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https://doi.org/10.20884/1.jm.2019.14.1.477

A Cytotoxic Compound from *n*-Hexane Fraction of *Lantana camara* Linn Leaves

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Received: 21 Feb 2019; **Accepted:** 28 May 2019; **Available online:** 5 Jun 2019

ABSTRACT. In this study, one triterpenoid compound from *n*-hexane fraction of *Lantana camara* Linn leaves has cytotoxic activity was isolated. Isolation was carried out using gravity chromatography column and purification by recrystallization method. Isolated compound obtained was white solid with melting point 252-253°C. The structure of isolated compound was elucidated using spectroscopic analysis Ultraviolet (UV), Infrared (IR), ^{1}H-Nuclear Magnetic Resonance (^{1}H-NMR), ^{13}C-Nuclear Magnetic Resonance (^{13}C-NMR), Heteronuclear Multiple Bond Connectivity (HMBC), Heteronuclear Multiple Quantum Correlation (HMQC), Distortionless Enhancement Polarization Transfer (DEPT) and comparative literature data, identified as Lantadene A (*22β-angeloyloxy-3-oxoolean-12-en-28-oic-acid*) with molecule formula $C_{35}H_{52}O_5$. The Lantadene A compound was evaluated for cytotoxic activity against shrimp larvae *Artemia Salina* Leach using *Brine Shrimp Lethality Test* (BSLT) method, showed strong cytotoxic activity with an LC_{50} value of 48.97 μg/mL.

Keywords : *Lantana camara* Linn, Lantadene A, *Artemia Salina* Leach, Cytotoxic.

INTRODUCTION

Lantana camara Linn plant has various activities such as antibacterial (Ganjewala, Sam, & Khan, 2009), anticancer (Pour, Latha & Sashidaran, 2011), antifungal (Passos et al., 2012), anti-inflamatory, antiviral, antitubercular, nematicidal, hepatotoxic, antimutagenic (Kumar, Katiyar, Singh, Surender & Tarun, 2016), antioxidant (Suryati, Santoni, M.Z, & Aziz, 2016), anti-diabetic (Kazmi et al., 2012), and cytotoxic activity (Ediruslan, Manjang, Suryati & Aziz, 2015).

Several compounds have been isolated from *Lantana camara* Linn such as 9-Hydroxy-lantadene A (M.Z, Suryati & Efdi, 2018), 24-Hydroxy-lantadene B, 24-Hydroxy-lantadene X, 24-Hydroxy-lantadene D, 22-Hydroxy-4-epi-hederagonic acid, 3β-Hydroxy-lantadene C (Abdjul, et al, 2017), ursolic acid (Jamal, Amir, Ali, & Mujeeb, 2018), oleanolic acid (Vyas & Argal, 2014), camaric acid, Lantadene C, camarinic acid, camaraside, 24-Hydroxy-3-oxours-12-en-28-oic acid (Kumar, et al., 2016).

Previous research, Ediruslan et al. (2015) has reported the cytotoxic of n-hexane, and ethyl acetate fractions of *Lantana camara* Linn leaves. *n*-Hexane and ethyl acetate fractions showed strong toxicity with LC_{50} values of 34 and 27 µg/mL, respectively. Ediruslan et al. (2015) has also reported triterpenoid compound (Lantanilic acid) show strong cytotoxic potential with LC_{50} values of 27.99 µg/mL of ethyl acetate, but not yet reported cytotoxic compound from n-hexane fraction of *Lantana camara* Linn leaves.

This paper reported the isolation of triterpenoid compound from n-hexane fraction of *Lantana camara* Linn leaves and its cytotoxic activity using *Brine Shrimp Lethality Test* (BSLT) method. The structure of isolated compound was elucidated using spectroscopic analysis UV, IR and NMR (1D and 2D).

EXPERIMENTAL SECTION

Material

Lantana camara Linn leaves were collected from area of Limau Manis, Pauh sub-district, Padang city, West Sumatera province, Indonesia. This plant has been identified in the Herbarium Laboratory Andalas University (ANDA), Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University with the specimen code 472/ K-ID/ANDA/XII/2017.

