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Original Article

Phytochemical study of *Waltheria viscosissima* and evaluation of its larvicidal activity against *Aedes aegypti*

Maria Denise Leite Ferreira [a], Diégina A. Fernandes [a], Fabíola C. Nunes [e], Yanna C.F. Teles [c], Yngred M. Rolim [b], Camila Macaúbas da Silva [c], Janderson B.L. de Albuquerque [d], Maria de Fátima Agra [e], Maria de F.V. de Souza [a,b,*]

[a] *Pós-graduação em Produtos Naturais e Sintéticos Bioativos, Centro de Ciências da Saúde, Universidade Federal da Paraíba, João Pessoa, PB, Brazil*
[b] *Pós-graduação em Desenvolvimento e Inovação Tecnológica em Medicamentos, Centro de Ciências da Saúde, Universidade Federal da Paraíba, João Pessoa, PB, Brazil*
[c] *Departamento de Química e Física, Centro de Ciências Agrárias, Universidade Federal da Paraíba, Areia, PB, Brazil*
[d] *Departamento de Ciências Farmacêuticas, Universidade Federal da Paraíba, Centro de Ciências da Saúde, João Pessoa, PB, Brazil*
[e] *Centro de Biotecnologia, Universidade Federal da Paraíba, João Pessoa, PB, Brazil*

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ABSTRACT

The species *Waltheria viscosissima* A.St.–Hil, Malvaceae, which is known as 'malva-branca', is traditionally used in the Brazilian northeast for the treatment of coughs. This research looks towards reporting the isolation of phytoconstituents of *W. viscosissima*, as well as the quantification of its phenolics, total flavonoid content, and free radical scavenging potential, along with an evaluation of its larvicidal activity against *Aedes aegypti* larvae. Chromatographic techniques were used to isolate the compounds and a structural elucidation was performed by 1D and 2D NMR. The quantification of total phenolics and flavonoids and the DPPH•radical scavenging activity was determined through spectrophotometric methods. Consequently, the phytochemical investigation led to the identification of fourteen compounds from the aerial parts of the *W. viscosissima*: steroids, triterpenes, alkaloids, and eight flavonoids previously reported in the literature. The quantification of compounds showed that the aerial parts extract possessed high concentration of flavonoids, while the roots extract were rich in other phenolic compounds. At the DPPH•free radical scavenging assay, the roots extract presented $EC_{50} = 77.32 \pm 4.37$ μg/ml and the aerial parts extracts showed $EC_{50} = 118.10 \pm 1.21$ μg/ml. *W. viscosissima roots extract showed the most potent larvicidal activity against Ae. aegypti* ($LC_{50} = 4.78$ mg/ml), with the potential of being used in effective and economically viable preparations that can be catered for domestic use towards controlling the vector insect of severe diseases, such as dengue and Zika.

Introduction

Malvaceae *sensu lato* comprises the traditional families that can be found within the Malvales order: Bombacaceae, Sterculiaceae, Tiliaceae e Malvaceae *sensu stricto* (APG IV, 2016). The *Waltheria* genus, Malvaceae, occurs in both Asia and America, including Brazil. It possess around sixty species, many of which are used in human health treatments to treat respiratory and dental infections, along with inflammation and cases of malaria (Zongo et al., 2013; Cretton et al., 2014, 2015; Esteves, 2015; Yougbare-Ziebrou et al., 2016; Veeramani and Alagumanivasagam, 2016; Silveira-Júnior et al., 2017; Mundo et al., 2017).

Based on the *Waltheria* genus, previous studies have reported the occurrence of quinolone alkaloids, (Hoelzel et al., 2005; Lima et al., 2009; Cretton et al., 2016), triterpenes, phenolics, and flavonoids (Monteillier et al., 2017; Caridade et al., 2018).

Waltheria viscosissima A.St.-Hil, Malvaceae, popularly known as 'malva-branca' and 'malva-viscosa', is endemic of the Northeast region of Brazil, with its aerial parts having been traditionally used as antitussive and expectorant. However, the phytochemical investigations on the species are scarce (Corrêa, 1974; Vasques et al., 1999). A preliminary phytochemical screening has detected the presence of triterpenes, steroids, phenolic compounds and saponins in the crude ethanol extract of *W. viscosíssima* (Vasques

* Corresponding author.
E-mail: mfvanderlei@ltf.ufpb.br (M.F. de Souza).

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et al., 1999), and one triterpene also has been identified (Soares et al., 1998).

Indeed, phytochemical researches allied to biological assays have raised the potential uses of plant extracts or isolated phytoconstituents as insecticides and/or larvicides, with many studies confirming the efficacy of these natural products (Santiago et al., 2005; Santos et al., 2012; Lakshmi et al., 2018). These insecticides and/or larvicides are especially relevant in tropical countries, where mosquitoes contribute to the occurrence of severe viral diseases, such as yellow fever, dengue, and Zika (Fernandes et al., 2018).

Considering the popular use of *W. viscosissima* in Brazil and the lack of consistent information with regards to its phytoconstituents, this study seeks to report on the isolation of compounds from aerial parts of *W. viscosissima,* as well as the quantification of phenolics and flavonoids in the obtained extracts. The wide occurrence of flavonoids in *Waltheria* and the larvicidal activity of Sterculiaceae species have been recently reported, justifying the interest in evaluating the antiradical and larvicidal activity of *W. viscosissima* (Caridade et al., 2018; Fernandes et al., 2018).

Materials and methods

General procedures and chemicals

Chromatographic glass columns were used for the purification of the compounds, packed with Silica gel 60 (Merck), Sephadex LH-20 (Merck) or Amberlite XAD-2 as stationary phases, and hexane, EtOAc and MeOH in increasing polarity mixtures as the mobile phase (Fig. 1).

Thin layer cchromatography (TLC) was carried out using silica plates and the resulting spots were visualized either under UV light (254 and 366 nm) or with a *p*-anisaldehyde acid solution. The isolated compounds were identified by 1D and 2D NMR (^{1}H - 500 MHz/^{13}C -125 MHz - Varian and ^{1}H - 400 MHz/^{13}C -100 MHz Bruker. ^{1}H - 300 MHz/^{13}C -75 MHz – Bruker Avance DPX-300), using deuterated solvents. The chemical shifts were expressed as parts per million (ppm) and the coupling constants of (J) in Hz.

