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Triterpenoids Betulinaldehyde and Betulinic Acid from The Stem Bark of *Dillenia ochreata* (Miq.) Teijsm. & Binn. ex Martelli

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ABSTRACT: *Dillenia ochreata* is a traditional medicinal plant used to treat scurvy. The pharmacological activity of *D. ochreata* as a scurvy drug was related to the content of secondary metabolite compounds. The research aimed to isolate and determine the structure of compounds from the stem bark of *D. ochreata* and assess their antibacterial activity. The extraction used the maceration method with graded polarity (n-hexane and ethyl acetate).The separation and purification using chromatography methods. The structures were determined by spectroscopic analyses using FT-IR and NMR spectroscopy. The compounds were tested for antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* using the diffusion method, and the minimum inhibitory concentration (MIC) was determined by the microdilution method. Two compounds, betulinaldehyde (**1**) and betulinic acid (**2**), have been isolated. Betulinaldehyde (**1**) showed weak antibacterial activity against *E. coli* with a minimum inhibition concentration (MIC) of 272 µM and moderate activity against *S. aureus* (MIC 34 µM), while betulinic acid (**2**) showed weak activity against both bacteria (MIC 272 µM). The betulinaldehyde and betulinic acid were first reported from *D. ochreata.*

Keywords: Antibacterial activity, *D. ochreata,* MIC, stem bark, triterpenoid.

INTRODUCTION

Dillenia belongs to the Dilleniaceae family, with about 100 species. A few species of the genus *Dillenia* have been used in traditional medicine to treat various conditions, including cancer, wounds, jaundice, fever, cough, diabetes mellitus, and diarrhoea (Sabandar et al., 2017). This plant is widely found in Southern Asia, Australia, and the Indian Ocean Islands (Sabandar, 2017). *Dillenia* spp have local names, such as *Red beech*, *golden guinea* (Australia), *Simpoh* (Malaysia), *Simpor* (Brunei), *San, Masan* (Thailand), and *Kadmon* (Philippines) (Lim, 2012). One species of *Dillenia* is *Dillenia ochreata* (synonym *Wormia ochreata* Miq.). In Indonesia, it is known as *semprawang*. *D. ochreata* has been traditionally used, especially in Musi Banyuasin, South Sumatra, Indonesia, to treat scabies (Muharni et al., 2017).

The therapeutic potential of a traditional medicine was associated with the presence of secondary metabolites, such as triterpenoids, steroids, alkaloids, flavonoids, and polyketides. (Qalbi et al., 2017; Husein, 2017). The leaves of *D. ochreata* were reported to contain terpenoids, steroids, and phenolic compounds that showed antibacterial activity. An antibacterial compound, sentulic acid, has been reported from *D. ochreata* leaves. (Muharni et al., 2023).

Scientific reports on other species of the genus *Dillenia* have reported the presence of many triterpenoid compounds. A few species of *Dillenia* have been reported to contain lupeol and betulinic acids as major components, with potential pharmacological effects (Barua et al., 2018). The triterpenoids betulinic acid, 3-oxoolean-12-en-30-oic, coetjapic acid, 3-oxoolean-12-en-30-oic acid, and a lupine derivative, betulinic acid, were reported from the *Dillenia serrata* (Jalil et al., 2015). Sultana et al. (2022) reported that the bark of *D. pentagyna* contains flavonoids such as kaempferol, quercetin, and isorhamnetin, as well as terpenoids including lupeol, retinaldehyde, betulin, β-sitosterol, stigmasterol, diploic acid, and malonic acid. Yadav et al. (2015) reported that the crude extracts of the bark of *D. pentagyna* in ether, ethyl acetate, and methanol exhibit antibacterial activity. Based on the literature, no information has been found on the chemical composition of *D. ochreata* stems. This study aims to investigate the chemical constituents of the stem bark extract of *D. ochreata* and their antibacterial activity.

EXPERIMENTAL SECTION

Instruments/equipment

The equipment was employed: distillation apparatus (Samheung energy SH-WB-6GDN), UV-Vis

spectrophotometry (Thermo scientific Orion Aquamate 8000), FTIR spectrometer (Perkin Elmer-FTIR), NMR (Agilent DD2 500 MHz for H-NMR and 125 MHz for 13C-NMR), rotary evaporator (Eyelletype N-1300V-WB), incubator, autoclave, oven (Memmert), analytical balance (Ohaus), and hotplate.

