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Research Article

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Structure elucidation of brine shrimp toxic compound from *Lantana camara* L. leaves

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ABSTRACT

A brine shrimp toxic compound has been isolated from Lantana camara L. leaves. Based on spectroscopic data (IR, ^{1}H- NMR, ^{13}C-NMR 1D and 2D), the structure of this compound was identified as lantanilic acid, $C_{35}H_{52}O_2$. In this study, the n-hexane, ethyl acetate and methanol extracts and isolated compound showed toxic effects (LC_{50} 34.2972; 27.4254; 133.1930 and 27.9903 µg/mL, respectively). The toxicity degree among the three extracts and isolated compound is isolated compound > ethyl acetate extract > n-hexane extract > methanol extract.

Keywords: Brine Shrimp Toxicity, *Lantana camara* L. leaves, lantanilic acid

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INTRODUCTION

Plants have been used in traditional medicine for thousand years. It correlated to secondary metabolites in the plants. However, some negative effects obtained in the use of local plants as source medicine are basically due to over dosage and lack in adequate knowledge of the detrimental by products obtained in some plants [1]. To solve this problem, some researchers have searched the toxicity of some medicinal plants. Brine shrimp Letahlity Test (BSLT) is used as an indicator for general toxicity [2-4] and also as a guide for the detection of in vitro cytotoxicity in marine natural products [4].

Lantana camara L have been used by folk healers in Asia and South America to treat various dermatological and gastrointestinal disease, tetanus, malaria and tumors [5]. Leaf extract of Lanatanacamara have been found to have an antimicrobial [6,7], fungicidal [8], insecticidal [9], antibacterial[10], and antidiabetic activity [11]. The study on Brine Shrimp Lethality test of this plant have been done on ethanolic fraction of the leaf extract [5] and methanol fraction of different parts extract of this plant [2]. On the other hand, there is no report related to its chemical constituents isolated from the leaves of this plant and bioactivity. In this report, the elucidation structure of the isolated compound from ethyl acetate fraction of *Lantana camara* L. leaves extracts and its brine shrimp toxicity are discussed

EXPERIMENTAL SECTION

Material and Methods

Fresh leaves of Lantana camara L. leaves (about 6 kg) were collected in Solok regency, west Sumatra, Indonesia. The plant were identified by comparing it with the herbarium specimens at Herbarium Universitas of Andalas, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Andalas, Padang, Indonesia.

This plant was washed with water and air dried in shade for about 4 weeks. Then air dried leaves were powdered by using grinder and kept at 20°C in closed plastic container.

Preparation of Plant Extracts:
About 3 kg of fine of plant sample was macerated with 4 L of n-hexane in brown bottle as much as 9 repetitions and each repetition for 72 hours while stirring a few times periodically. Collected filtrate filtered through filter paper and the concentrated by using a rotary evaporator at temperature 40°C. Then successive extraction done by using ethyl acetate and methanol respectively of 6 and 3 repetitions. Concentrated extracts subsequently treated for BSLT toxicity and ethyl acetate extract was used for isolation of compound. The isolated compound was also treated for BSLT toxicity. An alternative dilution procedure developed by Lilybeth F. Olowaand Olga M.Nuneza (2013) were adopted in the preparation of the different dilutions of the plant extracts and isolated compound for BSLA where 5 mg of each extracts and isolated compound was dissolved in 5 mL of the solvent to get 1000 µg/mL. Then this solution was diluted into different concentrations. The final concentrations were 100, 80, 60, 40 and 20 µg/mL. There were two replicates in each concentration. A control test was also prepared

Extraction and Isolation
The dried powder of leaves (3 kg), of lantana camara L was macerated sequentially with hexane, ethyl acetate and methanol at room temperature. The combined extracts were concentrated *in-vacuo*, to give the hexane extract (65 g), ethyl acetate (82,92 g) and methanol (35,7g). The ethyl acetate extract (18 g), was further chromatographic over silica gel eluted with hexane-ethyl acetate (10:0-0:10) to give 12 fraction (F_1 –F_{12}). On F_{12}, asolid yellowish mass was obtained and then washed with hexane to give white crystal (75 mg).

Brine Shrimp Toxicity Assay
Brine shrimp eggs were obtained from Kebun Tanaman Obat Laboratory, Department of Biology, Faculty of Mathematics and Natural Science, University of Andalas, Padang, Indonesia.

Sea water was obtained Bungus Offshore, Padang, West Sumatra for hatching the shrimp eggs.

