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A Triterpenoid from The Leaves of Tahi Ayam (*Lantana camara* Linn)

Kartika MZ[1], Suryati[1]*, Mai Efdi [1]

[1]*Department of Chemistry FMIPA Andalas University, Kampus Limau Manis, Padang. Sumatra Barat. 25163*

**Correspondence Author: suryati_chemua@yahoo.com*

Abstract

A triterpenoid has been isolated from the ethyl acetate fraction of Tahi ayam (*Lantana camara* Linn) leaves. The isolation was performed by chromatography of gravitation column with a gradient elution system using hexane, ethyl acetate and methanol. Purification by recrystallization produces white solid (20 mg) with a melting point of 288-289 °C. The results of elucidation of UV, IR, 1H-NMR, 13C-NMR, HMBC, HSQC, DEPT and COSY spectra data, it is known that the isolated triterpenoid compound is 22-angeloyloxy-9-hydroxy-3-oxo-olean-12-en-28-oic acid (9- hydroxy-Lantadene A) with molecular formula $C_{35}H_{52}O_6$.

Keywords: Tahi ayam *(Lantana camara* Linn), Triterpenoid, $C_{35}H_{52}O_6$

Abstrak (Indonesian)

Satu senyawa triterpenoid telah diisolasi dari fraksi etil asetat daun tumbuhan Tahi ayam (*Lantana camara* Linn). Isolasi dilakukan dengan metode kromatografi kolom grafitasi dengan sistem elusi bergradien menggunakan heksana, etil asetat dan metanol. Pemurnian dilakukan dengan rekristalisasi dan menghasilkan padatan putih (20 mg) dengan titik leleh 288-289 ^oC. Hasil elusidasi data-data spektrum UV, IR, ^{1}H-NMR, ^{13}C-NMR, HMBC, HSQC, DEPT dan COSY, diketahui bahwa senyawa triterpenoid hasil isolasi adalah asam 22-angeloiloksi-9-hidroksi-3-oxo-olean-12-en-28-oat (9- hidroksi-Lantadene A) dengan rumus molekul $C_{35}H_{52}O_6$.

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Kata Kunci: Tahi ayam *(Lantana camara* Linn), Triterpenoid, $C_{35}H_{52}O_6$

INTRODUCTION

Several secondary metabolite compounds have been reported from *Lantana camara* L. plants, among other triterpenoids, steroids, coumarins, phenolics, tannins, saponins, anthraquinones and cardiac glycosides [1-3]. The reported triterpenoids of this plant include camarolic acid, lantrigloylic acid, camaric acid, lanthanolic acid, lanthanolic acid, pomolic acid, camarinic acid, lantoic acid, camarin, lantacin, camarinin, and ursolic acid, betulonic acid, betulinic acid, lantadene A, lantadene B, icterogenin, ursolic acid, 3β, 17-dihydroxy-olean-12-ene, oleanolic acid, katonic acid, 3-hydroxy-10,19-en-urs-28-oic acid [4-9]. To complement the information of secondary metabolite content of *Lantana camara* leaf, this paper will be reported one triterpenoid compound from the ethyl acetate fraction of plant *Lantana camara* L. leaves. Determination of structure of triterpenoid was carried out through analysis of UV, IR, ^{13}C-NMR, ^{1}H-NMR, HMBC, HSQC, DEPT and COSY spectrum data.

MATERIALS AND METHODS

Materials

A. Plant material

Tahi ayam (*Lantana camara* L.) plant is obtained from the area of Limau Manis, Pauh sub-district, Padang city, West Sumatera province, Indonesia. This plant has been identified in the Laboratory Herbarium Andalas University (ANDA), Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University with the specimen code 472/ K-ID/ANDA/XII/2017. This study used leaf samples that have been dried and smoothed.

