



UNIVERSIDAD  
DE SANTIAGO  
DE CHILE

BOLETÍN LATINOAMERICANO Y DEL CARIBE  
DE PLANTAS MEDICINALES Y AROMÁTICAS

17 (3): 302 - 309 (2018)

© / ISSN 0717 7917 / [www.blacpma.usach.cl](http://www.blacpma.usach.cl)



Artículo Original | Original Article

## 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 gardineriana* Schauer]

Evaristo José P. Pereira<sup>1</sup>, Horlando C. Silva<sup>5</sup>, Carlos L. Holanda<sup>2</sup>, Jane Eire Silva A. de Menezes<sup>1,2</sup>,  
Sônia Maria C. Siqueira<sup>1,2</sup>, Tigressa Helena S. Rodrigues<sup>3</sup>, Raquel Oliveira. S. Fontenelle<sup>1,3</sup>,  
Jean Parcelli C. do Vale<sup>3</sup>, Priscila T. da Silva<sup>3</sup>, Gilvandete Maria P. Santiago<sup>4,5</sup> & Hécio S. Santos<sup>3</sup>

<sup>1</sup>Mestrado Acadêmico em Recursos Naturais, Universidade Estadual do Ceará, Fortaleza-CE, Brazil

<sup>2</sup>Curso de Química, Universidade Estadual do Ceará, Itapipoca-CE, Brazil

<sup>3</sup>Centro de Ciências Exatas e Tecnologia, Universidade Estadual Vale do Acaraú, Sobral-CE, Brazil

<sup>4</sup>Departamento de Farmácia, Universidade Federal do Ceará, Fortaleza-CE, Brazil

<sup>5</sup>Departamento de Química Orgânica e Inorgânica, Universidade Federal do Ceará, Fortaleza-CE, Brazil

Contactos / Contacts: Hécio S. SANTOS - E-mail address: [helciodossantos@gmail.com](mailto:helciodossantos@gmail.com)

**Abstract:** *Vitex gardineriana* 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<sub>50</sub> = 28.0 µg/mL). In the evaluation of the bioassay with *Artemia salina* the essential oil showed LC<sub>50</sub> = 98.11 µg/mL. In addition, the essential oil did not show cytotoxicity (IC<sub>50</sub> > 2.50 mg/mL) by the hemolysis assay.

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

**Resumen:** *Vitex gardineriana* 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<sub>50</sub> = 28.0 µg/mL). En la evaluación del bioensayo con *Artemia salina*, el aceite esencial demostró CL<sub>50</sub> = 98.11 µg/mL. Además, el aceite esencial no mostró citotoxicidad (IC<sub>50</sub> > 2.5 mg / mL) mediante el ensayo de hemólisis.

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

**Recibido | Received:** November 17, 2017

**Aceptado | Accepted:** April 9, 2018

**Aceptado en versión corregida | Accepted in revised form:** April 25, 2018

**Publicado en línea | Published online:** May 31, 2018

**Declaración de intereses | Declaration of interests:** The authors are grateful to the Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). EMBRAPA AGROINDÚSTRIA TROPICAL-Laboratório Multiusuário de Química de Produtos Naturais by obtaining the spectral data. H.S. Santos, Ph.D, acknowledges financial support from the PQ-BPI/FUNCAP (Grant#: BP2-0107-00026.01.00/15).

**Este artículo puede ser citado como / This article must be cited as:** EJP Pereira, HC Silva, CL Holanda, JESA de Menezes, SMC Siqueira, THS Rodrigues, ROS Fontenelle, JPC do Vale, PT da Silva, GMP Santiago, HS Santos. 2018. Chemical Composition, cytotoxicity and larvicidal activity against *Aedes aegypti* of essential oils from *Vitex gardineriana* Schauer. *Bol Latinoam Caribe Plant Med Aromat* 17 (3): 302 – 309.

### 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 250° C and 200° C, respectively. The column temperature was programmed from 35° C to 180° C at 4° C/min and then 180° C to 250° C at 10° 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<sub>2</sub>O/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<sub>50</sub> 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<sub>50</sub>, 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<sub>2</sub>. 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

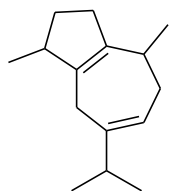
The LC<sub>50</sub> value of essential oil from leaves of *V. gardneriana* was calculated using the probit analysis (Finney, 1971) of the mortality data derived from bioassays.