The sea water was put in a small glass container (hatching chamber) with a partition for dark (covered) and light areas. Shrimp eggs were added into the dark side of the chamber while a lamp above of the other side (light) will attract the hatched shrimp, Two days were allowed for the shrimp to hatch and mature as nauplii (larva). After two days, when the shrimp larva are ready, 4 mL of the sea water was added to vial and ten brine shrimps were introduced into each vial. Thus, there were a total 20 shrimps per dilution. Then the volume was adjusted with sea water up to 5 mL per vial. The vial were left uncovered under the lamp. The number of died shrimps were counted and recorded after 24 hours .

Using probit analysis, the lethality concentration (LC50) was accessed at 95% confidence intervals. As mentioned by Meyer and others , LC50 value of less than 1000 µg/mL is toxic while LC_{50} value of greater than 1000 µg/mL is non-toxic [11]. The percentage mortality (%M) was also calculated by dividing the number of died nauplii by the total number, and then multiplied by 100%. This is to ensure that the death (mortality) of the nauplii is attributed to the bioactive compounds present in the plant extracts.

RESULTS AND DISCUSSION

Lantanilic acid,$C_{35}H_{52}O_2$, mp: 288-289 ºC, IR (vmax, cm-1): 3388,69 (OH); 1034,99 (C-O); 1647 (C=C), 1468,96 and 1379,89 (geminal dimethyl) and 1719,91 (C=O), the comparison of ^{1}H and ^{13}C-NMR data of lantanilic acid and lantanilic acid literature [12].

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 lantanilic acid are quite similar. The DEPT analysis of isolated compound gave 35 carbon consist of 8 CH_3,10 CH_2, 6 CH and 11 C quaternary, corresponded to lantanilic acid by comparing their chemical shift.

Table1. The comparison of ^{1}H and ^{13}C-NMR (1D, 2D) data of lantanilic acid and ^{13}C-NMR data of lantanilic acid reported by Ammar A. Al-Fadhli and Jamal A. Nasser (2014)

No	Lantanilic isolated compound						Literature
	δ_C (ppm)	DEPT	δ_H(ppm),(H multiplicity)		HMBC	COSY	δ_C, ppm
1	35,13	CH_2	1,26(9H)	2,12 (7H)			34,53
2	29,82	CH_2	1,66 (3H,m)				29,24
3	97,16	C					98,87
4	40,00	C					40,25
5	50,35	CH					50,16
6	19,56	CH_2	1,53 (5H,m)		26,85(C-23)		19,72
7	31,30	CH_2	1,4 (2H,m)	1,36 (2H)			30,95
8	38,39	C					38,26
9	41,96	CH	1,73 (3H,s)		16,97(C-26)		41,95
10	34,81	C					35,04
11	23,70	CH_2	1,98 (3H, m)				23,73
12	122,38	CH	5,34 (2H, s)				122,48
13	143.42	C					143,02
14	42,15	C					41,95
15	27,68	CH_2	1,53 (5H, m)				27,74
16	24,03	CH_2	1,73 (3H, s)	1,8 (3H)			24,11
17	50,35	C					50,70
18	39,53	CH	3,06 (11H,dd)			3,06(H-18)	39,15
19	45,72	CH_2	1,86 (6H, s)	1,19 (8H)	37,61(C-21),42,15(C-14)	1,19(H-18a) 1,86(H-19b)	45,78
20	29,93	C					30,07
21	37,61	CH_2	1,66 (3H, m)	1,54 (5H)			37,68
22	75,21	CH	5,00 (1H, s)				75,30
23	26,85	CH_3		1,00 (9H)	41,96(C-9);29,82(C-2);18,17(C-24)		27,43
24	18,17	CH_3	0,96 (4H, s)		40,00(C-4);26,85(C-23);50,35(C-5)		18,27
25	66,90	CH2	3,81 (1H, d)	4,21 (1H)	50,35(C-5); 97,16(C-3; 34,81(C-10)	3,81(H-25a) 4,21(H-25b)	67,67
26	16,97	CH_3	0,80 (3H, s)	0,8 (3H)	41,96(C-9); 31,30(C-7)		17,44
27	24,82	CH_3	1,19 (8H, s)		143,42(C-13);27,68(C-15);42,15(C-14)		25,30
28	174,32	C					177,98
29	33,15	CH_3	0,90 (3H, s)		45,72(C-19);37,62(C-21);26,41C-30		33,74
30	26,41	CH_3	1,86 (6H, s)		45,72(C-19);33,15(C-29);39,53(C-18)		7,20
1’	164,64	C					166,34
2’	116,06	CH	5,55 (2H)		25,94(C-5’)		115,94
3’	156,62	C					157,10
4’	19,27	CH_3	2,12 (7H)		156,62(C-3’), 116,06(C-2’)		20,22
5’	25,94	CH_3	1,86 (3H)		156,62(C-3’), 116,06(C-2’)		26,23

