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Larvicidal Activity of Extracts and Triterpenoids from *Lantana camara*

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Abstract

Extracts of the leaves, twigs, stems and roots of *Lantana camara* Linn. (Verbenaceae) were solvent-partitioned and screened for activity in the brine shrimp lethality test (BST). The active fractions yielded known oleanonic acid (**1**), lantadene A (**2**) and oleanolic acid (**3**), which were very toxic to brine shrimp larvae. Compounds **1–3** were not lethal to *Spodoptera littoralis* Biosduval (Lepidoptera: Noctuidae), *Clavigralla tomentosicollis* Stal. (Hemiptera: Coreidae) and *Aphis craccivora* Koch (Homoptera: Aphididae) when tested at 5000 µg/ml. Compound **2**, however, suppressed the fecundity of *C. tomentosicollis* at this concentration.

Keywords: *Lantana camara*, Verbenaceae, pest control, brine shrimp, triterpenes.

Introduction

Crude extracts of biomass constitute a rich library of structurally diverse compounds, some of which are active against agronomic pests. Our preliminary screening of plants for activity in the brine shrimp test detected strong larvicidal activity in the extracts of *Lantana camara* Linn. (Verbnaceae). As a result, we investigated *L. camara* for compounds that can be used against garden pests. There are about 150 species of *Lantana*, and the most toxic of them is *L. camara* (Pass, 1991). *Lantana* spp. flourish on waste agricultural land where they compete with grass for ecological dominance (Sharma & Sharma, 1989). *L. camara* is toxic to ruminants (Black & Carter, 1985; De Aluja, 1970; Keller & Coetzer, 1985; Sharma et al., 1981). Preparations from the *Lantana* plant have been reported to show activity against *Aphis gossypii* Glover, fungal pathogens, and a number of insect pests (Sharma & Sharma, 1989). Phytochemical investigations of *L. camara* have afforded the following triterpenes: ursolic, lantic, lantanolic, oleanolic, oleanonic acids and their derivatives, and some phenolic compounds (Barre et al., 1997; Pan et al., 1993a,b). Oleanonic acid derived triterpenes, the lantadenes, have been implicated in *Lantana* poisoning of ruminants (Pass, 1991; Pass et al., 1985; Uppal & Paul, 1982). The susceptibility of ruminants to *Lantana* poisoning is linked to oleanolic acid, a metabolic product of lantadene A in the liver (Pass et al., 1979; Sharma et al., 1980, 1981).

Compelled by the abundance of *L. camara* on wasteland, and its activity in the brine shrimp test (McLaughlin, 1991; Meyer et al., 1982), we embarked on systematic investigation of the plant for compounds that could be used to control the pests of cowpea and edible vegetables. The most damaging pests of cowpea and edible vegetables in sub-Sahara Africa include *C. tomentosicollis*, *S. littoralis* and *A. craccivora* (Jackai & Daoust, 1986; Singh & Jackai, 1995).

Materials and methods

General experimental procedures

^{1}H and ^{13}C NMR spectra were recorded on JEOL JNM EX-400 or Bruker 400 MHz spectrometer. Mass spectra were obtained on a JEOL JMS-5X102A. Structures were assigned on the basis of spectral evidence. Open column chromatography was carried out on Kieslgel (Riedel-deltaen) 70–230

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mesh, and TLC analysis was performed on Whatman precoated silica gel (60A K6F) plates. TLC bands were visualized under a UV lamp or by exposure to iodine vapor.

S. littoralis were field collected on lawns at the premises of IITA station, Kano. *C. tomentosicollis* were collected from the field and maintained in the laboratory by routine transfer on cowpea pods under ambient conditions. *A. craccivora* on infested leaves of eggplants were used for lethality tests. Samples were tested against pests at 5000 µg/ml. A 5000 µg/ml solution was made by adding 10 mg of test sample to 0.03 ml of Tween 20 and diluting to 2.0 ml with distilled water. In the control, the test sample was replaced with water.

Plant material

The leaves, twigs, stems and roots of *Lantana camara* were collected in December from abandoned farmland within Bayero University campus, Kano, Nigeria. A voucher specimen was deposited in the Herbarium at the Biological Sciences Department, Bayero University Kano, Nigeria.

