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Dammarane-Type Triterpenoids from Twigs of Aglaia Foveolata and Their Antibacterial Activity

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ABSTRACT. The *Aglaia* species, which contains triterpenoids, is the most numerous in the Meliaceae family. *Aglaia foveolata* (*A. foveolata*) is a type of plant that has many benefits, as medicinal ingredients. The potential of this plant is inseparable from the content of various bioactive compounds. This study aims to isolate, characterize the active compound from the twigs of *A. foveolata* and test its activity as an antibacterial. Three dammarane-type triterpenoids were isolated from the *A. foveolata* twigs which is, namely dammar-24-en-3*β*,20-diol (**1**), an epimeric mixture of shoreic and eichlerianic acid (**2**, **3**). Their chemical structures were determined based on spectroscopic data using infrared, high-resolution mass spectrometry, and including one and two-dimensional NMR techniques, as well as through data comparison of the reported compound. Compound **1** was reported for the first time to be successfully isolated from this species. All these substances were tested for the first time for their antibacterial activity against two Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* and two Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, through this study. Compound 1 was inactive, the epimeric mixture of **2** and **3** showed moderate antibacterial activity with a minimum inhibitory concentration (MIC) value ranging from 31.7 to 126.6 ppm, particularly against *S. aureus* with a MIC value of 31.7 ppm.

Keywords: Aglaia, Elucidation, Isolation, Spectroscopy, Bacterial

INTRODUCTION

Triterpenoids are a structurally diverse group of natural products that exist in a variety of organisms exhibiting extensive biological activities (Li et al., 2023; Ren & Kinghorn, 2019). *Aglaia* is the largest genus belonging to the family Meliaceae, comprising over 130 species distributed mainly in tropical forest and more than 65 grow in Indonesia (Mabberley & Pannel, 1995; Pannel, 1992; Pérez et al., 2014). Various phytochemicals of this genus have been reported with fascinating bioactivities including several rocaglate derivatives, triterpenoids, and steroids (Harneti & Supratman, 2021; Hutagaol et al., 2021, 2022, 2023). Previous phytochemical studies of the species *A. foveolata* reported a variety of compounds from leaves, bark, and stem bark, including flavaglines (e.g., silvestrol, bisamides, and rocaglamides) and dammarane-type triterpenoids (Pan et al., 2013; Salim et al., 2007). There are still limited reports regarding antibacterial activities of triterpenoids from the species of *A. foveolata*. An infectious disease is one of the serious diseases causing high mortality worldwide. For example, lower respiratory infections and diarrheal diseases remain the world's most deadly infectious diseases, ranked as the fourth and eighth leading causes of death, respectively. Therefore, the discovery of new antimicrobial agents, especially from natural sources in Indonesia, is a very important subject to study.

In our ongoing efforts to search for triterpenoids from the Indonesian *Aglaia* plants, we have further investigated the twigs of *A. foveolata*. As a result, three dammarane-type triterpenoids (**1**-**3**) were successfully isolated and elucidated (**Figure 1**). Based on literature search, this is the first time compound **1** has been successfully isolated from this species. These triterpenoid compounds were evaluated the antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), which have not been tested previously for these compounds and the results are reported in this article.

EXPERIMENTAL SECTION

Materials

Materials the twigs of *A. foveolata* was obtained from Timpah Village, Timpah District, Kapuas Regency, Central Kalimantan, Indonesia, with coordinate 1.2735° S, 114.5934° E. The voucher specimen was authenticated by the staff of the Bogoriense Herbarium, Research Centre for Biology, Indonesian Institute of Science, Bogor, Indonesia, and deposited at that herbarium (No. BO-1295312). Evaluation of antibacterial activity using ATCC bacteria, including two gram-positive bacteria (*Staphylococcus aureus* (*S. aureus*) ATCC 6538 and *Bacillus subtilis* (*B. subtilis*) ATCC 19659, and two gram-negative bacteria (*Escherichia coli* (*E. coli*) ATCC 8739 and *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 15442. All types of test bacteria were obtained from the Research Center for Pharmaceutical Ingredients and Traditional Medicine, National Research and Innovation Agency (BRIN), Kawasan PUSPIPTEK, Serpong, South Tangerang, Banten, Indonesia. Technical solvents were distilled before maceration; isolation and spectral grade solvents (*n*-hexane, ethyl acetate (EtOAc), methanol (MeOH), and dimethylene chloride (MTC) from Merck, Darmstadt, Germany and Smart lab, Indonesia) were employed for spectroscopic measurements.

