© 2016
Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 15 (2): 122 - 127
ISSN 0717 7917
www.blacpma.usach.cl

Artículo Original | Original Article

Chemical Composition and larvicidal activity against *Aedes aegypti* of essential oils from *Croton jacobinenesis* Baill.

[Composición química y actividad larvicida contra *Aedes aegypti* de los aceites esenciales de *Croton jacobinenesis* Baill.]

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Abstract: The chemical composition of essential oils from leaves, stalks and inflorescences of *Croton jacobinensis* obtained by hydrodistillation were analyzed by GC-MS. E-caryophyllene, 1,8-cineole, α-pinene, viridiflorene, δ-cadinene were the main components in essential oils from plant parts. Essential oils of leaves, stalks, and inflorescences were tested at different concentrations against instar III larvae of *Aedes aegypti* and showed LC_{50} of 79.3, 117.2, 65.8 µg/ml, respectively.

Keywords: *Croton jacobinensis*, essential oil, *Aedes aegypti*, larvicidal activity

Resumen: La composición química de los aceites esenciales de las hojas, los tallos y las inflorescencias de *Croton jacobinensis* obtenidos por hidrodestilación se analizaron mediante GC-MS. E-cariofileno, α-pineno, 1,8-cineol, viridifloreno, δ-cadineno fueron los principales componentes de los aceites esenciales de partes de la planta. Los aceites esenciales de las hojas, tallos e inflorescencias fueron probados en diferentes concentraciones en contra de instar III de las larvas de *Aedes aegypti* y mostraron LC_{50} de 79,3; 117,2; 65,8 µg/ml, respectively.

Palabras clave: *Croton jacobinensis*, aceites esenciales, *Aedes aegypti*, actividad larvicida

Recibido | Received: January 12, 2015
Aceptado | Accepted: September 28, 2015
Aceptado en versión corregida | Accepted in revised form: February 1, 2016
Publicado en línea | Published online: March 30, 2016
Este artículo puede ser citado como / This article must be cited as: CCC Pinto, JESA de Menezes, SMC Siqueira, DS Melo, CRS Feitosa, HS Santos. 2016. Chemical Composition and larvicidal activity against *Aedes aegypti* of essential oils from *Croton jacobinenesis* Baill.. **Bol Latinoam Caribe Plant Med Aromat** 15 (2): 122 – 127.

INTRODUCTION

Dengue is an acute febrile disease that affects humans and constitutes a serious public health problem worldwide. It is estimated that 50 to 100 million people become infected each year in more than 100 countries in all continents. Approximately 550.000 patients require hospitalization and 20.000 die because of complications. It is caused by four serotypes of arbovirus belonging to the Flavivirus belonging to the family Flaviviriade (serotypes 1, 2, 3 and 4) and the transmission to man is made by *Aedes* mosquitos, especially species *aegypti* and *albopictus* (Gubler, 2004; Simas *et al*., 2004).

The vector control is based on the use of larvicides. Organophosphate insecticides, such as, temephos, have been used as larvicide in several countries. The repetitive use of these insecticides has made the *A. aegypti* increasingly resistant, which is a major barrier to control the mosquito that transmits dengue. This resistance results in an increased frequency of insecticide application, increasing dosages, environmental damage and outbreaks of diseases when the vectors cannot be controlled. Thus, resistance to pesticides has guided research to find new methods to control *A. aegypti* (Cheng *et al*., 2003; Geris *et al*., 2008).

In recent years, search for efficient natural compounds with larvicidal activity and low environmental toxicity has increased, so natural products have been a promising alternative for pest control. Essential oils are outstanding candidates, since they are in some cases, highly active against *A. aegypti* (Araújo *et al*., 2003; Costa *et al*., 2004; Albuquerque *et al*., 2004; Menezes *et al*., 2006; Santos *et al*., 2006), readily available, and economically viable. A study conducted with essential oil components revealed that the most active compounds against *A. aegypti* are phenylpropanoids, monoterpenes and sesquiterpene alcohols (Lima *et al*., 2006).

Croton is an extensive genus comprising around 1.300 species from Euphorbiaceae family. This genus with a wide range of bioactive compounds has been found to exert vasorelaxant activity (Baccelli *et al*., 2007). Popular uses include treatment of cancer, constipation, diabetes, digestive problems, dysentery, external wounds healing, fever, hypercholesterolemia, hypertension, inflammation, intestinal worms, malaria, pain, ulcers, and weight-loss (Salatino *et al*., 2007).