Plant material

The plant material (aerial parts and roots) of the *Waltheria viscosissima* A.St.-Hil, Malvaceae, was collected in Santa Rita City, during the month of August 2013. Plant identification was performed by Prof. Maria de Fátima Agra (PgPNSB/UFPB) and a voucher species was deposited at the Prof. Lauro Pires Xavier Herbarium (M. F. Agra 21709). This research has been registered in the National System of Genetic Resource Management and Associated Traditional Knowledge (SisGen - A568B8A).

Extraction and isolation of compounds

The plant material, aerial parts, and roots were oven dried at 40 °C for 72 h. The material was ground separately, and 2,000 g of powdered aerial parts and 350.3 g of powdered roots was obtained. Both materials were macerated with ethanol (95%) for 72 h. The obtained solutions were filtered and concentrated in a rotatory evaporator to obtain 550 g of aerial parts crude extract (APCE) and 33.5 g of roots crude extract (RCE).

The APCE was solubilized using EtOH:H_2O (7:3) and the obtained solution was sequentially partitioned in a separation funnel using hexane (Hex), chloroform ($CHCl_3$), ethyl acetate (EtOAc) and *n*-butanol, which yielded 125 g of hexane phase (HP), 25 g of $CHCl_3$ phase (ChlP), 10 g of EtOAc phase (ACP), 40 g of *n*-butanol phase (BP), and 100 g of hydroalcoholic phase (HAP).

A sample of HP (50 g) was submitted to vacuum liquid chromatography (VLC) using silica gel and Hex, EtOAc, and methanol in increasing polarity mixtures. The obtained fractions were combined after TLC. The fractions eluted with Hex:EtOAc (7:3), Hex:EtOAc (1:1), Hex:EtOAc (4:6) and EtOAc were combined and named HX1. HX1 (12 g) was column chromatographed (CC) in silica flash using Hex, EtOAc and MeOH in increasing polarity mixtures, yielding 97 fractions. Those fractions were analyzed by TLC. Fractions 19–20 and 21 were found to be pure white solids, and were named as compounds **1** (210 mg) and **2** (140 mg). Fraction 22–23 was pure (colorless crystals), and named as compound **3** (110 mg).

The ChlP (12 g) was submitted to silica gel CC using Hex, EtOAc and MeOH to obtain 150 fractions which were combined after TLC. The combined fractions of 79–92 were submitted to Sephadex LH-20 CC eluted with MeOH to yield forty fractions. The fraction of 12–15 (yellow powder) was pure, and named as compound **4** (9 mg). The combined fractions of 110–150 were submitted to silica gel CC using Hex, EtOAc and MeOH, resulting in the purification of compound **5** (13 mg) as yellow powder.

The ACP (8 g) was chromatographed using Sephadex LH-20 eluted with MeOH and MeOH: $CHCl_3$, resulting in 89 fractions. After TLC, the fractions were combined and selected for the new Sephadex CC. From successive Sephadex columns, the compounds **6** (25 mg), **7** (23 mg), **8** (15 mg) and **9** (9 mg) were purified.

A sample of HAP was chromatographed with the use of XAD-2 as stationary phase. The solvents utilized were H_2O (100%), H_2O:MeOH (7:3), H_2O:MeOH (1:1), MeOH, Hex, acetone and EtOAc. The fraction H_2O:MeOH (7:3) precipitated a pure solid, which was named as compound **10** (35 mg).

From XAD-2 column, the fraction eluted with MeOH was rechromatographed in the Sephadex LH-20 CC, resulting in 25 fractions. The fractions 13–18 was a pure yellow powder, which was named compound **11** (15 mg).

A sample of APCE (75 g) was submitted to acid-basic alkaloid purification in the separation funnel to obtain the total alkaloid fraction (TAF). The TAF was submitted to an aluminum oxide CC, using Hex, EtOAc and MeOH as eluents. From the column, 55 fractions were obtained and analyzed by TLC. The fraction 39–43 was chromatographed in Sephadex CC, and the resulting purification of compound **12** (9 mg) was a yellowish oil.

Quantification of total phenolic compounds

The quantification of total phenolic compounds was determined by the spectrophotometric through the use of the Folin–Ciocalteu reagent and gallic acid as a standard according to Gulcin et al. (2004). The experiment was performed in triplicates.

Total flavonoids content

Total flavonoid content was determined by the spectrophotometric method as described by Maciel et al. (2016), using quercetin as a standard. Total flavonoid content was determined by the spectrophotometric method described by Maciel et al. (2016), which used quercetin as a standard. The analysis was evaluated in triplicates and the total flavonoid content was determined from the calibration curve which was constructed with quercetin solutions (1.25 to 40 μg/ml). The result was expressed in milligrams of quercetin equivalents per gram of extract (mg QE/g of extract).

Free radical scavenging activity assay

The *in vitro* antiradical activity of APCE and RCE was evaluated by the DPPH(2, 2-diphenyl-1-picrylhydrazyl) radical scavenging method which was described by Maciel et al. (2016). The experiment was performed in a triplicate.

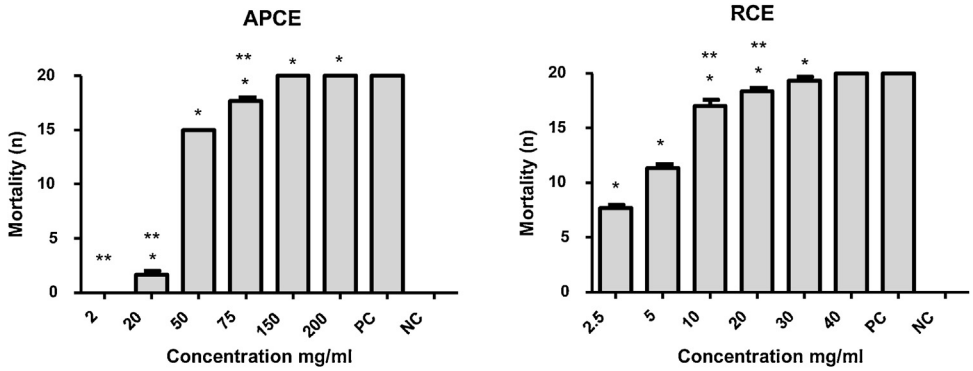


Fig. 1. Larvicidal activity of different concentrations of APCE and RCE of *Waltheria viscosissima* on *Ae. aegypti* larvae after 24 h. PC: Positive control; NC: Negative control. (*) statistically significant in relation to the NC. (**) statistically significant in relation to the PC (p-value < 0.05).

