Sesquiterpenoids from *Dysoxylum amooroides* Stem Bark: Isolation, Structure Determination, and Cytotoxicity Against MCF-7 Breast Cancer Cells

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Abstract: *Three sesquiterpenoids, guaianediol (**1**), alismol (**2**), and spathulenol (**3**), were isolated from the* n*-hexane and ethyl acetate extracts of the stem bark of* Dysoxylum amooroides*. The three compounds were found in* D. amooroides *species for the first time. The structures of the isolated compounds were identified and established based on an extensive spectroscopic analysis involving HR-TOF-ESI-MS, IR, and NMR data, as well as a comparison with the previously reported works of literature. Compounds **1**-**3** were further assessed for cytotoxic effects against MCF-7 breast cancer cells. Guaianediol (**1**) showed inactive activity with $IC_{50} > 100$ μM, alismol (**2**) showed weak activity with IC_{50} value of 82.1 μM and spathulenol (**3**) showed considerable activity with an IC_{50} value of 15.2 μM. A brief structure-activity relationship and comparison with the previous works were also discussed to understand better the role of guaiane- and aromadendrane-type sesquiterpenoids in the biological activity perspective.*

Keywords: *cytotoxic activity;* Dysoxylum amooroides*; MCF-7; sesquiterpenoids*

INTRODUCTION

Sesquiterpenoids are widely distributed in nature, especially within higher plants, and more than 10,000 sesquiterpenoids have been isolated. Sesquiterpenoids are natural organic compounds that belong to the terpenoid group. They comprise three isoprene units (C_5), generally containing 15 carbons and 24 hydrogens per molecule ($C_{15}H_{24}$). Since sesquiterpenoids are formed by the farnesyl pyrophosphate, a precursor that is produced by 2 building blocks, isopentenyl diphosphate and dimethylallyl diphosphate from 3 units of acetyl-CoA via the mevalonate pathway, these compounds exhibit abundant structural diversity, with many found in cyclic forms by the existence of 3 double bonds and more flexible carbon chains [1-3]. Sesquiterpenoids are often used as fragrance ingredients because they are the main constituents of essential oils. The biological potency of sesquiterpenoid compounds has been extensively reported, including antibacterial, antifungal, antimalarial, anti-inflammatory, anticarcinogenic, antitumor, cytotoxic, and immunomodulatory based on toll-like receptor 4 (TLR4) [4-19].

Meliaceae belongs to the order Sapindales and consists of tropical plants renowned for their high-quality wood and aromatic stems. This family encompasses 58 genera and approximately 740 species

[15]. Sesquiterpenoids are distributed in various genera in the Meliaceae family, namely *Aglaia* [20], *Dysoxylum* [21], *Chisocheton* [22], *Guarea* [23], *Trichilia* [24], and *Lansium* [25]. The *Dysoxylum* is distributed worldwide, predominantly in tropical and subtropical regions such as China, India, Malaysia, Northeast Australia, and various Southeast Asian countries. This genus has a characteristic tree height of ±36 m and comprises around 200 species of these compounds belonging to sesquiterpenoid [26], sesquiterpenoid dimers [27], diterpenoids [28-29], triterpenoids [30-33], limonoids [34-37], and macrolides [38]. Plants of the genus *Dysoxylum* are widely utilized in traditional medicine to treat various ailments. For instance, the leaves of *D. richii* are brewed into tea to alleviate pain and are often used as furniture because the wood has a high quality. It is also used in traditional medicine, such as *D. malabaricum*, known to cure rheumatism, and its oil is used as an eye and ear medicine [39]. Approximately 53 sesquiterpenoids have been successfully isolated from this genus [15]. 10β-hydroxy-4α,4β-dimethyl-5α*H*,7α*H*-eudesm-3-one, isolated from the stem bark of *D. parasiticum*, exhibits significant cytotoxic activity against MCF-7 cancer cells, with an IC_{50} value of 27.39 μM [40]. These results inspire us to explore further sesquiterpenoids from the *Dysoxylum* genus and their action in cytotoxic activity against breast cancer MCF-7 cells.

One species of the *Dysoxylum* that has not been extensively explored for its chemical compounds is *D. amooroides*. The investigation of the stem bark of *D. amooroides* yielded 3 compounds, including 1 previously described guaiane-type sesquiterpenoids guaianediol (**1**) and alismol (**2**), as well as 1 previously described aromadendrane-type sesquiterpenoid spathulenol (**3**). In this work, their isolation and structure elucidation will be described thoroughly to understand further the phytochemical study of sesquiterpenoids **1**–**3** from D. *amooroides*. A modest activity against breast cancer MCF-7 cells was obtained by inhibiting **3**, which was more potent than its reference drug cisplatin. This study also provides a brief structure-activity relationship based on the similarity of the basic framework in the biogenesis pathway that might be useful for developing drug discovery on the synthetic approach.