Instrumentation

Optical rotations were measured on a Perkin Elmer 341 Polarimeter (Waltham, MA, USA). UV was recorded on PerkinElmer UV WinLab Data Processor and Viewer Version 1.00.00. The IR spectra were recorded on a Perkin Elmer 1760 X FT-IR in KBr (Waltham, MA, USA). Mass spectra were obtained with a Waters Q-TOF. HR-MS XEV^{otm} mass spectrometer (Waters, Milford, MA, USA). NMR spectra of an epimer mixture (**2**,**3**) was obtained with a Bruker Avance Neo (BioSpin AG, Faellanden, Switzerland) at 700 MHz for ^{1}H and 175 MHz for ^{13}C-NMR and NMR JEOL JNM ECZ-600 (JEOL USA, INC.) spectrometer for **1**, (at 600 MHz for ^{1}H and 150 MHz for ^{13}C-NMR), with $CDCl_3$ as a solvent, chemical shift were given on a δ (ppm) scale and both using tetra methyl silane (TMS) as the internal standard. Chromatographic separations were carried out on silica gel 60 (Merck Kieselgel 60 PF 253 Art No. 7734.1000 and 9385.1000 with the particle size 0.063-0.200 mm and 0.040 – 0.063 mm). Thin Layer Chromatography (TLC) plates were precoated with silica gel GF_{254} (Merck, Darmstadt, Germany, 0.25 mm). Spots were visualized under UV light of 254 nm and 365 nm simultaneously and by spraying with 10% Sulfuric acid (H_2SO_4) in ethanol or vanillin reagent followed by heating.

Procedure

Extraction and isolation

The dried twigs (4.5 kg) of *A. foveolata* were extracted with MeOH exhaustively (40L), at room temperature for 5 days, 5 x 24 h. After removal of the solvent under vacuum, the viscous concentrated MeOH extract (233.5 g) was first suspended in water (H_2O) and then partitioned with *n*-hexane, EtOAc, and *n*-butanol, successively. The EtOAc extract fraction (46.3 g) was fractionated by vacuum liquid chromatography (VLC) on silica gel 60 eluting with a gradient of *n*-hexane:EtOAc: MeOH (100:0–0:100, 2.5% v/v) to produce 110 fractions (1-110). Fractions 9-11, white powder (6.68 g), were chromatographed on a column of silica gel, eluted with an isocratic eluent of *n*-hexane–EtOAc-CH_2Cl_2 (DCM) (9:0.5:0.5), to give 20 subfractions (9.01–9.20). The combination of subfractions 9.05-9.10 with the major spot as the target was chromatographed again on a silica gel column and repeated with the same process and eluent until a pure isolate of **1** (250) mg was obtained (**Figure 1**). Fractions 29-30 (510 mg) were subjected to column chromatography (CC) on silica gel (70–220 mesh) eluted with *n*-hexane: EtOAc (7:3) to produce eight subfractions (A-H) and the isolation process using CC was repeated until the final isolation stage where it was purified using non-polar silica gel, namely octa dodecyl silica (ODS) and eluted with MeOH eluent to produce a mixture of epimer compounds of **2** and **3** (12.5 mg), as shown in **Figure 1**.

Dammar-24-en-3*β*,20-diol (**1**). White amorphous powder. Retention factor (Rf) of **1** on TLC = 0.8 (7:2:1= *n*-hexane: EtOAc: MTC), IR vmax (cm^{-1}) 3343, 2921, 2852, 1743, 1465, 1376, 1070 cm^{-1}. $[\alpha]_D^{29.6}$-0,10^0 (c, 0.26, $CHCl_3$); No UV absorption was detected. ^{1}H-NMR ($CDCl_3$, 600 MHz) and ^{13}C-NMR spectral data ($CDCl_3$, 150 MHz) are shown in **Table 1**. HR-TOFMS m/z found at 467.3863 $[M+Na]^+$, (calculated for $C_{30}H_{52}O_2Na$ = 467.3865).

Epimeric mixture of shoreic acid (**2**) and **eichlerianic acid** (**3**). White amorphous powder. Rf of epimer **2** and **3** on TLC = 0.5 (7:3=*n* hexane: EtOAc), IR vmax (cm^{-1}) 3324, 2923, 2853, 1711, 1453, 1377, 1261, 1096, 1020. $[\alpha]_D^{28.7}$+0,25^0(c, 0.26, $CHCl_3$); Ultraviolet (UV) spectra showed absorbance at 196 nm. ^{1}H-NMR ($CDCl_3$, 700 MHz) and ^{13}C-NMR spectral data ($CDCl_3$, 175 MHz) are shown in **Table 1**. HR-TOFMS m/z found at 497.3601 $[M+Na]^+$ (calculated for $C_{30}H_{50}O_4Na$ = 497.3607).