Biological assay

The larvicidal activity of *W. viscosissima* extracts (APCE and RCE) have been evaluated by the World Health Organization (1970). The larvae of *Ae. aegypti* in the 4th stage (L4) of the Rockefeller strain for this study were obtained from the Biotechnology Applied to Parasites and Vectors Laboratory of the Biotechnology Center (Universidade Federal da Paraíba).

Twenty larvae (L4) were transferred to Falcon tubes containing 10 ml of APCE and RCE solutions. The concentrations used to determine the LC_{50} of APCE and RCE ranged from 2 to 200 mg/ml and from 2.5 to 40 mg/ml, respectively. The positive control group consisted of twenty larvae which were exposed to 1 ml of a commercial insecticide (Imiprotrin 0.02%, Permethrin 0.05% and Esbiotrin 0.1%). The negative control group consisted of twenty larvae exposed to distilled water. The experiments were performed in triplicate.

The experiment tubes containing the larvae were incubated for 24 h at 28 ± 4 °C, under a photoperiod comprising of 12 h of light and darkness. After 24 h, the larvae mortality was visually verified. Statistical analysis and CL_{50} calculation were performed using the GraphPad Prism version 5.0 for Windows program (GraphPad Software, San Diego, CA). Significant differences between the groups were analyzed by ANOVA and Tukey post-test ($p < 0.05$)

Results

Identification of isolated compounds

From the chromatographic procedures, along with the 1D and 2D NMR spectroscopic methods, fourteen compounds were identified from the aerial parts of *W. viscosissima,* including steroids, triterpenes, alkaloids and flavonoids.

Compound **1:** β-sitosterol e stigmasterol were identified based on spectral data and comparisons with the literature data (Maciel et al., 2016).

Compound **2:** 3-oxolup-20(29)-en-28-oico acid (betulonic acid): NMR ^{1}H (δ, $CDCl_3$, 400 MHz): 0.92 (s, 3H, H-23), 0.97 (s, 3H, H-24), 0.99 (s, 3H, H-25), 1.0 (s, 3H, H-26) 1.06, (s, 3H,H-27), 1.69 (s, 3H, H-28), 4.61 (bs, 1H, H-29), 4.74 (bs, 1H, H-29), 3.0 (1H, ddd, J^1 = 10.5 Hz, J^2 = 10.5 Hz and J^3 = 4.5 Hz, H-19), NMR ^{13}C (δ, $CDCl_3$, 100 MHz): 37.0 (C-1), 34.2 (C-2), 218.4 (C-3), 47.4 (C-4), 55.05 (C-5), 19.7 (C-6), 33.7 (C-7), 40.8 (C-8), 49.9 (C-9), 37.1 (C-10), 21.5 (C-11), 25.6 (C-12), 38.6 (C-13), 42.6 (C-14), 30.7 (C-15), 32.2 (C-16), 56.5 (C-17), 49.3 (C-18), 47,03 (C-19), 150.5 (C-20), 29.8 (C-21), 37.05 (C-22), 26.7 (C-23), 21.1 (C-24), 16.0 (C-25), 15.9 (C-26), 14.7 (C-27), 182.4 (C-28), 109.9 (C-29), 19.5 (C-30) (Caridade et al., 2018).

Compound **3:** 3-oxoolean-18-en-28-oic acid (**3a**) (Soares et al., 1998). **3b:** 3-oxoolean-12(13)-en-28-oic acid. NMR ^{1}H (δ, $CDCl_3$, 400 MHz): 0.89 (s, 3H, H-26), 0.92 (s, 3H, H-29), 0.96 (s, 3H, H-30), 1.03 (s, 3H, H-25) 1.07, (s, 3H, H-23), 1.13 (s, 3H, H-27), 2.37 (1H, dd, J = 6.8 and 3.6 Hz, H-2), 2.82 (1H, dd, J = 14.0 and 4.4 Hz, H-18), 5.28 (1H, t, J = 3.2 Hz, H-12), NMR ^{13}C (δ, $CDCl_3$, 100 MHz): 39.2 (C-1), 34.1 (C-2), 217.9 (C-3), 47.5 (C-4), 55.4 (C-5), 19.6 (C-6), 32.2 (C-7), 39.3 (C-8), 46.6 (C-9), 36.9 (C-10), 23.0 (C-11), 122.5 (C-12), 143.7 (C-13), 41.8 (C-14), 22.7 (C-15), 23.5 (C-16), 46.9 (C-17), 41.1 (C-18), 45.8 (C-19), 30.7 (C-20), 34.2 (C-21), 32.4 (C-22), 26.5 (C-23), 21.6 (C-24), 15.1 (C-25), 16.9 (C-26), 25.9 (C-27), 184.4 (C-28), 33.1 (C-29), 23.6 (C-30) (Kwon et al., 2011).

Compound **4:** 5,7-dihydroxy-4′-methoxy-flavone (acacetin); Compound **6:** 4′,5,7-trihydroxyflavon-3-ol (kaempferol); Compound **7:** 3′,4′,5,7-tetrahydroxyflavon-3-ol (quercetin) were identified based on spectral data and comparisons with the literature data (Fernandes et al., 2018; Chaves et al., 2017; Teles et al., 2015a).