Chemicals

The materials used were the stem bark of *Dillenia ochreata*, *n*-hexane, ethyl acetate, methanol, silica gel 60 G 70-230 mesh (Merck), TLC plate GF_{254} (Merck), nutrient agar (Merck), nutrient broth (Merck), *Staphylococcus aureus* ATCC 25923 (Indofarma), *Escherichia coli* ATCC 25922 (Indofarma), and dimethyl sulfoxide (DMSO) (Merck). The solvent was used in technical grade and was distilled before use.

Sample Preparation

The stem bark of *D. ochreata* was collected in Banyuasin, South Sumatra, Indonesia, in October 2019 and identified as *Dillenia ochreata* (Miq.) Teijsm. & Binn. Ex Martelli at Herbarium Bogoriense, Bogor, with register B-82/IV/D1.01/i/2021. Fresh stem bark was cut into pieces (±1×0.3 cm), initially sorted to separate the simplicia from impurities, and dried at room temperature until a constant weight was obtained. Furthermore, simplicia was ground to obtain stem bark powder.

Extraction

The simplicia powder (2 kg) was extracted using a solvent with increasing polarity (n-hexane and ethyl acetate) by the maceration method. The maceration for ±48 hours, with three repetitions. The macerations were filtered and concentrated using a rotary evaporator at 60 °C to yield crude extract of *n*-hexane and ethyl acetate. The yield percentage of each crude extract was calculated.

Separation and Purification of n-hexane Extract

The n-hexane extract was separated and purified using silica gel 60 (70-230 mesh) via gravity column chromatography method. The sample (3 g) was prepared by adsorption onto silica at a 1:1 sample-to-silica ratio (Muharni et al., 2023). The sample was eluted using an eluent with a graded-polarity: n-hexane: ethyl acetate (95:5-0:100). The eluate was collected and pooled based on their thin-layer chromatography (TLC) profiles. Based on the TLC profiles, four fractions, F1 -F4 were obtained. After purification, the F3 fraction yielded white crystals (Compound **1**, 34 mg). The isolated compound was characterized using FTIR and NMR Spectroscopy.

Separation and Purification of Ethyl Acetate Extract

The ethyl acetate extract (5 g) was separated using gravity column chromatography with silica gel 60 (70-230 mesh). The sample was eluted using an eluent with graded polarity (n-hexane 100%; n-hexane: ethyl acetate (9:1; 7:3; 5:5) and ethyl acetate: methanol (9:1; 8:2; 7:3). The eluate was collected and then analyzed by thin-layer chromatography (TLC). Based on their TLC profiles, five fractions (F1 - F5) were obtained. Furthermore, the F2 fraction was recolumn chromatography. Based on the TLC profiles, two fractions, F2.1 and F2.2, were obtained. From the fraction F2.1 was obtained a white solid form (compound **2**: 62 mg).

Antibacterial Activity Test

Preparation of bacterial cultures

The *S. aureus* and *E. coli* bacteria were each inoculated into agar medium using a single aseptic inoculation needle, then inoculated by scratching the agar medium. Furthermore, it was incubated for 24 hours at 37 °C.

Preparation of bacterial inoculum

Bacterial cultures in slant agar media were taken aseptically with a single loop, then inserted into 12 mL of NB media, shaken until homogeneous, and incubated for 24 hours at 37 °C. The amount of bacterial cells in the suspension was measured using a UV-Vis spectrophotometer with a wavelength of 625 nm and diluted until an absorbance of 0.08-0.1 McFarland was obtained (Balouiri et al., 2016).

Antibacterial activity

The agar diffusion method was used to test the antibacterial activity against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 bacteria (Ernilasari et al., 2021). The antibacterial activity was carried out three times. Each sample was dripped onto a paper disc containing five µL (concentration series of 1000, 500, 250, and 125 µg/mL), then placed on NA media inoculated with test bacteria. Incubation was carried out at 37 °C for 2×24 hours, and observations were made on forming an inhibition zone around the paper disc (Hossain et al., 2024).