Determination of minimum inhibitory concentration (MIC)

The MIC value of substances **1**-**3** was determined using a standard microdilution assay with some modifications (CLSI, 2022). A double dilution of the sample solution (100 µl) was added to a 96-well sterile microplate containing 100 µL Mueller-Hinton broth medium (MHB). An antibacterial test was conducted using a bacterial culture with titer 0.5 McFarland. For that, the bacterial culture titer was adjusted so that the OD value of 600 nm was 0.01 or equivalent to 1.5 x 10^7 CFU (Colony Forming Unit)/mL with the addition of the required volume of 0.9% NaCl and smeared

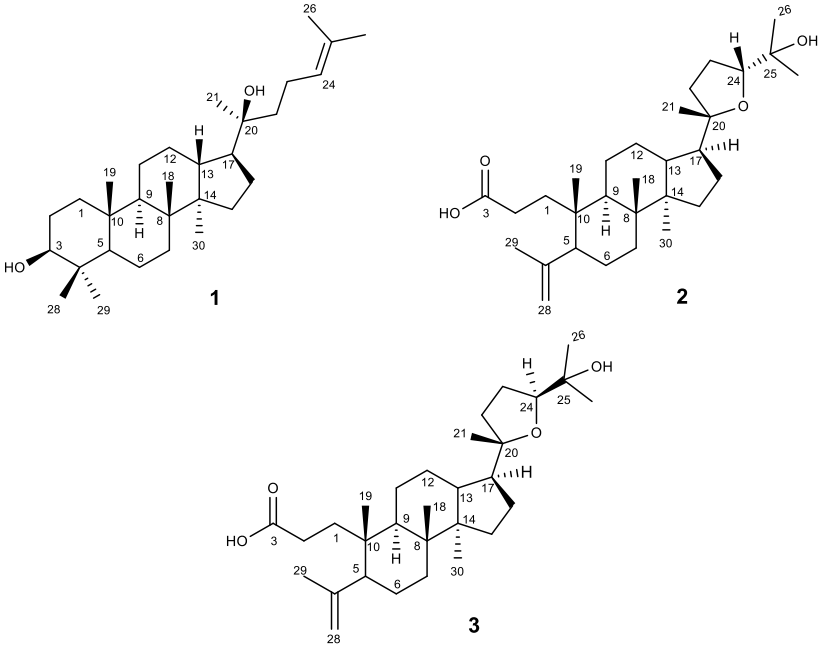

Figure 1. Compounds **1**- **3**

into each well. This particular plate was further incubated at 37 °C for 24 hours. The MIC values were determined to be the lowest concentration of the extract that could suppress the bacterial growth, observed by the clear medium of the well and the particular concentration with no bacterial growth observed on the medium, respectively. Tetracycline (Sigma-Aldrich) was used as the positive control.

RESULTS AND DISCUSSION

Three dammarane-type triterpenoids (**Figure 1**) were obtained by separating and purifying the ethyl acetate extract from the twigs of *A. foveolata* using the column chromatography technique. Compound **1** was obtained as a white powder. The molecular formula of compound **1** was determined as $C_{30}H_{52}O_2$ based on the HR-TOFMS spectrum of m/z found at 467.3863 $[M+Na]^+$, (calculated for $C_{30}H_{52}O_2Na$ = 467.3865) with the NMR data (Table 1) and thus obtained five degrees of unsaturation.

The UV spectrum of compound **1** showed no conjugated double bonds based on the maximum absorption at a wavelength above 200 nm. The IR spectrum of compound **1** showed the presence of hydroxyl groups (3343 cm^{-1}), aliphatic C-H (2921 and 2852 cm^{-1}), C=C (1743 cm^{-1}), gem-dimethyl (1458 and 1376 cm^{-1}), and C-O (1070 cm^{-1}) functionalities. The ^{1}H-NMR ($CDCl_3$, 600 MHz, ppm) spectrum of compound **1**) shows the presence of eight tertiary methyl signals resonating at δ_H 0.96 (H-18), 0.86 (H-19), 1.14 (H-21), 1.68 (H-26), 1.61 (H-27), 0.93 (H-28), 0.84 (H-29), and 0.89 (H-30), one oxygenated methine signal at δ_H 3.40 (1H, t, *J* = 4.5 Hz; H-3), and one sp^2 methyl proton signal resonates at δH 5.12 (1H, t, J = 5.5 Hz; H-24). The 13C-NMR (CDCl3, 150 MHz, ppm) spectrum of compound 1, detailed with DEPT 135° experiments, shows the presence of 30 carbon signals consisting of eight methyl, ten methylene, six methine, and six quaternary carbon signals.

The presence of eight methyl signals resonating at δ_C 15.5 (C-18), 16.0 (C-19), 25.4 (C-21), 25.8 (C-26), 17.7 (C-27), 28.3 (C-28), 22.1 (C-29), and 16.5 (C-30) ppm, one oxymethine signal at δ_C 76.3 (C-3) ppm, one oxygenated quaternary carbon signal at δ_C 75.4 (C-20) ppm, one sp^2 methine signal at δ_C 124.7 (C-24) ppm, and one sp^2 quaternary carbon signal at δ_C 131.6 (C-25) ppm. This function is counted as one of five degrees of unsaturation, the remaining four degrees of unsaturation are derived from the resin-type tetracyclic triterpenoid skeleton (Harneti et al., 2023; Joycharat et al., 2010).