Organic solvent such as *n*-hexane, ethyl acetate, methanol (Brataco) using distillate, Liebermann Burchard Reagent, Silica Gel 60 (Merck, 0.063-0.200 mm), Thin Layer Chromatography (TLC) (DC-Kiesegel 60 F_{254} Merck), and cytotoxic activity test such as shrimp larvae eggs *Artemia Salina Leach*, seawater, dimethyl sulfoxide (DMSO).

Apparatus

UV spectra recorded with spectrophotometer (Thermo Scientific, Genesys 10 UV-Vis), FT-IR spectra measured with spectrophotometer (Perkin Elmer, Frontier), NMR spectra collected using spectrometer (ECX400). ^{1}Hydrogen-Nuclear Magnetic Resonance (^{1}H-NMR, $CDCl_3$) ECX400 (400 MHz), ^{13}Carbon-Nuclear Magnetic Resonance (^{13}C-NMR, $CDCl_3$) ECX400 (100 MHz) with tetramethylsilane (TMS) as standard.

Extraction, Isolation and Structure Determination

Dry sample of *Lantana camara* Linn leaves (3 kg) macerated with methanol solvent. Maceration was performed repeatedly until the macerate obtained colorless.

It was then filtered and concentrated with rotary evaporator to obtain crude methanolic extract (400 g). The crude methanolic extract (400 g) was partitioned with *n*-hexane and obtained *n*-hexane fraction (40 g).

n-Hexane fraction (40 g) was fractionated by gravity chromatography column (0.063-0.200 mesh) with hexane:ethyl acetate (10:0;0:10) and ethyl acetate:methanol (10:0;0:10) as gradient eluent and obtained 13 fractions (F_1-F_{13}). Then, all fractions were evaluated for cytotoxic activity against shrimp larvae *Artemia salina* Leach. Fraction F_{13} (3 g) was rechromatographed on a silica gel column using hexane:ethyl acetate (10:0;0:10) and ethyl acetate:methanol (10:0;0:10) as the eluent and obtained 10 sub-fractions ($F_{13.1}$-$F_{13.10}$). Then, all sub-fractions were repeatedly evaluated cytotoxic activity against shrimp larvae *Artemia salina* Leach. Sub-fraction $F_{13.3}$ (1 g) was separated on silica gel column, eluted with *n*-hexane:ethyl acetate (10:0-0:10) and obtained 13 sub-fractions ($F_{13.3.1}$-$F_{13.3.13}$). Then, all sub-fractions were repeatedly evaluated cytotoxic activity against shrimp larvae *Artemia salina* Leach using *Brine Shrimp Lethality Test* (BSLT). Sub-fraction $F_{13.3.4}$ was purified using recrystallization with hexane:ethyl acetate, obtained pure compound (15 mg). Gave a triterpenoid positive test with Liebermann Burchard reagent and evaluated for cytotoxic activity. The isolated compound was carried out the melting point measurement and the structure was elucidated using spectroscopic analysis UV, IR, and NMR (1D and 2D).

Cytotoxic Activity Test

Sample preparation

The isolated compound (2 mg) was dissolved with *n*-hexane in 2 mL flask and obtained a stock solution (1000 µg/mL). Five different concentrations such as 250; 125; 62.5; 31.25 and 15.625 µg/mL were prepared in triplicate by dilution from a stock solution.

Hatching of shrimp larvae Artemia salina Leach

Seawater was put into a glass container such as the dark and light part. Shrimp larvae eggs *Artemia salina* Leach were put into the dark part of the container at temperature 24-26^{o}C (Vajha & Krishna, 2014). After 48 hours, the phototropic nauplii will move into the light part. These larvae were used as experimental animal in the cytotoxic test using *Brine Shrimp Lethality Test* (BSLT) method (Olowa & Nuñeza, 2013).