Brine shrimp lethality test (BST)

Test samples were evaluated for lethality to brine shrimp larvae using a standard protocol (McLaughlin, 1991; Meyer et al., 1982). The lethality data were analyzed with a Finney computer program to determine LC_{50} values and 95% confidence intervals, and to permit comparison of potencies of extracts and isolated compounds.

Lethality assay with *S. littoralis*

Solutions of compounds were tested against *S. littoralis* at 5000 µg/ml. Five or four *S. littoralis* and their feed (in this case grass on which they were collected) were wetted with test solution of sample once in 48 h and the mortality of *S. littoralis* in test and control experiments were recorded after 24 and 48 h. The experiment was performed in duplicate. The percent mortality after 48 h (Table 2) was computed, correcting for control deaths using Abbott's formula:

$$\% \text{ Control} = \frac{\% \text{ Dead (treated group)} - \% \text{ Dead (untreated group)}}{100 - \% \text{ Dead (untreated group)}} \times 100$$

Fecundity assay with *C. tomentosicollis*

Three pieces of cowpea pods, each 5 cm in length, were dipped in test solutions and placed in a small glass jar containing two egg-laying females (recognized by the abdominal shape) and two males. The pods were examined for eggs after 48 h. The experiment was performed in duplicate.

Lethality assay with *A. craccivora*

Leaves containing colonies of aphis were excised. The aphids on leaf surface were counted before application of test sample, and 24 h after application of sample, the survivals were counted in test and control experiments. Compounds **1** and **2** were not lethal to aphis at 5000 µg/ml.

Extraction and fractionation

Dried leaves, twigs, stems and roots of *L. camara* (200.0 g each) was separately extracted by maceration with 5 L of ethanol. The combined extract was evaporated below 40 °C on a rotavapor to give a residue, F001. F001 was partitioned between water and chloroform (400 ml, 1 : 1) in a separatory funnel. After removal of the chloroform layer, the water layer was further washed with chloroform (200 ml). The combined chloroform layers was concentrated under rotary vacuum below 40 °C to give the chloroform soluble residue (F002). The water soluble layer (F003) was discarded. F002 was further dissolved in 90% aqueous methanol (200 ml) and extracted twice with petroleum ether (200 ml). The two layers were separately evaporated to dryness to give the petroleum ether soluble residue (F004) and the aqueous methanol soluble residue (F005). The residues were weighed and screened for activity in the brine shrimp lethality test (BST). Table 1 shows the activity of F004 and F005 in the BST and their yield from ethanol extract, F001.

Extraction and isolation

Dried and ground stem (1.3 kg) of *L. camara* was extracted by maceration with ethanol. The extract was partitioned between water and chloroform to give 52 g of the chloroform soluble residue (F002). The chloroform soluble residue (25 g) was subjected to column chromatography over silica gel (300 g) and eluted successively with petroleum ether, chloroform, petroleum ether-ethyl acetate (1 : 1), chloroform-ethyl acetate (1 : 1), ethyl acetate, and methanol-ethyl acetate (1 : 3 and 1 : 1). Fractions were pooled according to their TLC patterns. A residue (10.6 g) from the chloroform pool, with BST LC_{50} value of 2.88 (0.25–7.33) µg/ml, was further chromatographed on silica gel (145 g) and eluted successively with petroleum ether (3.2 L), petroleum ether-chloroform (5 : 1) (4.0 L), (3 : 1) (4.0 L), (2 : 5) (800 ml) and chloroform (3.2 L) with collection of 400 ml fractions. Fractions 34–35 and 41–42 gave solid residues 5.05 g BST LC_{50} 12.72 (8.25–18.71) µg/ml and 50 mg BST LC_{50} 1.28 (0.04–2.36) µg/ml, respectively. A portion of Fraction 34–35 (103 mg) was further chromatographed on silica gel (20 g) and eluted successively with *n*-hexane (100 ml), ethyl acetate-hexane (1 : 9) (100 ml), ethyl acetate-hexane (1.5 : 8.5) (100 ml) and ethyl acetate-hexane (2 : 8) (300 ml) with collection of 10 ml fractions.