## RESULTS AND DISCUSSION

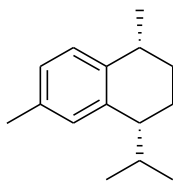
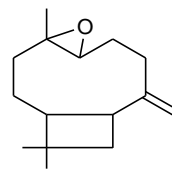
The essential oil from leaves of *V. gardneriana* was analyzed by GC/MS and GC/FID and was obtained with a yield of 0.06%. A total of 26 constituents (95.9%) organized in order of elution in a DB-5 column were identified, being 2 monoterpenes (0.4%) and 24 sesquiterpenes (95.5%) (Table No. 1). The chemical 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-guaiadiene 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 <sup>a</sup>	RI <sup>b</sup>	Percent Composition
$\alpha$ -Pinene	941	939	0.2
$\beta$ -Pinene	984	979	0.2
$\alpha$ -Cubebene	1354	1348	1.5
alfa-Copaene	1380	1376	4.3
$\beta$ -Bourbonene	1388	1388	0.1
$\beta$ -Cubebene	1393	1387	0.6
$\beta$ -Elemene	1395	1390	1.4
$\beta$ -Caryophyllene	1423	1419	2.8
6,9-Guaiadiene	1448	1444	14.5
$\alpha$ -Caryophyllene	1458	1457	0.8
<i>trans</i> -Cadina-1(6),4-diene	1477	1476	0.7
$\gamma$ -Muurolene	1484	1479	1.0
<i>trans</i> -Muurolo-4(14),5-diene	1494	1493	3.0
$\alpha$ -Muurolene	1502	1500	1.1
$\alpha$ -Bulnesene	1508	1509	0.3
$\gamma$ -Cadinene	1520	1513	1.7
<i>cis</i> -Calamenene	1527	1529	29.7
<i>trans</i> -Cadina-1,4-diene	1536	1534	2.4
$\alpha$ -Calacorene	1547	1547	4.4
$\beta$ -Calacorene	1568	1565	0.9
Caryophyllene oxide	1586	1583	14.0
1- <i>epi</i> -Cubenol	1632	1628	3.2
Cubenol	1646	1646	3.3
$\alpha$ -Cadinol	1659	1654	1.5
<i>cis</i> -Calamenen-10-ol	1664	1661	1.2
Cadalene	1679	1676	1.1
<b>Total</b>			<b>95.9</b>

<sup>a</sup>Retention indices on DB-5 column; <sup>b</sup>Literature retention indices

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. unguolata* essential oils showed LC<sub>50</sub> 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<sub>50</sub> 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<sub>50</sub> of 79.3, 117.2, 65.8 µg/ml (Pinto et al., 2016) and the essential oils from *Piperaceae* species studied showed LC<sub>50</sub> 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<sub>50</sub> value of 28.0 µg/mL, which can be considered very active, because in an earlier study, essential oils with LC<sub>50</sub> 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  $\alpha$ -pinene and  $\beta$ -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 <sub>50</sub> (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<sub>50</sub> 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<sub>50</sub> value obtained for essential oil was less than 1,000 µg/mL, presenting LC<sub>50</sub> of 98.11 µg/mL. This LC<sub>50</sub> 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. gardneriana* 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<sub>50</sub> > 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<sub>50</sub> 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<sub>50</sub> value was 98.11 µg/mL. In addition, the essential oil did not show cytotoxicity (IC<sub>50</sub> > 2.5 mg/mL) by the hemolysis assay.

## ACKNOWLEDGMENT

The authors are grateful to the Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). EMBRAPA AGROINDÚSTRIA TROPICAL-Laboratório Multiusuário de Química de Produtos Naturais by obtaining the spectral data. H.S. Santos, Ph.D, acknowledges financial support from the PQ-BPI/FUNCAP (Grant#: BP2-0107-00026.01.00/15).