Compound **5:** 7, 4′-di-*O*-metilisoscutellarein. NMR ^{1}H (δ, DMSO-d_6, 200 MHz): 12.97 (1H, s, 5—OH), 6.86 (s, 1H, H-3), 6.55 (1H, s, H-6), 8.11 (2H, d, J = 8,4 Hz, H-2′ and 6′), 7.13 (2H, d, J = 8.2 Hz, H-3′and 5′), 3.85 (s, OCH_3-4′), 3.90 (s, OCH_3-7). NMR ^{13}C (δ, DMSO-d_6, 50 MHz): 163.5 (C-2), 103.0 (C-3), 182.4 (C-4), 153.1 (C-5), 95.7 (C-6), 154.3 (C-7), 126.2 (C-8), 144.4 (C-9), 103.9 (C-10), 123.0 (C-1′), 128.5 (C-2′), 114.5 (C-3′), 162.4 (C-4′), 114.5 (C-5′), 128.5 (C-6′), 55.30 (OCH_3-4′), 56.50 (OCH_3-7) (Teles et al., 2015a, 2015b).

Compound **8:** 5,7-dihydroxy-4′-methoxyisoflavone. NMR ^{1}H (δ, $(CD_3)_2CO$, 500 MHz): 12.97 (1H, s, 5—OH), 8.17 (s, 1H, H-2), 6.28 (1H, d, J = 1.9 Hz, H-6), 6.41 (1H, d, J = 1,9 Hz, H-8), 7.55 (2H,d, J = 8.75 Hz, H-2′ and 6′), 7.00 (2H, d, J = 8.75 Hz, H-3′ and 5′), 3.83 (s, OCH_3-4′). NMR ^{13}C (δ, $(CD_3)_2CO$, 125 MHz): 154.6 (C-2), 123.8 (C-3), 181.5 (C-4), 163.9 (C-5), 99.80 (C-6), 165.1 (C-7), 94.5 (C-8), 159.0 (C-9), 106.1 (C-10), 124.2 (C-1′), 131.0 (C-2′), 114.5 (C-3′), 160.7 (C-4′), 114.5 (C-5′), 131.0 (C-6′), 55.50 (OCH_3-4′) (Almeida et al., 2008).

Compound **9:** tiliroside, ^{1}H RMN (500 MHz, DMSO-d_6) and ^{13}C (125 MHz, DMSO-d_6) in accordance with the literature data (Teles et al., 2015a).

Compound **10:** 5,7,4′-trihydroxyflavone-8-*C*-β-glucopyranoside (vitexin). ^{1}H NMR (400 MHz, DMSO-d_6): 13.16 (1H, s, 5—OH), 6.78 (1H, s, H-3), 6.26 (1H, s, H-6), 8.02 (2H, d, J = 8.8 Hz, H-2′/6′), 6.89 (2H, d, J = 8.8 Hz, H-3′/5′), 4.69 (1H, d, J 10.0 Hz, H-1″), 3.83 (dd, J = 9.2 and 9.6 Hz, H-2″), 3.51 (m, H-3″), 3.34 (m, H-4″), 3.24 (m, H-5″), 3.52 (1H, dd, J = 6.0 and 11.8, H-6″), 3.76 (1H, d, J = 11.0 Hz, H-6″). NMR ^{13}C (100 MHz, DMSO-d_6): 182.2 (C-4), 163.9 (C-2), 162.6 (C-7), 160.4 (C-5), 161.4 (C-4′), 156.0 (C-9), 128.9 (CH- 2′/CH-6′), 121.6 (C-1′), 115.8 (CH-3′/5′), 104.6 (C-8), 104.3 (C-10), 102.4 (C-3), 98.1 (C-6), 73.4 (CH-1″), 70.8 (CH-2″), 77.9 (CH-3″), 70.8 (CH-4″), 81.6 (CH-5″), 61.3 (CH_2-6″) (He et al., 2016).

Compound **11:** luteolin 7-*O*-β-D-glucopyranoside. ^{1}H NMR (400 MHz, DMSO-d_6): 12.95 (1H, s, 5—OH), 7.45 (d, J = 2.4 Hz, H-6′), 7.43 (d, J = 2 Hz, H-2′), 6.90 (d, J = 8.0 Hz, H-5′), 6.78 (d, J = 2.0 Hz, H-8), 6.74 (s, H-3), 6.43 (d, J = 2.0 Hz, H-6), 5.08 (d, J = 7.2 Hz, H-1″),

Table 1
Total phenolic, total flavonoids contents and DPPH free radical scavenging activity.

Waltheria viscosissima extract	Total phenolic (mg GAE/g ± SEM)[a]	Total flavonoid (mg QE/g ± SEM)[b]	DPPH (EC_{50})[c] (μg/ml ± SEM)
APCE (aerial parts)	131.01 ± 0.04	30.43 ± 0.02	118.10 ± 1.21
RCE (roots)	137.04 ± 0.01	8.25 ± 0.04	77.32 ± 4.37

All values are mean ± S.E.M (n = 3).
[a] GAE = mg of gallic acid equivalent per gram of sample.
[b] QE = mg of quercetin equivalent per gram of sample.
[c] Value defined as the concentration of sample that scavenged 50% of the DPPH.

3.70 (m, H-6″), 3.45 (m, H-3″, H-6″), 3.28 (m, H-2″), 3.18 (m, H-5″). NMR ^{13}C (100 MHz, DMSO-d_6): 181.8 (C-4), 164.5 (C-2), 162.9 (C-7), 161.1 (C-5), 156.9 (C-9), 150.01 (C-4′), 145.8 (C-3′), 121.3 (C-1′), 119.2 (CH-6′), 115.9 (CH-5′), 113.5 (CH-2′), 105.3 (C-10), 103.1 (CH-3), 99.9 (CH-1″), 99.5 (CH-6), 94.7 (CH-8), 77.1 (CH-3″), 76.3 (CH-2″), 73.1 (CH-5″), 69.5 (CH-4″), 60.5 (CH_2-6″) (Silva et al., 2006a).