B. Reagents and instruments

Distilled hexane, ethyl acetate and methanol (Brataco) solvents, Liebermann Burchard reagents, silica gel 60 (Merck, 0.063-0,200 mm), KLT plate

(Merck, DC-Alufolien Kiesegel 60 F_{25}). Macerator, rotary evaporator (Heidolp VV 2000), distillation apparatus, chromatography column, UV GL-58 (λ 254 and 365 nm) lamps, Stuart SMP 10 melting point apparatus, ultraviolet visible spectrophotometer (Thermo Scientific, Genesys 10s UV-Vis), FT-IR spectrometer (PerkinElmer, Frontier), NMR spectrometer (JEOL JNM-ECZ500R). The ^{1}Hydrogen-Nuclear Magnetic Resonance (^{1}H-NMR) spectra were recorded in chloroform-d on a JNM-ECZ500R/S1 spectrometer (500 MHz), while carbon-13 NMR (^{13}C-NMR) spectra were recorded in the same solvent on JNM-ECZ500R/S1 spectrometer at 125 MHz with tetramethylsilane as an internal standard.

Extraction, Isolation, and Purification

The powder leaves of *Lantana camara* L. (3 kg) was macerated with methanol. The extract was filtered and evaporated the solvent on a rotary evaporator to give a thick methanol extract [10]. The methanol extract was suspended in distilled water and fractionated with hexane. The aqueous layer of residual was fractionated with ethyl acetate and the ethyl acetate-soluble portion was concentrated to give the ethyl acetate fraction [11]. Further separation was carried out on the ethyl acetate fraction (50 g) by chromatographic column gravity technique with a gradient elution system using silica gel as a stationary phase and hexane:ethyl acetate (10-0 : 0-10) and ethyl acetate:methanol (10-0 : 0-10) as a mobile phase. From this separation obtained 11 sub-fractions (A-K sub-fraction). The sub-fraction F (15 g) was chromatographed again with chromatographic column gravity technique with a gradient elution system using silica gel and the result obtained 14 sub-fractions (F_1-F_{14}). F_8 obtained in the form of solid in recrystallization and it obtained a pure compound in the form of white solid (20 mg). The melting point test result, the isolated compound melted at 288-289 °C. The identification using Lieberman Buchard (LB) reagents against the purified compound of isolation showed a positive test of triterpenoid [12].

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RESULT AND DISCUSSION

Extraction, Isolation, and Purification

Extraction of dry leaves *Lantana camara* L. with methanol solvent obtained 490 g. Fractionation with hexane and ethyl acetate obtained by hexane fraction (45 g), ethyl acetate fraction (55 g) and residual fraction (387 g). Further purification of the ethyl acetate fraction obtained a pure compound of white solid (20 mg) with a melting point of 288-289 °C and provides a triterpenoid positive test with Liebermann Burchard reagent. Determination of this triterpenoid structure is done using UV, IR and NMR spectrum data.

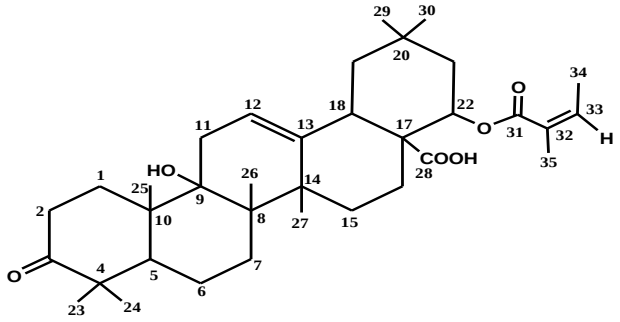

Figure 1. 22-angeloyloxy-9-hydroxy-3-oxo-olean-12-en-28-oic acid.

