SUPPLEMENTARY MATERIAL

Chemical composition and larvicidal activity of the essential oil from the leaves of *Onychopetalum periquino* (Rusby) D.M. Johnson & N.A. Murray
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The essential oil (EO) from the leaves of *Onychopetalum periquino*, obtained by hydrodistillation, was analyzed by gas chromatography coupled with mass spectrometry (GC-MS), and also was investigated for its larvicidal activity against *Aedes aegypti* larvae. Thirteen compounds, representing 91.31% of the crude oil, were identified. Major compounds were sesquiterpene, including β-elemene (53.16%), spathulenol (11.94%) and β-selinene (9.25%). The EO showed high larvicidal activity with a lethal concentration (LC\textsubscript{50}) of 63.75 μg/mL and 100% mortality at 200 μg/mL. These results represent the first report about the chemical composition of *O. periquino* and the first larvicidal evaluation with *Onychopetalum* species.

**Keywords:** *Aedes aegypti*, Annonaceae, β-elemene, spathulenol
1. Experimental

1.1. Plant material and hydrodistillation of the EO

Leaves of *O. periquino* were collected in May 2017 at the Catuaba Experimental Farm, Senador Guiomard, Acre state, Brazil (67°37'39.6" W 10°04'21.1" S). A voucher specimen under registration number 7552 was deposited in the herbarium of the Universidade Federal do Acre (UFAC). The access to genetic heritage was registered at Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen) under the code # AA42598. After collected, the leaves were dried at room temperature (25 °C) and pulverized. Approximately 100 g of the material was subjected to a hydrodistillation with a Clevenger-type apparatus for 4 hours. The EO was dried over anhydrous sodium sulphate, filtered and stored in amber glass vials at -15 °C. The yield was calculated based on the dry weight of plant material.

1.2. GC-MS analysis

The GC-MS analysis was carried out with a Trace Ultra gas chromatograph coupled to an ISQ single quadrupole mass spectrometer (Thermo Scientific). This system was equipped with a Tri Plus autosampler and a TR-5 capillary column (30 m x 0.25 mm x 0.25 μm). Helium was the carrier gas at 1.0 mL/minute flow rate. The injection solution was prepared by dissolving about 10 mg of oil in 1 mL of ethyl acetate, being 1 μL of this solution injected at a split ratio of 1:50. The column temperature program was: 40 °C/4 minutes, a rate of 4 °C/minute to 240 °C, then a rate of 10 °C/minute to 280 °C, and then 280 °C/2 minutes (Silva et al., 2013). The injection, interface and ion source temperatures were 250, 250 and 220 °C, respectively. Mass spectrometry acquisitions were performed at a mass range of *m/z* 40-600.

Identifications were performed based on comparison of the obtained mass spectra with those stored in the NIST library, and also by comparison of retention indexes (RI) with literature data (Adams, 2007). To obtain the RI, a homologous series of linear hydrocarbons (C_7-C_30) was injected, and the calculations performed according to the Van den Dool and Kratz equation (Van den Dool and Kratz, 1963).

1.3. Larvicidal assay

Larvae of *A. aegypti* were obtained from the insectarium of the Laboratório de Malária e Dengue, Instituto Nacional de Pesquisas da Amazônia (INPA). The larvicidal assay was
performed according to a previously reported method (Mesquita et al., 2018), as follows: larvae were kept in plastic trays under controlled temperature (26±2 °C) and humidity (70-80 %) until they reached the third final instar stage. Furthermore, 10 larvae were transferred to plastic cups with a 50-mL capacity, each containing 10 mL of mineral water and grinded fish food (TetraMin Tropical Flakes), followed by the addition of 100 μL solution of EO in dimethyl sulfoxide (DMSO) (25-500 μg/mL). After 24 hours, the number of dead larvae was counted and the lethal percentage calculated. All experiments were carried out in quintuplicate, including a negative control treatment with DMSO, mineral water, larvae, and grinded fish food. Larvicidal activities were reported as lethal concentration at 50% (LC50), representing the concentration in micrograms per milliliter that caused 50% larval mortality, within a confidence interval of 95%. Mortality data were assessed by probit analysis (Finney, 1971). The mortality data were treated by Polo plus® software (Robertson et al., 2003) with 95% confidence interval and values of P < 0.05 were considered statistically significant.

References

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Captions

Table S1. Chemical compositions of leaves EO from *O. periquino.*
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| R.I\(^a\) | R.I\(^b\) | Compounds              | % GC-MS |
|-----------|-----------|------------------------|---------|
| 1384      | 1387      | \(\beta\)-bourbonene   | 1.96    |
| 1391      | 1389      | \(\beta\)-elemene      | 53.16   |
| 1418      | 1417      | (E)-caryophyllene      | 0.76    |
| 1452      | 1452      | \(\alpha\)-humulene    | 2.37    |
| 1459      | 1458      | *allo*-aromadendrene   | 0.96    |
| 1474      | 1476      | \(\beta\)-chamigrene   | 0.76    |
| 1484      | 1489      | \(\beta\)-selinene     | 9.25    |
| 1494      | 1498      | \(\alpha\)-selinene    | 3.78    |
| 1503      | 1509      | \(\alpha\)-bulnesene   | 3.24    |
| 1576      | 1577      | spathulenol            | 11.94   |
| 1580      | 1582      | caryophyllene oxide    | 1.10    |
| 1596      | 1600      | guaiol                 | 0.51    |
| 1606      | 1608      | humulene epoxide II    | 1.52    |

|                     |           |                       |         |
|---------------------|-----------|------------------------|---------|
| Sesquiterpene hydrocarbons (%) | 78.86    |
| Oxygenated sesquiterpenes (%)     | 12.45    |
| Total identified (%)                     | 91.31    |

\(^{\text{a}}\)Retention index on TR5 column calculated according to Van den Dool and Kratz (1963);
\(^{\text{b}}\)Retention index according to Adams (2007)