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SHORT COMMUNICATION

ISOLATION AND CHARACTERIZATION OF LARVICIDAL COMPONENTS AGAINST MOSQUITO LARVAE (*AEDES AEGYPTI* LINN.) FROM *CALODENDRUM CAPENSE* THUNB

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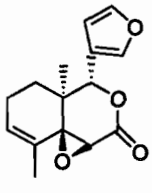
ABSTRACT. Chromatographic analysis of air-dried root barks and seeds of *Calodendrum capense* Thunb led to the isolation of calodendrolide (1), limonin (2) and limonin diosphenol (3) whose structures were elucidated using physical and spectroscopic techniques. The compounds and the crude extract were then tested against mosquito 2nd instar larvae of the species *Aedes aegypti* Linn *senso stricto*, a yellow fever vector at concentrations of 25, 50, 75, and 100 ppm. Calodendrolide (1) was the most toxic as it killed all the larvae even at the lower concentration of 25 ppm. In addition, concentrations of 15, 10, 5 and 1 ppm of this compound retained moderate efficacy. Calodendrolide (1), *C. capense* root bark crude, limonin (2), and limonin diosphenol (3) had LC_{50} values of 13.1, 29.2, 71.6 and 217.1 ppm, respectively.

KEY WORDS: *Aedes aegypti*, Limonoids, *Calodendrum capense*, 2nd instar larvae, Limonin, Limonin diosphenol

INTRODUCTION

It has been known for centuries that many trees and plants have evolve elaborate defence mechanisms against harmful insect attack [1]. Besides the potential use of such compounds as agricultural insect pest control agents, their use in mosquito larvae control is an interesting prospective. The possibility of relying on plant extracts has previously been investigated by [2], [3], and [4] among many others.

The pronounced insecticidal and antifeedant activity of some limonoids has recently prompted attempts to produce structurally simpler derivatives which retain biological activity [5]. It is in this light that calodendrolide (1) (a structurally simpler compound) was isolated from *Calodendrum capense* Thunb, a plant belonging to Rutaceae family which is known to contain limonoids. Isolation yielded calodendrolide (1), limonin (2) and limonin diosphenol (3). The compounds and the crude were tested against mosquito larvae (*Aedes aegypti* Linn.) for efficacy.



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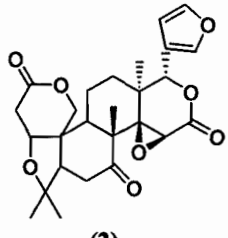

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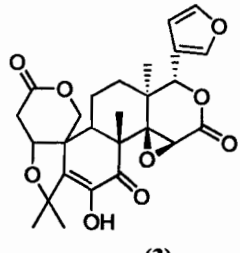

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EXPERIMENTAL

General

Melting points were determined using Stuart melting point apparatus. Infrared spectra were recorded on Model 408 Shimadzu spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Varian Mercury-VX Spectrometer operating at 200 MHz for proton and 50 MHz for carbon-13.

Extraction

(i) *Calodendrum capense* root bark was collected from Uasin Gishu district, Eldoret, Kenya. The plant was identified at the Botany Department Herbarium, Moi University where voucher specimens were deposited. Preliminary analysis was done to detect limonoids by thin layer chromatography (TLC) as per Dreyer, [6]. The root barks collected were air dried at room temperature for four weeks before being chopped into small pieces. A quantity of 2.8 kg of the dried bark was soaked in 4 L of acetone for 3 days. The extract was filtered and the dark brown filtrate was then concentrated to a semi solid using a rotavapor. The obtained concentrate weighed 49.28 g and was labeled (C.C.R.B.).

(ii) *C. capense* seeds were harvested from the same source and analyzed using the same protocol [6]. They were crushed and air-dried at room temperature for 4 weeks. The dry crushed seeds weighed 3 kg. They were then defatted by soaking in 5 L n-hexane for 4 days. The extract was filtered and the filtrate was discarded. The residue was soaked in 4 L of acetone. The extract was then filtered and the filtrate was concentrated to a semi solid weighing 46.20 g and labeled (C.C.S.).

Chromatographic separation

(i) C.C.R.B.: A 12.05 g portion of C.C.R.B. was pre-adsorbed on 11.5 g silica gel (70-30 mesh, Merck) and subjected to column chromatography on silica gel, using n-hexane for packing. Elution of the column with n-hexane led to the isolation of two pure compounds. TLC development was performed using 6:4 (hexane:acetone). Two compounds were isolated and labeled as **A** and **B** of which compound **A** was a limonoid since it responded to Erhlich's reagent.

(ii) C.C.S.: A 12.56 g portion of C.C.S. was pre-adsorbed on 10.4 g silica gel (70-30 mesh, Merck) and subjected to a column packed under n-hexane with silica gel. Elution of the column with n-hexane followed with an increase in polarity by addition of acetone up to a ratio of 60:40 (hexane:acetone) led to the isolation of two pure compounds. TLC development was done using 6:4 (hexane:acetone). The compounds were labeled as **C** and compound **D**. Compound **C** was recrystallized from acetone/hexane to give 24 mg as white needles. Compound **D** was recrystallized in acetone/hexane to give 27 mg as white needles.