Larvicidal activity of essential oils from Northeastern Brazilian plants showed good results for *Croton zehntneri*, essential oils of leaves, stalks, inflorescences and phenylpropanoid derivative *E*-anethole were tested at different concentrations against instar III larvae of *A. aegypti* and showed LC_{50} 56.2, 51.3, 57.5 and 69.2 μg/mL, respectively (Santos *et al*., 2007). Likewise, the hydrolates of stalk and leaf from *C. nepetaefolius* and *C. zehntneri* and leaf hydrolate of *C. argyrophylloides* presented 100% mortality against larvae, the main compounds of this hydrolates are oxygenated phenylpropanoids, monoterpenes and sesquiterpenes, compounds recognized larvicidal properties (Lima *et al*., 2006).

Croton jacobinensis, popularly known as "white quince" has a pleasant aroma. Ethnopharmacological information refers to this plant as having healing, anti-inflammatory and vasodilator properties as well as insect repellent properties (Oliveira *et al*., 2001). As part of a program to evaluation of essential oils from northeastern Brazil flora, the present here in report for the first time, composition and larvicidal activity of essential oil from leaves, stalks and inflorescences against *A. aegypti* of *C. jacobinensis*.

MATERIALS AND METHODSs

Plant material

Leaves, stalks and inflorescences of *Croton jacobinensis* Baill. were collected in April, 2013 in Itapipoca County, State of Ceará, Northeast of Brazil. A voucher specimem (43.045) was deposited at the Herbário Prisco Bezerra, at the Biology Department, at Federal University of Ceará, Brazil.

Extraction of the essential oils

The fresh leaves (900 g), stalks (1,500 g) and inflorescences (121 g) of *C. jacobinensis* were subjected to hydrodistillation in a Clevenger-type apparatus for 2 hours to afford 0.80% (w/w), 0.70% (w/w) and 0.05% (w/w) of pale yellow oils, respectively. The yield (w/w) was determined by the mass ratio of the oil obtained and the mass of fresh plant material used in the extraction. After being filtered and dried over anhydrous sodium sulfate, the isolated oils were stored in sealed glass vials, which

were maintained under refrigeration at 4° C until GC-MS and GC-FID analysis (Leandro *et al.*, 2014).

Gas Chromatography- Flame Ionization Detection

GC-FID for the quantitative analysis was carried out on a Shimadzu GC-17A gas chromatograph using a dimethylpolysiloxane DB-5 fused silica capillary column (30 mm x 0.25 mm, film thickness 0.25 μm). H_2 was used as the carrier gas at a flow rate of 1 mL/min and 30 psi inlet pressure; split, 1:30; temperature program: 35-180° C at 4° C/min, then heated at a rate of 17° C/min to 280° C and held isothermal for 10 min; injector temperature, 250° C; detector used FID, detector temperature, 250° C.

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., 0.25 μm 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 35° C to 180º C at 4º C/min and then 180° 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 (Feitosa *et al.*, 2009) searches using retention indices as a preselection routine (Alencar *et al*., 1990), as well as by visual comparison of the fragmentation pattern with those reported in the literature (Adams, 2001).

Larvicidal bioassay

Essential oils were placed in beakers and dissolved in 20 mL H2O/DMSO 1.5% (v/v) at concentrations of 50-500 μg/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 (Santiago *et al*., 2006; Feitosa *et al.*, 2009; Sousa *et al.*, 2012).

RESULTS AND DISCUSSION

The essential oils extracted from leaves, stalks and inflorescences of *C. jacobinensis* were analyzed by CG/MS and quantified by GC-FID. A total of 40 compounds were identified in three oil samples and they are arranged in Table 1 in the order of elution from a DB-5 column.

Twenty five constituents (96.7%) were identified in the oil from leaves, representing seven monoterpenes (38.7%) and eighteen sesquiterpenes (58.0%). In the oil from stalks, twenty two constituents (95.5%) were identified, comprised of five monoterpenes (15.6%) and seventeen sesquiterpenes, which represented (79.9%). Finally, twenty three constituents (98.7%) were identified in the oil from inflorescences: seven monoterpenes (30.0%) and sixteen sesquiterpenes (68.7%). The monoterpenes 1,8-cineole and *α*-pinene followed by sesquiterpenes *E*-caryophyllene and viridiflorene are the main components in the essential oils from leaves and inflorescences, while the sesquiterpenes *δ*-cadinene and *E*-caryophyllene are the main components in the essential oils from stalks of *C. jacobinensis*.

Essential oils from leaves, stalks and inflorescences from *C. jacobinensis* were evaluated against instar larvae of *A. aegypti* in order to determine their potential as larvicidal agent. The results of the tests are presented in Table 2. Results assessment of larvicidal evaluation showed that the essential oils were considered a natural agent against larvae of *A. aegypti* with LC values of leaves (LC_{50} = 79.3 μg/mL), stalks (LC_{50} = 117.2 μg/mL), and inflorescences (LC_{50} = 65.8 μg/mL), thus helping in the prevention of dengue fever.

