STRUCTURE ELUCIDATION OF ANTIBACTERIAL COMPOUND FROM *Ficus deltoidea* Jack LEAVES

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ABSTRACT

An antibacterial compound has been isolated from Ficus deltoidea Jack leaves. Based on spectroscopic data (IR, ^{1}H-NMR, ^{13}C NMR 1D and 2D and MS), the structure of this compound was identified as 3β-hydroksilup-20(29)-en, (lupeol), $C_{30}H_{50}O$. This compound showed antibacterial activities against E. coli, B. subtilis and S. aureus. The minimum inhibition concentration (MIC) against E. coli, B. subtilis and S. aureus are 150, 220 and 130 µg/mL respectively.

Keywords: *Antibacterial activity, Ficus deltoidea Jack, lupeol*

INTRODUCTION

Ficus deltoidea Jack is an epiphytic shrub which is native and widely distributed in several countries of the Southeast Asia. It is easily found in the coastal, but not in mangrove area. Different parts of the plant are used traditionally to treat various kinds of ailments. The fruits are chewed to relieve headache, toothache and cold. Powdered root and leaves of the plant has been applied externally to wounds and sores and for relief of rheumatism [1]. Decoction from the whole plants is well known as traditional herbal drink for women after childbirth to help strengthen the uterus [2]. The plant sap was used to detach wart from the skin [3]. Moreover it improves blood circulation and pharmacologically blood glucose [4]. On the other hand, there is no report related to its chemical constituent and bioactivity. In this report, the elucidation structure of the isolated compound from ethyl acetate fraction of *F. deltoidea* Jack leaves extracts and its antibacterial activity against *E. coli, B. subtilis* and *S. aureus* are discussed.

EXPERIMENTAL SECTION

Materials

Ficus deltoidea Jack leaves, were collected from Kambang, West Sumatera. The plant was identified at Herbarium of the Biology Department, Andalas University (ANDA), and a voucher specimen (MM 001), had been deposited at the Herbarium.

Instrumentation

Vacuum Liquid Chromatography (VLC), using silica gel PF_{254} Merck (7749), column chromatography, using silica gel 7734 (70-230 mesh) and silica gel 9385 (230-400 mesh) (Merck). IR spectrum was measured with FT-IR Perkin Elmer 1650. ^{1}H and ^{13}C-NMR spectra were recorded with a JEOL JNM ECA-500, at 500 MHz (^{1}H) and 125 MHz (^{13}C). TLC analysis was performed on precoated Si Gel plates (Kiesegel $60F_{254}$, Merck). MS spectra (EI-MS) were obtained on Finnigan LCQ-Deca 70 eV. The melting points were measured on Fisher John Melting point apparatus.

Procedure

Extraction and Isolation

The dried powder of leaves (1 kg), of *Ficus deltoidea* Jack was macerated sequentially with hexane, ethyl acetate and methanol at room temperature. The combined extracts were concentrated *in-vacuo*, to give the hexane extract (47 g), ethyl acetate (16 g) and methanol (29 g). The ethyl acetate extract (16 g), was further fractionated by VLC with gradient elution, using hexane-ethyl acetate (10:0–0:10) afforded 5 fractions (F1-F5). Fraction F4 (1.4 g) was rechromatographed on silica gel eluted with hexane-ethyl acetate (10:0–0:10), to give 4 subfraction (F4.1-F4.4). F4.3 (48 mg), was rechromatographed on silica gel eluted with hexane:ethyl acetate 8:2, and yellowish solid mass, was obtained and then washed with hexane to give white crystal (21 mg).

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Table 1. The comparizon of ^{1}H and ^{13}C-NMR (1D, 2D) data of lupeol and ^{13}C-NMR data lupeol reported by Mahato and Kundu (1994).