Compound **12a:** waltherione a ^{1}H NMR (300 MHz, CD_3OD): 7.57 (1H, d, *J* = 8.8 Hz, H-7), 7.42 (1H, d, *J* = 8.8 Hz, H-8), 4.74 (m, H-10), 1.99-2,06 (2H, m, H-11), 2.26-2,41 (2H, m, H-12), 6.68 (1H, dl, *J* = 6.19 Hz, H-13), 7.10 (dl, *J* = 8.1 Hz, H-3′), 7.25 (ddd, *J* = 8.10, 7.81 and 1.57 Hz, H-4′), 6.73 (ddd, *J* = 7.65, 7.81 and 1.35 Hz, H-5′), 6.31 (dd, *J* = 7.65 and 1.35 Hz, H-6′), 3.81 (s, OCH_3-3), 4.00 (s, OCH_3-2′), 2.47 (s, 2−CH_3). NMR ^{13}C (75 MHz, CD_3OD): 14.3 (2−CH_3), 23.09 (C-11), 35.2 (C-12), 56.0 (OCH_3-3), 60.3 (OCH_3-2′), 76.9 (C-13), 79.2 (C-9), 81.4 (C-10), 112.4 (C-3′), 118.02 (C-8), 119.9 (C-4a), 121.2 (C-5′), 128.9 (C-4′), 132.7 (C-7), 132.4 (C-6), 133.6 (C-6′), 81.3 (C-10), 143.1 (C-3), 143.5 (C-2), 140.5 (C-5), 142.6 (C-8a), 158.2 (C-2′), 176.01 (C-4) (Gressler et al., 2008).

Compound **12b:** waltherione b. ^{1}H NMR (300 MHz, CD_3OD): 7.58 (1H, d, *J* = 8.8 Hz, H-7), 7.42 (1H, d, *J* = 8.8 Hz, H-8), 4.76 (m, H-10), 1.99-2,06 (2H, m, H-11), 2.26-2,41 (2H, m, H-12), 6.68 (1H, dl, *J* = 6.19 Hz, H-13), 7.02 (d, *J* = 8.98 Hz, H-3′), 7.25 (ddd, *J* = 8.10, 7.81 and 1.57 Hz, H-4′), 6.73 (ddd, *J* = 7.65, 7.81 and 0.7 Hz, H-5′), 6.81 (dd, *J* = 8.93 and 3.07 Hz, H-6′), 3.95 (s, OCH_3-3), 3.52 (s, OCH_3-2′), 2.47 (s, 2−CH_3). NMR ^{13}C (75 MHz, CD_3OD): 14.4 (2−CH3), 23.3 (C-11), 32.0 (C-12), 55.5 (OCH3-3), 59.7 (OCH_3-2′), 76.9 (C-13), 79.1 (C-9), 81.44 (C-10), 112.4 (C-3′), 118.02 (C-8), 119.9 (C-4a), 121.2 (C-5′), 128.93 (C-4′), 132.7 (C-7), 132.4 (C-6), 133.6 (C-6′), 81.44 (C-10), 152.3 (C-3), 143.5 (C-2), 140.5 (C-5), 142.6 (C-8a), 154.5 (C-2′), 176.01 (C-4) (Gressler et al., 2008).

Quantification of phenolics, flavonoids, and free radical scavenging activity of Waltheria. viscosissima

Spectrophotometric methods were used to quantify the total phenolic compounds and total flavonoids in the extracts of *W. viscosissima*.

A calibration curve was constructed with gallic acid to quantify the phenolic compounds. The value of the coefficient of linearity found was R^2 = 0.9963, and the total phenolic compounds was calculated from the obtained equation of the line (y = 0.000993696x − 0.00218). To quantify the flavonoid content, a calibration curve was built using quercetin, which obtained the coefficient of linearity R^2 = 0.9997 and equation of the line: y = 0.00215x − 0.00243.

To calculate the concentration of extracts that were able to induce the reduction of 50% in DPPH·concentration (EC_{50}), crescent concentrations of APCE and RCE were used according to the described method. A calibration curve was built for each extract that was in mixture with DPPH: Y = −0.001921.x + 0.4705 and Y = −0.0008214.x + 0.4725 for APCE and RCE, respectively (Table 1).

Table 2
Mean of mortality of *Ae. aegypti* larvae (L4) exposed to different concentrations of APCE of *Waltheria viscosissima.*

Concentrations (mg/ml)	Mean mortality (n)	Standard deviation (triplicate)
2.0	0	0
20.0	4.3 (21.6%)	0.47
50.0	13.0 (65%)	0.81
75.0	17.6 (88%)	0.47
150.0 (a)[a]	20.0 (100%)	0
200.0 (a)	20.0 (100%)	0
Negative control	0	0
Positive control	20.0 (100%)	0

[a] Means followed by the same letter are not significantly different by Tukey test, at a level of 5% of probability.

Table 3
Mean of mortality of *Ae. aegypti* larvae (L4) exposed to different concentrations of RCE of *Waltheria viscosíssima.*

Concentrations (mg/ml)	Mean mortality (n)	Standard deviation (triplicate)
1.0	0	0
2.5	7.6 (38.3%)	0.47
5.0	11.3 (56.6%)	0.47
10.0 (a)[a]	17.0 (85%)	0.81
20.0 (a) (b)	18.3 (91.6%)	0.47
30.0 (b) (c)	19.3 (96,6%)	0.47
40.0 (c)	20.0 (100%)	0
Negative control	0	0
Positive control	20.0 (100%)	0

[a] Means followed by the same letter are not significantly different by Tukey test, at a level of 5% of probability.

Biological assay

The mortality of L4 larvae in different extract concentrations of *W. viscosissima* is shown in Tables 2 and 3. The concentration of 150.0 mg/ml of APCE killed 100% of larvae. Concentrations of 75, 50, 20, and 200 mg/ml killed 88%, 65%, 21.6% and 0%, respectively. The tested concentrations of 200 and 150 mg/ml were not found to differ statistically ($p < 0.05$). The calculated LC_{50} of APCE was 38.37 mg/ml (Figure 2).

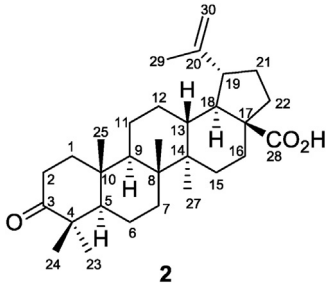
For RCE, the concentration of 40 mg/ml was able to kill 100% of larvae. Concentrations of 30, 20, 10, 5, 2.5 and 1 mg/ml showed a mortality of 96.6%, 91.6%, 85%, 56.6%, 38.3% and 0%, respectively. The concentrations of 5 and 2.5 mg/ml were statistically different ($p < 0.05$), and the calculated LC_{50} for RCE was 4.78 mg/ml (Figure 2).