The signal at δ_H 5,34 ppm (2H,s) belongs to olefinic proton at C-12 (δ_C 122,38 ppm), This supported the double bond between C-12 and quaternary carbon , C-13 (δ_C 143,42 ppm).This is also supported by HMBC correlation between H‒27 to C-13 (Table 1, Fig. 2).

The signal at δ_H 4,21 ppm (1H, d) and 3,81 ppm (1H, d) belong to methylene proton at C-25 (δ_C 66,90 ppm) that was coupled each other. This supported single bond between C-25 and quaternary carbon, C-10 (δ_C 34,81 ppm). This is also supported by HMBC correlation between H-25b to C-10, H-25b to C-3, H-25a to C10 and H-25a to C-5. This is also supported by 1H-1H COSY correlation between H-25a to H-25b (Table 1, Fig. 2).

The signal at δ_H 0,96 ppm belongs to methyl proton at C-24 (δ_C 18,17 ppm). This supported single bond between C-24 with C-4, C-4 with C-5, C3 with C-4 and C-4 with C-23. This is also supported by HMBC correlation between H-24 to C-3, H-24 to C-23 and H-24 to C-5 (Table 1, Fig. 2, Fig.3).

The signal at δ_C 97,16 ppm belongs to hemiketal carbon at C-3. This supported double bond between C-3 with OH, C-3 with C-4, C-3 with C-2 and C-3 with O-CH_2. This also supported by HMBC correlation between H-25 to C-3 and H-24 to C-3 (Table 1, Fig.2).

The signal at 0,90 ppm (3H, s) belongs to methyl carbon at C-29 (δ_C 33,15). This supported single bond between C-29 with C-20, C-20 with C-19, C-20 with C-21 and C-20 with C-30. This is also supported by HMBC correlation

between H-29 to C-19, H-29 to C-21 and H-30 to C-29, H-30 to C-18 and H-30 to C-29 (Table 1, Fig. 2).This is also supported by ^{1}H-^{1}H COSY correlation between H-18 to H-19, H-19a to H-19b

The signal at δ_H 5,55 ppm (2H,s) belongs to olefinic proton at C-2'(δ_C 116,06 ppm). This supported double bond between C-2' with C-3'(δ_C 156,62 ppm), C-3' with C-4'(δ_C 19,27 ppm) and C-3' with C-5'(δ_C 25,94 ppm). This is also supported by HMBC correlation between H-2' to C-4', H-2' to C-5', H-5' to C-3' and H-5' to C-4'.

Table 2. LC50 value of n-hexane, ethyl acetate and methanol extractsand isolated compound

	Extract	Regression	LC_{50}(mg/L)
1	n-Hexane	Y = 1.489X+ 2.714	34.2972
2	Ethyl Acetate	Y = 1.148X+3.349	27.4254
3	Methanol	Y = 1.205X+2.440	133.1930
4	Isolated compound	Y = 1.713X + 2.636	27.9903

