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Larvicidal and growth-inhibiting activities of extract and benzopyrans from *Hypericum polyanthemum* (Guttiferae) against *Aedes aegypti* (Diptera: Culicidae)

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ABSTRACT

Aedes aegypti is a mosquito responsible for transmitting dengue and urban yellow fever viruses with an estimated 3.5 billion people at risk of infection. For this reason, control of larvae is necessary to avoid outbreaks of such diseases. The benzopyrans HP1–HP3, the major compounds of *Hypericum polyanthemum* Klotzsch ex Reichardt, are structurally similar to precocenes, known for their insecticidal activity. In this way, the lipophilic extract obtained from the aerial parts of this plant and its main metabolites were tested in order to determine the larvicidal and growth regulating activity against *A. aegypti*. Both extract (LC_{50} = 121 μg/mL) and benzopyrans showed larvicidal activity. HP1 demonstrated the best activity (LC_{50} = 3.4 μg/mL). HP2 and HP3 were also active, but with a LC_{50} tenfold higher than HP1. Regarding the growth regulating activity, the extract inhibited the pupae formation and adults emergence of *A. aegypti* at LC_{10} and LC_{20}. Therefore, the results demonstrated the potential of *H. polyanthemum* as source of new insecticides.

1. Introduction

The *Aedes aegypti* is an urban mosquito worldwide distributed. This species is responsible for transmitting dengue and urban yellow fever viruses (Gubler, 2005), both diseases that cause great suffering to the population in tropical and subtropical countries. In these countries it is estimated that 3.5 billion people are at risk of dengue infection (Kyle and Harris, 2008). Furthermore, the mosquito has also been responsible for transmitting other arboviral diseases (Schwartz and Albert, 2010; Singh and Unni, 2011).

Control of *A. aegypti* can be accomplished by eliminating the breeding sites of larvae, besides the biological and chemical control with the use of insecticides. The constant use of insecticides, however, can induce resistance in *Aedes* spp. populations (Magdalena et al., 2004; Montella et al., 2007; Tikar et al., 2009; Polson et al., 2010; Dusfour et al., 2011; Lima et al., 2011; Prophiro et al., 2011a), and justifies the prospecting for new alternatives. Some species of *Hypericum* genus (Guttiferae) have been tested against disease vectors and agricultural pests. The hydrodistillate from *H. scabrum* showed larvicidal activity against the vector of the West Nile virus, *Culex pipiens* (Diptera: Culicidae) (Cetin et al., 2011). The aqueous extract of *H. perforatum* presented effects on egg hatching in different life stages of the sweet potato whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) (Al-mazra'awi and Ateyyat, 2009), while the acetone:methanol extract of this plant demonstrated insecticidal and growth regulating activities to milkweed bug, *Oncopeltus fasciatus* (Hemiptera: Lygaeidae) (Alexenizer and Dorn, 2007). Nevertheless, *Hypericum* species have never been investigated against *A. aegypti*.

The lipophilic extract of *Hypericum polyanthemum* presented as the main compounds the benzopyrans 6-isobutyryl-5,7-dimethoxy-2,2-dimethyl-benzopyran (HP1), 7-hydroxy-6-isobutyryl-5-methoxy-2,2-dimethyl-benzopyran (HP2) and 5-hydroxy-6-isobutyryl-7-methoxy-2,2-dimethyl-benzopyran (HP3) (Ferraz et al., 2001). According to literature, these metabolites demonstrated to inhibit the activity of some monoamine oxidases (Gnerre et al., 2001). Besides, these compounds are

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structurally similar to the precocenes, which were described as antifeedant (Szczepanik et al., 2005) and inhibitor of juvenile hormone in several insect species (Bowers et al., 1976; Oliveira Filho et al., 1980; Wilson et al., 1983; Bitsch and Bitsch, 1984; Ayoade et al., 1996) including *A. aegypti* (Cupp et al., 1977; Pridantseva et al., 1981). Growth regulators or anti-juvenile hormone are considered alternative insecticides since they act in the development and reproduction of insects (Braga and Valle, 2007).

Thus, considering the biological effect of the *Hypericum* species and structural aspects of the benzopyrans, the aim of this study was to evaluate the larvicidal and growth regulating activity of the lipophilic extract and benzopyrans from *H. polyanthemum* against *A. aegypti*.

2. Materials and methods

2.1. Plant material

The aerial parts of *H. polyanthemum* Klotzsch ex Reichardt were collected in the flowering period (December, 2008) in Caçapava do Sul, Rio Grande do Sul, Brazil. The plant was identified by Dr. Sérgio Bordignon (UNILASALLE, RS, Brazil) and the voucher specimen (Bordignon 1520) was deposited in the Herbarium of the Universidade Federal do Rio Grande do Sul (ICN). Plant collection was authorized by Conselho de Gestão do Patrimônio Genético (CGEN) and Instituto Brasileiro do Meio Ambiente (IBAMA – 003/2008 P 02000.001717/2008 – 60).

2.2. Extraction and benzopyrans isolation

The dried and powered plant material (aerial parts, 80 g) was extracted with *n*-hexane (5 × 24 h at 20 °C) by static maceration. Subsequently, the extract was evaporated to dryness under reduced pressure and treated with acetone in order to remove waxes and insoluble impurities. The solvent was evaporated providing a benzopyrans enriched fraction (EFHP) (Cargnin et al., 2010).

