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ORIGINAL ARTICLE

# CHEMICAL COMPOSITION, LARVICIDAL ACTIVITY AND RESIDUAL EFFECT OF *Pterodon polygalaeflorus* (BENTH.) BENTH. (FABACEAE) FRUIT OIL EXTRACTS AGAINST *Aedes aegypti* (DIPTERA: CULICIDAE)

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## ABSTRACT

The purpose of this study was to investigate the larvicidal activity of *Pterodon polygalaeflorus* oil extract against the mosquito vector *Aedes aegypti*. For this, crushed *P. polygalaeflorus* fruit underwent solvent extraction to obtain the oil extract. The chemical characterization was performed by gas chromatography coupled to mass spectrometry. For the bioassays third instar larvae of *Ae. aegypti* were utilized. Tests were carried out to determine the larvicidal activity and the residual effect under laboratory conditions, as well as field screening (small scale). The major components of *P. polygalaeflorus* oil extract were, respectively, E-caryophyllene, germacrene D and bicyclogermacrene. Lethal concentrations of 50% and 90% were 36.5 and 64.8 µg/mL respectively. The solution presented a residual effect for seven days and the efficiency of the product was preserved under field conditions. The results encourage continuing studies with the oil extract of *P. polygalaeflorus* as a research target for bioinsecticides.

KEY WORDS: Bioinsecticide; sucupira; terpenes; vector control.

## INTRODUCTION

*Pterodon polygalaeflorus*, popularly known as sucupira, sucupira-lisa or faveiro azul, is widely distributed in the Cerrado in Goiás (Lorenzi & Matos, 2002). Its Pterocarpus fruit present a honeycomb arrangement filled with oil extracts that cover the fruit (Arriaga et al., 2000). Phytochemical studies detected the presence of alkaloids, isoflavones and triterpenes in the wood (Marques et al., 1998), as well as terpenes, isoflavones and alcohols present in the seed oil extract (Arriaga et al., 2000; Spindola et al., 2011; Bavaresco et al., 2016; Coelho-de-Souza et al., 2018). Among the main chemical constituents

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found in the species of the genus *Pterodon* are the vouacapane diterpenes, which cause different types of biological activities (Oliveira et al., 2017a). The oil extract of *P. polygalaeflorus* fruit showed antirheumatic activity (Hoscheid & Cardoso 2015); anti-inflammatory and nociceptive activities (Moraes et al., 2012; Coelho-de-Souza et al., 2018); antispasmodic activity (Leonhardt et al., 2010); $Ca^{2+}$ like channel blockers and $Na^{+}$-dependent electromechanical coupling (Evangelista et al., 2007; Reis et al., 2015) as well as immune system modulators, interfering with cell migration and interleukin inhibition (Velozo et al., 2013, Alberti et al., 2014, Leal et al., 2018).

Several plant-based substances have shown promising activity in insect control (Viegas-Júnior, 2003; Rattan, 2010; Zoubiri & Baaliouamer, 2014), especially against Culicidae vectors of important diseases (Arruda et al., 2003; Barreto et al., 2006; Geris et al., 2008; Guissoni et al., 2013; Oliveira et al., 2016; Romano et al., 2018). Previous studies have shown that *P. polygalaeflorus* has a larvicidal activity on the species *Aedes aegypti*, the mosquito vector of the yellow fever, dengue, chikungunya and zika viruses (Arriaga et al., 2000; Omena et al., 2006; Pimenta et al., 2006). However, there are no estimates regarding the persistence of the lethal effect of these substances, not even under field conditions. Thus, the objective of this research was to evaluate the residual effect and the larvicidal activity of the oil extract of *P. polygalaeflorus* against *Ae. aegypti* under field conditions.

## MATERIALS AND METHODS

### *Plant material*

*P. polygalaeflorus* fruit were collected in the Serra da Mesa lake area (14° 13’24’2’S – 48° 12’33’7’W), Northwest of Goiás, Brazil, in September 2016. An exsiccata was authenticated by Dr. José Ângelo Rizzo and deposited in the Herbarium of the Conservation Unit, under No. UFG60048, in the Botany Department of the Federal University of Goiás (UFG).

