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ISOLATION AND CHARACTERIZATION OF LANTANILLIC ACID FROM *LANTANA CAMARA* L. IN BANGLADESH

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Abstract

Lantana camara L. is an important medicinal plant with potential medicinal values and rich phytochemical composition. Lantanillic acid (**1**), an oleanane type triterpene, was isolated from the ethyl acetate extract of leaves of *L. camara* grown in Bangladesh. The structure of this compound was established by analysis of ^{1}H NMR, ^{13}C NMR and mass spectroscopic data and comparison with published values.

Introduction

Lantana camara L. is an aromatic, evergreen shrub that is widely distributed as an ornamental flowering plant throughout the tropical and subtropical areas around the world. It is reported to possess antioxidant, anti-inflammatory, hepato-protective and anti-diabetic properties. The plant has traditional uses in the treatment of cancers, asthma, ulcers, swellings, skin disease, high blood pressure, bilious fevers, rheumatism and malaria (Al-Fadhli *et al.* 2014, Nayak *et al.* 2009, Lonare *et al.* 2012). The plant has been found to contain several important phytochemicals such as triterpenoids, flavonoids, alkaloids, saponins, steroids, and tannins (Khan *et al.* 2023). Lantana oil is used as antiseptic in the treatment of itching, wounds, leprosy and scabies (Kurade *et al.* 2010, Seth *et al.* 2012). It is fascinating to determine whether their traditional uses are reinforced by actual pharmacological effect or simply based on folklore. In recent works, the ethyl acetate extract of *L. camara* leaf showed prominent activity against *Escherichia coli*, *Bacillus subtilis*, *Pneumococcus* and *Klebsiella* (Khan *et al.* 2023). Therefore, the present study was designed to isolate and purify bioactive molecules from *L. camara* leaf in Bangladesh.

Materials and Methods

Leaves of *L. camara* were collected from the Dhaka University campus in 2019. The leaves were washed with distilled water to remove debris. The samples were oven dried at 45°C and ground to coarse powder with a grinding machine.

About 22 g of dry powder of *L. camara* leaf was soaked in 100% ethyl acetate. The extract was concentrated using rotary evaporator at 45°C. The crude extract thus obtained was subjected to column chromatography over silica gel 60 (230-400 mesh, Carl Roth GmbH) column (40 cm x5 cm ID) using isocratic elution with *n*-hexane and ethyl acetate (80:20). A total of 47 fractions were collected, each 100 ml. Fraction 9 upon evaporation at room temperature gave the clear crystalline substances which upon washing with *n*-hexane-ethyl acetate (80:20) afforded compound 1 in pure crystalline form.

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Result and Discussion

Extensive chromatographic analysis of the ethyl acetate extract of *Lantana camara* leaves provided a crystalline mass of compound **1** which showed a pseudo molecular ion peak at 569.2 in the ESIMS, compatible with the molecular formula $C_{35}H_{52}O_6$. After mass spectroscopy, the compound **1** was also analyzed by both ^{1}H NMR and ^{13}C NMR spectroscopy, and NMR data are represented in Tables 1 and 2, respectively. The ^{13}C NMR spectrum (100 MHz, $CDCl_3$) demonstrated 36 carbon resonances including a carbonyl carbon at δ 165.3 and a carboxylic acid group at δ 177.3, while the DEPT-135 spectra indicated that out of the 36 carbons, 21 had attached protons. Thus, the compound **1** had eight methyl (δ 33.7, 27.3, 27.2, 26.2, 25.3, 20.2, 18.3, 17.4), nine methylene (68.7, 45.8, 37.8, 34.7, 31.2, 29.3, 27.8, 24.1, 23.7, 19.7), 3 methine (δ 50.3, 41.9, 39.3) and six quaternary carbons (δ 50.7, 42.1, 40.3, 38.4, 35.1, 30.0) (Table 2).

Table 1. ^{1}H NMR (400 MHz, $CDCl_3$) spectral data of compound 1 and lantanillic acid.