■ EXPERIMENTAL SECTION

Materials

The plant of *D. amooroides* Miq. stem bark was collected from the Pangandaran Nature Reserve, Pangandaran, West Java province, Indonesia, in February 2021. The plant was identified and made by Mr. Joko Kusmoro and was deposited at the Plant Taxonomy Laboratory, Department of Biology, Universitas Padjadjaran (specimen No. 42/HB/07/2021).

Instrumentation

The instruments used are lab glassware and in the separation process vacuum liquid chromatography and common column chromatography (CC) were performed on silica gel 60 (Merck KGaA, Darmstadt, Germany, 70–230 and 230–400 mesh) and octadecyl silane (ODS, Chromatorex C_{18} DM1020T, Fuji Sylisia Chemical Ltd., Japan, 100–200 mesh). The isolation guidance was utilized in a thin layer chromatography (TLC) using silica gel 60 F_{254} (Merck KGaA, Darmstadt, Germany) plates of the normal phase and the reverse one using reverse phase RP-18 F_{254S} plates (Merck KGaA, Darmstadt, Germany). The TLC plates were analyzed under UV light at 254 and 365 nm wavelengths and sprayed with 10% H_2SO_4 in EtOH. Infrared spectra were measured on a Perkin-Elmer Spectrum 100 with KBr disk (Waltham, Massachusetts, USA). Mass spectra were measured with a waters XEVO HR-TOF-ESI-MS (Milford, MA) complemented with ESI+ mode. The NMR spectra were recorded with internal standard as a TMS on a Bruker Ascend spectrometer, involving ^1H at 700 MHz, ^{13}C at 175 MHz, DEPT at 135 MHz, ^1H-^1H COSY, HMBC, HSQC, and NOESY. A cytotoxic assay was conducted using a microplate reader Infinite M200 (TECAN, Switzerland) at λ_{max} 570 nm, a 96-well plate (Thermo Fisher Scientific Inc., USA), and an incubator.

Procedure

Extraction and isolation

The stem bark of *D. amooroides* was prepared in a preliminary step by drying and powdering (2.8 kg) and was then macerated with EtOH at room temperature (3 × 12 L, 24 h each). The macerates were then evaporated, and the solvent under an evaporator decompression was obtained as a crude extract of EtOH (491.3 g), eluted in H_2O, and partitioned sequentially based on polarity with *n*-hexane and EtOAc. The solvent was removed by an evaporator to afford *n*-hexane (30 g) and EtOAc (110.6 g) extracts.

The concentrated *n*-hexane extract of 30 g was separated by vacuum liquid chromatography using a polar stationary phase (on silica gel) with the eluent *n*-hexane-EtOAc (100:0–0:100, 10% v/v) to obtain 9 fractions (Fr. A–I). Furthermore, 2.63 g of Fr. C was further separated by CC with *n*-hexane:EtOAc (60:1) as eluent to afford 11 subfractions (C1–C11). Using CC with *n*-hexane:EtOAc (60:1) as eluent, subfraction C5 (420 mg) was separated into 5 subfractions (C5a–C5e). Subfraction C5b (22.4 mg) was purified by ODS CC (acetonitrile:H_2O, 7:3) to afford compound **2** (11.3 mg). Compound **3** was purified by flash ODS CC (acetonitrile:H_2O, 7:3) from subfraction C5c, weighing 17.3 mg.

The concentrated EtOAc extract of 110.6 g was separated by vacuum liquid chromatography of silica gel with *n*-hexane:EtOAc and EtOAc:MeOH (100:0–0:100, 10% v/v) gradient elution to yield 5 fractions (Fr. A-E). Furthermore, Fr. B weighing 680 mg was further separated with CC with *n*-hexane:EtOAc (8.5:1.5) to get 11 fractions (B1–B11). Then, subfraction B6 (32.7 mg) was separated by CC on ODS with MeOH:H_2O (7:3) eluent to produce 5 fractions (B6a–B6e). Then, purified fraction B6b by flash ODS CC (MeOH:H_2O, 4:6) to obtain compound **1** (4.1 mg).

Compound 1. Colorless oil. IR (KBr) ν_{max} 3300, 2900, 1600, 1383, 1384, 1150 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 700 MHz) is presented in Table S1 and ^{13}C-NMR ($CDCl_3$, 175 MHz) data is presented in Table 1. HR-TOF-ESI-MS *m/z* 239.2013 $[M+H]^+$ (calc. for $C_{15}H_{26}O_2$, *m/z* 239.2011).

