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DEHYDRO-α-LAPACHONE OBTAINED FROM *HANDROANTHUS INCANUS* SPECIES DISPLAYS *AEDES AEGYPTI* LARVICIDAL ACTIVITY

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ABSTRACT

Handroanthus incanus wood from certified logging concession, afforded two active compounds against *Aedes aegypti* larvae: lapachol (**1**) and dehydro-*α*-lapachone (**2**). The larvicidal action of lapachol observed in this work is compatible with published data. This is the first publication of a dehydro-*α*-lapachone larvicidal assay revealing it as a potential natural larvicidal agent, since this compound is considered non-toxic and exhibits high activity (LC_{50} 43.38 ppm) according to the criteria commonly used in the literature. Larvicidal assays of *Peltogyne lecointei*, *Martiodendron elatum*, *Dipteryx odorata*, *Erisma uncinatum* and *Allantoma decandra* crude extracts showed no larvicidal activity.

Keywords: *Handroanthus incanus*; *Aedes aegypti*; lapachol; dehydro-*α*-lapachone

INTRODUCTION

Aedes aegypti is a cosmopolitan insect considered the main vector for serious diseases such as dengue, chikungunya, Zika and urban yellow fever **[1]**. The occurrence of these diseases has grown dramatically worldwide in recent decades with more than 50 million people infected annually by dengue alone **[2]**. In addition to vaccines, which are not available for all of the afore mentioned diseases, the simplest preventive action is mosquito elimination **[3]**. As such, synthetic insecticides such as temephos, malathion, fenitrothion and cypermethrin have been used in Brazil over the years **[4]**. However, the use of these products is problematic in terms of their non-selectivity, toxicity and persistence in the environment **[5]**. Furthermore, effective mosquito control using traditional insecticides is threatened by the increasing resistance to the compounds used **[4]**. This scenario underlines the need for the development of new insecticides, especially those of natural origin which can be less harmful to the environment **[6]**, humans and non-target organisms **[7]**. Some plants produce insecticidal phytochemicals to protect themselves from herbivorous insects **[8]** with this feature extended to the wood.

More than half of Brazil (approximately 57%) is occupied by natural forest **[9]**. The rich biodiversity of this forest combined with the uncounted active compounds existing in wood make this raw material an immeasurable source of bioactive products, including those with insecticidal activity.

P. lecointei, *M. elatum*, *D. odorata*, *E. uncinatum*, *A. decandra* and *H. incanus* are all native species in Brazil **[10]**. *H. incanus* stands out in that it belongs to a genus traditionally used to treat syphilis, fever, malaria, cutaneous infections and stomach disorders in the Brazilian Amazon **[11]**. A number of scientific reports documented biological properties for *Handroanthus* species, such as antitumor **[12]**, anti-inflammatory **[13]** and anti-depressant activities **[14]**. In fact, some of the activities presented by these plants were attributed to the secondary metabolite lapachol and other active compounds **[15]**.

MATERIALS AND METHODS

Collection and preparation of samples

Wood samples were collected in Jacundá National Forest, Rondônia State, Brazil, in June 2017 (ICMBio Authorization n° 60658-3). The plants were identified by botanical taxonomist Carlos Alberto da Silva and Luiz Carlos Lobato, both of the Emílio Goeldi Museum. Voucher specimens were deposited in the Brazilian Forest Service under numbers: 1575- *Allantoma decandra* (Lecythidaceae); 1590- *Dipteryx odorata*

(Fabaceae); 1597-*Handroanthus incanus* (Bignoniaceae); 1601- *Martiodendron elatum* (Fabaceae); 1603- *Erisma uncinatum* (Vochysiaceae), and 1610- *Peltogyne lecointei* (Fabaceae).

Wood samples from each species were air-dried and powdered using a knife mill to obtain 1200 g. These plant material were submitted to extraction using ultrasound equipment and 2 L of extractor solvent hexane:ethyl acetate:ethanol: dichloromethane (4:4:1:1 *v/v*). This extraction procedure was carried out 3 times and the fluid extracts concentrated by a rotary evaporator, yielding 12 g of each crude extract.

The major compounds of the *H. incanus* active crude extract (12 g) were isolated by fast chromatography on silica gel 60 using 100% hexane, increasing the polarity with ethyl acetate and finishing the separation process utilizing 100% methanol. A total of 122 fractions (300 mL each) were collected and monitored by thin-layer chromatography. Fraction 20 (400 mg) was re-chromatographed on a silica gel column yielding 150 mL fractions using an isocratic solvent system with hexane:dichloromethane (85:15%). Compounds **1** (80 mg) and **2** (40 mg) were obtained from fractions 20.2 and 20.4, respectively. The structure of both compounds was determined by LCMS and NMR analyses.

