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Characterization antibacterial constituent from *Ficus deltoideus* Jack leaves

Karakterisasi konstituen antibakteri dari daun *Ficus deltoideus* Jack

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Abstract

An antibacterial constituent, has been isolated from *Ficus deltoideus* 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 Olean-12en-3β-ol, (β-amyrin), $C_{30}H_{50}O$. This compound showed antibacterial activities against *E. coli*, *B. subtilis* and *S. aureus*. The minimum inhibition concentration (MIC) agains *E. coli*, *B. subtilis* and *S. aureus* are 230, 380 and 460 (µg/mL) respectively.
Key words : Antibacterial activity, *Ficus deltoideus* Jack, β-amyrin

Abstrak

Suatu konstituen antibakteri, telah diisolasi dari daun *Ficus deltoideus* Jack. Karakterisasi struktur menggunakan data spektroskopi IR, ^{1}H-NMR, ^{13}C-NMR, 1D dan 2D serta data MS, menunjukkan bahwa konstituen hasil isolasi adalah Olean-12en-3β-ol, (β -amyrin), $C_{30}H_{50}O$. Uji aktivitas antibakteri terhadap *E. coli*, *B.subtilis dan S. aureus* menunjukkan bahwa β-amyrin dapat menghambat pertumbuhan bakteri, dengan nilai MIC 230 µg/mL untuk *E. coli*, 380 µg/mL untuk *B. subtilis*, dan 460 µg/mL untuk bakteri *S. aureus*.
Kata kunci: Aktivitas antibakteri , *Ficus deltoideus* Jack, β-amirin

Introduction

Ficus deltoideus Jack is an epiphytic shrub which is native and widely distributed in several countries of the Southeast Asia (Musa and Lip, 2007). 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 headche, tootache and cold. Powdered root and leaves of the plant has been applied externally to wounds and sores and for relief of rheumatism (Musa and Lip, 2007). Decoction from the whole plants is well known as traditional herbal drink for women after childbirth to help strengthen the uterus (Sulaiman, *et al.,* 2007). The plant sap was used to detach wart from the skin (Burkill, 1966). Moreover it improves blood circulation and pharmacologically blood glucose (Aminudin, *et al.,* 2007). On the other hand, there is no report related to its chemical constituent and bioactivity. In this report, the structur elucidation of the isolated compound from ethyl acetate fraction of *F.deltoideus* Jack leaves extracts and its antibacterial activity againts *E. coli, B.subtilis* and *S. aureus* are discussed.

Methodology

General

Vaccum 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.

Table I. The comparizon of ^{1}H and ^{13}C-NMR (1D,2D) data of β-amyrin and ^{13}C-NMR data of β-amyrin

	β-amyrin isolated compound					Literature[8]
No	δ_C (ppm)	DEPT	δH(ppm), (ΣH multiplisity)	HMBC	COSY	δ_C β-amyrin (ppm)
1	38.9	CH_2	1.67 (2H, t)	47.9 (C-9)		38.7
2	27.5	CH_2	1.60 (2H, m)		3.21 (H-3)	27.3
3	79.2	CH	3.21 (1H, t)		1.60 (H-2)	79.0
4	39.7	C				38.8
5	55.4	CH	0.75 (1H, t)			55.3
6	18.5	CH_2	1.52 (2H, m)	37.3 (C-10); 39.8 (C-8)		18.5
7	33.1	CH_2	1.54 (2H, t)	17.0 (C-26)		32.8
8	39.8	C				38.8
9	47.9	CH	1.53 (1H, t)	23.5 (C-11)	1.90 (H-11)	47.7
10	37.3	C				37.6
11	23.5	CH_2	1.90 (2H, dd)	124.6 (C-12); 139.8 (C-13); 47.9 (C-9)	1.53 (H-9); 5.12 (H-12)	23.6
12	124.6	CH	5.12 (1H, t)	42.3 (14); 47.9 (C-9)	1.90 (H-11)	121.8
13	139.8	C				145.1
14	42.3	C				41.8
15	26.8	CH_2				26.2
16	28.2	CH_2				27.0
17	33.9	C				32.5
18	47.8	CH				47.4
19	47.0	CH_2				46.9
20	31.3	C				31.1
21	37.1	CH_2				34.8
22	37.0	CH_2				37.2
23	28.3	CH_3	0.99 (3H, s)	55.4 (C-5); 79.2 (C-3)		28.2
24	15.8	CH_3	0.78 (3H, s)	55.4 (C-5); 79.2 (C-3); 28,3 (C-23)		15.5
25	15.9	CH_3	0.95 (3H, s)	55.4 (C-5); 38.9 (C-1); 47.9 (C-9)		15.6
26	17.0	CH_3	1.00 (3H, s)	42.3 (C-14); 47.9 (C-9); 33.1 (C-7)		16.9
27	21.6	CH_3	0.91 (3H, s)	39.8 (C-8)		26.0
28	28.9	CH_3	0.79 (3H, s)	33.9 (C-17)		28.4
29	33.5	CH_3	0.86 (3H, s)	47.0 (C-19); 23.4 (C-30); 31.3 (C-20)		33.3
30	23.4	CH_3	1.06 (3H, s)	26.8 (C-21)		23.7