## REFERENCES

- Adams RP. 2017. **Identification of essential oil components by gas chromatography/qua-drupole massa spectroscopy**. US (United States): Allured Publ Corp Carol Stream, IL, USA.
- Andrade SEO, Maracajá PB, Silva RA, Freires GF, Pereira AM. 2012. Estudo etnobotânico de plantas medicinais na Comunidade Várzea Comprida dos Oliveiras, Pombal, Paraíba, Brasil. **Revista Verde** 7: 46 - 52.
- Aghraz A, Wanner J, Schmidt E, Aitdra L, Aitsidibrahim M, Tabanca N, Ali A, Nafis A, Hassani L, Markouk M, Jirovetz L, Larhsini M. 2017. Chemical composition, *in vitro* antioxidant, antimicrobial and insecticidal activities of essential oil from *Cladanthus arabicus*. **J Essent Oil Bear Pl** 20: 601 - 609.
- Bhargava S. 2016. Mosquito repellency potential of the essential oil from *Mentha* Spp. **Int J Pharm Pharm Res** 6: 509 - 522.
- Botas GS, Cruz RAS, De Almeida FB, Duarte JL, Araújo RS, Souto RNP, Ricardo F, Carvalho JCT, Santos MG, Rocha L, Pereira VLP, Fernandes CP. 2017. *Baccharis reticularia* DC. and limonene nanoemulsions: Promising larvicidal agents for *Aedes aegypti* (Diptera: Culicidae) control. **Molecules** 22: 1990 - 1914.
- Callmander M, Phillipson P, Schatz G. 2014. Towards a revision of the genus *Vitex* L. (Lamiaceae) in Madagascar I: a distinctive new species from Northeastern Madagascar. **Candollea** 69: 141 - 147.
- Carroll JF, Demirci B, Kramer M, Bernier UR, Agramonte NM, Baser HC, Tabanca N. 2017. Repellency of the *Origanum onites* L. essential oil and constituents to the lone star tick and yellow fever mosquito. **Nat Prod Res** 31: 2192 - 2197.
- Cheng SS, Chua MT, Chang EH, Huang CG, Chen WJ, Chang ST. 2009. Variations in insecticidal activity and chemical compositions of leaf essential oils from *Cryptomeria japonica* at diferente ages. **Bioresour Technol** 100: 465 - 470
- Costa VJP, Gonçalves FB, Da Silva PT, Bandeira PN, Teixeira EH, Vasconcelos MA, Sampaio AH, Sampaio SS, Braz-Filho R, Santos HS. 2017. Isolation and antioxidant activity of chemical constituents from *Vitex gardneriana* Schauer. **J Pharmacogn Phytochem** 6: 1806 - 1811.
- Finney DJ. 1971. **Probit Analysis**, Cambridge University Press, Cambridge, UK.
- Gois RWS, De Sousa LM, Lemos TLG, Arriaga AMC, Andrade-Neto M, Santiago GMP, Ferreira YS, Alves PB, de Jesus HCR. 2011. Chemical composition and larvicidal effects of essential oil from *Bauhinia acuruana* (Moric) against *Aedes aegypti*, **J Essent Oil Res** 23: 59 - 62.
- Govindarajan M. 2010. Chemical composition and