### *Extraction and chemical composition analysis*

For the extraction of the oil extract of *P. polygalaeflorus*, 80.4 g of fruit were ground in an analytical mill. The solvent extraction process was applied, using absolute ethanol as the extractive solution at room temperature. The extracted solution was concentrated in a rotary evaporator and the oil extract obtained was subjected to gas chromatographic analysis coupled to mass spectrometry (GC/MS) in a Shimadzu apparatus, model GC-MSQP5050A, with capillary silica column SBD-5 (30 m × 0.25 mm × 0.25 m). The temperature

was programmed as follows: 60-240°C at 3°C/min, 280°C at 10°C/min and 10 min at 280°C at a flow rate of 1 mL/s. The injection port was set at 225°C. Other operating parameters: interface temperature 240°C; electron ionization at 70 eV with a scanning mass band of 40-350 m/z and a sampling rate of 1 scan/s. Retention rates were calculated by co-injecting C9-C26 n-alkanes. The chemical components of *P. polygalaeflorus* oil extract were identified by comparison with mass spectra and retention indices reported in the literature (Adams, 2007).

*Bioassays*

*Larvicidal activity*

Bioassays were carried out in the Laboratory of Insect Biology and Physiology (IPTSP/UFG) in a biological chamber climatized at 25°C ± 1°C, relative humidity of 85% ± 5%. The 3rd instar larvae ($L_3$) of *Ae. aegypti* were utilized. The tests followed the guidelines proposed by the World Health Organization (WHO, 2005). For this test, a stock solution with pre-solubilized *P. polygalaeflorus* oil extract was prepared in 0.4 mL of dimethyl sulfoxide (DMSO) and distilled water to a final concentration of 100 µg/mL. Bioassays were performed in serial dilutions up to 5 µg/mL in polystyrene containers containing 25 mL of solution and 20 $L_3$ added thereafter. Mortality was verified after 24 hours of exposure of the larvae to the solutions, confirmed by the absence of response to mechanical stimuli and body darkening. The negative control was performed with a solution of water and DMSO and the positive control with temephos (Abate®) at 0.012 µg/mL.

Residual effect

To verify the persistence of the larvicidal effect of *P. polygalaeflorus* oil extract, 20 L3 of *Ae. aegypti* were exposed to 200 mL of 90% lethal concentration (LC) test solution in polystyrene containers under the above-mentioned laboratory conditions. The test solution utilized in the residual effect bioassay was prepared as described in the previous topic. After 24 hours of exposure mortality events were quantified and the larvae replaced with other recent $L_3$ without renewal of the test solution. The exposure and counting schemes followed until total loss of the lethal effect (Romano et al., 2018). For the negative control, water and DMSO were used and for the positive control, temephos (Abate®) at 0.012 µg/mL was used. All the bioassays were performed in triplicate.

*Larvicidal activity in the field (small-scale)*

In order to test the effectiveness of *P. polygalaeflorus* oil extract in extra laboratory conditions, small-scale field trials with oil extract solution in the $LC_{90}$ were performed in three types of containers simulating the most common breeding sites: plastic, glass, and tire (WHO, 2005). Each container received 150 mL of the test solution and 20 $L_3$ of *Ae. aegypti*. The containers were distributed in isolated places in the courtyard of the Institute of Tropical Pathology and Public Health (IPTSP/UFG) in Goiânia, GO, in July 2017. The chosen sites were in the shade and out of the way of animals and/or humans. The larvicidal activity was evaluated 24 hours after exposure of the larvae to the test solution. For all assays, there was a negative control with water and DMSO and positive control with temephos (Abate®) at 1 µg/mL. Bioassays were performed in triplicate.

*Statistical analysis*

The data obtained in the bioassays of larvicidal activity were submitted to a linear regression of Probit to obtain lethal concentrations (LC's) of 50, 90 and 99% ($\alpha = 0.05$). Statistical differences in mortality among breeders were calculated by the $\chi$-square test ($\alpha = 0.05$) by the software STATISTICA 12.0 (StatSoft, 2013).

## RESULTS AND DISCUSSION

The extraction procedure of *P. polygalaeflorus* oil extract yielded 31.1%. Chromatographic analysis showed 15 chemical compounds (Figure 1; Table 1), of which E-caryophyllene, germacrene D and bicyclogermacrene were major. More than 95% of the compounds detected in *P. polygalaeflorus* oil extract are sesquiterpenes. The oil extract presented the diterpene voucapane 6α-hidroxyvouacapane-7,17β-lactone, characteristic of the species. Favareto et al. (2017) isolated different voucapane diterpenes in samples of *Pterodon* fruit oil extract. According to the authors, the extraction method may interfere with the presence of these compounds in the final product. A study evaluating the chemical composition of the essential oil extract of *P. polygalaeflorus* found four compounds common to those found in this research: α-humulene, aromadendrene, allo-aromadendrene and bicyclogermacrene (Evangelista et al., 2007; Coelho-de-Souza et al., 2018). Terpenes are produced by plants for protection against herbivory, their apolar character facilitates passage through membranes, being generally associated with toxicity to herbivores (Rattan, 2010).