Discussion

Identification of compounds

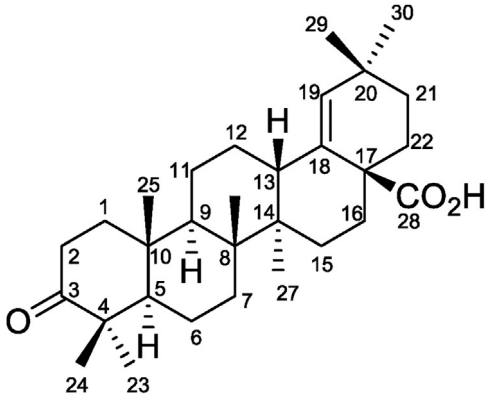
Compound **1** was identified as a mixture of the phytosteroids: β-sitosterol and stigmasterol, which is widely isolated from plants and known by their biological role as vegetal cell membrane constituents (Maciel et al., 2016).

Compound **2** was identified as the lupane-type triterpene 3-*oxolup-20*(29)-en-28-oic acid (betulonic acid) that was previously isolated from *Waltheria cinerencens* (Caridade et al., 2018). The betulonic acid was demonstrated to possess leishmanicidal activity and anti-proliferative activity against cell cancer lineages (Alakurtti et al., 2010; Yang et al., 2015).

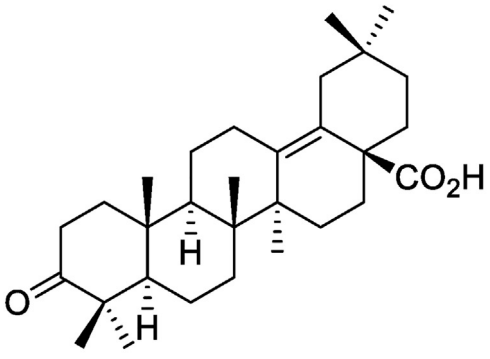


2

The ^{1}H NMR of compound **3**, showed a triterpene aspect, as shown in compound **2**. Absorptions for two hydrogen, at δ_H 5.28 (1H, t, J = 3.2 Hz) and δ_H 5.15 (1H, bs), indicate the presence of double bonds, suggested that the sample could be a mixture of two triterpenes. The ^{13}C NMR spectra presented signals for sixty carbons, corroborating the claim regarding the presence of two triterpenes in the sample. The olefinic carbons at δ_C 122.5 (C-12) and δ_C 143.7 (C-13), characteristic of oleanan-type triterpenes, allowed an identification of the compounds in the mixture as 3-oxo-olean-12(13)-en-28-oic acid and the 3-oxo-olean-18-en-28-oic acid (moronic acid). The moronic acid was previously isolated from *W. viscosíssima* (Soares et al., 1998; Kwon et al., 2011).

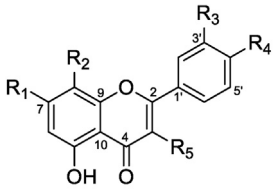


3a



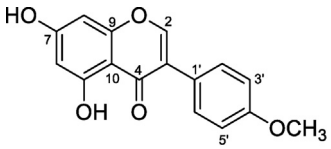
3b

The NMR spectra of compounds **4**, **5**, **6** and **7**, isolated as yellow powder, showed signals in the aromatic region that was compatible with flavonoids. By analyzing the 1D and 2D NMR of them, it was possible to identify the compounds as acacetin (**4**), 7,4′-di-*O*-methyl-isoscutellarein (**5**), kaempferol (**6**) and quercetin (**7**). The presence of these compounds in Malvaceae *sensu lato* species is in agreement with the literature (Silva et al., 2006a; Costa et al., 2008; Chaves et al., 2017; Gomes et al., 2011; Dixit et al., 2011; Fernandes et al., 2018). Flavonoids have been shown to be the major compounds in several species from the Sterculiaceae family, including species from the *Waltheria* genus (Muqarrabun and Ahmat, 2015; Cretton et al, 2015, 2016; Teles et al., 2015b; Caridade et al., 2018).



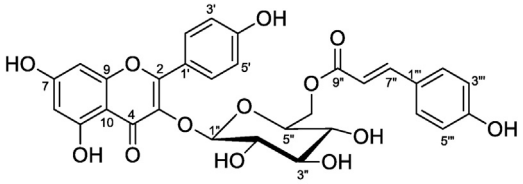
4 R_1=OH; R_2=R_3=R_5=H; R_4=OCH_3
5 R_1=R_4=OCH_3; R_2=OH; R_3=R_5=H
6 R_1=R_4=R_5=OH; R_2=R_3=H
7 R_1=R_3=R_4=R_5=OH; R_2=H

Compound **8** was isolated as orange powder. The ^{1}H NMR showed a couple of doublets coupling *meta*, which were characteristic of 5, 7-di-substituted flavonoids. The hydroxyl at position 5 was found at em δ_H 12.97 (s). A singlet was detected at δ_H 8.17, suggesting an isoflavonoid structure for compound **8**. The ^{13}C NMR showed fourteen signals, including one methoxyl. Among the signals, the highlights include the carbons at δ_C 154.4 and 123.8 assigned to the presence of a double bond between C-2 and C-3; the carbonyl, found at δ_C 181.5; and the high intensity carbons of a *para* substituted B-ring at 114.5 (C-3′ and 5′), 131.0 (C-2′ and 6′). The 2D spectral data and comparison with the literature led to the identification of compound **8** as the isoflavan: 5,7-dihydroxy-4′-methoxyisoflavone (biochanin A) (Almeida et al., 2008). The compound is being reported for the first time in the Sterculiaceae family. In previous studies, it has demonstrated anticancer and anti-hyperlipidemic activities (Harini et al., 2012; Muqarrabun and Ahmat, 2015; Xiao et al., 2017).