Minimum Inhibition Concentration

The microdilution method was used to determine the minimum inhibitory concentration (MIC) value (CLSI, 2019). The NB medium (180 µL) was inoculated into a microplate (hole 1), and 100 µL was inoculated into holes 2–8. The samples diluted with DMSO (20 µL) with a concentration of 120 µg/mL were inserted into hole 1, stirred until homogeneous, then 100 µL solution from hole 1 was inserted into hole 2 and to be continued until the well 10, (final sample concentration: 120; 60; 30; 15; 7.5; 3.75; and 1.67 µg /mL). Hole 8 was inserted with a negative DMSO 10 µg / mL control. Furthermore, the bacterium (30 µL) was placed into holes 1-8. The culture was incubated for 24 hours at 37°C. The MIC of each sample was defined as the lowest concentration that completely inhibited bacterial growth (Guefac et al., 2022).

Data Analysis

The antibacterial activity was analyzed using analysis of variance (ANOVA) at $\alpha = 0.05$, followed by a Duncan new multiple range test (DNMRT) at $\alpha = 0.05$, using SPSS. (Willey, 2014).

RESULTS AND DISCUSSION

The extraction of *D. ochreata* stem bark powder (2 kg) after concentration yielded crude extracts of n-hexane (3.46 g) and ethyl acetate (52.5 g), with yields of 0.17% and 2.63%, respectively. The separation and purification of the n-hexane extract (3 g), a white crystalline (34 mg), to obtain compound **1** (white crystalline 34 mg), and from the ethyl acetate extract (5 g), after purification, to obtain compound **2** (white solid 62 mg)

Structure Elucidation

Analysis of the FTIR spectrum of compound **1** showed a peak at 3357.1 cm^{-1} (-OH group), and a supported peak at 1083.7 cm^{-1} (C-O functional group). The Absorption at 2942.1 cm^{-1} and 2867.1 cm^{-1} represented C-H stretching and asymmetric and symmetric groups of aliphatic C-H. Absorption at 1715.9 cm^{-1} represented the absorption of the carbonyl group (-CH=O aldehyde). The absorption at 1642.5 cm-1 is specific to the C=C group, while those at 1387.3, 1468.1, and 1376.1 cm^{-1} are specific to cycloaliphatics. This data indicated the compound is a triterpenoid. The ^{1}H NMR spectrum (500 MHz) in Deuterated chloroform ($CDCl_3$) solvent of the compound **1** showed six methyl signals at the chemical shifts δ_H 0.74, 0.81, 0.88, 0.96, 0.97, and 1.69 ppm, a typical signal for methyl protons. ^{1}H NMR spectrum also showed a signal at δ_H 2.86 ppm (1H, *m*), a signal for CH sp^3 bound to C sp^2. The signal at δ_H H 3.18 ppm (1H, *m*) is a signal of H bound to a carbon that binds an electronegative group (O atom), characteristic of triterpenoids with OH at the C-3 position. The signals at δ_H of 4.62 ppm (1H, *d*, J=1.5 Hz) and 4.75 ppm (1H, *d*, J=1.5 Hz), which are signals for two vinylic protons >C=CH_2 and a signal on δH 9.67 ppm (1H, *d)*, a characteristic signal for aldehyde protons (CH=O group).

The ^{13}C-NMR spectrum (**Figure 1**) showed some signals accumulated in the chemical shifts below δC 60 ppm. These signals are characteristic of C, CH, CH_2, and CH_3 in the cycloaliphatic structure of the triterpenoid compound. The signal at δ_C 79.2 ppm is a typical signal for the C-3 position of triterpenoid compounds (Abdelrahman & Jogaiah, 2020). The signals at δ_C 110.4 and 150.0 ppm are signals for carbon with double bonds (C = C), while the signal at δ_C 207.0 ppm is a typical signal for C carbonyl in an aldehyde. The ^{13}C-NMR spectrum (**Figure 1**) was widened in the region < δ_C 60 ppm, and 26 signals were observed, so the total carbon signal from the compound is 30. This data indicated that the compound is a triterpenoid. Measurements Distortionless Enhancement by Polarisation Transfer (DEPT) 135 spectrum, the compound **1** had 6 CH_3 signals at δ_C 14.4; 15.3; 16.0; 16.3; 19.2; 28.2 ppm, 11 CH_2 signal at δ_C 18.5; 20.8; 25.7; 27.6; 28.9; 29.5; 30.0; 33.4; 34.5; 38.8; 110.4 ppm, 7 CH signals at δ_C 38.9; 47.7; 48.2; 50.6; 55.4; 79.1; 206.9 ppm and 6 C quaternary signals at δ_C 39.0; 40.9; 37.3; 42.7; 59.5; 149.9 ppm.