***Brine Shrimp Lethality Test* (BSLT)**

The isolated compound with different concentrations such as 250; 125; 62.5; 31.25 and 15.625 µg/mL were put into vial and dried. Further, 50 µL DMSO was added to each vial until homogeneous (Sivasankar et al., 2013). Then, ten shrimp larvae were put to each vials using pasteur pipette and added 5 mL of seawater in vial (Musa, 2012). Each vial contain shrimp larvae were placed in the light and after 24 hours shrimp larva *Artemia salina* Leach observed and calculated total of dead larvae in each vial. The percentage of mortality of shrimp larvae *Artemia salina* Leach on each concentration were determined using probit analysis. *Lethality Concentration* (LC_{50}) value determined using a regression equation.

RESULTS AND DISCUSSION

The result (40 g) of *n*-hexane fraction was fractionated by silica gel gravity chromatography column and purification by recrystallization, obtained pure compound (15 mg) with melting point 252-253^{o}C. The structure of isolated compound was elucidated using spectroscopic analysis UV, IR, and NMR (1D and 2D) and identified by comparative literature data (**Table 1**).

The data value the chemical shift of triterpenoid isolated compound was compared by published Lantadene A data (**Table 1**), the chemical shift value of the isolation has high similarity with comparative Lantadene A data, from the comparative data identified as Lantadene A (*22β-angeloyloxy-3-oxoolean-12-en-28-oic-acid*) (**Figure 1).**

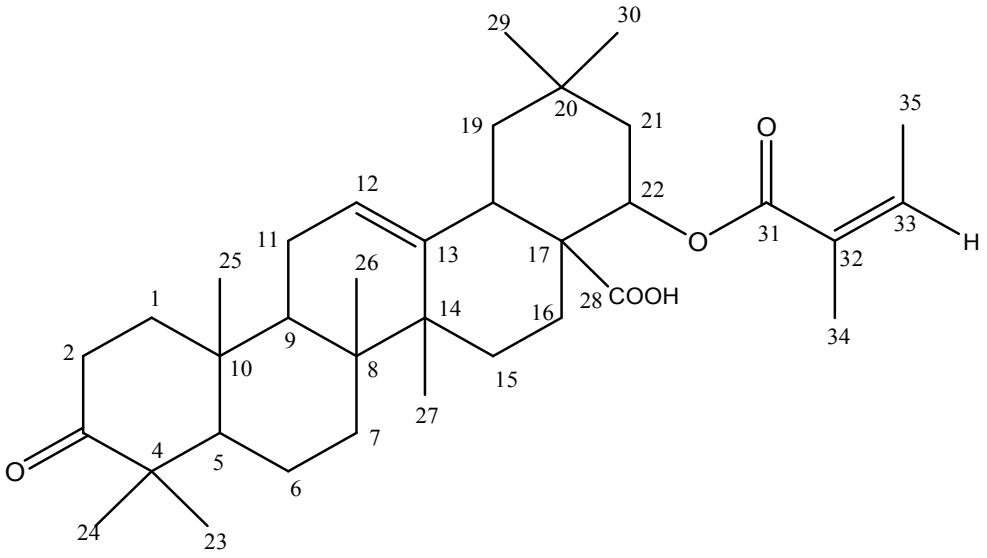

Figure 1. Chemical structure of Lantadene A (*22β-angeloyloxy-3-oxoolean-12-en-28-oic-acid*)

Table 1. ^{1}H-NMR data (400 MHz, $CDCl_3$) and ^{13}C-NMR data (100 MHz, $CDCl_3$) isolated compound and comparative *Lantadene* A data (Manu, Sharma, Bansal & Singh, 2007).