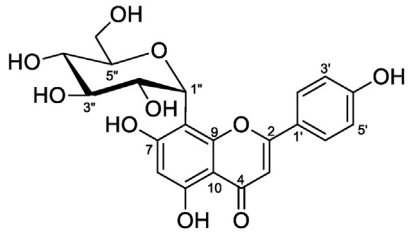
8

Compound **9** was identified as kaempferol-3-*O*-β-D-(6″-*E*-*p*-coumaroyl) glucopyranoside (tiliroside). Tiliroside is widely produced by Malvaceae *sensu lato* and has demonstrated many pharmacological properties, such as vasorelaxant, antioxidant, hypolipidemic, antinociceptive and anti-inflammatory activities (Barbosa et al., 2007; Orhan et al., 2009; Zhang et al., 2015).

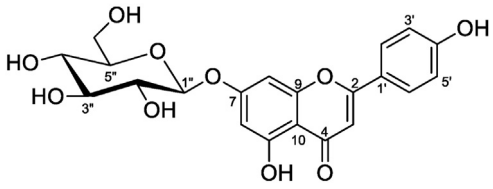


9

The NMR data obtained for compounds **10** and **11**, along with comparisons with literature, led to them being identified as two glucosyl flavones: vitexin (**10**) and luteolin 7-*O*-D-glucopyranoside (**11**). Both of them have pharmacological properties and have been isolated from the species *Pterospermum acerifolium* and *Theobroma cacao*, Sterculiaceae (Silva et al., 2006a; Dixit et al., 2011; Cuong et al., 2015; He et al., 2016).



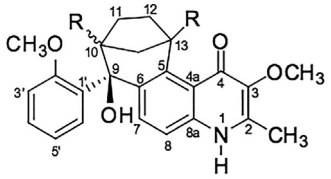
10



11

Compound **12**, which was isolated as yellowish oil, showed signals at NMR spectra that were compatible with quinoline alkaloids. The ^{1}H NMR spectra presented a set of signals δ_H 6.31–7.59 which was compatible with tetra substituted ring of quinoline alkaloids. The chemical shifts in addition to integrals values led to suggest that this would be a mixture of two substances in the proportion of 65:35. The nucleus of 10,13-oxo-*bicyclo* heptane was suggested by the absorptions for oxymethine protons at δ_H 4.74 (m) and 6.68 (d, J = 6.19 Hz), besides the multiplets at 1.99–2.06 (m) and 2.26–2.41 (m). The ^{13}C NMR spectra confirmed the proposal of a mixture of two compounds by showing carbons with very different intensities. Relevant signals for elucidating the structure of the compounds were α,β-unsaturated ketone carbonyl at δ_C 176.6; oxymethine carbons δc 82.44 (C-10, 12b); 81.39 (C-10, 12a) and 76.10 (C-13, 12a and 12b).

The analysis of the 2D spectra, compilation of chemical shifts, and comparisons with literature data, allowed an identification of compound **12** as a mixture of the alkaloids waltherione a (**12a**) and waltherione b (**12b**) in the proportion (65:35), respectively, along with antimicrobial compounds previously reported from *Waltheria douradinha* (Hoelzel et al., 2005; Gressler et al., 2008).



12a R=β-H
12b R=α-H

Quantification of phenolics, flavonoids and free radical scavenging activity of Waltheria viscosíssima

It has been well reported that plants with a high production of phenolic compounds, such as phenolic acids derivatives, tannins, flavonoids, etc., have been established to have great antioxidant activity (Singh et al., 2016). The quantification of *W. viscosíssima* phenolic compounds demonstrated 131.01 ± 0.04 mg de EAG/g of APCE and 137.04 ± 0.01 mg de EAG of RCE. The total phenolic content of other species of Malvaceae *sensu lato,* such as *Sterculia striata, Sidastrum micranthum* and *Sida rhombifolia,* have been determined by the same method. The *W. viscosíssima* extracts (both APCE and RCE) were shown to possess a concentration of phenolic compounds that were at least twice as great as the other investigated species (Costa et al., 2009; Oliveira et al., 2012).

Flavonoids are compounds that have garnered the interest of researchers in biological field due their huge pharmacological potential and their common occurrence in medicinal plants and in functional foods (Teles et al., 2015a). In this research, eight flavonoid structures have been identified, justifying our interest in quantifying the total content of flavonoids in *W. viscosíssima* extracts. The obtained results showed a greater concentration of flavonoids in the APCE than the RCE. The result is in agreement with literature that reports the accumulation of these compounds in surface cells of vegetal organs that have a high exposure to sun light (Agati et al., 2012). The flavonoids have been shown to act as protective agents against light-induced oxidative damage, since they absorb UV and visible light. Indeed, studies have demonstrated that sun light up-regulates the flavonoids biosynthesis. Thus, the accumulation of flavonoids in external vegetal structures is consistent with UV and visible light protection functions (Havaux and Kloppstech, 2001; Agati et al., 2012).

Due the ability of phenolic and flavonoid compounds to prevent oxidative stress and inflammation (Tao et al., 2016; Herrera-Calderon et al., 2016; Hou et al., 2017), the wide occurrence of flavonoids that were previously reported in other *Waltheria* (Caridade et al., 2018) and reported here in the aerial parts of *W. viscosissima* might be related to the popular use of the *Waltheria* species as an anti-inflammatory (Yougbare-Ziebrou et al., 2016).

In this study, we demonstrated that the RCE presented greater free radical scavenging activity against DPPH·radical than the APCE. The ability of free radicals reduction is one of several cell mechanisms that prevent oxidative damage (Li et al., 2012). According to our results, greater reductive activity was carried out by the RCE. Studies have demonstrated that the free radical scavenging activity is more intense for phenolic compounds with *ortho*-dihydroxy aromatic rings (Rice-Evans et al., 1996). In fact, just two out of the eight flavonoids identified in APCE possess *ortho*-dihydroxy rings in their structures. Thus, we can attribute the greater scavenging activity to the presence of other phenolic compounds in the RCE, rather than the flavonoids that have been shown to be in very low concentration in the RCE. When taken into consideration with other Malvaceae *sensu lato* species that were previously investigated, *W. viscosissima* extracts have shown greater free radical scavenging activity (Oliveira et al., 2012). Indeed, when compared to the other *Waltheria* species previously investigated, *Waltheria ovata* has presented the best result, with EC_{50} of 3.50 μg/ml (Herrera-Calderon et al., 2016).