The NMR compound **1** were compared with the NMR data of the betulinaldehyde compound from the bark of the *Avicennia officinalis* (Haque et al., 2006) (**Table 1**). This indicates that compound **1** refers to betulinaldehyde. Betulinaldehyde was reported to be present in the bark of *Dillenia pentagyna* (Sultana et al., 2020). According to Nahar et al. (2025), betulinaldehyde from *D. Indica* has hematoprotective activity. Betulinaldehyde isolated from the bark of *Plantanus kerrii* showed moderate activity against two Hep-G2 and MCF-7 cancer cell lines with IC_{50} values of 50.07 µg/mL and 85.63 µg/mL, respectively (Ngoe and My, 2018).

Analysis of the FTIR spectrum of compound **2** showed a peak of 2939 – 2867 cm^{-1} (C-H aliphatic) and 1448 - 1375 cm^{-1} (gem dimethyl absorption (C-$(CH3)_2$) which was characteristic for triterpenoid compounds. Next, absorption at 1684 cm^{-1} (C=O group), and 1644 cm^{-1} (conjugated C=C group). The ^{1}H NMR spectrum showed three signals for vinylic protons (-CH=CH_2) at δ_H 4.68 (1H, s) and 4.55 (1H, s), and δ_H 4.29 (1H, s) for the proton at position C-3 in the triterpenoid compound that binds -OH. The signals at δ_H 1.0 - 2.5 ppm, with integration of 1H and 2H, and multiplet peaks are signals from cycloaliphatic CH and CH_2. In addition, there are six signals CH_3 group. The methyl signal at δ_H 1.63 (3H, s) is typical for methyl bound to C sp^2.