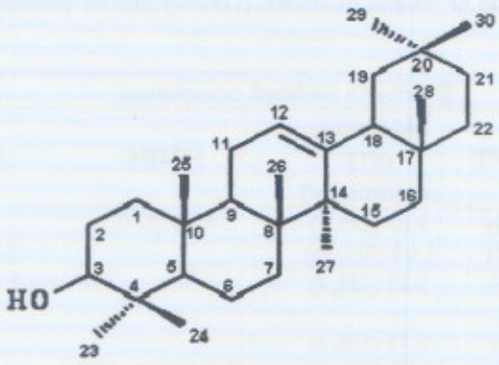

Figure 1. Structure of β-amyrin.

Table II. Inhibition zone (cm) and MIC of β-amyrin againts *E. coli*, *B. subtilis*, and *S. Aureus* bacteria

Compound	Bacteria	Concentration % (b/v) in ethyl acetate					MIC (µg/mL)
		Control	0.25	0.50	1.00	2	
β-Amyrin	*E. coli*	0.0	0.9	1.0	1.2	1.3	230
	B. subtilis	0.0	0.9	0.9	1.0	1.1	380
	S. aureus	0.0	0.8	0.9	0.1	1.2	460

^{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) was obtained on Finnigan LCQ-Deca,70 eV. The melting points were measured on Fisher John Melting point apparatus.

Plant Material

Ficus deltoideus 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), is deposited at the Herbarium.

Extraction and Isolation

The dried powder of leaves (1 kg), of *Ficus deltoideus* 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 F2 (2.8 g) was rechromatographed on silica gel eluted with hexane-ethyl acetate (10:0 – 0:10), to give 4 subfraction (F2.1-F2.4). F2.3 (98 mg), was rechromatographed on silica gel eluted with hexane : ethyl acetate 9 : 1, the yellowish solid mass, was obtained and then washed with hexane to give white crystal (14 mg).

Bioassay

Antibacterial activity test was carried out by measuring growth inhibition zone of *E. coli*, *B. subtilis*, and *S. Aureus*, at various concentration, using disk diffusion susceptibility method (Rojas, *et al.*, 2008 ; Zakaria, *et al.*, 2006). The minimum inhibition concentration (MIC), was determined by dilution method (Edberg, *et al.*, 1986).

β-amyrin, $C_{30}H_{50}O$

(Fig.1.): mp:198-199 °C ; IR (ν_{maks}, cm^{-1}) : 3362 (OH), 2927 and 2865 (C-H alifatik), 1139 (C-O), 1456 and 1377 (methyl and methylene) ; EI-MS (m/z) : 426 (M^+), 411, 218 (base peak), 203, 189 and 175. The comparizon of ^{1}H and ^{13}C-NMR data of β-amyrin and ^{13}C-NMR β-amyrin literature[8], see Table I, and antibacterial activity, see Table II.

