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Chemical composition, cytotoxicity and larvicidal activity against *Aedes aegypti* of essential oils from *Vitex gardineriana* Schauer

[Composición química, citotoxicidad y actividad larvicida contra *Aedes aegypti* de aceites esenciales de *Vitex gardneriana* Schauer]

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Abstract: *Vitex gardneriana* Schauer (Lamiaceae) popularly known as "jaramataia", is a shrub commonly found in caatinga biome located in Northeast Brazil. In folk medicine, its leaves have been used as analgesic and anti-inflammatory agents. The chemical composition of the essential oil from leaves obtained by hydrodistillation was analyzed and identified by GC-MS and GC-FID and showing a total of 26 constituents (95.9%) being 2 monoterpenes (0.4%) and 24 sesquiterpenes (95.4%). The main constituents identified were cis-calamenene (29.7%), 6,9-guaiadiene (14.5%) and caryophyllene oxide (14.0%). The essential oil has been demonstrated high larvicidal activity against *Aedes aegypti* (LC_{50} = 28.0 µg/mL). In the evaluation of the bioassay with *Artemia salina* the essential oil showed LC_{50} = 98.11 µg/mL. In addition, the essential oil did not show cytotoxicity (IC_{50} > 2.50 mg/mL) by the hemolysis assay.

Keywords: *Vitex gardineriana*; Essential oil; Larvicidal activity; Cytotoxicity

Resumen: *Vitex gardneriana* Schauer (Lamiaceae) popularmente conocido como "jaramataia", es un arbusto que se encuentra comúnmente en el bioma de caatinga ubicado en el noreste de Brasil. En medicina popular, sus hojas se han utilizado como analgésicos y agentes antiinflamatorios. La composición química de los aceites esenciales de las hojas obtenidas por hidrodestilación fue analizada e identificada por GC-MS y GC-FID y mostrando un total de 26 constituyentes (95.9%) siendo 2 monoterpenos (0.4%) y 24 sesquiterpenos (95.4%). Los componentes principales fueron cis-calamenene (29.7%), 6,9-guaiadiene (14.5%) y caryophyllene oxide (14.0%). El aceite esencial ha demostrado una alta actividad larvicida contra *Aedes aegypti* (CL_{50} = 28.0 µg/mL). En la evaluación del bioensayo con *Artemia salina*, el aceite esencial demostró CL_{50} = 98.11 µg/mL. Además, el aceite esencial no mostró citotoxicidad (IC_{50} > 2.5 mg / mL) mediante el ensayo de hemólisis.

Palabras clave: *Vitex gardineriana*; Aceites esenciales; Actividad larvicida; Citotoxicidad.

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Gas Chromatography-Mass Spectrometry

GC-MS for the analysis of the volatile constituents was carried out on a Hewlett-Packard Model 5971 GC/MS using a non-polar DB-5 fused silica capillary column (30 mm x 0.25 mm i.d., 0.25m film thickness); carrier gas helium, flow rate 1 mL/min and with split ratio 1:1. The injector temperature and detector temperature were 250º C and 200º C, respectively. The column temperature was programmed from 35º C to 180º C at 4º C/min and then 180º C to 250º C at 10º C/min. Mass spectra were recorded from 30 - 450 *m/z*. Individual components were identified by matching their 70 eV mass spectra with those of the spectrometer data base using the Wiley L-built library MS (Santos *et al*., 2017) searches using retention indices as a preselection routine, as well as by visual comparison of the fragmentation pattern with those reported in the literature (Adams, 2017).

Larvicidal bioassay

Essential oils were placed in beakers and dissolved in 20 mL H_2O/DMSO 1.5% (v/v) at concentrations of 50-500 mg/mL, followed by the addition of 50 larvae at the third-instar. For each experiment, both positive (Temephos at 3.22 µg/mL) 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 (Sousa *et al*., 2012).

Toxicity front Artemia salina Leach.

The toxicity test for *A. salina* was performed following the methodology proposed by Meyer *et al.* (1982), which consists of the hatching of *A. salina* eggs in artificial saline water, and then the larvae were collected for the bioassays. The dissolution of the samples and of the blank test were performed with 3.9 mL of saline water, 1mL of saline water with *A. salina* and 0.1 mL of concentrated DMSO. The procedure was performed in triplicate at concentrations of 1000, 100, 10 and 1 µL, and added 10 *Artemia* larvae in each vial, the survivors count being after 24 hours. The negative control was 100 µL to 2% of DMSO and 4.9 mL of distilled water. Experiment performed in triplicate. After analyzing the results, the procedure is repeated at intermediate concentrations in an attempt to find the lethal concentration, capable of killing 50% of microcracks. For the calculation of LD_{50}, Microsoft Excel Program was used by calculating linear regression.