The determination of the location of the functional groups from the structure of compound **1** was confirmed by HMBC experiments. The correlations between methyl protons CH_3-21 (δ_H 1.14 ppm), and H-22 (δ_H 1.45 ppm) with C-20 indicate the –OH group is bound to C-20. Other –OH groups bound to C-3 are evidenced by the correlations between H-3 (δ_H 3.40) with C-1 (33.6) and C-5 (49.5), CH_3-28 (δ_H 0.93), and CH_3-29 (δ_H 0.84) with C-3 (76.3), C-4 (37.6), and C-5 (49.5). The correlations observed in CH_3-26 (δ_H 1.68), and CH_3-27 (δ_H 1.61) with C-25 (131.6), and C-24 (124.7), and H-24 (δ_H 5.12) with C-23 (22.5), C-26 (25.8), and C-27 (17.7) indicate the presence of a double bond in the side chain of compound **1**, at the C-24/C-25 position as shown in **Figure 2.**

Table 1. NMR data of compounds **1**–**3** in $CDCl_3$ (δ in ppm, 175 MHz ^{13}C-NMR, 700 MHz ^{1}H-NMR for **2, 3** and 150 MHz, 600 MHz for **1**)

	1		2		3	
No	δ_C	δ_H (Integ., mult, *J*=Hz)	δ_C	δ_H (Integ., mult, *J*=Hz)	δ_C	δ_H (Integ., mult, *J*=Hz)
1	33.6	1.38 (1H, m) 1.42 (1H, m)	34.3	1.61 (2H, m)	34.3	1.63 (2H, m)
2	24.8	1.43 (1H, m) 1.74 (1H, m)	28.2	2.18 (1H, m) 2.38 (1H, m)	28.2	2.18 (1H, m) 2.38 (1H, m)
3	76.3	3.40 (1H, t, 4,5)	179.4	-	179.2	-
4	37.6	-	147.5	-	147.5	-
5	49.5	1.23 (1H, m)	50.9	1.97 (1H, m)	49.8	1.96 (1H, m)
6	18.2	1.39 (2H, m)	24.6	1.38 (2H, m)	24.6	1.36 (2H, m)
7	35.1	1.25 (1H, m) 1.55 (1H, m)	33.9	-	33.9	-
8	40.6	-	40.1	-	40.1	
9	50.4	1.43 (1H, m)	41.2	1.47 (1H, m)	41.2	1.47 (1H, m)
10	37.2	-	39.1	-	39.1	-
11	21.4	1.51 (2H, m)	22.1	1.29 (2H, m)	22.3	1.27 (2H, m)
12	25.4	1.91 (1H, m) 1.52 (1H, m)	27.2	-	26.9	-
13	42.2	1.60 (1H, s)	43.0		42.9	
14	50.4		50.4		50.4	
15	31.1	1.05 (1H, m) 1.49 (1H, m)	31.5	1.47 (2H, m)	31.5	1.48 (2H, m)
16	27.5	1.78 (2H, m)	25.7	1.48 (2H, m)	25.8	1.48 (2H, m)
17	49.8	1.68 (1H, m)	49.5	1.88 (1H, m)	49.8	1.87 (1H, m)
18	15.5	0.96 (3H, s)	16.4	0.89 (3H, s)	16.3	0.89 (3H, s)
19	16.0	0.86 (3H, s)	20.2	0.85 (3H, s)	20.2	0.86 (3H, s)
20	75.4	-	86.4	-	86.5	-
21	25.4	1.14 (3H, s)	23.5	1.13 (3H, s)	27.1	1.13 (3H, s)
22	40.5	1.45 (1H, s)	35.8	1.67-1.81 (2H, m)	34.8	1.70-1.82 (2H, m)
23	22.5	2.05 (2H, m)	26,2	1.80 (2H, m)	26.4	-
24	124.7	5.12 (1H, t, 5,5)	83.3	3.73 (1H, t, 7.5)	86.6	3.62 (1H, t, 5.10)
25	131.6	-	71.6	-	70.3	-
26	25.8	1.68 (3H, s)	27.4	1.19 (3H, s)	27.8	1.15 (3H, s)
27	17.7	1.61 (3H, s)	24.3	1.12 (3H, s)	24.1	1.11 (3H, s)
28	28.3	0.93 (3H, s)	113.5	4.66 (1H, brs) 4.85 (1H, brs)	113.5	4.66 (1H, brs) 4.85 (1H, brs)
29	22.1	0.84 (3H, s)	23.2	1.73 (3H, s)	23.2	1.73 (3H, s)
30	16.5	0.89 (3H, s)	15.3	1.00 (3H, s)	15.4	1.02 (3H, s)