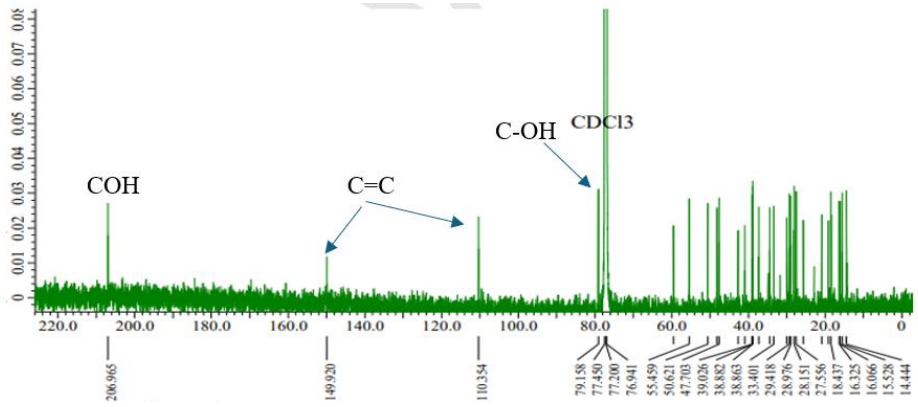

Figure 1. ^{13}C-NMR spectrum of compound **1**

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

No. C	δ_H (ppm) , mult, *J* (Hz), Compound **1**	δ_H (ppm), multi, *J* (Hz) Betulinaldehyde	δ_C(ppm) Compound **1**	δ_C(ppm) Betulinaldehyde
1			38.9	39.1
2			27.6	27.6
3	3.18 (1H, *dd*, *J*= 4.5 Hz)	3.18 (1H, *dd*, 4.5 Hz)	79.1	79.0
4			39.1	39.1
5			55.4	55.4
6			18.5	18.3
7			33.4	34.4
8			40.9	40.7
9			50.6	50.6
10			37.3	37.7
11			20.8	20.9
12			25.7	25.5
13			38.8	38.1
14			42.7	42.4
15			28.9	30.5
16			30.0	32.5
17			59.5	56.2
18			47.7	47.0
19	2.86 (1H, *m*)	2.86 (1H, *m*)	48.2	49.3
20			150.0	150.0
21			29.5	29.8
22			34.5	37.2
23	0,96 (3H, *s*)	0.96 (3H, *s*)	28.2	27.9
24	0,74 (3H, *s*)	0.75 (3H, *s*)	15.3	15.4
25	0,88 (3H, *s*)	0.91 (3H, *s*)	16.0	16.2
26	0,81 (3H, *s*)	0.82 (3H, *s*)	16.3	16.3
27	0,97 (3H, *s*)	0.97 (3H, *s*)	14.4	14.6
28	9.67 (1H,*s*)	9.68 (1H, *s*)	207.0	180.0
29	4.75 (1H, *d*, *J*= 1.5) 4.62 (1H, *d*, *J*= 1,5)	4.75 (1H, *d*, *J*= 1.5) 4.63 (1H, *d*, *J*= 1.5)	110.4	108.0
30	1.69 (3H, *s*)	1.69 (3H, *s*)	19.2	19.6

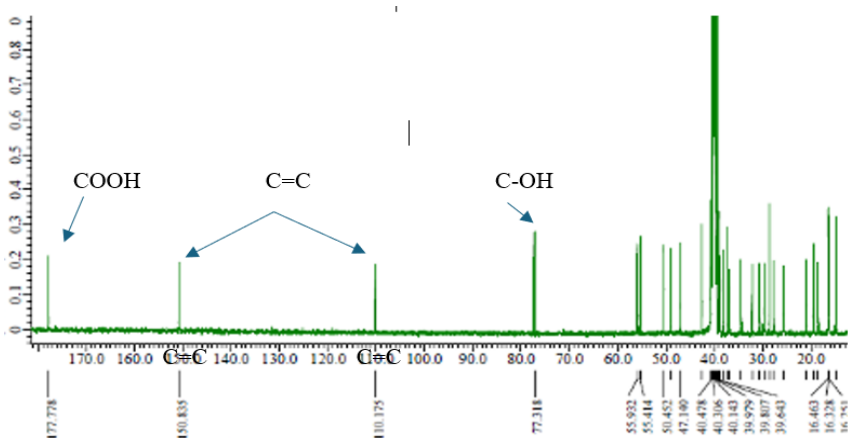

Figure 2. ^{13}C NMR Spectrum of compound **2**

Table 2. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) data of **2** in $CDCl_3$ solvent.

No. C	δ_H, *m, J* (Hz) Compound **2**	δ_H, *m, J* (Hz) Betulinic acid	δ_C (ppm) Compound **2**	δ_C (ppm) Betulinic acid
1			38.6	39.0
2			29.7	28.0
3	4,29(1H, *s*)	3,44(1H, *t*, *J*=8,05 Hz)	77.2	77.8
4			39.0	39.2
5			55.5	55.6
6			18.5	18.5
7			34.4	34.5
8			40.7	40.8
9			50.5	50.7
10			37.2	37.2
11			21.0	20.9
12			25.5	25.8
13			38.1	38.3
14			42.6	42.5
15			30.6	30.0
16			32.2	32.6
17			55.9	56.3
18			49.0	49.5
19			47.2	47.5
20			150.8	151.9
21			27.7	30.9
22			36.8	37.3
23	0.93 (*s*)	1.21(3H, *s*)	28.6	28.4
24	0.75 (*s*)	0.99(3H, *s*)	16.2	16.1
25	0.64 (*s*)	0.80(3H, *s*)	1.5	16.1
26	0.86 (*s*)	1.04(3H, *s*)	16.4	16.1
27	0,86 (*s*)	1.05(3H, *s*)	14.9	14.6
28			177.8	178.4
29	4.68 (1H,*s*); 4.55 (1H, *s*)	4.75(1H, *s*); 4.93(1H, *d*) *J* = 2.10 Hz	110.2	109.7
30	1.63 (3H, *s*)	1.77(3H, *s*)	19.5	19.2