The UV triterpenoid spectra data of isolated compound shows a maximum absorption at a wavelength of 203.40 nm. Infrared spectra data showed the presence of vibration of the dimethyl geminal group bonds at wave numbers 1455 cm^{-1} and 1372 cm^{-1} supporting the isolated triterpenoid skeleton. Infrared spectra data also showed the presence of binding vibration in wave numbers 1730 cm^{-1} (C = O), 1293 cm^{-1} (C-O ester) and 3450 cm^{-1} (OH), 1159 cm^{-1} (C-O carboxylate). This data supports the presence of ester and carboxylic groups attached to the triterpenoid skeleton. Determination of subsequent structures by analysis of spectra data ^{1}H–NMR, ^{13}C–NMR, DEPT-135, HMBC, HSQC, COSY [13]. Based on the spectra data, the isolated compound was established as 22-angeloyloxy-9-hydroxy-3-oxo-olean-12-en-28-oic acid (9- hydroxy-Lantadene A) with molecular formula $C_{35}H_{52}O_6$ (Figure 1).

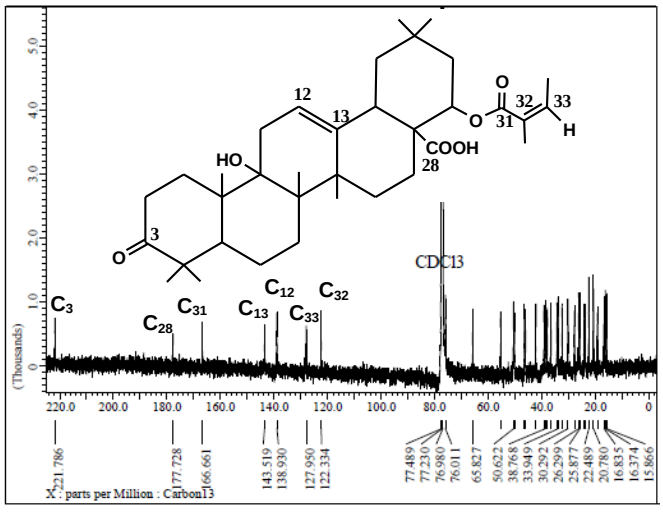

Figure 2. ^{13}C-NMR spectra data of isolated compound.

Table 1. Data ^{1}H(500 MHz in $CDCL_3$), ^{13}C(125 MHz in $CDCL_3$) NMR data of isolated compound and data ^{1}H (400 MHz in $CDCL_3$), ^{13}C (400 MHz in $CDCL_3$) NMR data of comparative compound (Lantadene A) [14].

C	Isolated Compound					Comparative Compound	
	δ_C (ppm)	DEPT	δ_H (ppm)	HMBC	COSY	δ_C (ppm)	δ_H (ppm)
1	38.58	CH_2	1.50		2.38 (H_2)	38.41	
2	34.38	CH_2	2.38	38.58 (C_1), 221.78 (C_3), 36.66 (C_{10})	1.50 (H_1)	34.11	
3	221.78	C				217.66	
4	46.51	C				47.42	
5	55.46	CH				55.29	
6	19.27	CH_2				19.59	
7	32.49	CH_2				32.17	
8	39.27	C				39.21	
9	50.29	C/C-OH	5.09	39.27 (C_8), 36.66 (C_{10})	1.8 (H_{11})	47.45	
10	36.66	C				36.76	
11	23.86	CH_2	1.8			23.5	
12	122.33	CH	5.37	38.77 (C_{18}), 42.21 (C_{14})	1.8 (H_{11})	122.46	5.38
13	143.51	C				143.1	
14	42.21	C				41.97	
15	27.86	CH_2				27.57	
16	24.25	CH_2				24.18	
17	50.62	C				50.6	
18	38.77	CH	3.05		1.25 (H_{19})	38.41	3.4
19	46.19	CH_2	1.25		3.05 (H_{18})	46.87	
20	30.29	C				30.03	
21	37.97	CH_2	1.88		3.99 (H_{22})	37.71	
22	65.82	CH	3.99	50.62 (C_{17})	1.88 (H_{21})	75.88	5.09
23	26.29	CH_3	0.98	221.78 (C_3), 55.46 (C_5)		26.44	
24	22.48	CH_3	1.25	221.78 (C_3), 55.46 (C_5)		21.45	
25	15.86	CH_3		38.58 (C_1), 36.66 (C_{10}), 55.46 (C_5)		15.09	
26	16.83	CH_3				16.83	
27	23.87	CH_3		42.21 (C14), 39.27 (C_8), 143.51 (C_{13}), 27.86 ($_{15}$)		25.79	
28	177.72	C				180.1	
29	33.94	CH_3	0.84	30.29 (C_{20}), 46.19 C_{19})	0.98 (H_{30})	33.67	
30	26.29	CH_3	0.98	30.29 (C_{20}), 37.97 (C_{21})	0.84 (H_{29})	26.13	
31	166.66	C				166.26	
32	127.95	C				127.61	
33	138.93	CH	5.98	166.66 (C_{31}), 16.37 (C_{35})	1.95 (H_{34}), 1.76 (H_{35})	138.88	5.98
34	20.78	CH_3	1.95	127.95 (C_{32}), 138.93(C_{33})	5.98 (H_{33})	20.56	1.96
35	16.37	CH_3	1.76	166.66 (C_{31}), 127.95 (C_{32}), 138.93(C_{33})	5.98 (H_{33})	15.64	1,76