The ^{1}H-^{1}H COSY correlations of compound **1** indicates the presence of a basic framework of dammarane triterpenoid compounds. The stereochemistry of compound **1** was determined through a comparative study with the literature, where the chemical shift (ppm) of carbon and proton of compound **1** at C-3 [76.3; 3.40 (1H, J = 4.5Hz)], this indicates that the position of protons 2 and 3 are axially positioned, the hydroxyl group at C-3 is equatorially positioned (3*β*) (Zhang et al., 2010), and based on the biogenesis approach to the existence of dammarane triterpenoids in the Genus Aglaia. Thus, compound **1** was identified as 3*β*,20*S*-dihydroxy-dammar-24-en (**Figure 1**). Compound **1** has previously been isolated from the species *Aglaia elliptica* (Meliaceae), where the H and C NMR data obtained as a comparative reference can be seen in **Table 2** (Farabi et al., 2022).

Compounds **2** and **3** were obtained as an inseparable mixture of epimers in a ratio of around 3:1, with **2** as a major compound. Epimeric mixture was obtained as a white amorphous powder, with the molecular formula $C_{30}H_{50}O_4$. Its molecular composition was established from the HR-TOF MS found m/z 497.3601[M+Na]$^+$ (calculated for $C_{30}H_{50}O_4Na$, m/z 497.3607) and NMR data (**Table 1**). The Hydrogen Deficiency (HD) index was calculated using the equation HD = ∑C − ∑H/2 + 1, yielding an HD index of six for compounds **2** and **3** each. The IR spectra showed the presence of a -OHst (3324 cm^{-1}), a sp^3-CH3 (2962 and 2923 cm^{-1}), C=Ost (1711 cm^{-1}), a gem-dimethyl (1457 and 1377 cm^{-1}), C-Ost (1261, 1096, and 1020 cm^{-1}).

Table 2. NMR reference data for compounds **1**–**3** in $CDCl_3$ (δ in ppm, 75 MHz ^{13}C-NMR, 400 MHz ^{1}H-NMR for **2**, **3**, and 125 MHz, 500 MHz for **1**)

	1 (3*β*,20*S*-dihydroxy-dammar-24-en, (Farabi et al., 2022))		**2** (Shoreic acid,(Roux et al., 1998))		**3** (Eichlerianic acid, (Roux et al., 1998))	
No	δ_C	δ_H (Integ., mult, *J*=Hz)	δ_C	δ_H *(J=Hz)*	δ_C	δ_H (*J*=Hz)
1	33.7	1.37 (1H, m) 1.40 (1H, m)	34.0		34.1	
2	24.9	1.43 (2H, m)	28.1	2.18 2.38	28.1	2.18 2.38
3	76.4	3.37 (1H, t, J = 4.5)	179.5		179.5	
4	37.7		147.2		147.1	
5	49.6	1.23 (1H, m)	50.5		49.7	
6	18.3	1.38 (2H, m)	24.3		24.4	
7	35.2	1.24 (1H, m 1.55 (1H, m)	33.6		33.7	
8	40.7		39.8		39.9	
9	50.4	1.42 (1H, m)	40.9		41.0	
10	37.3		38.8		38.9	
11	21.4	1.52 (2H, m)	22.9		22.2	
12	25.4	1.53 (1H, m) 1.91 (1H, m)	27.0		26.7	
13	42.3	1,58 (1H, m)	42.7		42.8	
14	50.5		50.1		50.2	
15	31.2	1.04 (1H, m) 1.45 (1H, m)	31.2		32.0	
16	27.6	1.77 (2H, m)	25.4		25.6	
17	49.8	1.69 (1H, m)	49.2		49.6	
18	15.6	0.93 (3H, s)	16.1	0.86 s	16.1	0.87 s
19	16.1	0.82 (3H, s)	19.9	0.82 s	20.0	0.84 s
20	75.5		86.1		86.4	
21	25.5	1.13 (3H, s)	23.9	1.11 s	27.0	1.13 s
22	40.6	1.44 (2H, m)	35.5		34.6	
23	22.6	2.02 (2H, m)	25.9		26,2	
24	124.8	5.10 (1H, t, J = 5.4)	83.1	3.71 dd (7.7)	86.2	3.62, dd (5.5, 10)
25	131.7		71.4		70.2	
26	25.9	1.66 (3H, s)	27.1	1.18 s	27.6	1.17 s
27	17.8	1.59 (3H, s)	24.3	1.10 s	23.9	1.09 s
28	28.4	0.91 (3H, s)	113.2	4.63 brs 4.82 brs	113.3	4.64 *brs* 4.83 *brs*
29	22.2	0.81 (3H, s)	23.2	1.70 s	23.1	1.71 s
30	16.6	0.86 (3H, s)	15.0	0.97 s	15.2	0.99 s