Proton position	Compound **1** δ_H (ppm)	Lantanillic acid* δ_H (ppm)
H-12	5.37 (1H, m)	5.37 (1H, m)
H-18	3.03 (1H, dd, J = 9.2, 4.6 Hz)	3.04 (dd, J = 13.8, 4.0 Hz
H-23	1.01, s	1.03, s
H-24	0.94, s	0.96, s
H-25a	3.87 (1H, d, J = 7.8 Hz)	3.88 (1H, dd, J = 9.1, 3.2 Hz)
H-25b	4.22 (1H, d, J = 7.8 Hz)	4.25 (1H, dd, J = 9.1, 3.2 Hz)
H-26	0.76, s	0.77, s
H-27	1.14, s	1.16 s
H-29	0.86, s	0.88, s
H-30	0.98, s	1.01, s
H-2′	5.56 (1H, m)	5.57 (1H, m)
H-4′	1.83 (3H, d, J = 1.1 Hz)	1.85 (3H, d, J = 1.1 Hz)
H-5′	2.12 (3H, d, J = 1.1 Hz)	2.14 (3H, d, J = 1.1 Hz)

*Delgado-Altamirano *et al.* (2019).

The ^{1}H NMR spectrum of compound **1** showed a pair of doublets (J = 7.8 Hz), each of one proton intensity at δ 4.22 and 3.87, due to two nonequivalent methylene protons, H-25a and H-25b, respectively. The broad singlet at δ 5.37 could be ascribed for the olefinic proton at C-12. The ^{1}H NMR signal at δ 5.00 (δ_C 75.3) was assigned to the oxymethine proton at H-22. The ^{1}H NMR spectrum of compound **1** demonstrated six three proton singlets at δ 0.76, 0.86, 0.94, 0.98, 1.01 and 1.14 for six tertiary methyl groups of the triterpenoid carbon skeleton. The multiplet at δ 5.56 was assigned to the olefinic proton H-2′ and the three proton signals δ 1.83 and 2.12 (3H, d, J = 1.1 Hz) were assigned to the *β*-methyl groups (Table 1). The remaining ^{1}H NMR and ^{13}C NMR signals matched well with the published data for lantanillic acid. Based on the above information, compound **1** was identified as lantanillic acid (Delgado-Altamirano *et al.* 2019). Although lantanillic acid was isolated previously from *L. camara* by Siddiqui *et al.* (1995), but this is the first report of its isolation from *L. camara* leaves in Bangladesh.

Table 2. ^{13}C NMR (100 MHz, $CDCl_3$) spectral data of compound 1 and lantanillic acid.

Carbon position	Compound **1** δ_C (ppm)	Lantanillic acid* δ_C (ppm)
1	34.7	34.8
2	29.3	29.3
3	98.6	98.9
4	40.3	40.4
5	50.3	50.4
6	19.7	19.8
7	31.2	31.0
8	38.4	38.4
9	41.9	42.1
10	35.1	35.2
11	23.7	23.9
12	122.6	122.8
13	143.0	143.0
14	42.1	42.2
15	27.8	27.9
16	24.1	24.2
17	50.7	50.9
18	39.3	39.4
19	45.8	45.9
20	30.0	30.5
21	37.8	37.9
22	75.2	75.3
23	27.3	27.3
24	18.3	18.4
25a 25b	68.7	68.3
26	17.4	17.4
27	25.3	25.4
28	177.3	177.2
29	33.7	33.8
30	26.2	26.3
1′	165.3	165.5
2′	116.0	116.0
3′	157.0	157.5
4′	27.3	27.5
5′	20.2	20.3

*Delgado-Altamirano *et al.* (2019).

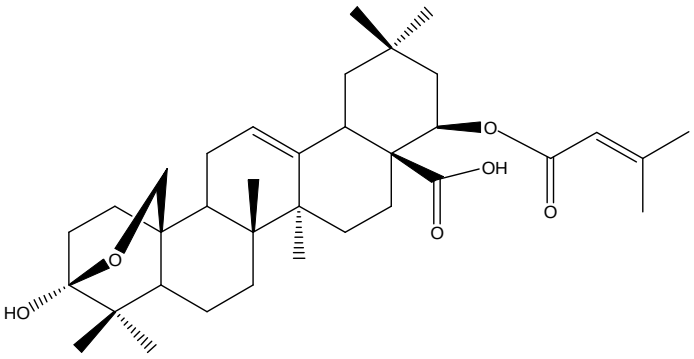

Fig.1. Structure of compound **1** isolated from *Lantana camara* leaves

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