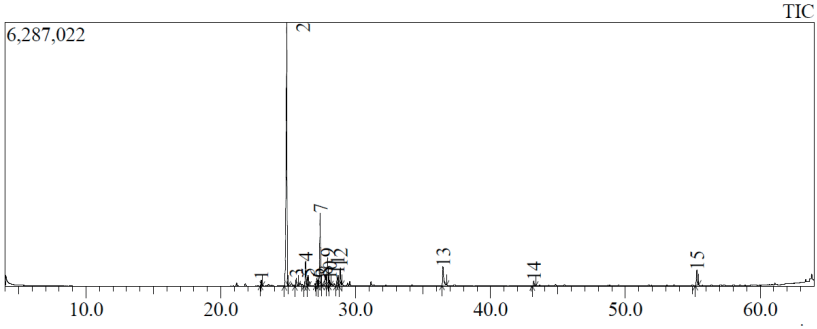


*Figure 1.* Chromatogram obtained of *Pterodon polygalaeflorus* fruit oil extract by CG-MS method. Gas chromatographic analysis coupled to mass spectrometry (GC/MS)

*Table 1.* Chemical constituents in *Pterodon polygalaeflorus* fruit oil extract obtained by solvent extraction.

| Compound | KI | RT | % |
|---|---|---|---|
| α ylangene[1] | 1365.0 | 1373 | 1.0 |
| E-caryophyllene[1] | 1409.8 | 1417 | 53.5 |
| aromadendrene[1] | 1426.8 | 1439 | 1.4 |
| α humulene[1] | 1444.0 | 1452 | 4.9 |
| allo-aromadendrene[1] | 1448.0 | 1458 | 1.7 |
| 9-epi-E-caryophyllene[1] | 1464.0 | 1464 | 1.3 |
| germacrene D[1] | 1470.0 | 1484 | 13.1 |
| γ-amorphene[1] | 1479.5 | 1495 | 3.0 |
| biciclogermacrene[1] | 1483.6 | 1500 | 5.5 |
| α-muurolene[1] | 1487.1 | 1500 | 1.0 |
| γ-cadinene[1] | 1500.8 | 1513 | 1.9 |
| δ-cadinene[1] | 1506.6 | 1522 | 3.1 |
| 2E, 6Z-farnesal[1] | 1705.3 | 1715 | 4.6 |
| Z, Z-Geranyl linalol[2] | 1898.7 | 1960 | 0.9 |
| 6α-hidroxyvouacapane-7,17β-lactone[2]* | 2294.9 | 2308 | 3.2 |
| Total | | | 100.1 |

KI – Kovatz Index; RT – Retention time in literature; [1] - Sesquiterpenes; [2] - Diterpenes; * - Founded in Favareto et al. 2017.

In the evaluation of the biological activity against *Ae. aegypti* the following were obtained: $LC_{50}$ = 36.5 µg/mL (CI: 29.0 - 43.9 µg/mL), $LC_{90}$ = 64.9 µg / mL (CI: 58.7 - 71.0 µg/mL) and $LC_{99}$ = 71.3 µg/mL (CI: 64.2 - 78.3 µg/mL). Dead larvae observation showed a reduction in the length of the larva treated in relation to the larva in the negative control. Pimenta et al. (2006) evaluated the larvicidal activity of hexanic extract as well as of the 6-α-acetoxyivouacapane obtained from *P. polygalaeflorus* fruit against *Ae. aegypti*, yielding $LC_{50}$ of approximately 24 µg/mL and 180 µg/mL. Omena et al. (2006) investigated the larvicidal activity of three vouacapane diterpenes isolated from *P. polygalaeflorus* fruit oil extract, one of them being 6α-hydroxyvouacapane-7,7β-lactone, showing $LC_{50}$ close to 50 µg/mL. Promising results have also been reported for the seed oil extract of *Pterodon emerginatus* against *Ae. aegypti* and *Culex quinquefasciatus* (Oliveira et al., 2016; Oliveira et al., 2017b). The mechanism of action of *P. emarginatus* oil extract appears to be strongly linked to neurotoxicity due to the inhibition of acetylcholinesterase (Oliveira et al., 2016), as well as alterations in the integument of larvae exposed to treatment, which, according to the authors, could contribute to the lethal effect (Oliveira et al., 2017b).