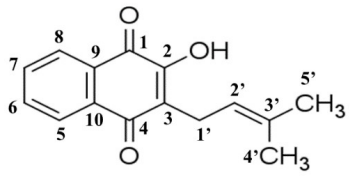
Larvicidal bioassay

Third-instar (L3) *Ae. aegypti* larvae (Rockefeller strain) aged 72 - 96 h were collected from a mosquito colony maintained in the Laboratório de Farmacognosia Insectarium at the Universidade de Brasília without exposure to any insecticide. For each bioassay, the temperature was maintained at 28 ± 2 °C, with a relative humidity of 70 ± 10% and a 12 h photoperiod.

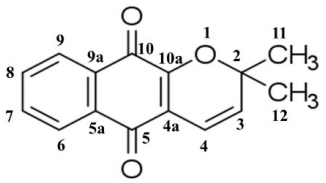
Triplicate assays were conducted in 12-well plates, with each well containing 10 L3 larvae, 3 mL of water and the respective crude extract at 250 ppm. For the LC_{50} calculation of active species, the lethality of samples was tested in quadruplicate, repeated 2 to 3 times using different larvae lots from the same colony, at different concentrations (from 10 to 200 ppm). After 24 h, 48 h and 72 h of exposure, all wells were inspected, the number of dead larvae recorded, and the mortality percentage determined. Larvae with no movement, confirmed by light plate agitation, were considered dead. Samples causing ≥ 80% mortality were considered active. The negative control consisted of exposing 10 larvae to water only. The dose-response curves and LC_{50} calculation, with 95% reliability, were performed using the GraphPad Prism 7.05 Program.

RESULTS & DISCUSSION

The larvicidal assay of *P. lecointei*, *M. elatum*, *D. odorata*, *E. uncinatum* and *A. decandra* crude extracts showed no larvicidal activity against *Ae. aegypti*. However, the *H. incanus* crude extract showed significant activity (LC_{50} 96.03 ppm). Fractionation of this extract resulted in two active compounds **1** and **2 (Figure 1)**. The ^{1}H NMR (600 MHz, $CDCl_3$) spectra of **1** displayed similar signals to those found in the literature for the compound 2-hydroxy-3-(3'-methylbut-2'-enyl)naphthalene-1,4-dione (lapachol) **[16]**. The UPLC-PDA-MS data corroborated the identification with $[M+H]^+$*m/z* 243.1002 (calculated $[M+H]^+$*m/z* 243.1016) and the same retention time as commercial lapachol (Sigma-Aldrich). The chromatographic purity was 97%. The ^{1}H and ^{13}C NMR signals of **2** displayed similarity with the literature data for 2,2-dimethylbenzo[g]chromene-5,10-dione (dehydro-*α*-lapachone) **[17]**. The molecular formula ($C_{15}H_{12}O_3$) was confirmed by UPLC-PDA-MS with $[M+H]^+$*m/z* 241.0852 (calculated $[M+H]^+$*m/z* 241.0859). The chromatographic purity was 95%.



(1)



(2)

Figure 1: Structures of the compounds isolated from *H. incanus* wood

Lapachol **(1)** showed high larvicidal activity against *Ae. aegypti* as found in the literature, although the LC_{50} value (6.41 ppm) was slightly different **[16, 18]**. Methodological differences may have caused this difference in the results. However, in this study using the same methodology, the result found for the isolated lapachol was similar to commercial lapachol (LC_{50} 9.96 ppm).

No similar investigation was found in the literature for dehydro-*α*-lapachone **(2)**. This compound displayed larvicidal activity against *Ae. aegypti* with LC_{50} 43.38 ppm. Adopting several criteria available in the literature, including the most stringent, the LC_{50} result classifies dehydro-*α*-lapachone as a highly active compound against *Ae. aegypti* larvae **[19-22]**. This result combined with the non-toxicity in mice reported in a previous study **[23]** warrant further studies on the use of this compound as a model for new larvicides.

CONCLUSION

The natural origin of dehydro-*α*-lapachone **(2)** and its non-toxicity may provide several advantages in relation to the use of non-natural larvicides, such as

biodegradability and less toxicity to the environment. Therefore, further studies of this raw material are suggested to evaluate the effects on non-target organisms, together with a field evaluation and a commercial viability study.

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