Limonoids as Larvicidal Components against Mosquito Larvae (*Aedes aegypti* Linn.)

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The study focussed on four limonoids (calodendrolide, harrisonin, pedonin and pyroangolensolide) as larvicidal components against mosquito 2nd instar larvae of the species *Aedes aegypti* Linn. *senso stricto*. Since pyroangolensolide is close to calodendrolide in structure, it was synthesized through reduction of calodendrolide with chromium(II) chloride in acetone. Harrisonin and pedonin were extracted with cold methanol from the root bark of *Harrisonia abyssinica* while calodendrolide was extracted with the same solvent from the root bark of *Calodendrum capense*. The structure of pyroangolensolide was elucidated using physical and spectroscopic techniques. 25, 50, 75, and 100 μM of each compound were tested against the mosquito larvae. Calodendrolide was the most toxic since 100% mortality was registered at all concentrations, while pyroangolensolide showed 100% mortality up to 50 μM and for contents of 25 μM, a mortality of 70% was registered. As a result of this toxicity, lower concentrations (5, 10 and 15 μM) were tested for both calodendrolide and pyroangolensolide. Toxicity of harrisonin and pedonin was lower. The relative toxicity was in the order: calodendrolide > pyroangolensolide > harrisonin > pedonin with LC_{50} values of 13.2, 16.6, 28.1 and 59.2 μM, respectively.

Key words: Aedes aegypti, Limonoids, 2nd Instar Larvae

Introduction

Limonoids are a group of chemically related triterpene derivatives found in Rutaceae, Meliaceae and Simaroubaceae (Ourison *et al*., 1964). They have been named after the first known compound of this type, limonin (Maier *et al*., 1977).

Recently, limonoids have attracted much attention because of their marked insect antifeedant and growth regulating (IGR) activity of azadirachtin and related highly oxidized C-*seco* limonoids from the neem tree, *Azadirachta indica* A. Juss. and the chinaberry tree, *Melia azedarach* L. (Jacobson, 1988; Ascher and Schmutterer, 1987).

Besides the potential use of such compounds as insect pest control agents, their use in mosquito larvae control is an interesting perspective. The possibility has previously been investigated (Chavan *et al.*, 1979; Attri and Prasad, 1980; Chavan, 1984). Calodendrolide has also been reported to be a potent larvicide (Kiprop *et al.*, 2005).

The pronounced insecticidal and antifeedant activity of some limonoids has recently prompted attempts to produce structurally simpler derivatives which retain biological activity (Klocke and Yamasaki, 1989). It was in this light that calodendrolide was derivatized to pyroangolensolide. These compounds, calodendrolide and pyroangolensolide (structurally simpler compounds), with harrisonin and pedonin (Fig. 1) were assayed for larvicidal activity against 2nd instar larvae of *Aedes aegypti* Linn.

Information on biological activities of over seventy other limonoids has been published over the last two decades (Champagne *et al*., 1992).

Experimental

Melting points were determined using a Stuart melting point apparatus. Infra-red spectra were recorded on a model 408 Shimadzu spectrometer.

From the methanol extract of dried ground root barks of *Harrisonia abyssinica* and *Calodendrum capense*, the larvicidal compounds harrisonin, pedonin and calodendrolide were isolated.

Calodendrolide weighing 100 mg was treated with chromium(II) chloride (20 mL) in acetone (20 mL) *in situ* for 45 min. The solution was evaporated giving a residue, which was pyroangolensolide.

Results and Discussion

Pyroangolensolide

Melting point of pyroangolensolide was 144–146 °C. The IR spectrum of the compound had five peaks, which were observed at 2950, 1730, 1500, 1280, and 875 cm^{-1}. These were characteristic for a C–H stretch of an alkane, a lactone, a double bond of an aromatic system, a C–O stretch of an oxymethylene and a furan, respectively. The data agreed with those reported in the literature (Tokoroyama *et al.*, 1988).