Hemolytic Analysis

This test was performed in 96-well plates using 1% fresh blood erythrocytes suspension in 0.85% NaCl containing 10 mM $CaCl_2$. The essential oil was assayed at concentrations ranging from 0.07 to 2.0 mg/mL. After 1h incubation, the plate was centrifuged and the supernatant containing hemoglobin was measured spectrophotometrically for the absorbance 540 nm (Multimode Detector DTX 880, Beckman Couter) (Jimenez *et al*., 2003).

Statistical analysis

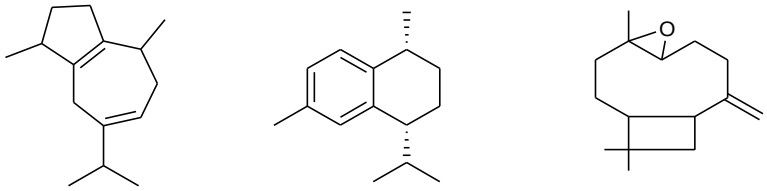
The LC_{50} value of essential oil from leaves of *V. gardneriana* was calculated using the probit analysis (Finney, 1971) of the mortality data derived from bioassays.

RESULTS AND DISCUSSION

The essential oil from leaves of *V. gardneriana* was analyzed by GC/MS and GC/FID and was obtained with a yield of 0.06%. A total of 26 constituents (95.9%) organized in order of elution in a DB-5 column were identified, being 2 monoterpenes (0.4%) and 24 sesquiterpenes (95.5%) (Table No. 1). The chemical composition of the essential oil showed greater abundance of sesquiterpenes and the main constituents identified were *cis*-calamenene (29.7%), 6,9-guaiadiene (14.5%) and caryophyllene oxide (14.0%) (Figure No. 1).

This result are in agreement with previous reports on the chemical composition of essential oils from *Vitex* genus. For instance, the chemical composition of essential oil from *V. agnus castus* and *V. negundo* showed the compounds 1,8-cineole, β-phellandrene, α-terpinyl acetate, β-caryophyllene, bicyclogermacrene, δ-elemene, β-eudesmol, carene, caryophyllene oxide, 1-oceten-3-ol, α-pinene, 4-terpineol, γ-terpinene, viridiflorol and β-eudesmol (Lal *et al.*, 2007; Khokra *et al.*, 2008). It is possible to observe a diversity of terpenoids, mainly sesquiterpenes which were present in great abundance (Stojković *et al.*, 2011).

Figure No. 1
The main constituents of the essential oil of the leaves of *V. gardneriana*



6,9-Guaiadiene *cis*-Calamenene Caryophyllene oxide

The chemical composition of volatile metabolite profiles to 13 other *Vitex* species showed a large number of terpenes, especially sesquiterpenes with gem-dimethylcyclopropyl subunits on seven-member ring compounds like the sesquiterpenoid 6,9-guaidiene one of the major constituents of essential oil leaves from *V. gardneriana*, which can be a good chemosystematic biomarker for the *Vitex* genus (Sena Filho *et al*., 2017).

Table No. 1
Chemical composition of essential oil from leaves of *V. gardneriana*

Compound	RI[a]	RI[b]	Percent Composition
α-Pinene	941	939	0.2
β-Pinene	984	979	0.2
α-Cubebene	1354	1348	1.5
alfa-Copaene	1380	1376	4.3
β-Bourbonene	1388	1388	0.1
β-Cubebene	1393	1387	0.6
β-Elemene	1395	1390	1.4
β-Caryophyllene	1423	1419	2.8
6,9-Guaiadiene	1448	1444	14.5
α-Caryophyllene	1458	1457	0.8
trans-Cadina-1(6),4-diene	1477	1476	0.7
γ-Muurolene	1484	1479	1.0
trans-Muurola-4(14),5-diene	1494	1493	3.0
α-Muurolene	1502	1500	1.1
α-Bulnesene	1508	1509	0.3
γ-Cadinene	1520	1513	1.7
cis-Calamenene	1527	1529	29.7
trans-Cadina-1,4-diene	1536	1534	2.4
α-Calacorene	1547	1547	4.4
β-Calacorene	1568	1565	0.9
Caryophyllene oxide	1586	1583	14.0
1-epi-Cubenol	1632	1628	3.2
Cubenol	1646	1646	3.3
α-Cadinol	1659	1654	1.5
cis-Calamenen-10-ol	1664	1661	1.2
Cadalene	1679	1676	1.1
Total			**95.9**

[a]Retention indices on DB-5 column; [b]Literature retention índices

In recent years, search for efficient natural compounds with larvicidal activity and low environmental toxicity has increased, essential oils have been a promising alternative for pest control (Pavela, 2015). For instance, the larval bioassay against *A. aegypti* of *Bauhinia pulchella* and *B. ungulata* essential oils showed LC_{50} values of 105.9 ± 1.5 and 75.1 ± 2.8 µg/mL (Sousa *et al*., 2016). The *A. aegypti* larvae mortality rate of 100% was obtained after 24 h of treatment with the essential oil of *Eugenia candolleana* at concentrations of 0.50 µg/µL and the LC_{50} at this time was estimated to be 0.30 µg/µL (Neves *et al*., 2017). Essential oils of leaves, stalks, and inflorescences from *C. jacobinensis* were tested at different concentrations against *A. aegypti* and showed LC_{50} of 79.3, 117.2, 65.8 µg/ml (Pinto *et al*., 2016) and the essential oils from *Piperaceae* species studied showed LC_{50} of 34-55 µg/mL (Santana *et al*., 2015).