Fractions 19–22 gave a residue (17.4 mg) LC-34-1, identified as oleanonic acid (**1**). ^{1}H NMR ($CDCl_3$) δ 5.30 (1H, m, 12-H), 2.82 (1H, dd, J = 14.0, 4.0 Hz, 18-H), 1.14, 1.08, 1.05,

1.03, 0.93, 0.91, 0.81 ($CH_3 \times 7$); ^{13}C NMR ($CDCl_3$) δ 39.08, 34.13, 217.72, 47.42, 55.29, 19.53, 32.13, 39.25, 46.87, 36.80, 23.46, 122.38, 143.63, 41.72, 27.67, 22.88, 45.76, 41.00, 46.56, 30.67, 33.78, 33.04, 26.52, 21.43, 14.99, 16.99, 26.43, 183.89, 32.39, 23.56 (C-1 to C-30); EIMS *m/z* (%) 454 (M^+, 14), 408 (10), 248 (100), 203 (40), 189 (7), 83 (4); HREIMS *m/z* 454.3472 (M^+) (calcd 454.3447 for $C_{30}H_{46}O_3$). Fractions 29–44 gave a residue (33.4 mg) LC-34-2, identified as lantadene A (**2**). ^1H NMR ($CDCl_3$) δ 5.98 (1H, dd, $J = 7.19$ and 1.20 Hz, 3′-H), 5.38 (1H, br, 12-H), 5.09 (1H, br, 22-H), 3.40 (1H, dd, $J = 14.39$, 4.0 Hz, 18-H), 1.96 (3H, dd, $J = 7.6$, 1.6 Hz, 4′-(CH_3), 1.76 (3H, d, $J = 1.6$ Hz, 5′-CH_3), 1.18, 1.05, 1.04, 1.02, 1.00, 0.90, 0.86 ($CH_3 \times 7$); ^{13}C NMR ($CDCl_3$) δ 38.41, 34.11, 217.66, 47.42, 55.29, 19.59, 32.17, 39.21, 47.73, 36.76, 23.50, 122.46, 143.10, 41.97, 27.57, 24.18, 50.60, 38.41, 46.87, 30.03, 37.71, 75.88, 26.44, 21.45, 15.09, 16.83, 25.79, 180.10, 33.67, 26.13 (C-1 to C-30), 166.26, 127.61, 138.88, 20.56, 15.64 (C-1′ to C-5′); EIMS *m/z* (%) 452 ($M^+ - C_5H_8O_2$, 100), 408 (10), 248 (25), 246 (18), 201 (15), 83 (75), 55 (11).

Fraction LC-41-42 (50 mg) gave oleanolic acid (**3**) as a pure compound. ^1H NMR (C_5D_5N) δ 5.5 (1H, m, 12-H), 3.4 (1H, m, 18-H), 3.3 (1H, m, 3-H), 1.33, 1.25, 1.03, 1.02, 1.01, 0.93, 0.89 (each 3H, s, $CH_3 \times 7$); ^{13}C NMR (C_5D_5N) δ 38.92, 28.04, 78.04, 39.34, 55.80, 18.76, 33.27, 39.72, 48.10, 37.34, 23.76, 122.51, 144.77, 42.12, 28.27, 23.67, 46.62, 42.00, 46.45, 30.94, 34.20, 33.14, 28.70, 16.51, 15.53, 17.39, 26.15, 180.10, 33.10, 23.80 (C-1 to C-30); EIMS *m/z* (%) 456 (M^+, 5), 438 (2), 248 (100), 203 (55); HREIMS *m/z* 456.3643 (M^+) (calcd 456.3603 for $C_{30}H_{48}O_3$).

Results and discussion

Extracts of the twigs and stems of *L. camara* are more active than the extracts of the roots or leaves (Table 1). The differences in activity level between the twigs or stems and the roots or leaves in the brine shrimp test are significant since the LC_{50} values at 95% confidence intervals did not overlap.

BST-directed chromatography of the 90% aqueous methanol soluble residue (F005) of the stem on a silica gel column yielded oleanonic acid (**1**), its derivative lantadene A (**2**), and oleanolic acid (**3**), as the most active compounds (Fig. 1). The structural identity of compounds **1–3** was quickly ascertained by correlation of their NMR and EIMS spectral data with literature values (Pan et al., 1993). Oleanolic acid (**3**) is the most toxic compound to brine shrimp larvae.