Compound 2. Colorless oil. IR (KBr) ν_{max} 3372, 2958, 2862, 1638, 1461, 1379 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 700 MHz) is presented in Table S1 and ^{13}C-NMR ($CDCl_3$, 175 MHz) data is presented in Table 2. HR-TOF-ESI-MS *m/z* 259.1453 $[M+K]^+$ (calc. for $C_{15}H_{24}O$, *m/z* 259.1464).

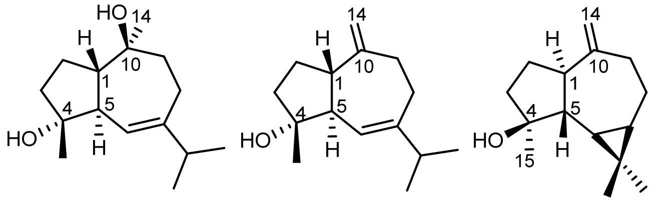
Compound 3. Colorless oil. IR (KBr) ν_{max} 3396, 2926, 2856, 1636, 1457, 1376 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 700 MHz) is presented in Table S1 and ^{13}C-NMR ($CDCl_3$, 175 MHz) data is presented in Table 3. HR-TOF-ESI-MS *m/z* 259.1453 $[M+K]^+$ (calc. for $C_{15}H_{24}O$, *m/z* 259.1464).

Cytotoxic activity assay

All the isolated compounds **1-3** were evaluated for cytotoxicity against human MCF-7 breast cancer cells using the resazurin method by measuring cell viability with PrestoBlue® reagent. The cells were cultured in RPMI-1640 medium, consisting of 50 µL/50 mL of antibiotic (1% penicillin) and 10% fetal bovine serum. The cells were cultured in 96-well plates and incubated for 24 h at 37 °C in 5% CO_2 gas. RPMI media was disposed of, and then the sample media and positive control cisplatin in DMSO with required concentrations (500.00; 250.00; 125.00; 62.50; 31.25; 15.63; 7.81; 3.91 µg/mL) were added, respectively. Cells were incubated for 48 h after being treated with samples and positive controls. The media containing the sample was discarded and then incubated for 2 h with PrestoBlue® reagent until a color change occurred. The samples were measured using a multimode reader to determine their absorbance at 570 nm, and absorbance was converted into cell viability values to determine the IC_{50} value of each compound [34].

■ RESULTS AND DISCUSSION

The *n*-hexane and EtOAc extracts were repeatedly separated and purified by normal and reversed-phase CC [15,20-21,27], producing 3 sesquiterpenoids **1-3** (Fig. 1). Compound **1** was isolated as a colorless oil with a yield of 4.1 mg, isolated from EtOAc extract, with a molecular formula of $C_{15}H_{26}O_2$ established by HRTOF-ESI-MS *m/z* 239.2013 (calc. for $[M+H]^+$ *m/z* 239.2011),



1 **2** **3**

Fig 1. Structure of sesquiterpenoid compounds **1**-**3**

which represents 3 unsaturation degrees. The IR spectrum (Fig. S2) showed absorption bands of hydroxyl group (3300 cm^{-1}), CH *sp*^{3} group (2900 cm^{-1}), C=C group (1600 cm^{-1}), *gem*-dimethyl group (1383 and 1384 cm^{-1}) and C–O group (1150 cm^{-1}). The ^{1}H-NMR data showed 2 tertiary methyl at δ_H (ppm) 1.21 (*s*, CH$_3$-14) and 1.27 (*s*, CH$_3$-15), 2 doublet signals of secondary methyl at δ_H 0.98 (*d*, CH$_3$-13) and 0.99 (*d*, CH$_3$-12), and 1 olefin proton at δ_H shift 5.48 (*br.s*, CH-6). Furthermore, ^{13}C-NMR data (Table 1) supported with DEPT and HSQC, showed 15 carbons signals, including 4 methyl at δ_C (ppm) 21.1 (C-14), 21.3 (C-13), 21.4 (C-12) and 22.5 (C-15), 4 *sp*^{3} methylene at δ_C 21.5 (C-2), 25.1 (C-8), 80.3 (C-4) and 42.6 (C-9), 3 *sp*^{3} methine at δ_C 37.3 (C-11), 50.3 (C-1) and 50.3 (C-5), 1 *sp*^{2} methine at δ_C 121,3 (C-6), 2 oxygenated quaternary carbons present at δ_C 75.3 (C-10), 80.3 (C-4), and 1 *sp*^{2} quaternary carbon at δ_C 149.6 (C-7). The above data suggested that compound **1** is a sesquiterpenoid compound with 3 degrees of unsaturation. The existence of one pair double bond (δ_C 121.3, 149.6) indicated that the remaining unsaturation in **1** was attributed to 2 rings (bicyclic system) of sesquiterpenoid.