- larvicidal activity of leaf essential oil from *Clausena anisata* (willd.) Hook. F. Benth (Rutaceae) against three mosquito species. **Asian Pacific J Trop Med** 3: 874 - 877.
- Jimenez PC, Fortier SC, Lotufo TMC, Pessoa C, Moraes MEA, Moraes MO, Costa-Lotufo LV. 2003. Biological activity in extract of ascidians (*Tunicata ascidiacea*) from the Northeastern Brazilian coast. **J Exp Mar Bio Ecol** 287: 1 - 9.
- Kerdudo A, Chandre F, Ellong EN, Adenet S, Burger P, Rochefort K, Gonnot V, Michel T, Boyer L, Xavier F. 2017. Chemical composition, antimicrobial and insecticidal activities of flowers essential oils of *Alpinia zerumbet* (Pers.) from Martinique Island. **Chem Biodiv** 14: 1 - 11.
- Khokra SL, Prakash O, Jain S, Aneja KR, Dhingra Y. 2008. Essential oil composition and antibacterial studies of *Vitex negundo* Linn. Extracts. **Ind J Pharm Sci** 70: 522 - 526.
- Lal S, Prakash O, Jain S, Ali M. 2007. Volatile constituents of the fruits of *Vitex negundo* Linn. **J Essent Oil Bear Pl** 10: 247 - 250.
- Magalhães LAM, Lima MP, Marques MOM, Facanali RAC, Pinto S, Tadei WP. 2010. Chemical composition and larvicidal activity against *Aedes aegypti* of essential oils from four *Guarea* species. **Molecules** 15: 5734 - 5741.
- Mar JM, Silva LS, Azevedo SG, França LP, Goes AFF, Dos Santos AL, Bezerra JA, Nunomura RCS, Achado MB, Sanches EA. 2018. *Lippia organoides* essential oil: An efficient alternative to control *Aedes aegypti*, *Tetranychus urticae* and *Cerataphis lataniae*. **Ind Crops Prod** 111: 292 - 297.
- Mendes LA, Martins GF, Valbon WR, Tercio SS, Menini L, Ferreira A, Ferreira MFS. 2017. Larvicidal effect of essential oils from Brazilian cultivars of guava on *Aedes aegypti* L. **Ind Crops Prod** 108: 684 - 689.
- Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DJ, McLaughlin JL. 1982. Brine shrimp: a convenient general bioassay for active plant constituents. **Planta Med** 45: 31 - 34.
- Miyazaki CMS, Hirota BCK, Lima CP, Verdum MCS, Paula CS, Chaves SC, Pavan PMN, Miguel MD, Miguel OG. 2013. Coumarin isolation and comparative study of biological activities of *Pterocaulon alopecuroides* DC and *Pterocaulon lorentzii* Malme. **Int J Phytomed** 5: 298 - 301.
- Monteiro LCCF, Araujo EIM, Oliveira AMS, Alves LA, Bertini LM. 2015. Atividade antioxidante, teor de fenóis e atividade larvicida frente ao *Aedes aegypti* de *Vitex gardineriana* Schauer. **Blucher Chem Proc** 3: 292 - 300.
- Nascimento AMD, Maia TDS, Soares TES, Menezes LRA, Scher R, Costa EV, Cavalcanti SCH, La Corte R. 2017. Repellency and larvicidal activity of essential oils from *Xylopi laevigata*, *Xylopi frutescens*, *Lippia pedunculosa*, and their individual compounds against *Aedes aegypti* Linnaeus. **Neotrop Entomol** 46: 223 - 230.
- Neves IA, Rezende SRF, Kirk JM, Pontes EG, De Carvalho MG. 2017. Composition and larvicidal activity of essential oil of *Eugenia candolleana* DC. (Myrtaceae) against *Aedes aegypti*. **Rev Virtual Quim** 9: 2305 - 2315.
- Panyakaew J, Sookkhee S, Rotarayanont S, Kittiwachana S, Wangkarn S, Mungkornasawakul P. 2017. Chemical variation and potential of *Kaempferia* oils as larvicide against *Aedes aegypti*. **J Essent Oil Bear Pl** 20: 1044 - 1056.
- Pavela R. 2015. Essential oils for the development of eco-friendly mosquito larvicides: A review. **Ind Crops Prod** 76: 174 - 187.
- Perumalsamy H, Kim NJ, Ahn AJ. 2009 Larvicidal activity of compounds isolated from *Asarum heterotropoides* against *Culex pipiens pallens*, *Aedes aegypti*, and *Ochlerotatus togoi* (Diptera: Culicidae). **J Med Entomol** 46: 1420 - 1423.
- Pinto CCC, de Menezes JESA, Siqueira SMC, Melo DS, Feitosa CRS, Santos HS. 2016. Chemical Composition and larvicidal activity against *Aedes aegypti* of essential oils from *Croton jacobinensis* Baill. **Bol Latinoam Caribe Plant Med Aromat** 15: 122 - 127.
- Rajeswary M, Kadaikunnan S, Govindarajan M, Khaled JM, Alharbi NS, Benelli G. 2017. *Zingiber cernuum* (Zingiberaceae) essential oil as effective larvicide and oviposition deterrent on six mosquito vectors, with little non-target toxicity on four aquatic mosquito predators. **Environ Sci Pollut Res** 24: 1 - 11.
- Rani A, Sharma A. 2013. The genus *Vitex*: A review.



