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Short Report

Chemical Composition and Larvicidal Activity of *Rollinia leptopetala* (Annonaceae)

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No presente trabalho descreve-se a composição química dos óleos essenciais de *Rollinia leptopetala* R.E. Fries (Annonaceae) e as atividades larvicidas dos óleos essenciais, do extrato metanólico das raízes desta espécie e do alcalóide oxoaporfinico, liriodenina (**1**), frente às larvas no terceiro estágio do mosquito *Aedes aegypti*. O extrato metanólico mostrou-se ativo com CL_{50} 64,6 ± 1,5 ppm e uma forte atividade foi exibida para o composto (**1**), CL_{50} 3,6 ± 0,4 ppm. Os óleos essenciais das folhas e galhos também mostraram atividade com CL_{50} 104,7 ± 0,2 and 34,7 ± 0,3 ppm, respectivamente. Estes dados sugerem que *R. leptopetala* é fonte potencial de larvicidas naturais. A composição química do óleo essencial e as atividades descritas são comunicadas pela primeira vez.

The aim of present study was to describe the chemical composition of the essential oils from the leaf and stem of *Rollinia leptopetala* R. E. Fries (Annonaceae) and to evaluate the larvicidal activities of these essential oils, of the methanol extract from roots of this plant and of the oxoaporphine alkaloid, liriodenine (**1**) against the third-instar of *Aedes aegypti* larvae. The methanol extract from the roots showed larvicidal activity with LC_{50} 64.6 ± 1.5 ppm. Higher activity was observed for the isolated alkaloid liriodenine (**1**), LC_{50} 3.6 ± 0.4 ppm. The essential oils from the leaves and stems, also exhibited larvicidal activity with LC_{50} 104.7 ± 0.2 and 34.7 ± 0.3 ppm, respectively. These results suggest *R. leptopetala* as a source of natural larvicidal compounds. This is the first report about the chemical composition and larvicidal activity of the leaf and stem essential oils of *R. leptopetala*.

Keywords: *Rollinia leptopetala*, Annonaceae, essential oils, liriodenine, *Aedes aegypti*

Introduction

Aedes aegypti is the principal mosquito vector of dengue fever, including the hemorrhagic form, which is endemic to South East Asia, Central and South America, and West Africa.[1] Between 50 and 100 million of cases are registered each year, causing thousands of deaths.[2] The disease has high levels of mortality, and also inflicts great economic losses and social disruption in Brazil.[3] Although some viral diseases, such as yellow fever, have been brought reasonably under control with a vaccine, no vaccine is so far available for dengue. Today, the only way of decreasing the incidence of this disease is by controlling the *Aedes aegypti* proliferation. This is not an easy task because the mosquitoes have developed resistance to the current synthetic insecticides that, in addition, are toxic to humans and the environment. The mosquito population control in the larval stage is much easier than in the adult

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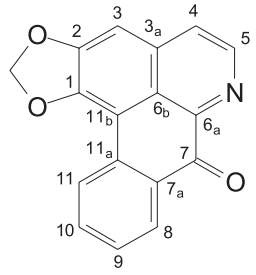
stage, and then new strategies are needed for controlling the proliferation of the larvae of *A. aegypti*. Several studies have focused on natural products as insecticides for controlling *A. aegypti* larvae. Alkaloids and essential oils from herbal plants have demonstrated larvicidal activity,[4-8] which motivated our group to search for new insecticides from Brazilian plants.

Rollinia leptopetala R. E. Fries, popularly known as *pinha brava*, is an endemic shrub in Brazil and shows strong toxicity when eaten by animals.[9] The phytochemical investigation of *Rollinia* species has been reported in the literature revealing acetogenins, steroids, lignans and alkaloids, as secondary metabolites.[10-13] Previous studies on liriodenine showed prominent antibacterial and cytotoxic biological activities.[14,15] In this paper, the larvicidal activities of *R. leptopetala* and of the alkaloid liriodenine (**1**) against the larvae of *Aedes aegypti* are reported.