	Lupeol isolated compound					Literature[8]
No	δC (ppm)	DEPT	δH(ppm),(ΣH multiplicity)	HMBC	COSY	δ_C lupeol (ppm)
1	38.9	CH_2				38.6
2	27.6	CH_2	1.62 (2H, m)		3.18 (H-3)	27.3
3	79.2	CH	3.18 (1H, t)		1.62 (H-2)	78.9
4	39.0	C				38.8
5	55.5	CH	0.67 (1H, t)		1.38 (H-6)	55.2
6	18.5	CH_2	1.38 (2H, m)		0.67 (H-5)	18.2
7	34.4	CH_2				34.2
8	41.0	C				40.7
9	50.6	CH	1.25 (1H, t)			50.3
10	37.3	C				37.1
11	21.1	CH_2				20.9
12	25.3	CH_2				25.0
13	38.2	CH				38.0
14	43.0	C				42.7
15	27.7	CH_2				27.4
16	35.8	CH_2				35.5
17	43.2	C				42.9
18	48.5	CH	1.35 (1H, dd)	43.0 (C-14)	2.36 (H-19)	48.2
19	48.2	CH	2.36 (1H, m)		1.35 (H-18)	47.9
20	151.2	C				150.8
21	30.0	CH_2				29.8
22	40.2	CH_2				39.9
23	28.2	CH_3	0.96 (3H, s)	55.5 (C-5); 78.9 (C-3); 15.6 (C-24)		27.9
24	15.6	CH_3	0.75 (3H, s)	55.5 (C-5); 78.9 (C-3); 28.2 (C-23); 39.0 (C-4)		15.3
25	16.2	CH_3	0.82 (3H, s)	50.6 (C-9)		15.9
26	16.3	CH_3	1.02 (3H, s)	50.6 (C-9); 34.4 (C-7)		16.1
27	14.7	CH_3	0.94 (3H, s)	27.7 (C-15)		14.5
28	18.1	CH_3	0.78 (3H, s)	43.3 (C-17); 48.5 (C-18); 35.8 (C-16); 40.2 (C-22)		17.9
29	109.5	CH_2	a. 4.68 (1H, d) b. 4.56(1H, d)	48.2 (C-19); 19.5 (C-30) 48.2 (C-19); 19.5 (C-30)	4.68 (H-29 a) 4.56 (H-29 b)	109.3
30	19.5	CH_3	1.67 (3H, s)	109.5 (C-29); 151.2 (C-20); 48.2 (C-19)		19.2

Table 2. Inhibition zone (cm) and MIC of lupeol againts *E. coli*, *B. subtilis*, and *S. aureus* bacteria

Compound	Bacteria	Concentration % (b/v) in ethyl acetate					MIC
		Control	0.25	0.50	1.00	2.00	(µg/mL)
Lupeol	*E. coli*	0.0	1.0	1.0	1.3	1.5	150
	B. subtilis	0.0	0.9	0.9	1.2	1.4	220
	S. aureus	0.0	1.1	1.1	1.4	1.5	130

Bioassay

Antibacterial activity test was carried out by measuring growth inhibition zone of *E. coli*, *B. subtilis*, and *S. aureus,* at various concentrations, using disk diffusion susceptibility method [5-6]. The minimum inhibition concentration (MIC), was determined by dilution method [7].

RESULT AND DISCUSSION

Lupeol, $C_{30}H_{50}O$ (Fig.1): mp: 215-216 °C; IR ($v_{maks,}$ cm^{-1}): 3370 (OH), 2936 and 2865 (C-H aliphatic), 1139 (C-O), 1456 and 1379 (methyl and methylene); EI-MS (m/z): 426 (M^+), 218 (base peak) and 279, the comparison of ^{1}H and ^{13}C-NMR data of lupeol and ^{13}C-NMR lupeol literature [8], see Table 1, and antibacterial activity, see Table 2.