- Pharmacogn Rev** 7: 188 - 198.
- Santana HT, Trindade FTT, Stabeli RG, Silva AAE, Militão JSLT, Facundo VA. 2015. Essential oils of leaves of *Piper* species display larvicidal activity against the dengue vector, *Aedes aegypti* (Diptera: Culicidae). **Rev Bras Plant Med** 17: 105 - 111.
- Santos HS, Bandeira PN, Lemos TLG, Santiago GMP. 2017. Chemical composition and larvicidal activity against *Aedes aegypti* L. (Diptera: Culicidae) of essential oils from leaves, stalks and roots of the *Croton nepetaefolius* Baill (Euphorbiaceae) **Int J Mosquito Res** 4: 19 - 22.
- Sayes CM, Reed KL, Warheit DB. 2007. Assessing toxicity of fine and nanoparticles: comparing in vitro measurements to in vivo pulmonary toxicity profiles. **Toxicol Sci** 97: 163 - 180.
- Sena Filho JG, Barreto IC, Soares Filho AO, Nogueira PCL, Odoro AV, Cruz da Silva AV, Xavier HS, Rabbani ARC, Spakowicz DJ, Durringer JM. 2017. Volatile metabolomic composition of *Vitex* species: chemodiversity insights and acaricidal activity. **Frontiers in Plant Science** 8: 1931.
- Silva IMA, Faro RRN, Cristaldo PF, Martins GF, Blank AF, Melo CR, Alves PB, Araújo APA, Santana AS, Picanço MC, Bacci L. 2018. Alternative control of *Aedes aegypti* resistant to pyrethroids: lethal and sublethal effects of monoterpene bioinsecticides. **Pest Manag Sci** 74: 1001 - 1012.
- Sobrinho ACN, De Souza EB, Rocha MFG, Albuquerque MRJR, Bandeira PN, Dos Santos HS, Fontenelle ROS. 2016. Chemical composition, antioxidant, antifungal and hemolytic activities of essential oil from *Baccharis trinervis* (Lam.) Pers. (Asteraceae). **Ind Crops Prod** 84: 108 - 115.
- Sousa LGSS, Almeida FJQM, Santiago GMP, Filho RB, Lemos TLG, Gomes CL, Nascimento RF. 2012. Constituintes químicos de *Capraria biflora* (Scrophulariaceae) e atividade larvívica de seu óleo essencial. **Quím Nova** 35: 2258 - 2262.
- Sousa LM, de Carvalho JL, Gois RWS, Da Silva HC, Santiago GMP, Lemos TLG, Arriaga AMC, Alves PB, de Matos IL, Militão GCG, da Silva PBN, da Silva TG. 2016. Chemical composition, larvicidal and cytotoxic activities of the essential oils from two *Bauhinia* Species. **Rec Nat Prod** 10: 341 - 348.
- Stojković D, Soković M, Glamočlija J, Džamić A, Ćirić A, Ristić M, Grubišić D. 2011. Chemical composition and antimicrobial activity of *Vitex agnus-castus* L. fruits and leaves essential oils. **Food Chem** 128: 1017 - 1022.
- Valle D, Pimenta DN, Aguiar R. 2016. Zika, dengue e chikungunya: desafios e questões. **Epidemiol Serv Saude** 25: 419 - 422.
- Vasanth-Srinivasan P, Senthil-Nathan S, Thanigaivel APA, Edwin ES, Selin-Rani S, Chellappandian M, Pradeepa V, Lija-Escaline J, Kalaivani K, Hunter WB, Duraipandiyam V, Al-Dhabi NA. 2017. Comparative analysis of mosquito (Diptera: Culicidae: *Aedes aegypti* Liston) responses to the insecticide Temephos and plant derived essential oil derived from *Piper betle* L. **Ecotoxicol Environ Saf** 139: 439 - 446.