The ^{13}C-NMR spectrum (**Figure 2**) of compound **2** showed 30 C signals, indicating that compound **2** is a triterpenoid. In spectrum showed four signals at δ_C 60 - 180 ppm, the signal at δ_C 177.8 ppm is signal for carbonyl group (C = O) in acid form, δ_C 150.8 ppm and δ_C 110.2 ppm were signals from vinylic carbon (C = C) and signal at δ_C 77.2 ppm is a C signal that binds the OH group at C-3 position in the triterpenoid compound. Other signals in the region below δ_C 60 ppm correspond to the types of carbon sp^3.

The DEPT 135 spectrum was measured to determine the signal for carbon CH_3, CH_2, CH, or C. Analysis of the DEPT 135 spectrum compound **2** showed the presence of 6 CH_3 signals at δ_C 28.6; 19.5; 16.5; 16.4; 16.2; and 14.9 ppm, 11 CH_2 signals at δ_C 110.2; 38.6; 36.8; 34.4; 32.2; 30.6; 29.7; 27.7; 25.5; 21.0; and 18.5 ppm; 6 CH signal at δ_C 77.2; 55.5; 50.5; 49.0; 47.2; and 38.1 ppm and seven quaternary C signals at δ_C 150.8; 177.8; 55.9; 42.6; 40.7; 39.0; 37.2 ppm. The ^{13}C NMR data of compound **2** were compared with the NMR data of triterpenoid betulinic acid from *Dillenia suffruticosa* (Abubakar et al., 2019). Data (**Table 2**) showed that the data for compound **2** were almost identical to those of the NMR reference compound, betulinic acid (**Figure 3**).

Betulinic acid compounds have been reported from the root bark of *D. serrata* (Jalil et al., 2015), the methanol extract of *D. indica* fruit (Song et al., 2025), and the dichloromethane fraction of *D. suffruticosa* leaves (Abubakar et al., 2019; Rahayu et al., 2025); however, it was reported from the bark of *D. ochreata* for the first time. Betulinic acids have been reported to have potential pharmacological activity (Lou et al., 2021).

Antibacterial Activity of Isolated Compounds

The antibacterial activity of the compounds (betulinaldehyde and betulinic acid) was evaluated against *Escherichia coli* and *Staphylococcus aureus* (**Table 3**). The diameter of the inhibition zone of compound **1** of *E. coli* bacteria is 8.81 ± 0.28 - 9.46 ± 0.67 mm and *S.aureus* bacteria is 9.42 ± 0.89 - 7.41 ± 0.90 at a concentration of 125-1000 µg/mL while compound **2** exhibited inhibition zone diameter of 7.26 ± 0.32- 9.59 ± 0,28 against *E.coli* and 7.56 ± 0.26 - 10,23 ± 0,37 against *S. aureus* at

a concentration of 125-1000 µg/mL Besari et al. (2023) reported that antibacterial activity with an inhibition zone ≤ 5 mm was categorized as weak, 6-10 mm as moderate, 11-20 mm as strong, and ≥20 mm as very strong. Accordingly, the inhibitory activity of the compounds **1** and **2** was assigned to the moderate category against *E. coli* and *S. aureus* bacteria at 125-1000 µg/ml. The antibacterial activity of both compounds was lower than the positive control tetracycline. The positive control tetracycline at a concentration of 50 µg/mL showed antibacterial activity with an inhibition zone of 17.66 ± 0.76 at *E. coli* and 15.33 ± 0.91 at *S. aureus*. Statistical analysis showed that the antibacterial activity of the isolated compounds is homogeneously distributed, and the one-way ANOVA test revealed no significant difference ($p > 0.05$) in the concentrations for both bacteria, and a significant difference ($p < 0.05$) between the bacteria test (*E. coli* and *S. aureus* bacteria) with the positive control (tetracycline).

