H.P., 2013, A new carotane sesquiterpene from *Walsura robusta*, *Chin. J. Nat. Med.*, 11 (1), 84–86.

[8] Fu, L.R., Ma, Q.Y., Huang, S.Z., Dai, H.F., Guo, Z.K., Yu, Z.F., and Zhao, Y.X., 2014, Terpenoids and their anti-feedant activity from *Cipadessa cinerascens*, *J. Asian Nat. Prod. Res.*, 16 (11), 1054–1059.

[9] Nugroho, A.E., Sugiura, R., Momota, T., Hirasawa, Y., Wong, C.P., Kaneda, T., Hadi, A.H.A., and Morita, H., 2015, Dysosesquiflorins A and B, sesquiterpenoids from *Dysoxylum densiflorum*, *J. Nat. Med.*, 69 (3), 411–415.

[10] Huang, P.Q., Deng, J.W., Li, Y., Liao, Z.B., Zhao, E., Tian, Y.C., Tu, Y.D., Li, D.L., Jin, J.W., Zhou, C.X., Wu, R.H., and Gan, L.S., 2022, Terpenoids from the twigs and leaves of *Aglaia elaeagnoidea* and their chemotaxonomic significance, *Biochem. Syst. Ecol.*, 103, 104427.

[11] Fadhilah, K., Wahyuono, S., and Astuti, P., 2021, Fractions and isolated compounds from *Lansium domesticum* fruit peel exhibited cytotoxic activity against T-47D and HepG2 cell lines, *Biodiversitas*, 22 (9), 3743–3748.

[12] Fadhilah, K., Wahyuono, S., and Astuti, P., 2020, A bioactive compound isolated from duku (*Lansium domesticum* Corr) fruit peels exhibits cytotoxicity against T47D cell line, *F1000Res.*, 9, 3.

[13] Fadhilah, K., Wahyuono, S., and Astuti, P., 2020, A

sesquiterpene aldehyde isolated from ethyl acetate extract of *Lansium Domesticum* fruit peel, *Indones. J. Pharm*., 3 (2), 394–398.

[14] Naini, A.A., Mayanti, T., Nurlelasari, Harneti, D., Maharani, R., Safari, A., Hidayat, A.T., Farabi, K., Lesmana, R., Supratman, U., and Shiono, Y., 2022, Cytotoxic sesquiterpenoids from *Dysoxylum parasiticum* (Osbeck) Kosterm. stem bark, *Phytochem. Lett.*, 47, 102–106.

[15] Milawati, H., Sukmawati, W., Harneti, D., Maharani, R., Nurlelasari, N., Hidayat, A.T., Darwati, D., Supratman, U., and Shiono, Y., 2020, Cytotoxic sesquiterpenoids from the stem bark of *Aglaia harmsiana* (Meliaceae), *Indones. J. Chem*., 20 (6), 1448–1454.

[16] Milawati, H., Harneti, D., Maharani, R., Nurlelasari, N., Hidayat, A.T., Azmi, M.N., Shiono, Y., and Supratman, U., 2019, Caryophyllene-type sesquiterpenoids from the stembark of *Aglalia harmsiana* and their cytotoxic activity against MCF-7 breast cancer cells, *Molekul*, 14 (2), 126–132.

[17] Lubis, M.F., Hasibuan, P.A., Syahputra, H., and Astyaka, R., 2022, A Review on phytochemicals and pharmacological activities as ethnomedicinal uses of duku (*Lansium domesticum* Corr.), *Open Access Maced. J. Med. Sci*., 10 (F), 57–65.

[18] Mayanti, T., Apriantini Y.P., Soidah, S., Sianturi, J., Darneti, D., Julaeha, E., Sumiarsa, D., Nurlelasari, and Maharani, R., 2018, Antibacterial triterpenoids from the barks and leaves of *Lansium domesticum* Corr cv. Kokossan (Meliaceae), *J. Chem.*, 12 (1), 54–58.

[19] Soares, L.R., de Queiroz e Silva, A.C., Freire, T.V., Garcez, F.R., and Garcez, W.S., 2012, Sesquiterpenos de sementes de *Guarea guidonia* (Meliaceae), *Quim. Nova*, 35 (2), 323–326.

[20] Zhang, H.J., Tan, G.T., Santarsiero, B.D., Mesecar, A.D., Van Hung, N., Cuong, N.M., Soejarto, D.D., Pezzuto, J.M., and Fong, H.H.S., 2003, New sesquiterpenes from *Litsea verticillata*, *J. Nat. Prod*., 66 (5), 609–615.

[21] Xu, M., McCanna, D.J., and Sivak, J.G., 2015, Use the viability reagent PrestoBlue in comparison with alamarBlue and MTT to assess the viability of human corneal epithelial cells, *J. Pharmacol. Toxicol. Methods*, 71, 1–7.

[22] Azizi, K., Hamedi, A., Azarpira, N., Shahini, M., and Pasdaran, A., 2021, A new cytotoxic sesquiterpene lactone from *Euphorbia microsphaera* Boiss against human breast cancer (MCF-7) and human fibrosarcoma (HT1080) cells, *Toxicon*, 202, 60–66.