No	Isolated compound				Literature
	δc (ppm)	DEPT	HMQC	HMBC	δc (ppm)
1	39.1	CH_2	1.5 (H_1)	23.5 (C_{11})	38.4
2	34.2	CH_2	2.3 (H_2)	39.1 (C_1)	34.1
3	217.7	C			217.6
4	47.5	C			47.4
5	55.3	CH	1.3 (H_5)	26.5 (C_{23})	55.2
6	19.8	CH_2			19.5
7	32.2	CH_2			32.1
8	39.3	C			39.2
9	46.9	CH			47.7
10	36.8	C			36.7
11	23.5	CH_2	1.2 (H_{11})	143.1 (C_{13}); 15.7 (C_{25})	23.5
12	122.6	CH	5.3 (H_{12})	42. 1 (C_{14})	122.4
13	143.1	C			143.1
14	42.1	C			41.9
15	27.6	CH_2	1.0 (H_{15})	143.1 (C_{13}); 42.1 (C_{14})	27.5
16	24.2	CH_2	0.9 (H_{16})	42.1 (C_{14})	24.1
17	50.6	C			50.6
18	38.6	CH	3.0 (H_{18})		38.4
19	46.0	CH_2	1.6 (H_{19})	38.6 (C_{18})	46.8
20	30.1	C			30.0
21	37.8	CH_2			37.7
22	75.9	CH	5.0 (H_{22})	30.1 (C_{20})	75.8
23	26.5	CH_3	1.2 (H_{23})		26.4
24	21.5	CH_3	1.1 (H_{24})	217.7 (C_3)	21.4
25	15.1	CH_3	1.0 (H_{25})		15.0
26	16.8	CH_3	0.8 (H_{26})	32.2 (C_7); 39.3 (C_8); 46.9 (C_9)	16.8
27	25.8	CH_3	0.9 (H_{27})		25.7
28	178.4	C			180.1
29	33.7	CH_3	0.9 (H_{29})	46.0 (C_{19}); 30.1 (C_{20}); 37.8 (C_{21}); 26.2 (C_{30})	33.6
30	26.2	CH_3	1.0 (H_{30})		26.1
31	166.3	C			166.2
32	127.6	C			127.6
33	139.1	CH	6.0 (H_{33})	20.6 (C_{34}); 166.3 (C_{31})	138.8
34	20.6	CH_3	1.7 (H_{34})	166.3 (C_{31}); 127.6 (C_{32}); 139.1 (C_{33})	20.5
35	15.7	CH_3	1.9 (H_{35})	166.3 (C_{31});127.6 (C_{32})	15.6

Ultraviolet (UV) spectrum data showed the absorption at 210. At 210 nm wavelength, show a double bond (C = C) that was not conjugated, suitable for C_{12} and C_{32}.

Infrared (IR) spectrum data showed absorption bands at 1457.10 and 1371. 38 cm^{-1}, suitable the absorption of gem dimethyl at C_{24}; C_{23}; C_{29} and C_{30} (**Figure 1**) (Mayanti et al., 2011). Absorption at 3579.32 cm^{-1} showed the presence of O-H (C_{28}), supported by the vibration of atom C-O at 1034.03 cm^{-1}, and at 1715.66 cm^{-1} showed the presence (C=O) carbonil (C_3 and C_{31}).

^{1}H-NMR spectrum data (400 MHz) showed nine methyl proton signals at δ_H (ppm); 1.2 (H_{23}); 1.1 (H_{24}); 1.0 (H_{25}); 0.8 (H_{26}); 0.9 (H_{27}); 0.9 (H_{29}); 1.0 (H_{30}); 1.7 (H_{34}) and 1.9 (H_{35}), two olefinic proton signals at δ_H (ppm); 5.3 (H_{12}); 6.0 (H_{33}) and methine proton signals at δ_H (ppm); 1.3 (H_5) ppm; 5.0 (H_{22}) supported by DEPT 135 data.