The ^{1}H NMR ($CDCl_3$, 700 MHz, ppm) spectrum of **2** displayed the presence of three tertiary methyl groups at δ_H 0.89 (H-18), 0.85 (H-19), and 1.00 (H-30), as well as three secondary methyl groups at δ_H 1,73 (H-29), 1.13 (H-21), 1.19 (H-26) and 1.12 (H-27). The presence of seven methyl protons indicates that compound **2** is a triterpenoid compound (Harneti et al., 2012). The presence of a characteristic oxygenated methine at δ_H 3.73 (1H, t, *J* = 7.5 Hz, H-24) indicates the presence of a tetrahydrofuran ring in the side chain of a triterpenoid compound of the dammarane group (Roux et al., 1998). In compound **2**, a methylene sp^2 signal was also observed at δ_H 4.66 and 4.85 ppm (1H, s, H-28).

The ^{13}C NMR ($CDCl_3$, 175 Hz, ppm) spectrum of **2**, showed 30 carbon resonances. These resonances were classified by their chemical shifts, DEPT, and HMQC spectra as follows: 7 methyl groups (three tertiary at δ_C 16.4 (C-18), 20.2 (C-19), and 15.3 (C-30) ppm. 4 secondary at δ_C 23.2 (C-29), 23.5 (C-

21), 27.4 (C-26), and 24.3 (C-27) ppm. 11 methylene groups of which one methylene olefinic, five methine groups of which one is oxygenated methine, seven quaternary carbons of which two are oxygenated quaternary carbons at 86.4 (C-20) and 71.6 (C-25) ppm which indicated the presence of a typical tetrahydrofuran ring in the side chain of dammarane-type triterpenoid (Roux et al., 1998). In the spectrum, the signals of sp^2 quaternary carbon at δ_C 147.5 (C-4) and sp^2 methylene at δ_C 113.5 (C-28) as well as carboxylic acid carbonyl at δ_C 179.4 (C-3) were also observed, indicating the presence of a ring in the open dammarane triterpenoid framework in compound **2** (Szoka et al., 2024). The existence of these 2 functional groups accounted for 2 out of the total 6 hydrogen deficiency index. The remaining 4 hydrogen deficiencies were consistent were consistent with the tetracyclic triterpenoid structure of **2**.

The functional group's position of **2** was deduced from the HMBC spectra. The existence of open cyclic A at positions 3 and 4 in compound **2** can be shown by the absence of observed correlation between H-2 (δ_H 2.38) to C-4 or correlations between H-29 (δ_H 1.73) to C-3. In addition, the presence of a double bond at C-4/C-28 can be seen by the correlations between H-28 (δ_H 4.66 and 4.85 ppm) with C-4 and C-5 as well as the correlations between H-29 (δ_H 1.73) with C-4, C-28, and C-5. The presence of correlations between the methyl protons H-21 (δ_H 1.15) with C-17, C-22, and C-20, as well as correlations between H-27 with C-24, C-26, and C-25, is also seen in the spectrum of the correlations between H-26 (δ_H 1.21 ppm) with C-24, C-27, and C-25, which indicates the presence of a tetrahydrofuran ring located at C-17 (**Figure 1**). The positions of the methyl groups seen embedded at C-10, C-8, and C-14 are respectively indicated by the correlations between H-19, H-18, and H-30. The entire HMBC correlation of compound 2 can be seen as in **Figure 2.**

The ^{1}H-^{1}H COSY correlation of compound **2** indicates the presence of a basic framework of dammarane triterpenoid compounds (**Figure 2**). The presence of a cross peak between H1-H2 indicates an open dammarane triterpenoid A ring, the cross peak between H5-H6-H7 indicates an open dammarane triterpenoid A ring at C-3/C-4. Thus, compound **2** has a planar structure of 20,24-epoxy-25-hydroxy-3,4-secodamar-4(28)-en-3-oic acid (shoreic acid) and its complete correlations are shown in **Figure 2**.

To determine the stereochemistry of asymmetric carbons it can be determined through the chemical shift values of ^{1}H and ^{13}C-NMR. Compound **2** shows chemical shifts for C-24 and H-24 respectively δ_C 83.3 and δ_H 3.73 ppm; dd, J = 4.8 and 10.8 Hz, and the chemical shift of C-20 is 86.4 ppm, this indicates the configuration of C-20 is *S* and C-24 is *R* (Roux et al., 1998). Further support was obtained from the results of the comparison of NMR data between compound **2** and shoreic acid in **Tables 1** and **2**. Based on the spectral data obtained, previous research data, and the approach to the biogenesis of dammarane triterpenoid compounds, compound **2** was identified as shoreic acid (Seger et al., 2008).