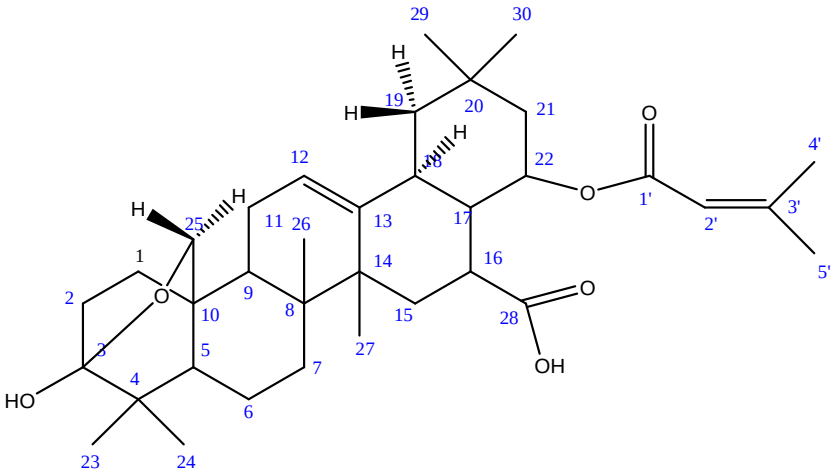

Fig 1.The structure of lantanilic acid

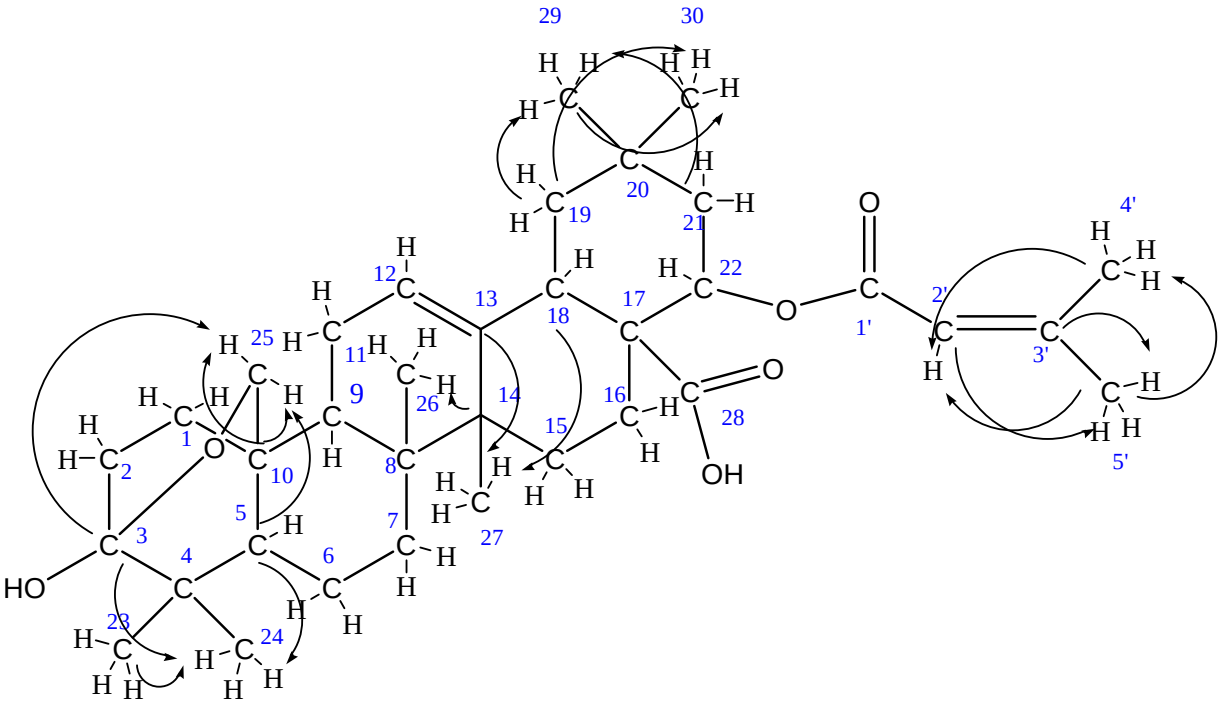

Fig 2. HMBC correlation in the structure of isolated compound

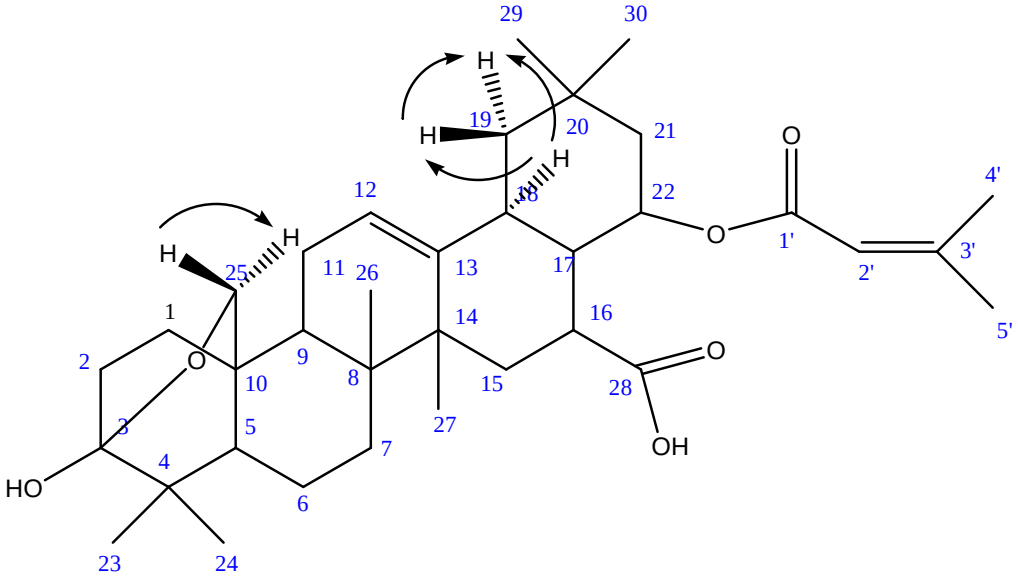

Fig 3.^{1}H-^{1}H COSY correlation in the structure of isolated compound

The n-hexane, ethyl acetate and methanol extracts and compound isolated from ethyl acetate extract of *Lantana camara* L. leaf showed good brine shrimp larvacidal activity. The lethality concentration (LC_{50}) of n-hexane, ethyl acetate, methanol extract and isolated compound were 34,2972µg/mL, 27,4254µg/mL, 133,1930 µg/mL and 27,9903 µg/mL respectively (Table 2). The degree of lethality was directly proportional to the concentration of the extract. Maximum mortalities (90%) were observed at a concentration of 100 µg/mL incompound isolated. Based on the results, the brine shrimp lethality of the three extracts and isolatedcompound were found tobe concentration dependent. The observed lethality of the three extracts and isolated compound