Furthermore, the minimum inhibitory concentration (MIC) value was determined using the microdilution method (**Table 4**). According to Cate et al. (2024), bacterial growth was inhibited when wells were filled with a clear solution, but it still occurred when wells showed turbidity. The antibacterial activity obtained from the pure compounds was classified as significant activity if the MIC <10 µg/mL, moderate activity 10 µg/mL < MIC ≤ 100 µg/mL, and weak activity MIC > 100 µg/mL (Demgne et al., 2021; Guefack et al., 2022). The MIC values for compound **1** are 120 µg/mL (272 µM) against *E. coli*, which is in weak activity, and 15 µg/mL (34 µM) against *S. aureus*, which is in moderate activity. In comparison, compound **2** has an MIC of 120 µg/mL (272 µM) against both bacterial *strains, E. coli and S. aureus, and was assigned* weak activity.

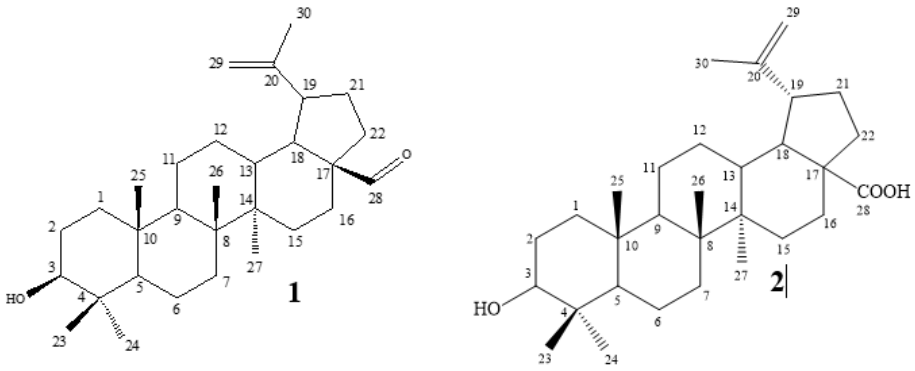

Figure 3. Structure of betulinaldehyde (**1**) and betulinic acid (**2**)

Table 3. Diameter of the inhibition zone

Compounds	Concentration (µg/mL)	Inhibition zone (mm) Average ± SD	
		E. coli	*S. aureus*
Betulinaldehyde **(1)**	1000	9.46 ± 0.67	9.42 ± 0.89
	500	9.36 ± 0.27	8.67 ± 0.16
	250	9.01 ± 0.22	8.05 ± 0.44
	125	8.81 ± 0.28	7.41 ± 0.90
Betulinic acid **(2)**	1000	9.59 ± 0.28	10.23 ± 0.37
	500	8.90 ± 0.15	8.79 ± 0.39
	250	7.28 ± 0.44	7.88 ± 0.19
	125	7.26 ± 0.32	7.56 ± 0.26
Tetracycline	50	17.66 ± 0.76*	15.33 ± 0.91*

*inhibition zone indicates a significant difference with isolated compounds

Table 4. MIC values of pure compounds.

Compounds	Bacteria	MIC value (µg/mL)	Classified activity
Betulinaldehyde (**1**)	*E. coli*	120	Weak
	S. aureus	15	Moderate
Betulinic acid (**2**)	*E. coli*	120	Weak
	S. aureus	20	Weak

Betulinaldehyde showed higher antibacterial activity against Staphylococcus aureus than betulinic acid due to differences in their functional groups at the C-28 position, which affect their interactions with the bacterial cell. The presence of an aldehyde group (-CHO) at the C-28 position in betulinaldehyde makes it more chemically reactive compared to the carboxylic acid group (-COOH) in betulinic acid. This increased reactivity allows for potentially stronger interactions with bacterial cellular targets at the aldehyde group. Besides that, in betulinaldehyde is more hydrophobic and can penetrate the bacterial cell well (Chung et al., 2011).

CONCLUSIONS

Two compounds, betulinaldehyde (**1**) and betulinic acid (**2**), were isolated from the stem bark of *D. ochreata.* Betulinaldehyde (**1**) showed weak antibacterial activity against *E. coli* (MIC 272 µM) and moderate activity against *S. aureus* (MIC 15 µg/mL), while betulinic acid (**2**) showed weak activity against both bacteria (MIC 272 µM). The betulinaldehyde and betulinic acid were first reported from *D. ochreata.*

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