RESULTS AND DISCUSSION

Structure elucidation

Structures of the compounds isolated were elucidated by comparing their physical and spectroscopic data with those compounds already isolated and reported in the literature.

Calodendrolide (**1**). The melting point of compound **A** was 147-149 ^{0}C. The IR spectrum had five characteristic functional groups. Bands were observed at 2950, 1730, 1500, 1280 and 875 cm^{-1}, which were characteristic of a C-H stretch of an alkane, a lactone, a double bond of an aromatic system, a C-O stretch of an epoxide and a furan, respectively. The position and the chemical shifts of the protons and carbons of the compound are in agreement with those reported by [7].

Limonin (**2**). The melting point of compound **C** was 294-296 ^{0}C. The IR spectrum had six characteristic functional groups. Bands were observed at 2950, 1750, 1700, 1500, 1280 and 875 cm^{-1}, these were; a C-H stretch of an alkane, a lactone, a carbonyl, a double bond of an aromatic system, a C-O stretch of an epoxide and a furan, respectively. The position and the chemical shifts of the protons of the compound are similar with those reported by [8].

Limonin diosphenol (**3**). The melting point of compound **D** was 312-318 ^{0}C. The IR spectrum had eight characteristic functional groups. Bands were observed at 3500, 2950, 1760, 1730, 1705, 1500, 1280 and 875 cm^{-1}, these were; an alcohol, a C-H stretch of an alkane, two lactones, a carbonyl, a double bond of an aromatic system, a C-O stretch of an epoxide and a furan, respectively. The position and the chemical shifts of the protons of the compound are exactly as reported by [8].

Larvicidal test

The method according to Zebitz [9] was adopted for larvicidal assay. To each jar holding 40 mL of 0.9% sodium chloride, ten 2nd instar mosquito larvae were introduced and immediately treated with 25, 50, 75, 100 ppm of limonin (**2**), limonin diosphenol (**3**), calodendrolide (**1**) and *Calodendrum capense* root bark crude each in triplicate. Limonin diosphenol (**3**) produced negligible toxicity even at 100 ppm. Calodendrolide (**1**) was the most active as it killed all the larvae even at 25 ppm. Further lower concentrations of 1, 5, 10 and 15 ppm for calodendrolide (**1**), which was very potent, were also tested. Calodendrolide (**1**) had an LC_{50} of 13.1 ppm. Calodendrolide (**1**), *C. capense* root bark crude, limonin (**2**), and limonin diosphenol (**3**) had LC_{50} values of 13.1, 29.2, 71.6 and 217.1 ppm, respectively.

The control experiment contained the 0.9% sodium chloride solution alone. The experiment was carried on for ten days and the data analyzed for LC_{50} values with 95% confidence interval for significant comparison of potencies. The results of this assay on day 10 are summarized in Table 1.

Toxicity on mosquito larvae was lacking or minimal in limonin (**2**) and limonin diosphenol (**3**) up to 75 ppm, although possible acute toxic effects may express themselves physiologically later. An interesting observation from the study was that calodendrolide (**1**) though simple in chemical structure, displayed high activity as compared to the more complex limonoids, limonin (**2**), and limonin diosphenol (**3**). This may imply that there may be simpler structural variants of calodendrolide (**1**), which may display activity.

Log P calculation

Log P values were calculated for the entire compounds tested using ACD/Log P version 1.0. This indicated that there was no correlation between larvicidal activity and solubility. Limonin (**2**) had a value of 1.44±0.77, limonin diosphenol (**3**) 1.98±0.76 and calodendrolide (**1**) 2.03±0.51.

Calodendrolide (**1**) has potential use as a larvicide for control of mosquitoes. This compound can reduce our dependence on the toxic industrial chemicals, and thus offer an alternative for a

new generation of eco-friendly larvicides that will potentially not pollute our environment, poison our food, or harm non-pest species.

Table 1. Larvicidal effect of some limonoids against *A. aegypti* after 10 days treatment at 2nd instar.

Compound	Concentration. (ppm)	No. of larvae alive	No. of Larvae dead	Mortality (%)
Calodendrum capense root bark crude	100	0	10	100
	75	0	10	100
	50	3	7	70
	25	6	4	40
Calodendrolide	100	0	10	100
	75	0	10	100
	50	0	10	100
	25	0	10	100
	15	6	4	40
	10	5	5	50
	5	9	1	10
	1	10	0	0
Limonin	100	0	10	100
	75	8	2	20
	50	9	1	10
	25	10	0	0
Limonin diosphenol	100	8	2	20
	75	9	1	10
	50	9	1	10
	25	10	0	0
Control	-	10	0	0

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