showed the presence of lantanilic acid in this plant. The toxicity degree among n-hexane, ethyl acetate and methanol extract and isolated compound is isolated compound > ethyl acetate extract >n-hexane extract > methanol extract. According to Meyer etal., crude plant extract is toxic (active) if it has an LC50 value ofless than 1000 µg/mL while non-toxic (inactive) if it is greaterthan 1000 µg/mL.

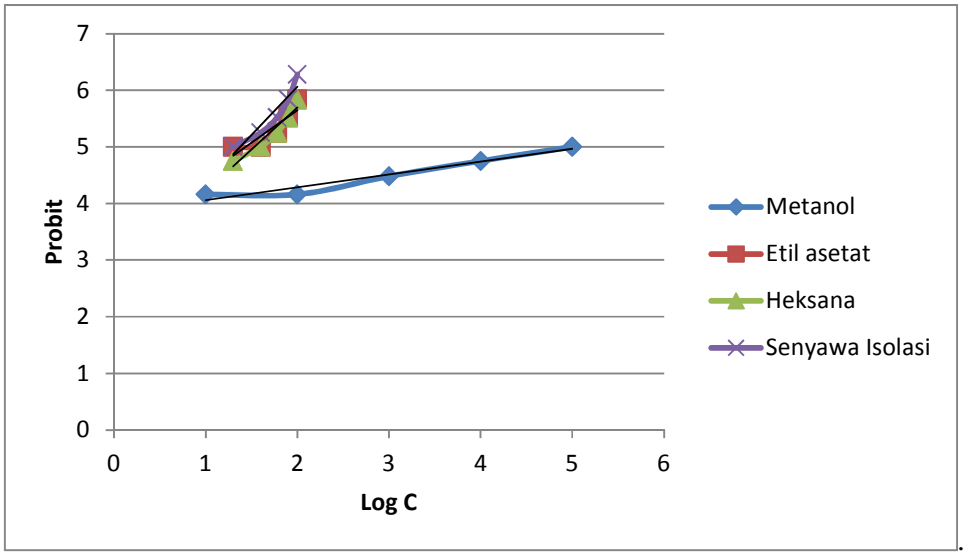

Fig 4.Probitmorthality curve of n-hexane, ethyl acetate and methanol extracts and isolated compound

CONCLUSION

Abrine shrimp toxic constituent, lantanilic acid ($C_{35}H_{52}O_2$) has been isolated from lantana camara L. leaves. In this study, the n-hexane, ethyl acetate,methanol extract and isolated compound showed toxic effects (LC_{50} 34.2972; 27.4254; 133.1930 and 27.9903 µg/mL, respectively). The toxicity degree among the three extracts and isolated compound is isolated compound > ethyl acetate extract > n-hexane extract > methanol extract.

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