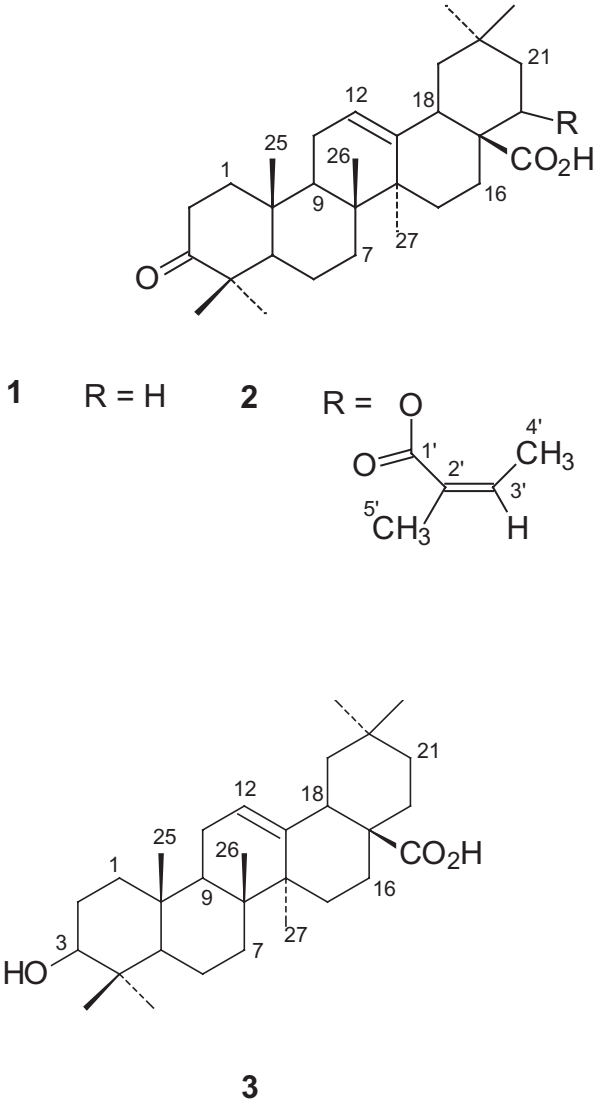

Figure 1. Structures of isolated bioactive compounds.

Table 1. BST activity and recovery of plant parts.

	Methanol soluble (F005)		Petroleum ether soluble (F004)	
Plant Parts	BST LC_{50} µg/ml[a]	% Recovery from F001	BST LC_{50} µg/ml[a]	% Recovery from F001
Leaves	18 (9–13)	19	54 (32–90)	6
Twigs	0.3 (0–4)	19	62 (31–117)	19
Stems	0.3 (0–4)	33	3.6 (0.1–10)	26
Roots	17 (7–36)	39	47 (25–108)	39

[a] At 95% confidence intervals.

Table 2. Assay of fractions against *S. littoralis*.

Sample	Dosage µg/ml	Number of *S. littoralis*	Mortality 24 h	after 48 h	% Control[a]
Oleanonic acid **1**	5000	10	0	2	20
Lantadene A **2**	5000	10	0	4	40
Oleanolic Acid **3**	5000	10	1	3	30
Control		10	0	0	

[a] $\%\ \text{Control} = \dfrac{\%\ \text{Dead (treated group)} - \%\ \text{Dead (untreated group)}}{100 - \%\ \text{Dead (untreated group)}} \times 100.$

Table 3. Fecundity Assay results with *C. tomentosicollis*.

Sample	Dosage µg/ml	Total number of *C. tomentosicollis*	Number of eggs after 48 h
Lantadene A **2**	5000	8	4
Oleanolic Acid **3**	5000	8	19
Control		8	20

However, compound **2** caused 40% control lethality of *S. littoralis* at 5000 µg/ml (Table 2), and suppressed the fecundity of *C. tomentosicollis* relative to **3** at the same concentration (Table 3). Compound **2** had no effect on aphis. In conclusion, the bioactive components of *L. camara* are concentrated in the twigs and the stems. Triterpenoids **1–3** are not sufficiently potent to be useful for rapid control of aphis and bugs on crops.

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