The ^{13}C-NMR spectra data (Figure 2) shows the presence of 35 carbon signals appearing at a chemical shift (δ_C) of 15.6 to 221.5 ppm and from the DEPT spectra data it is known that the 35 carbon atoms comprise 9 methyl carbons (CH_3), 5 methine carbons (CH), 9 methylene carbons (CH_2) and 12. quaternary carbons (C). The value of carbon chemical shift at δ_C

221.7 ppm (C_3) is suitable for the ketone group (R_2C = O), δ_C 177.7 ppm (C_{28}) suitable for carboxylate (R-CO_2H), 166.6 ppm (C_{31}) suitable for esters (R-CO_2R), δ_C 138.9 ppm (C_{33}), δ_C 127.9 ppm (C_{32}) and δ_C 122.3 ppm (C_{12}) corresponding to alkenes (C = C), δ_C 65.8 ppm (C_{22}) according to C-OR, δ_C 50.29 ppm (C_9) suitable for C-OH. The chemical shift data of the isolated compounds are shown in Table 1.

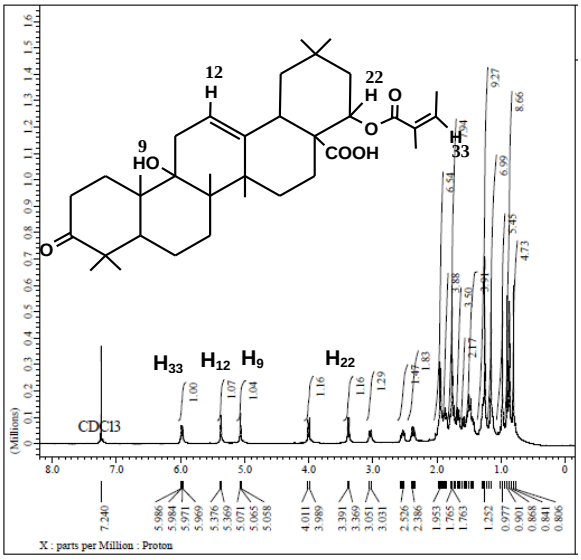

Figure 3. ^{1}H–NMR spectra data of isolated compound.