CONCLUSIONS

Therefore, the essential oils of the leaves and inflorescences from *C. jacobinensis* were more active than the essential oils of the stalks. These results can be explained by the presence of the sesquiterpene 1,8-cineole in these essential oils and because

monoterpenes and sesquiterpenes present in these oils (e.g. α-pinene, β-pinene, camphor and borneol) have been reported to be active against A. aegypti. Some reports also suggest that monoterpenes and sesquiterpenes found in these oils may also act synergistically (Santiago *et al.*, 2006).

The larvicidal activity against *A. aegypti* of these compounds can be explained because terpenes are substances that increase the transmembrane absorption of lipophilic drugs (El-Kattan *et al.*, 2001), which can kill instar III larvae of *A. aegypti*. The use of essential oils from leaves, stalks and inflorescences of *C. jacobinensis* as a natural insecticide against *A. aegypti*, since these essential oils are biodegradable and non toxic to the environment.

Table 1
Chemical composition of essential oil from leaves, stalk and inflorescences of *C. jacobinensis.*

Compounds	RI[a]	Leaves [%]	Stalks [%]	Inflorescences [%]
Monoterpenes				
α-Pinene	**939**	**7.9**	**4.8**	**10.9**
Camphene	**954**	**-**	**3.9**	**-**
β-Pinene	**979**	**7.4**	**-**	**2.2**
***p*-Cymene**	**1024**	**-**	**2.0**	**-**
Limonene	**1027**	**1.6**	**-**	**1.8**
1,8-Cineole	**1031**	**16.9**	**-**	**24.3**
Terpinolene	**1088**	**0.8**	**-**	**0.5**
Camphor	**1146**	**-**	**3.8**	**-**
Borneol	**1169**	**-**	**1.1**	**-**
Terpinen-4-ol	**1177**	**2.0**	**-**	**1.8**
α-Terpineol	**1188**	**2.1**	**-**	**1.2**
Sesquiterpenes				
Isoledene	**1376**	**1.1**	**2.8**	**0.9**
α-Copaene	**1379**	**-**	**2.3**	**-**
β-Elemene	**1390**	**-**	**3.7**	**-**
Longifolene	**1407**	**1.3**	**-**	**0.8**
α-Gurjunene	**1409**	**-**	**1.6**	**-**
***E*-caryophyllene**	**1419**	**15.6**	**9.7**	**17.6**
Aromadendrene	**1441**	**0.8**	**-**	**0.8**
6,9-Guaiadiene	**1444**	**-**	**5.6**	**-**
α-Humulene	**1454**	**2.5**	**3.0**	**3.2**
Alloaromadendrene	**1460**	**4.5**	**0.6**	**4.9**
9-*epi*-*E*-caryophyllene	**1466**	**1.7**	**-**	**1.6**
Ishwarane	**1468**	**1.3**	**-**	**1.2**
γ-Muurolene	**1479**	**-**	**8.7**	**-**
α-Amorphane	**1484**	**-**	**1.3**	**-**
Viridiflorene	**1496**	**14.8**	**5.2**	**17.0**
α-Muurolene	**1500**	**-**	**4.3**	**-**
α-Cuprenene	**1505**	**1.5**	**0.9**	**1.1**
γ-Cadinene	**1513**	**-**	**8.0**	**-**
δ-Cadinene	**1523**	**-**	**20.0**	**-**

α-Calacorene	**1545**	**-**	**1.4**	**-**
Elemol	**1549**	**2.1**	**-**	**-**
Caryolan-8-ol	**1572**	**-**	**0.8**	**-**
Spathulenol	**1578**	**0.7**	**-**	**0.8**
Globulol	**1590**	**1.3**	**-**	**1.6**
Viridiflorol	**1592**	**1.9**	**-**	**2.0**
Guaiol	**1600**	**0.9**	**-**	**0.5**
γ-Eudesmol	**1632**	**1.5**	**-**	**1.2**
α-Eudesmol	**1653**	**2.3**	**-**	**-**
β-Eudesmol	**1650**	**2.2**	**-**	**0.8**
Total Identifield		**96.7**	**95.5**	**98.7**

[a] Retention indices

Table 2
LC_{50} values for larval mortality caused by the essential oils

Essential oil	LC_{50} (μg/mL)
Leaves	79.3 ± 0.3
Stalks	117.2 ± 0.1
Inflorescences	65.8 ± 0.5
Themephos	1.4 ± 0.2

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