^{13}C-NMR spectrum data (100 MHz) showed 35 carbon signals. Through DEPT 135 analysis, it's known that from 35 carbon signals were nine methyl carbon (ppm); δc 26.5 (C_{23}); δc 21.5 (C_{24}); δc 15.1 (C_{25}); δc 16.8 (C_{26}); δc 25.8 (C_{27}); δc 33.7 (C_{29}); δc 26.2 (C_{30}). δc 20.6 (C_{34}); δc 15.7 (C_{35}). Nine methylene carbon signals were showed at δc (ppm); δc 39.1 (C_1); δc 34.2 (C_2). δc 19.8 (C_6); δc 32.2 (C_7); δc 23.5 (C_{11}); δc 27.6 (C_{15}); δc 24.2 (C_{16}); δc 46.0 (C_{19}); δc 37.8 (C_{21}), Six methyne carbon signals were showed at δc (ppm); δc 55.3 (C_5); δc 46.9 (C_9); δc 122.6 (C_{12}); δc 38.6 (C_{18}); δc 75.9 (C_{22}); δc 139.1 (C_{33}). Eleven quartener carbon signals were showed at δc (ppm); δc 217.7 (C_3); δc 47.5 (C_4); δc 39.3 (C_8); δc 36.8 (C_{10}); δc 143.1 (C_{13}); δc 42.1 (C_{14}); δc 50.6 (C_{17}); δc 30.1 (C_{20}); δc 178.4 (C_{28}); δc 166.3 (C_{31}); δc 127.6 (C_{32}). The signal at δc 178.4 ppm showed chemical shift suitable for the carbon carboxylic (C_{28}), signal at δc 166.3 ppm chemical shift suitable for the carbon carboxylic (C_{31}) and signal at δc 217.7 ppm showed chemical shift suitable for the carbon carboxylic (C_3).

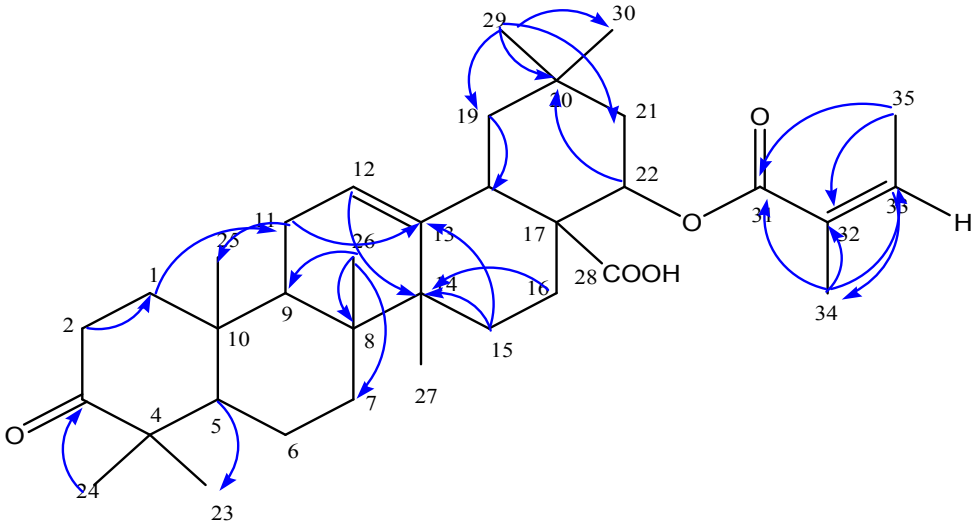

Figure 2. HMBC correlation of Lantadene A

Olefinic carbon signals (C_{12} and C_{13}), which appears at δc 122.6 ppm and δc 143. 1 ppm) and olefinic carbon (C_{32} and C_{33}), which appears at δc 127. 6 ppm and δc 139.1 ppm supported by HMQC correlation H_{12} proton (δ_H 5.3 ppm) with C_{12} (δc 122.6 ppm) and H_{33} (δ_H 6.0 ppm) with C_{33} (δc 139.1 ppm).

HMBC correlation spectrum data (**Figure 2**), showed correlation between H_1 (δ_H 1.5 ppm) with C_{11} (δc 23.5 ppm); H_5 (δ_H 1.3 ppm) with C_{23} (δc 26.5 ppm); H_{11} (δ_H 1.20 ppm) with C_{13} (δc 143.1 ppm) and C_{25} (δc 15.7 ppm); H_{15} (δ_H 1.1 ppm) with C_{13} (δc 143.1 ppm) and C_{14} (δc 42.1 ppm); H_{16} (δ_H 0.9 ppm) with C_{14} (δc 42.1 ppm); H_{24} (δ_H 1.0 ppm) with C_3 (δc 217.7 ppm); H_{26} (δ_H 0.8 ppm) with C_7 (δc 32.2 ppm); C_8 (δc 39.3 ppm); and C_9 (δc 46.9 ppm); H_{29} (δ_H 0.9 ppm) with C_{19} (δc 46.0 ppm); C_{20} (δc 30.1 ppm); C_{21} (δc 37.3 ppm); and C_{30} (δc 26.2 ppm); H_{35} (δ_H 1.9 ppm) with C_{31} (δc 166.3 ppm) and C_{32} (δc 127.6 ppm).