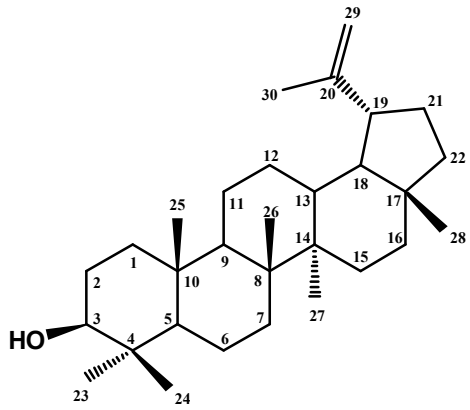

Fig 1. Structure of lupeol

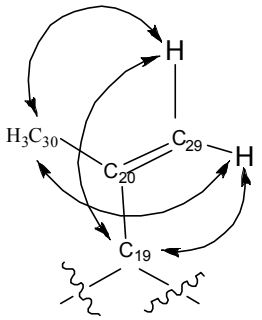

Fig 2. HMBC correlation proton vynilic

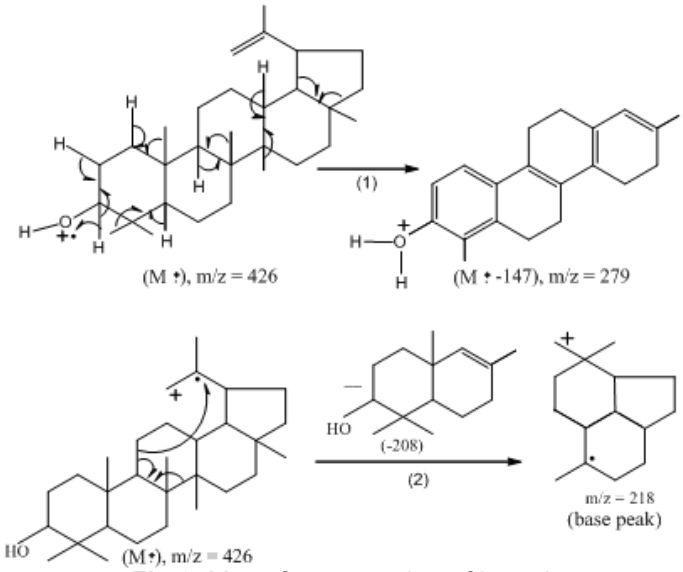

Fig 3. Mass fragmentation of lupeol

The ^{1}H and ^{13}C-NMR spectra of isolated compound showed a characteristic pattern to triterpenoid. It is shown in Table 1, the NMR spectral data of isolated compound and lupeol are quite similar. The DEPT analysis of isolated compound gave 30 carbon, consist of 7 CH_3, 11 CH_2, 6 CH and 6 C quarternary, corresponded to lupeol by comparing their carbon chemical shift. The signal at δ_H 4.68 ppm (1H, d) and 4.56 ppm (1H, d) belong to proton vynilic at C-29 (δ_C 109.5 ppm) that was coupled each other this supported the double bond between C-29 and quarternary carbon, C-20 (δ_C 151.2 ppm), this is also supported by HMBC correlation between $H_{29} \rightarrow C_{19}$ and $H_{29} \rightarrow C_{30}$ (Fig.2).

The HMBC correlation of methyl protons $H_{23} \rightarrow C_{24}$, $H_{24} \rightarrow C_{23}$, $H_{23} \rightarrow C_3$ & C_5, and $H_{24} \rightarrow C_3$ & C_5, supported the gem-dimethyl position at C_4. (Table 1, Fig. 1). The signal at δ_H 3.18 ppm (1H, t), belongs to methyneoxy proton C-3 (δ_C 79.2 ppm; ν_{C-O} = 1179 cm^{-1}). This methyneoxy proton H-3 was coupled by methylene protons H-2 at 1.60 ppm (2H, m), this correlation between H-3 and H-2 also established by COSY analysis.

The mass spectrum of this compound showed the molecular ion, (M^+) at m/z: 426. The fragment ion at m/z: 218 (100%) is characteristic to triterpenoid fragmentation. The other fragment at m/z: 279 supported the proposed structure (Fig. 3).

Based on the antibacterial activity test, it is shown that this compound is more sensitive against *S. aureus* than *E. coli* and more sensitive than *B. subtilis*. The minimum inhibition concentration (MIC) against *E. coli*, *B.* subtilis and *S. aureus* are 150, 220 and 130 µg/mL respectively.

The minimum inhibition concentration (MIC) of lupeol againts *S. Aureus* (MIC: 130 µg/mL) and *E. coli* (MIC: 150 µg/mL) were found to be more sensitive than which were reported (*S. aureus*, MIC 250 µg/mL) and (*E. coli*, MIC > 200 µg/mL) [9]. The MIC of lupeol against *B. subtilis* was not reported yet.

As describe early, there is no report the isolated compound from *F. deltoidea* and the related plants in the same genus. Although lupeol is a well known compound, but this is the first report of this compound from *F. deltoidea* Jack.

CONCLUSION

An antibacterial constituent, lupeol ($C_{30}H_{50}O$), has been isolated from *Ficus deltoidea* Jack leaves. The minimum inhibition concentration (MIC) against *E. coli*, *B. subtilis* and *S. aureus* are 150, 220 and 130 µg/mL respectively.

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