The 2D-NMR analysis showed HMBC correlations of H-5 (δ_H 2.16) to C-4 (δ_C 80.3) and C-1 (δ_C 50.3), suggesting that **1** is a guaiane-type sesquiterpenoid. Two tertiary methyl by hydroxyl attachments at C-4 and C-10 was confirmed by HMBC correlations of CH$_3$-15 (δ_H 1.27) to C-4 (δ_C 80.3) and C-3 (δ_C 40.5) and CH$_3$-14 (δ_H 1.21) to C-9 (δ_C 42.6), C-10 (δ_C 75.3) and C-1 (δ_C 50.3), respectively. The isopropyl moiety at C-7 as well as a pair double bond positioned at C-6/C-7 were assigned based on the observed correlations of CH$_3$-13 (δ_H 0.98)/CH$_3$-12 (δ_H 0.99) to C-11 (δ_C 37.3) and C-7 (δ_C 149.6) and H-1 (δ_H 1.88) to C-6 (δ_C 121.3). In addition, the ^{1}H-^{1}H COSY

Table 1. ^{13}C-NMR data for compound **1** and their related literature

Position of carbon	**1**		Guanaediol [41]*
	δ_H ppm (ΣH, mult., *J* = Hz)	δ_C (mult.)	
1	1.88 (1H, *m*)	50.3 (d)	50.7 (d)
2	1.64 (1H, *m*) 1.77 (1H, *m*)	21.5 (t)	21.5 (t)
3	1.71 (1H, *m*) 1.61 (1H, *m*)	40.5 (t)	40.5 (t)
4	-	80.3 (s)	80.2 (s)
5	2.16 (1H, *m*)	50.3 (d)	50.3 (d)
6	5.48 (1H, *brs*)	121.3 (d)	121.3 (d)
7	-	149.6 (s)	149.6 (s)
8	2.20 (1H, *m*) 1.92 (1H, *m*)	25.1 (t)	25.1 (t)
9	1.82 (1H, *m*) 1.47 (1H, *m*)	42.6 (t)	42.6 (t)
10	-	75.3 (s)	75.3 (s)
11	2.25 (1H, *m*)	37.3 (d)	37.3 (d)
12	0.99 (3H, *d*, 5.5)	21.4 (q)	21.4 (q)
13	0.98 (3H, *d*, 5.5)	21.3 (q)	21.3 (q)
14	1.21 (3H, *s*)	22.5 (q)	22.5 (q)
15	1.27 (3H, *s*)	21.1 (q)	21.1 (q)

*(CDCl$_3$, 125 MHz)

cross-peaks of H-1/H-2/H-3, H-1/H-5/H-6, and H-9/H-8 convinced the planar structure of **1** by 2 adjacent vicinal proton systems. The fusion of five and seven-membered rings with 2 hydroxyls and one pair of olefinic bond groups in **1** was deduced. Since the guaiane compound in **1** shows several stereocenter carbons, the relative configuration is mandatorily required to be established. A NOESY experiment was then performed to observe the key cross-peaks between 2 protons from asymmetrical carbons in a particular space. In the ^{1}H-^{1}H NOESY spectrum, the key cross-peaks between H-5 (α-oriented) to CH_3-14 and H-2a indicated that CH_3-14 and H-5 are α-oriented. Conversely, the cross-peak of H-2b/CH_3-15 suggested the β-orientation of CH_3-15 and α-orientation of hydroxyl at C-4. A comparison of 1D-NMR data between **1** and the related literature showed a significant resemblance to the guaianediol compound. Hence, compound **1** was identified as a guaianediol like previous literature [41]. The MS, FTIR, ^{1}H-NMR, ^{13}C-DEPT NMR, HSQC, HMBC, ^{1}H-^{1}H COSY, and NOESY spectra were presented in Fig S1-S8.