Lantadene A compound has one chiral center at C_8, proven by DEPT 135 data. Where at position C_8 bound to four different groups at C_{26} (methyl carbon), C_7 (methylene carbon), C_9 (methyne carbon) and C_{14} (quaterner carbon) with each of chemical shift at δc 16.8 (C_{26}), δc 32.2 (C_7), δc 46.9 (C_9) and δc 42.1 (C_{14}), but this paper only compare the similarity of the chemical shift from isolated compound with published compound, not explain the absolute configuration of Lantadene A compound.

Cytotoxic Activity

The result of the cytotoxic activity Lantadene A isolated compound against shrimp larvae *Artemia salina* Leach (**Table 2**). The percentage of mortality of each shrimp larvae *Artemia salina* Leach in the test solution, calculated using the equation:

$$\% \text{ Mortality} = \frac{\text{total shrimp larvae die}}{\text{total shrimp larvae}} \text{ x } 100\%$$

From the percentage of mortality value obtained, it is determined the probit value using the probit analysis table. Then, the LC_{50} value was determined using a regression equation between log concentration variations with the probit values (**Figure 3**).

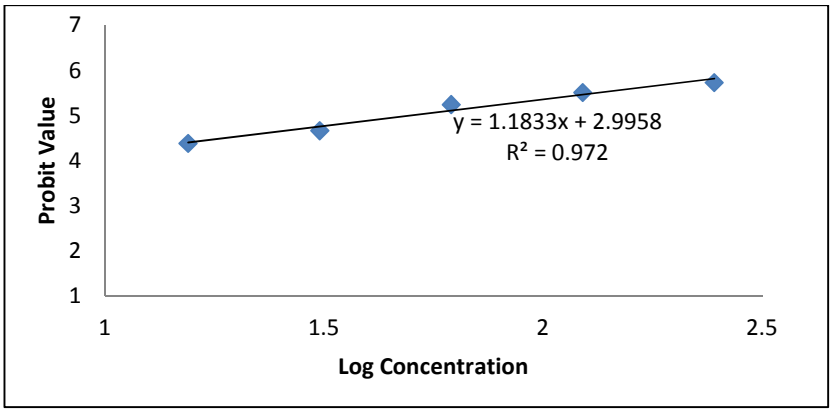

Figure 3. Regression Equation of Log Concentration vs Probit Value of Lantadene A

Table 2. Data of cytotoxic activity result Lantadene A

No	Concentration (µg/mL)	Log Concentration (X)	Total shrimp larvae (tail)	Total shrimp larvae die (tail)	Percentage of Mortality (%)	Probit Value (Y)
1.	15.625	1.19	30	8	27	4.39
2.	31.25	1.49	30	11	37	4.67
3.	62.5	1.79	30	18	60	5.25
4.	125	2.09	30	21	70	5.52
5.	250	2.39	30	23	77	5.74

From regression equation y = 1.183x + 2.995 **(Figure 3)**, obtained the LC_{50} value of Lantadene A isolated compound was 48.97 µg/mL. Pure compound was declared toxic if has LC_{50} value < 200 µg/mL (Handayani, Rasyid, Rustini, Zainudin & Hertiani, 2018). Lantadene A compound was isolated from *Lantana camara* Linn leaves showed strong cytotoxic activity.

CONCLUSIONS

The triterpenoid isolated compound from n-hexane fraction of *Lantana camara* Linn leaves, identified as *Lantadene* A (*22β-angeloyloxy-3-oxoolean-12-en-28-oic-acid*). *Lantadene* A, showed strong cytotoxic activity with LC_{50} value of 48.97 µg/mL.

ACKNOWLEDGMENTS

Thanks to the Chemistry and Biomolecular Science, Mamoru Koketsu Laboratory for measuring *Nuclear Magnetic Resonance* (NMR) spectrometer.

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