The ^{1}H-NMR spectra data of the isolated compound (Figure 3) showed a proton shift value of δ_H 0.81 ppm to 5.98 ppm. The shift value δ_H 5.98 ppm (m, 1H) corresponds to H_{33}, this proof is also observed in the correlation of HSQC spectra data where this H_{33} proton (δ_H 5.98) correlates with methine carbon (C_{33}, δ_c 138.9 ppm). Proton H_{12} appears at δ_H 5.37 ppm (s, 1H). This data is also proved by HSQC spectra data where the value of δ_H 5.37 ppm correlates with δ_c 122.3 ppm (C_{12}). The chemical shift value at δ_H 5.09 ppm is thought to be a proton of OH bound to C_9. This proof is supported by carbon C_9 known as quaternary carbon in DEPT spectra data. This assumption is also supported by HSQC spectra data where there is no HSQC spectra data correlation between δ_H 5.09 ppm with carbons. Further evidence with HMBC spectra data shows that the proton at δ_H 5.09 ppm has correlation with carbon δ_c 36.66 ppm (C_{10}) and δ_c 39.27 ppm (C_8). Based on COSY spectra data, it is known that proton at δ_H 5.09 ppm has correlation with proton δ_H 1.8 ppm (H_{11}).

Furthermore, the proton at a chemical shift of δ_H 3.99 ppm (H_{22}) appears as a duplet duplet signal. HSQC spectra data, showing correlation with δ_c 65.82 ppm (C_{22}). Based on spectra data of isolated compound, there is a difference between chemical shift of H_{22} (δ_{H22}) and chemical shift of C_{22} (δ_{C22}) of isolated compound with δ_{H22} and δ_{C22} of comparative compound. This is due to the stereochemistry from carboxylate group of isolated compound structure. Then, proton signals at a shift of δ_H 2.52 ppm to 0.80 ppm are suitable for proton signals attached to the basic skeleton of the triterpenoid structure. The structure of the proposed triterpenoid compound also corresponds to the correlation shown on the HMBC and COSY spectrum data of the isolated compound (Figure 4).

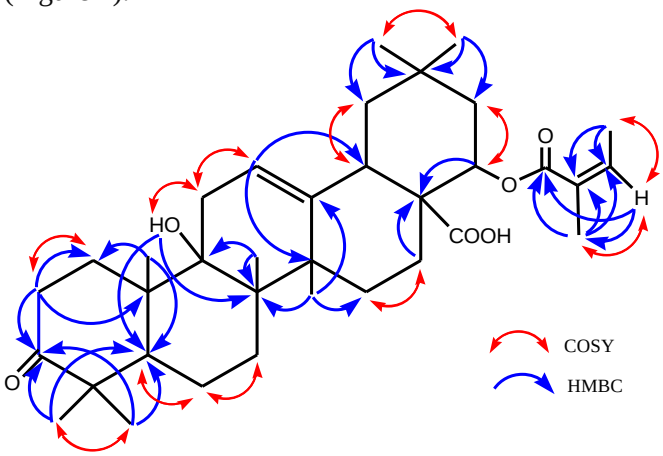

Figure 4. Key HMBC and H-H COSY correlations of isolated compound.

The spectra data of the isolated triterpenoid compound were also compared with the previously reported Lantadene A compound spectra data [14]. These data show almost no difference with the isolated triterpenoid compound (Table 1). Differences occur in C_9 where in Lantadene A the proton chemical shift occurs at a value of δ_H 5.09 ppm and a carbon chemical shift at 47.45 ppm (methine carbon), while in the triterpenoid compound C_9 results appear as quaternary carbon at δ_c 50.29 ppm. The presence of a hydroxyl group attached to C_9 is also evidenced by the DEPT spectra data, in which carbon C_9 appears as a quaternary carbon. The chemical shift value (δ_H 5.09 ppm) in the ^{1}H-NMR spectrum corresponds to a chemical shift of hydroxyl (-OH) protons attached to quaternary carbon (C-OH) [13].

CONCLUSION

A triterpenoid isolated from the ethyl acetate fraction of Tahi ayam (*Lantana camara* L.) leaves were obtained in the form of white solid (20 mg) with a melting point of 288-289 °C. Determination structure of isolated triterpenoid compound by elucidation of

UV, IR and NMR spectrum data, it is concluded that the isolated triterpenoid compound is 22-angeloyloxy-9-hydroxy-3-oxo-olean-12-en-28-oic acid (9- hydroxy-Lantadene A) with molecular formula $C_{35}H_{52}O_6$.

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