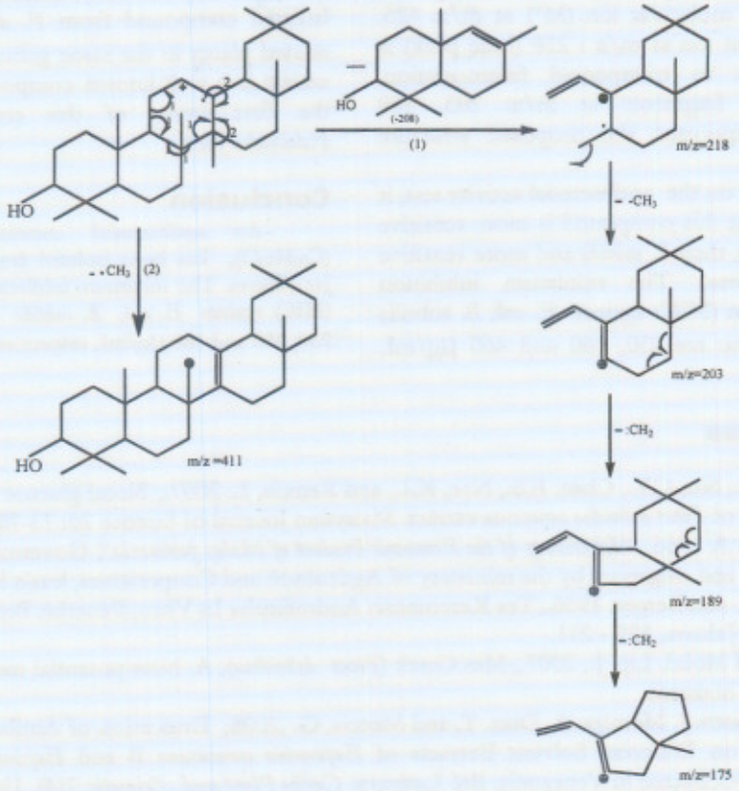

Figure 2. Mass fragmentation of β-amyrin.

Result and Discussion

The ^{1}H and ^{13}C-NMR spectra of this compound is identical to the triterpenoid. The 8 methyl protons signal (0-2 ppm) was assigned (s,3H) at δ_H(ppm) : 0.78 (H-24); 0.79 (H-28); 0.86 (H-29); 0.91 (H-27); 0.95 (H-25); 0.99 (H-23); 1.00 (H-26) and 1.06 (H-30), respectively. The ^{13}C NMR showed 30 carbon atom signals, related to triterpenoids. DEPT analysis gave 8 CH_3, 10 CH_2, 5 CH and 7 C quarternary carbon signals. All of these signals are identical with β-amyrin carbon signals[8] (Table I, Fig. 1). The olefinic proton at δ_H 5.12 ppm (H-12;1H,t) was coupled by two neighboring methylene protons at δ 1.90 ppm (H-11;2H,dd). Correlation between H-11 and H-12 was supported by COSY analysis. So that, the double bond must be located at C-12 (δ_C 124.6) and C-13 (δ_C139.8) (Table I, Fig. 1). The signal at δ_H 3.21ppm (1H,t), belongs to methyneoxy proton C-3 (δ_C 79.2 ppm; $\nu_{C\text{-}O}$ = 1179 cm^{-1}). This methyneoxy proton H-3 was coupled by methylene protons H-2 at 1.60 ppm (2H,m). Correlation between H-3 and H-2 also established by COSY analysis. HMBC correlation between to methyl group protons (H-23 and H-24) with carbon C-5 and C-3, established a gem-dimethyl system at C-4. The methyl groups position at C-10, C-8, C-17 and C-20, was also established in the same manner (Table I, Fig.1).

The mass spectrum of this compound showed the molecular ion (M^+) at m/z: 426. The fragment ion at m/z : 218 (base peak) is characteristic to triterpenoid fragmentation. The other fragments at m/z: 203, 189 and 175 supported the proposed structure (Fig. 2).

Based on the antibacterial activity test, it is shown that this compound is more sensitive against *E.coli*, than *B. subtilis* and more sensitive than *S. aureus*. The minimum inhibition concentration (MIC) against *E. coli*, *B.* subtilis and *S. aureus* are 230, 380 and 460 µg/mL respectively.

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

Conclusion

An antibacterial constituent, β-amyrin ($C_{30}H_{50}O$), has been isolated from *Ficus deltoideus* Jack leaves. The minimum inhibition concentration (MIC) against *E. coli*, *B. subtilis* and *S. aureus* are 230, 380 and 460 µg/mL respectively.

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