Compound **2** was isolated as a colorless oil of 11.3 mg from *n*-hexane extract. Its molecular formula as $C_{15}H_{24}O$ was determined by HRTOF-ESI-MS *m/z* 259.1453 (calc. for $[M+K]^+$ *m/z* 259.1464), which represents 4 degrees of unsaturation. The IR spectrum (Fig. S10) implied the existence of hydroxyl (3372 cm^{-1}), the aliphatic of C-H sp^3 (2958 and 2869 cm^{-1}), C=C sp^2 (1638 cm^{-1}), and *gem*-dimethyl (1461 and 1379 cm^{-1}) groups. ^{1}H-NMR data (Table 2) showed 3 methyl, including 2 secondary methyl at δ_H (ppm) 0.98 (3H, *d*, *J* = 6.0 Hz, CH_3-13), 0.99 (3H, *d*, *J* = 6.0 Hz, CH_3-12) and 1 tertiary at δ_H 1.25 (3H, *s*, CH_3-15). The presence of a sp^2 methylene at δ_H 4.73 and 4.76 (1H, *s*, H-14a/H-14b) and a methine sp^2 at δ_H 5.55 (1H, *s*, H-6) were also observed. The ^{13}C-NMR data, with the aid of DEPT and HSQC spectrum, retrieved 15 carbons, which consists of 3 sp^3 methyl at δ_C 21.2 (C-12), 21.5 (C-13), and 24.0 (C-15), 4 sp^3 methylene at δ_C 24.8 (C-2), 40.3 (C-3), 30.0 (C-8), and 37.1 (C-9), 1 sp^2 methylene at δ_C 106.5 (C-14), and 3 methine sp^3 at δ_C 47.3 (C-1), 55.1 (C-5), and 37.1 (C-11), 1 sp^2 methine at δ_C 121.5 (C-6), as well as 1 oxygenated

Table 2. ^{13}C-NMR data for compound **2** and their related literature

Position of carbon	2		Alismol [42]*
	δ_H ppm (ΣH, mult., *J* = Hz)	δ_C (mult.)	
1	2.28 (1H, *t*)	47.3 (d)	47.3 (d)
2	1.73 (2H, *m*)	24.8 (t)	24.8 (t)
3	1.75 (2H, *t*)	40.3 (t)	40.3 (t)
4	-	80.8 (s)	80.6 (s)
5	2.29 (1H, *d*)	55.1 (d)	55.1 (d)
6	5.55 (1H, *s*)	121.5 (d)	121.4 (d)
7	-	148.7 (s)	149.6 (s)
8	2.02 (1H, *m*) 2.21 (1H, *m*)	30.0 (t)	30.0 (t)
9	2.05 (1H, *m*) 2.5 (1H, *m*)	37.1 (t)	37.1 (t)
10	-	154.1 (s)	153.9 (s)
11	2.26 (1H, *m*)	37.6 (d)	37.4 (d)
12	0.99 (3H, *d*, 6.0)	21.2 (q)	21.3 (q)
13	0.98 (3H, *d*, 6.0)	21.5 (q)	21.5 (q)
14	1.25 (3H, *s*)	106.5 (q)	106.4 (t)
15	4.73 (1H, *s*) 4.76 (1H, *s*)	24.0 (q)	24.1 (q)

*($CDCl_3$, 100 MHz)

non-protonated carbon at δ_C 80.8 ppm (C-4). The presence of 2 non-protonated sp^2 carbons at δ_C 148.7 (C-7) and 154.1 (C-10) confirmed that **2** bears two pairs of double bonds. The above data showed that **2** was also a bicyclic guaiane-type sesquiterpenoid. The 2D-NMR analysis (Fig. 2 and 3) revealed that **2** resembled **1**, in which the methylene olefinic group replaced hydroxy methyl at C-10 in **1**. This assignment was proved by HMBC correlations from H-14 at δ_H 4.76 (1H, *s*) and 4.73 (1H, *s*) to C-10 (δ_C 154.1), C-9 (δ_C 37.1), and C-1 (δ_C 47.3). The relative configuration of **2** was also assigned as **1** by NOE correlations of CH_3-15 to H-1 (β-oriented), and no observed cross-peaks between H-5 to β protons. Further comparison of 1D-NMR data between **2** and the related literature showed high similarity with the known compound alismol. Thus, compound **2** was identified as the same as in previous literature [42]. The MS, FTIR, ^{1}H-NMR, ^{13}C-DEPT NMR, HMBC, ^{1}H-^{1}H COSY, and NOESY spectra were presented in Fig S9-S15.