The oil extract of *P. polygalaeflorus* presented residual effect for seven days (Figure 2) with 100% dead larvae up to the fourth day of exposure. Similar results were found in the bioassays with Cashew Nut Shell Liquid (CNSL) of the species *Anacardium humile* (Romano et al., 2018). Other plant oil extracts that had a residual effect between seven and nine days were *Curcuma zedoaria* (Champakaew et al., 2007) and *Annona coriacea* (Dill et al., 2012), respectively. The persistence of the lethal effect presented by *P. polygalaeflorus* may be considered promising as it is similar to the time necessary for the complete development of the insect (Romano et al., 2018).

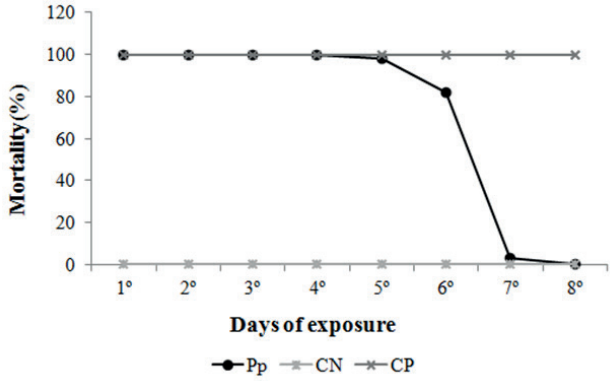


*Figure 2.* Residual effect of *Pterodon polygalaeflorus* fruit oil extract against 3rd instar larvae of *Aedes aegypti*
*Pterodon polygalaeflorus* (Pp), Negative control (CN), Positive control (CP)

Field bioactivity tests were performed during the dry season in order to avoid rainfall interference in the results. The mean temperature in the study area was 19.9°C, relative humidity 63.9%, without precipitation records. The oil extract of *P. polygalaeflorus* presented 88.3% efficient larvicidal activity in the tire, 98.3% in the glass and 100% in the PET plastic container. There was a reduction in the percentage of dead larvae in the tire and glass tests. The variation in mortality among containers was not statistically significant when compared with the 1 ppm temephos solution (positive control) or when compared among each other (Figure 3). There was no mortality in larvae exposed to the negative control solution. The reduction in mortality in the tire type containers had been observed in other studies, suggesting that the type of container may interfere with product stability (Carvalho & Silva, 1999; Forattini & Brito, 2003; Oliveira et al., 2015). Oliveira et al. (2015) evaluated the interference of breeding sites in the determination of LC's for fractions of *Copaifera langsdorffii* oil extract resin, where they noted an increase in LC's of all fractions in the tire-type breeding site.

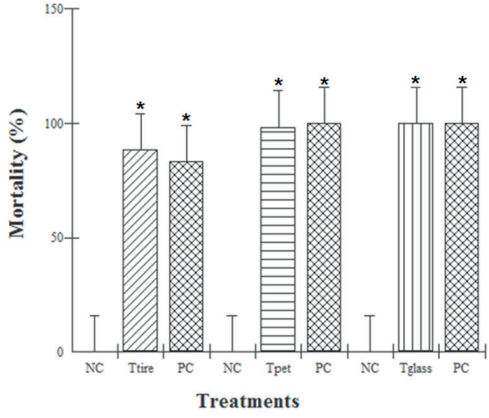


*Figure 3.* In field bioactivity results of *Pterodon polygalaeflorus* fruit oil extract against 3rd instar larvae of *Aedes aegypti*. *no significance (p value < 0,005).

Considering that most botanical products are composed of complex mixtures that can cause toxicity by different mechanisms, there is an alternative to the control of vector insects (Rattan, 2010), in particular, to mitigate the effects of the emergence of synthetic insecticide resistant strains. However, although promising, very little is known about the mechanism of action of these products (Zoubiri & Baaliouamer, 2014). In this sense, the results obtained with the oil extract of *P. polygalaeflorus* fruit indicate that it can be considered a potential target in the research for new insecticides to control *Ae. aegypti* as its $LC_{50}$ is less than 50 µg/mL, and the oil extract presents residual

effect and persistence of the lethal effect under field conditions. Thus, further studies should be performed to elucidate possible mechanisms involved in the lethal effect, as well as evaluation of the field activity on a large scale.

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