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Results and Discussion

The methanol extract from the roots of *R. leptopetala* showed activity against *A.aegypt*i larvae with LC_{50} 64.6 ± 1.5 ppm. Fractionation of this extract by column chromatography gave the alkaloid liriodenine (**1**), which was identified by comparing its NMR and MS data with those previously reported in the literature.[13,16-18]

The alkaloid was evaluated and showed strong larvicidal activity against *A. aegypti*, with LC_{50} 3.6 ± 0.4 ppm. Although no similar investigation was found in the literature for oxoaporphine alkaloids, some nitrogenated compounds have been presented as promising larvicides.[19-22]

The chemical composition of the essential oils from the leaves and stems of *R. leptopetala* showed quantitative and qualitative variation. The leaf oil consisted mainly of oxygenated monoterpenes (47.7%), with linalool and 1,8-cineole as the major compounds. The stem oil was devoid of monoterpenes, whereas sesquiterpenes, mainly oxygenated, were abundant (76.2%). Spathulenol (63.9%) was found in high concentration. The retention indices and relative percentages of the constituents of the oils are shown in Table 1.

Table 1. Chemical composition of essential oils from *Rollinia leptopetala*

Constituents[a]	Retention index	Leaves %	Stems %
α-Thujene	930	1.4	-
α-Pinene	939	4.9	-
β-Pinene	979	2.6	-
Myrcene	991	3.0	-
1,8-Cineole	1031	16.5	-
β-Ocimene	1037	3.4	-
α-Terpinolene	1089	1.1	-
Linalool	1097	18.7	-
α-Terpineol	1189	10.4	-
Geraniol	1253	2.1	-
β-Caryophyllene	1419	5.0	9.3
Bicyclogermacrene	1500	7.6	14.4
δ-Cadinene	1523	0.5	-
Germacrene B	1561	1.6	-
Spathulenol	1578	1.1	63.9
Caryophyllene oxide	1583	-	3.0
Globulol	1585	-	5.2
Guaiol	1601	0.7	
epi-α-Muurolol	1642	-	4.1
Total		80.6	99.9
Monoterpenes %		64.1	0
Sesquiterpenes %		16.5	99.9

[a]Constituents listed in order of elution of DB-5 column.

The essential oil from the leaves showed moderate activity against the third-instar of *Aedes aegypti* larvae (LC_{50} 104.7 ± 0.2 ppm) but higher larvicidal activity (LC_{50} 34.7 ± 0.3 ppm) was found for essential oil from the stems, which confirmed a higher concentration of oxygenated sesquiterpenes. As no reports on the larvicidal activity were found for spathulenol, and this sesquiterpene was not isolated from the oil to be assayed, we can not suggest the higher activity found in the essential oil from stems is associated to spathulenol.

The quite high larvicidal activity of liriodenine (**1**) suggests this alkaloid as a model for development of new insecticides for *A. aegypti* control. The biological activity found for the essential oils, particularly for the essential oil from the stems, corroborates the importance of the investigation of essential oils from plants as potential insecticides and larvicides for controlling *Aedes* mosquitoes.[5,8,23]

Experimental

Plant material

R. leptopetala was collected, at the flowering stage, in Guaraciaba do Norte, in the Northeast of Brazil, in February

2004. The plant was identified by Dr. F. S. Cavalcanti and Prof. E. P. Nunes from the Herbário Prisco Bezerra (EAC), Universidade Federal do Ceará, Brazil where a voucher specimen, # 33496, is deposited.