Compound **3** was isolated, having the characteristics of colorless oil of 17.3 mg, isolated from *n*-hexane extract. Its molecular formula of $C_{15}H_{24}O$ was determined based on HRTOF-ESI-MS *m/z* 259.1453 (calc. for $[M+K]^+$ *m/z* 259.1464), representing 4 unsaturation degrees. The IR spectrum (Fig. S17) suggested the presence of hydroxyl group (3396 cm^{-1}) with typical bandwidth, C-H sp^3 (2926 and 2856 cm^{-1}), C=C sp^2 (1636 cm^{-1}), and *gem*-dimethyl (1457 and 1376 cm^{-1}) groups. The ^{1}H-NMR data showed 3 methyl at δ_H 1.03 (CH_3-12), 1.04 (CH_3-13), and 1.28 (3H, *s*, CH_3-15), and a pair sp^2 methylene at δ_H 4.68 (1H, *s*, H-14a) and 4.70 (1H, *s*, H-14b). Furthermore, the ^{13}C-NMR corroborated with DEPT data possessed 15 carbons resonances, involving 3 sp^3 methyl at δ_C 16.5 (C-12), 28.8 (C-13), and 26.8 (C-15), 4 sp^3 methylene at δ_C 26.7 (C-2) 41.9 (C-3) 24.4 (C-8) and 39.0 (C-9), 1 methylene olefinic at δ_C 106.4 ppm (C-14), 4 sp^3 methine at δ_C 53.5 (C-1), 54.9 (C-5), 30.0 (C-6), and 27.6 (C-7), 1 sp^3 non-protonated carbon at δ_C 20.4 ppm (C-11), 1 oxygenated at δ_C 81.1 ppm (C-4), and 1 sp^2 non-protonated carbon at δ_C 153.6 (C-10). Together with the fact that no other olefinic bonds were present, compound **3** was then deduced to share a tricyclic sesquiterpenoid scaffold. The presence of a sp^3 quaternary carbon at δ_C 20.4 ppm (C-11) and the shielded protons at δ_H 0.44 (1H, *dd*, *J* = 9.2, 10.7 Hz, H-6), 0.71 (1H, *m*, H-7) firmly pointed out that **3** is an aromadendrane-type sesquiterpenoid by a cyclopropane moiety positioned at C-6/C-7/C-11. Based on the above assignment, compound **3** was deduced as an aromadendrane bearing 3 tertiary methyls with one attached to hydroxyl quaternary carbon and a pair of

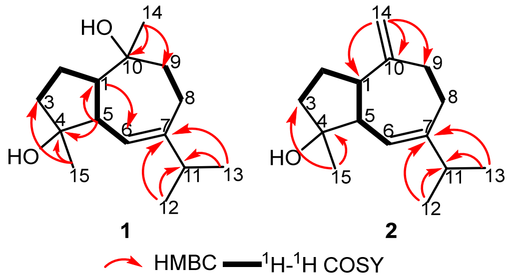


Fig 2. Selected HMBC and ^{1}H-^{1}H COSY Correlation of compounds **1** and **2**

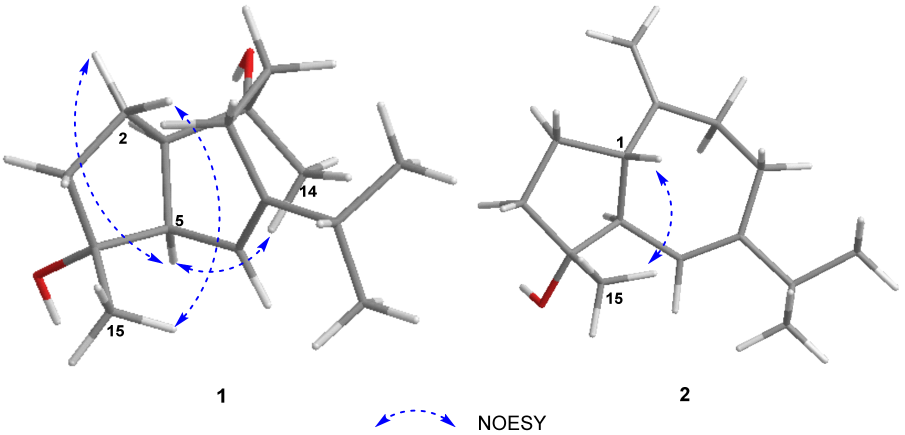


Fig 3. NOESY correlation of compounds **1** and **2**

olefinic bonds constituted by the [$-CH_2=C-$] system. Further literacy to compound **3** with a known aromadendrane-type sesquiterpenoid spathulenol disclosed that their 1D-NMR was nearly identical (Table 3). Therefore, compound **3** was identified as 4β-hydroxy-10-en-aromadendran spathulenol [43]. The MS, FTIR, ^{1}H-NMR, and ^{13}C-DEPT NMR spectra were presented in Fig S16-S19.