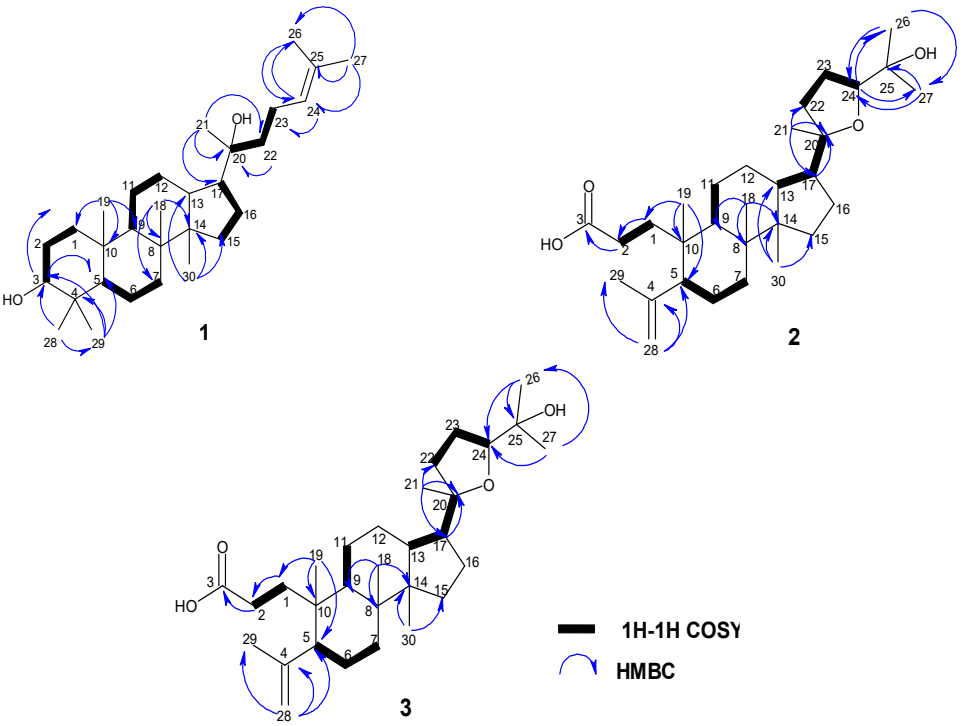

Figure 2. Selected HMBC and ^{1}H-^{1}H COSY correlations of **1** – **3**

Based on the reference search that has been done, the mixed epimer compounds **2** and **3** have a structure similar to the aglinin A compound that has been successfully isolated from several species, such as *A. forbesii*, *A. smithii* was also found in the form of a mixture of its epimers, 24*R* and 24*S*. The tetrahydrofuran ring with hemiketal in molecule **2** might be interconvertible with an intermediate 20-ol-24-one form (through a ring opening and closing procedure under protic conditions, which results in the epimer mixture **2** and **3** being inseparable (Feng et al., 2013).

The 1D NMR chemical shift and spectrum of **3** in the epimeric mixture as can be seen in **Table 1**, shows duplication in almost all signals but with the signal of compound **2** being more dominant than compound **3** with a ratio of approximately 3:1, that was indicating the presence of two epimeric compounds. The ^{13}C NMR spectrum of compounds **3** and **2,** as shown in **Table 1** shows duplication in 29 signals from 30 carbon signals, which indicates the presence of two epimeric compounds. In the 1D NMR spectrum only one significant difference is seen at the C-24 position, where compound **2** shows a chemical shift for C-24 and H-24 respectively δ_C 83.3 ppm and δ_H 3.73 ppm (1H, t, J = 7.5 Hz), while the chemical shift at C-20 is 86.4 ppm, this indicates that the configuration of C-20 is *S* and C-24 is *R* for compound **2.**

Meanwhile, compound **3** shows the chemical shift δ_C 86.6 ppm and δ_H 3.62 ppm (1H, t, J = 5.10 Hz), while the chemical shift at C-20 is 86.5 ppm, this indicates that the configuration of C-20 is *S* and C-24 is *S* for compound **3**, it can be seen that **2** and **3** is an epimeric isomer compound at C-24. Shoreic acid (**2**), whose stereochemistry has been determined by X-ray (Lavie et al., 1984), is the 24*R* isomer, while **3** is the 24*S* isomer. Experiments with 2D NMR allow to obtain the correlations and structure of the two compounds as shown in **Figures 1** and **2** and also the exact ^{13}C and ^{1}H chemical shift data of **2** and **3** as shown in **Table 1**, and the comparative NMR data can be seen as in **Table 2.** Based on the spectral data obtained, which is in accordance with the literature, the name of compound **3** was confirmed to be eichlerianic acid.