*Extraction and isolation of liriodenine (**1**)*

The air-dried and pulverized roots (500 g) of *R. leptopetala* were washed with 20% (v/v) aqueous NH_4OH, and extracted with CH_2Cl_2 in a Soxhlet apparatus. The organic solution was distilled under reduced pressure giving a brown viscous syrup (50 g). This material was chromatographed on a silica gel column (63-200 μm, Vetec), previously washed with $NaHCO_3$, and eluted with a gradient system (0→30%) of CH_2Cl_2/MeOH. Fractions were pooled according to TLC analysis. From the combined fractions eluted with an 80:20 mixture of CH_2Cl_2:MeOH, a residue was obtained which was submitted to exclusion chromatography on Sephadex™ LH-20 (Amersham Biosciences, Sweden) by elution with a mixture of MeOH/CH_2Cl_2 to yield liriodenine (**1**, 78.0 mg, 0.16% yield), 278.0-280.4 °C. Liriodenine (**1**) was also isolated from the methanol extract (18.5 g) of the roots (500 g), which was obtained by extraction of roots with cold MeOH for 72 h at room temperature. This material was chromatographed on a silica gel column (63-200 μm, Vetec) using hexane, $CHCl_3$, EtOAc and MeOH as eluents. The combined fractions eluted with $CHCl_3$ (2.23 g) were chromatographed using a gradient system (0→30%) of CH_2Cl_2/MeOH. The fractions pooled with CH_2Cl_2/MeOH (90:10 v/v), provided **1** (12.7 mg, 0.07% yield). Recrystallization of **1** was from MeOH and structural determination was performed by spectral analysis, including comparison with literature data.[16] ^1H and ^{13}C NMR spectra were recorded on a Bruker Advance DRX 500 spectrometer and the mass spectrum was obtained from a VG Auto M Spec Fisions Instruments spectrometer.

Essential oil isolation and chemical analysis

The oils from the fresh leaves (500 g) and stems (500 g) of *R. leptopetala* were extracted by hydrodistillation for 4 h, using a modified Clevenger-type apparatus with a water-cooled oil receiver to reduce distillation overheating artifacts. Pale yellowish oils were obtained in 0.03 and 0.05% yield from the leaves and stems, respectively, which were dried over anhydrous sodium sulfate and kept at 4 °C until GC-MS and GC analysis.

The essential oil constituents were quantified by Analytical GC-FID, that was carried out on a Shimadzu GC-17A gas chromatograph using dimethylpolysiloxane DB-5 fused silica capillary column (30 m × 0.25 mm × 0.25 μm film thickness). Hydrogen was used as carrier gas at a flow rate of 1 mL min^{-1} and 30 psi inlet pressure; split, 1:30; temperature programmed, 35-180 °C at 4 °C min^{-1}, then heated at a rate of 17 °C min^{-1} to 280 °C and held isothermal for 10 min; injector temperature, 250 °C; detector used FID, detector temperature, 250 °C. The analysis of the oils was performed by GC-MS analysis on a Hewlett-Packard 5971 GC-MS instrument employing the following conditions: column: dimethylpolysiloxane DB-5 fused silica capillary column (30 m × 0.25 mm × 0.1 μm film thickness); carrier gas: helium (1mL min^{-1}); injector temperature: 250 °C; detector temperature: 200 °C; column temperature: 35°-180 °C at 4 °C min^{-1} then 180-250 °C at 10 °C min^{-1}; mass spectra: electron impact 70 eV. Individual components were identified using the Wiley L-built library and two other computer libraries[24,25] MS searches using retention indices as a pre-selection routine, as well as by visual comparison of the fragmentation patterns with those reported in the literature.[26,27] No linear retention index was calculated and no standard was used. The chemical components identified in the essential oils are presented in Table 1.

Screening for larvicidal activity

Liriodenine (**1**), essential oils, and the methanol extract were placed in beakers (50 mL) and dissolved in H_2O/DMSO 1.5% (v/v) at concentrations of 1-500 ppm, followed by the addition of 50 larvae at the third-instar. For each experiment, both positive (Temephos® at 3.22 ppm) and negative (distilled water containing 1.5% DMSO) control assays were carried out. Mortality was recorded after 24 h of exposure, during which no nutritional supplement was added. The experiments were carried out at 28 ± 2 °C. Each test was performed in triplicate. Data were evaluated through regression analysis. From regression line, the LC_{50} values were read representing the lethal concentration for 50% larval mortality of *A. aegypti*. The bioassays were performed at the Laboratório de Entomologia, Núcleo de Endemias, Secretaria de Saúde do Estado do Ceará, Brazil.

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Supplementary Information

Supplementary information are available free of charge at http://jbcs.sbq.org.br, as PDF file.

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