The benzopyrans were isolated and purified from the EFHP by successive column chromatography on silica gel 60 (70–230 Mesh, Merck, Darmstadt, Germany) using as eluent mixtures of *n*-hexane and dichloromethane in increasing polarity. All solvents employed were analytical grade from F. Maia (Cotia, São Paulo, Brazil). The purified process furnished 0.28, 0.15 and 0.19% (wt compound/wt plant) of HP1, HP2 and HP3, respectively. The benzopyrans were characterized by ^{1}H-NMR (60 MHz) spectroscopy (Eft-60®, Anasazi Instruments) and compared with literature data (Ferraz et al., 2001). Additionally, melting point of HP1 (63–66 °C), HP2 (68–69 °C) and HP3 (65–67 °C) were also evaluated.

2.3. Test solutions

The EFHP (100 mg) and the benzopyrans HP1–HP3 (50 mg each one) were dilute in mineral water with 1% ethanol and 0.025% polysorbate 80 (vehicle) in a total volume of 100 mL (Prophiro et al., 2011b).

2.4. Bioassays of larvicidal activity

Larval susceptibility of *A. aegypti* (Strain: Rockefeller) was determined at 25 °C with relative humidity of 80 ± 10% in an incubator model 132FC Eletrolab®. For the test, 1125 larvae of late third and early fourth instar were exposed to EFHP in a concentration range of 66–200 μg/mL. The assays (n = 3) were conducted in triplicate with 225 larvae (75 per replicate) in each concentration. The benzopyrans (HP1–HP3) were similarly evaluated using 1200 larvae and eight concentrations (5–100 μg/mL). The triplicates were conducted with 150 larvae (50 per replicate) in each concentration. For control, the larvae were exposed to vehicle (1% ethanol and 0.025% polisorbate 80 in water) and water. Mortality was checked after 24 h of exposure to solution tests. Larvae unable to reach the water surface when touched were considered as dead (WHO, 1981a,b).

2.5. Growth-inhibiting activity

EFHP and its main compound, HP1, were selected to evaluate the effect on the development of *A. aegypti*. Sub-lethal concentrations determined by linear regression analysis were used to evaluate the effect of the EFHP (LC_{10} = 53 μg/mL and LC_{20} = 71 μg/mL) and the benzopyran HP1 (LC_{10} = 1.0 μg/mL and LC_{20} = 1.6 μg/mL) in the development of *A. aegypti*. Three replicates were prepared, each one containing 500 mL of solution in plastic containers with a capacity of 1000 mL. In each triplicate, 150 larvae of late third and early fourth instar were placed and puppy food crushed (0.36 g) for larvae feeding (450 larvae per concentration). Pupae formation and adult emergence were checked every 48/72 h during 16 days. Every 96 h food was added to the treated and control group.

2.6. Statistical analysis

Values of lethal concentration, χ^2 test, the slope and confidence intervals were determined by the GW-Basic Probit analysis (Finney, 1971). Differences were analyzed by one or two way ANOVA followed Tukey test according to experimental design. The results were considered statistically significant when $P < 0.05$. Data analyses were performed using the GraphPad Prism 5.0 software.

3. Results and discussion

3.1. Larvicidal activity

The toxicity of the EFHP and benzopyrans HP1, HP2 and HP3 were investigated on larvae of *A. aegypti*. All samples showed larvicidal activity in a range of dose–response concentrations (Tables 1 and 2). The lethal concentrations (LC_{50}) and respective confidence intervals are shown in Table 3. According to these results, the larvicidal activity of the EFHP can be attributed to the benzopyrans since these compounds showed higher toxicity to larvae of *A. aegypti* (Tables 1 and 2). These metabolites are also the main compounds present in the lipophilic extract of *H. polyanthemum* (HP1 = 11.7%, HP2 = 6.3% and HP3 = 8.3%) (Cargnin et al., 2010). When the effect of the benzopyrans is compared, HP1 showed a LC_{50} about 10 times lower than HP2 and HP3 (Table 3), showing clearly its great larvicidal potential. Additionally, no statistical difference between the treatments with HP2 and HP3 was observed (Table 2). Chemically, the benzopyrans HP1, HP2 and HP3 differ regarding the presence and position of the methoxyl and hydroxyl groups (Fig. 1). Despite this, no difference in the

Table 1
Larval toxicity effect of the enriched fraction of *H. polyanthemum* (EFHP) against *A. aegypti* (75 larvae at each triplicate, 225 larvae in each concentration).

Concentration (μg/mL)	Larval mortality (mean % ± SD) EFHP
0[d]	0
66	13.78 ± 5.39
100	43.11 ± 8.14[a,c]
133	68.00 ± 1.88[a,c]
166	81.33 ± 5.65[a,b,c]
200	84.67 ± 6.59[a,b,c]

Significantly different values were detected by one-way ANOVA followed by Tukey test with $P < 0.05$.
[a] Compared to treatment at 66 μg/mL.
[b] Compared to treatment at 100 μg/mL.
[c] Compared to their control group.
[d] Control group: 1% ethanol with 0.025% of polisorbate 80.