The activity of compounds **1**-**3** were evaluated against MCF-7 breast cancer cells by using the resazurin method with the positive control of cisplatin, as shown in Table 4. The results showed that all compounds had varying toxicity from inactive to moderate. The cytotoxicity compounds were classified on the basis of a previous literature report for pure compounds as highly active ($IC_{50} < 2$ μM), moderately active ($IC_{50} < 10$ μM), and inactive ($IC_{50} > 100$ μM) [44]. Compound **3** showed the strongest activity with an IC_{50} value of 15.2 μM, followed by **2** with weak activity (an IC_{50} value of 82.1 μM), while **1** was inactive with an IC_{50} value of > 100 μM against MCF-7 cancer cells. The potent inhibition played by compound **3** in this study attracts us to verify its activity further. A previous study by Naini et al. [40] agreed that spathulenol (**3**) was more potent than cisplatin, with an IC_{50} value of 12.2 μM. Furthermore, a significant enhancement activity of **2** compared to **1** in a guaiane-type revealed that [$-CH_2=C-$] at C-10/C-14 might facilitate the response of **2**, while a pair olefinic bond [$-CH=C-$] at C-6/C-7 did not contribute against breast cancer cell's growth MCF-7. Beyond that, the formation of cyclopropane at C-6/C-7/C-11 yielded an aromadendrane-type in **3**, resulting in a superior activity more than 5-fold compared to **2** (Fig. 4). These findings sharpen the study of the discovery of sesquiterpenoids with biological activity as cytotoxic agents against cancer cells, especially the naturally occurring aromadendrane framework with considerable activity.

Table 4. Cytotoxicity of **1**-**3** against MCF-7 cell lines

Compound	IC_{50} (μM)
Guaianediol (**1**)	> 100
Alismol (**2**)	82.1
Spathulenol (**3**)	15.2
Cisplatin (positive control)	53.0

Table 3. ^{13}C-NMR data for compound **3** and its related literature

Position of carbon	**3**		spathulenol [43]*
	δ_H ppm (ΣH, mult., J = Hz)	δ_C (mult.)	
1	1.31 (1H, *m*)	53.5 (d)	53.4 (d)
2	1.88 (1H, *dd*, 6.0, 12.0) 1.64 (1H, *dd*, 6.0, 12.0)	26.7 (t)	26.7 (t)
3	1.76 (1H, *m*), 1.54 (1H, *m*)	41.9 (t)	41.7 (t)
4	-	81.1 (s)	81.0 (s)
5	1.31 (1H, *m*)	54.5 (d)	54.3 (d)
6	0.44 (1H, *dd*, 9.0, 10.7)	30.0 (d)	29.9 (d)
7	0.71 (1H, *m*)	27.6 (d)	27.5 (d)
8	1.96 (2H, *m*)	24.4 (t)	24.8 (t)
9	2.41 (1H, *dd*, 6.0, 13.5) 2.04 (1H, *dd*, 6.0, 13.5)	39.0 (t)	38.9 (t)
10	-	153.6 (s)	153.4 (s)
11	-	20.4 (s)	20.3 (s)
12	1.03 (3H, *s*)	16.4 (q)	16.3 (q)
13	1.04 (3H, *s*)	28.8 (q)	28.7 (q)
14	4.68 (1H, *s*), 4.70 (1H, *s*)	106.4 (t)	106.3 (t)
15	1.28 (3H, *s*)	26.8 (q)	26.1 (q)

*($CDCl_3$, 150 MHz)

Since compounds **1**-**3** share the same 5/7-bicyclic fused ring skeleton with a diverse activity in inhibiting the proliferation of cancer cells, a brief exploration with those of previous works was prompted to gain more understanding of the existence of this group in the biological activity window. As mentioned, guaianediol (**1**) has not demonstrated significant activity against breast cancer cells. However, according to the previous work conducted by Li et al. [45], compound **1** showed potential antimicrobial activity, particularly against *Staphylococcus aureus*, with minimum inhibitory concentrations (MIC) recorded at 32 μg/mL. These findings suggest that guaianediol (**1**) may be helpful in representing a potent candidate for further development as an antibacterial agent.

Regarding its cytotoxic effects, alismol (**2**) provides a revealed inhibition compared to **1** by forming a terminal olefinic bond to replace the hydroxyl methyl at C-10/C-14. These results were supported by a previous work that **2** has a notable cytotoxicity against HT-29, A-549, and A-2058 cancer cells, with the IC_{50} consecutively exhibiting values of 29.8 ± 3.0, 25.4 ± 3.6, and 26.2 ± 2.9 μM [46]. Regardless of the cytotoxicity of alismol (**2**), which was categorized as moderately active against several human cancer cells [44], its potency as a naturally cytotoxic compound is worth considering. Moreover, alismol (**2**) was reported to potentially reduce lung inflammation, which mechanistically activates the nuclear factor erythroid 2-related Ffctor 2 (Nrf2) pathway and induces Nrf2-dependent gene expression without inhibiting NF-κB and also demonstrated as a potent compound in the treatment of respiratory disorders [47].