The essential oil from leaves of *V. gardneriana* was evaluated for its activity against instar III larvae of *Aedes aegypti* using Temephos® (O,O'-(thiodi-4,1-phenylene) bis (O,O-dimethyl phospho-rothioate) as positive control. The mortality percentages were calculated after 24 h. The larvicidal effect of this essential oil is shown in Table No. 2. The essential oil showed LC_{50} value of 28.0 µg/mL, which can be considered very active, because in an earlier study, essential oils with LC_{50} values smaller than 100 µg/mL are promising larvicidal agents against *Aedes aegypti* (Cheng *et al.*, 2003).

The highly larvicidal activity showed by essential oil from leaves of *V. gardneriana* can be explained by the presence of the monoterpenes *α*-pinene and *β*-pinene in this essential oil, which have been reported to be active against *A. aegypti* (Cheng *et al*., 2009; Perumalsamy *et al*., 2009; Govindarajan, 2010) and for predominance of sesquiterpenes in the essential oil, once several studies have shown that essential oils containing high levels of sesquiterpenoid compounds possess significant larvicidal activities (Magalhães *et al*., 2010; Gois *et al*., 2011; Sousa *et al.*, 2016). The results showed a possible relationship between larvicidal activity and presence of sesquiterpenes in the essential oil, since these substances can serve to increase the transmembrane absorption of lipophilic drugs which can kill larvae of *A. aegypti* and that may also act synergistically (Santos *et al*., 2017).

Table No. 2
Larval mortality (%) of essential oils against third-instar of *Aedes aegypti* larvae

Concentration (µg/mL)	Average (%) of dead larvae after 24 h (%)	LC_{50} (µg/mL)
500	99.9	28.0
250	99.9	
100	92.0	
50	75.3	

Lethality bioassays with *A. salina* allow the evaluation of general toxicity and therefore it is considered essential as a preliminary bioassay in the study of compounds with potential biological activity. In the evaluation of the bioassay with *A. salina*, LC_{50} values below 1000 µg / mL represent non-toxic products (Meyer *et al*., 1982). In this way, it can be suggested that essential oil extracted from the leaves of *V. gardneriana* is bioactive against *A. salina*, since the LC_{50} value obtained for essential oil was less than 1,000 µg/mL, presenting LC_{50} of 98.11 µg/mL. This LC_{50} result allows correlation with other potential biological activities such as larvicidal and antifungal activity (Sobrinho *et al.*, 2016).

The tests to determine the *in vitro* hemolytic activity of the essential oil from *V. gardineriana* was performed at the concentrations of 0.07 to 2.0 mg/mL. Based on the observed results, we can say that essential oil did not present hemolytic activity (IC_{50} > 2.50 mg/mL). The hemolytic action of a natural product or drug can occur by various mechanisms, from dissolving or increasing the permeability of cell membranes to complete cell lysis (Sayes *et al*., 2007). According to Miyazaki *et al.* (2013), methods for determining the hemolytic activity *in vitro* consist of checking for potential damage caused by the substances present in essential

oils to the membranes of erythrocytes, which when undergoing lysis release hemoglobin.

CONCLUSION

The chemical characterization of the *V. gardneriana* oil allowed to identify a total of 26 constituents (95.9%) being 2 monoterpenes (0.4%) and 24 sesquiterpenes (95.5%). The essential oil showed greater abundance of sesquiterpenes and the main constituents were *cis*-calamenene (29.7%), 6,9-guaiadiene (14.5%) and caryophyllene oxide (14.0%). The essential oil of the leaves from *V. gardneriana* has been demonstrated high larvicidal activity against *A. aegypti* LC_{50} value of 28.0 μg/mL, which can be explained by a possible relationship between larvicidal activity and the presence of monoterpenes and sesquiterpenes, since these substances can serve to increase the transmembrane absorption of lipophilic drugs which can kill larvae of *A. aegypti* and synergistically effects. In the evaluation of the bioassay with *A. salina* it can be suggested that essential oil extracted from *V. gardneriana* is bioactive against *A. salina*, since the LC_{50} value was 98.11 μg/mL. In addition, the essential oil did not show cytotoxicity (IC_{50} > 2.5 mg/mL) by the hemolysis assay.

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