Larvicidal test

The method according to Zebitz (1984) was adopted for the larvicidal assay. The procedure was as follows: 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 μM of calodendrolide, harrisonin, pedonin and pyroangolensolide (Fig. 1), each in triplicate.

Lower contents of 5, 10 and 15 μM were also tested for calodendrolide and pyroangolensolide. The control experiment contained the 0.9% sodium chloride solution and the 2nd instar mosquito larvae only. The experiment was monitored for ten days, thereafter data was 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 I.

Calodendrolide was the most active of all the compounds. 100% Mortality was registered within the 1st day at concentrations of 100, 75, 50 and 25 μM. Pyroangolensolide attained 100% mortality by the 2nd day at concentrations of 100 μM and 75 μM while 70% mortality was recorded with 25 μM on the 10th day.

Calodendrolide, pyroangolensolide, harrisonin and pedonin had LC_{50} values of 13.2, 16.6, 28.1 and 59.2 μM, respectively. The LC_{50} value of 13.2 μM for calodendrolide is high compared with that of 50 μM for larval mortality using *Melia volkensii* extracts on the same mosquito larvae

Table I. Limonoids against *A. aegypti* after 240 hours of treatment at 2nd instar stage.

Compound	Content [μM]	Number of larvae alive	Number of larvae dead	Mortality (%)
Calodendrolide	100	0	10	100
	75	0	10	100
	50	0	10	100
	25	0	10	100
	15	4	6	60
	10	5	5	50
	5	7	3	30
Harrisonin	100	0	10	100
	75	0	10	100
	50	0	10	100
	25	6	4	40
Pedonin	100	2	8	80
	75	3	7	70
	50	6	4	40
	25	8	2	20
Pyroangolensolide	100	0	10	100
	75	0	10	100
	50	0	10	100
	25	3	7	70
	15	6	4	40
	10	7	3	30
	5	9	1	10
Control	–	10	0	0

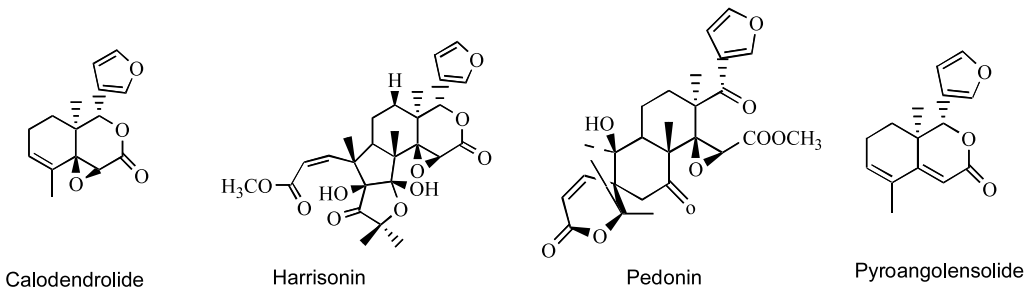

Fig. 1. Structures of the assayed compounds.

(Mwangi and Rembold, 1988). The toxicological data obtained indicates that both calodendrolide and pyroangolensolide at 5 μM contents caused larval mortality of 30% and 10%, respectively, which is higher than the 2.6% larval mortality caused by a 20 μM methanolic extract of neem seed kernel on the same larvae.

Toxicity of both harrisonin and pedonin was lower and not significantly different from each other. It was observed that though calodendrolide was highly toxic, its efficacy was not significantly different from that of pyroangolensolide. Both calodendrolide and pyroangolensolide, though simple in chemical structure, displayed higher larvicidal activity compared to the more complex compounds harrisonin and pedonin.

Log P calculation

Log P values were calculated for the compounds tested so as to check for any correlation between solubility and larvicidal activity. This was done using ACD/Log P version 1.0. Calodendrolide, harrisonin, pedonin and pyroangolensolide had values of (2.03 ± 0.51), (2.72 ± 0.81), (2.77 ± 1.03) and (2.92 ± 0.41), respectively. This showed that there was no correlation between solubility and larvicidal activity.

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