In addition to the strong activity against human breast MCF-7 cancer cells, spathulenol (**3**) was found to have modest activity against HT-29, A-549, and A-2058 cancer cells, with its IC_{50} values of 25.4 ± 4.6, 22.9 ± 2.8, and 21.6 ± 2.0 μM, respectively [46]. Although the previous findings disclosed lower cytotoxicity results than those against MCF-7 cells concluded in this work, spathulenol (**3**) deserves to be considered a potent cytotoxic agent with selective activity against several panel cancer cells. A versatile biologically active compound from nature in **3** was also proved by a strong antibacterial activity against *Mycobacterium tuberculosis*, with both MIC and minimum bacterial concentration (MBC) values of 6.25 μg/mL [48]. Thus, spathulenol (**3**) provided a broad-spectrum activity, which might be useful as a "gold-mine" compound for drug development.

Based on the current cytotoxicity results, structure-activity relationship analysis, and previous works, this information significantly contributes to the growing research on sesquiterpenoids. The potent biological properties of these natural sesquiterpenoids, especially those with guaiane-type, such as guaianediol (**1**) and alismol (**2**), and aromadendrane-type skeleton such as spathulenol (**3**) indicate their potential for further exploration. Furthermore, research on the structure-activity relationship of sesquiterpenoids offers

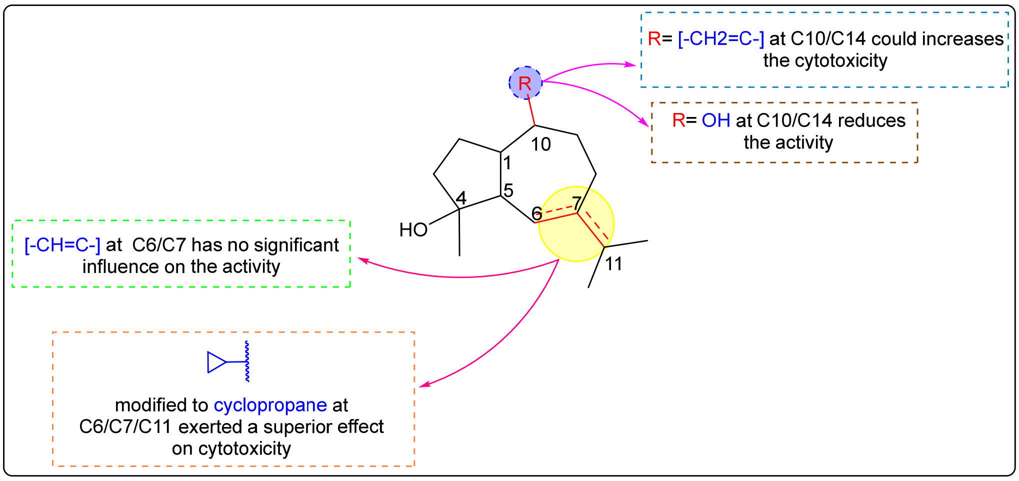


Fig 4. The structure-activity relationship of sesquiterpenoids **1**–**3**

excellent opportunities to optimize these compounds for drug discovery and therapeutic applications.

CONCLUSION

The phytochemical study of *D. amooroides* stem barks resulted in 3 sesquiterpenoid compounds, which were identified as the known compounds guaianediol (**1**), alismol (**2**), and spathulenol (**3**). The structure elucidation was performed based on an extensive spectroscopy method and a comparison with 1D-NMR data from previously reported compounds. Compounds **2** and **3** were obtained from the *n*-hexane extract, while **1** was isolated from the ethyl acetate. The cytotoxic assay exhibited that **3** was the strongest against breast cancer MCF-7 cells with an IC_{50} value of 15.2 μM, while **2** possessed weak activity with an IC_{50} value of 82.1 μM. Compound **3** was more potent than their positive control cisplatin (IC_{50} 53.0 μM). The structure-activity relationship implied that the presence of an olefinic bond [$-CH_2=C-$] at C-10/C-14 in a guaiane type, also an alteration from an isopropyl moiety in the guaiane skeleton to a cyclopropane ring in the aromadendrane core can boost its activity. In addition, according to both current and previous works, the naturally occurring guaiane- and aromadendrane-type sesquiterpenoids are worth further investigation for drug development, including anticancer, antibacterial, and inflammatory diseases.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, Latifah Gunawan, Al Arofatus Naini, Unang Supratman; methodology, software, validation, formal analysis, investigation, resources, data curation, writing—original draft preparation, writing—review and editing, Latifah Gunawan, Hidayat Nurul Mustofa, Al Arofatus Naini, Desi Harneti, Ace Tatang Hidayat, Nurlelasari, Sofa Fajriah, Khalijah Awang, Mohamad Nurul Azmi; visualization, supervision, project administration, Rani Maharani, Tri Mayanti, Khalijah Awang, Mohamad Nurul Azmi, Unang Supratman. All authors have read and agreed to the published version of the manuscript.

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