The antibacterial activities of the isolated compounds were evaluated against normal ATCC bacteria strains (**Tables 3**). To assess the potential of antimicrobial compounds in this study, the broth microdilution method was employed, which yields quantitative data, specifically the MIC value (Swebocki et al., 2023). MIC is the lowest concentration of an antimicrobial agent that can inhibit the growth of certain bacteria, which can be observed with the naked eye through changes in turbidity (Hossain, 2024). Turbidity values are measured by inserting a microplate into a plate reader and reading the OD_{600} value from the wells. Then, the data is collected and the MIC value is calculated (Kadeřábková et al., 2024).

The epimeric mixture of **2** and **3** showed moderate antibacterial activity with a minimum inhibitory concentration value ranging from 31.7 to 126.6 ppm, particularly against *S. aureus* with a MIC value of 31.7 ppm, while compound **1** was inactive against all types of bacteria. Based on the activity data of the triterpenoid compounds, it is suspected that the functional groups, namely (-COOH) and the double bond in the open cyclic A found in the mixed compounds of epimers **2** and **3**, play a role in increasing their activity.

The epimers **2** and **3** are more active against gram-positive bacteria, particularly *S. aureus*, than gram-negative bacteria. This is also thought to be because gram-negative bacteria have a double-layered membrane structure, a lipopolysaccharide layer, membrane proteins, and porins that are not found in gram-positive bacteria, thus inhibiting the activity of compounds (Hickson et al., 2025; Ji et al., 2022; Woods et al., 2025). *S. aureus* is a Gram-positive species that belongs to the family Micrococcaceae. It is commonly found on human skin and in the nose (Rajput et al., 2024).

Based on this research, these epimer compounds have the potential to be further researched and developed as an antibiotic compound against *S. aureus*. The electron-withdrawing group (-COOH) bound to the open cyclic A is thought to contribute to the increased antibacterial activity (Purwantiningsih et al., 2020). Carboxylic organic acids have antimicrobial potential due to the lipophilic nature of the undissociated acid form (RCOOH) which can penetrate the microbial plasma membrane only through passive diffusion (Mira et al., 2024). Organic acids seem to have varied putative antibacterial mechanisms involved in cytoplasmic acidification, osmotic stress, membrane disintegration, and cytoplasmic enzyme destabilization (Yoon et al., 2024). The antibiotic tetracycline, used as a positive control, showed strong activity against all four bacterial species, with an MIC value of 1.55 ppm. Tetracycline inhibits protein biosynthesis by targeting ribosomal subunit 305. Tetracycline inhibits the binding of tRNA to the ribosomal A site, ultimately inhibiting protein synthesis (Halawa et al., 2023).

Based on several references that have been successfully obtained, it is known that the antibacterial activity of triterpenoid compounds is influenced by the presence and position of functional groups such as carboxylic acids and double bonds contained in the compound. Two triterpenoid compounds without carboxylic acid functional groups, lanosterol and lupeol, have been successfully isolated from *Euphorbia arbuscula* and tested for their antibacterial activity against *S. aureus*, *E. coli*, and *P. aeruginosa*. The isolated compounds showed weak or no antibacterial activity. (Al-Ansi et al., 2024).

Table 3. MIC values of the isolated compound against ATCC strains.

Compound	Bacterial tested			
	P. aeruginosa	*E. coli*	*S. aureus*	*B. subtilis*
	MIC (ppm)			
1	> 225	> 225	> 225	> 225
Epimeric mixture of **2** and **3**	126.6	63.3	31.7	63.3
Tetracycline (Control)	1.55	1.55	1.55	1.55

Three tetracyclic triterpenoic acid compounds having carboxylic acid groups in their structure, namely densiflorinic acid A-C, have been successfully isolated from *Dysoxylum densiflorum* and tested for their antibacterial activity. These compounds were tested against seven bacterial species using the microdilution method. They exhibited only weak antibacterial properties, with densiflorinic A exhibiting the highest activity against *B. subtilis*, with an MIC of 26.5 µM (Komang et al., 2016).

CONCLUSIONS

The ethyl acetate preparation of *A. foveolata* twigs produced three dammarane-type triterpenoids and one steroid, which were identified as dammar-24-en-3*β*,20-diol (**1**), an epimeric mixture of shoreic acid (**2**); eichlerianic acid (**3**). The antibacterial activities of the isolated compounds were evaluated against four normal ATCC bacteria strains, including two gram-positive bacteria (*S. aureus*, and *B. subtilis*) and two gram-negative bacteria (*E. coli*, and *P. aeruginosa*). An epimeric acid mixture of shoreic (**2**) and eichlerianic acid (**3**) was highly active (MIC 31.7 ppm) against *S.aureus* and active to inhibit other normal bacterial strains.

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CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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