Larvicidal activity

Concentrations of 2 mg/ml APCE and 1 mg/ml of RCE were used to perform the bioassays. Using the cited concentrations, no activity was observed. As such, the concentrations were gradually increased to get a mortality that was statistically different from the negative control. The mortality is observed when the larvae presented compromised mobility, lethargy and complete paralysis (Ravikumar et al., 2011; Oliveira et al., 2013; Santos, 2015).

After a statistical analysis, it was observed that in the *W. viscosissima* APCE, the utilized concentrations statistically differed, with the exception of the 200 mg/ml and 150 mg/ml, as observed in Table 2. For the RCE sample, concentrations of 10 mg/ml and 20 mg/ml did not differ statistically, and this was similar with the concentrations of 20 mg/ml, 30 mg/ml, and 40 mg/ml (Table 3). After 24 h of exposure, the mortality reached 100% for both extracts. The concentrations of 150 mg/ml for APCE and 40 mg/ml for RCE were significantly more effective when compared against other concentrations and the negative control.

The two extracts exhibited larvicidal activities with different LC_{50}, which were calculated based on the statistical results obtained. For APCE, the LC_{50} was 38.7 mg/ml and the closest concentration tested was 50 mg/ml, which killed 13 larvae (mean), the equivalent of 65%. For the RCE, the LC_{50} was 4.78 mg/ml and the closest concentration was 5 mg/ml, which killed 11.3 larvae (mean), and this was equivalent to 56.6% (Table 3).

The RCE possess more significant larvicidal activity than the APCE. Indeed, different parts of plants contain a complex mixture of compounds with certain biological activities (Veni et al., 2016; Govindarajan et al., 2008a, b,c). The activities are usually related to the presence of toxins and secondary metabolites that act as larvicides (Niraimathi et al., 2010). The difference display by the LC_{50} when comparing different parts of a vegetal has been reported by Satana (2012), and has also been shown to affect the leaves and roots of the *Murraya koenigii* (Tennyson et al., 2012), seeds and leaves of *Calophyllum inophyllum* (Pushpalatha and Muthukrishnan, 1999), and stem and leaves of *Guettarda grazielae* (Oliveira et al., 2010).

The concentration of RCE (LC_{50} = 4.78 mg/ml) was lower than other larvicidal activities previously reported. The LC_{50} found for the species *Croton linearifolius*, Euphorbiaceae, and *Albizzia amara*, Fabaceae, were 17.42 and 7.10 mg/m, respectively (Murugan et al., 2007; Silva et al., 2014).

The concentration of APCE (LC_{50} = 38.70 mg/ml) were also lower than *Cymbopogon citratus* (LC_{50} = 63.89 mg/ml) and *Ocimum gratissimum* (LC_{50} = 71.27 mg/ml). The Sterculiaceae *Helicteres velutina* have been evaluated and showed an LC_{50} of 138.89 mg/ml (stem) and 171.68 mg/ml (roots) (Santos et al., 2012). However those concentrations were higher than the ones shown by aerial parts of *Helicteres velutina* (LC_{50} = 2.98 mg/ml) and *Sida acuta* ($LC_{50} = 4.28 \times 10^{-2}$ mg/ml) (Govindarajan, 2010; Fernandes et al., 2018).

According to Guarda et al. (2016) e Simões et al. (2010), the presence of high levels of polyphenols and flavonoids in the extracts may be related to larvicidal activity. Those compounds are known to have toxicity that affects insects and larvae (Santiago et al., 2005; Simões et al., 2010).

Based on the obtained larvicidal results of tested plant extracts, it can be speculated that the compounds can thus act as a potent phyto-complex with synergic effect, and may show greater bioactivity as a mixture than in its isolated constituents (Sumroiphon et al., 2006). It is through this notion that researchers have supported the use of the plant crude extracts, instead of isolated compounds, as insecticides and larvicides (Veni et al., 2016; Guarda et al., 2016).

RCE showed the greater larvicidal activity. Due the low yield of the root extract it was not possible to perform columns for isolation of compounds. Thus, we analyzed its chemical composition by H^1 NMR and TLC, in order to know better the chemical profile of compounds in RCE. In the NMR spectra (Figure 5, supplementary material), it has been verified the presence of typical signals for terpenes, steroids and phenolic compounds, compatible with the spots found on TLC dyed with *p*-anisaldehyde acid or acetic anhydride solutions. The total phenolic compounds and flavonoids from RCE were quantified by spectrophotometry.

The different results showed for separated parts of plants extracts are often showed in literature. The ethanolic extract of the leaves and roots of *Piper alatabaccum*, Piperaceae, and *Azadirachta indica*, Meliaceae, showed greater larvicidal potential for leaves than for the roots after 48 h of exposure, demonstrating that different parts of the plant may show particular larvicidal response because of their chemical composition (Nour et al., 2012; Guarda et al., 2016; Castillo et al., 2017).

This is the first study that has reported the larvicidal activity of plants from the *Waltheria* genus against *Aedes aegypti*. Based on the better activity demonstrated by the roots extract, we suggest that the extract can be used in effective and economically viable preparations for domestic use to control the vector insect of severe diseases, such as dengue and Zika (Caridade et al., 2018; Fernandes et al., 2018; Lakshmi et al., 2018).

Considering the larvicidal potential of *W. viscosissima* extracts, the sub-fractions and isolated compounds of *W. viscosissima* are being tested in a bio-monitoring study against the *Ae. aegypti* in order to identify the bioactive compounds responsible for this activity (Fernandes et al., 2018b). Previous studies have shown that the substances may perform their larvae toxic effects in several ways, such as suppression of reproduction, fertility, and the inhibition of growth (Silva et al., 2015).

Conclusions

The present study contributed to the phytochemical knowledge of the species *W. viscosissima*. Fourteen compounds have been identified, including a mixture of steroids, two triterpenes, two alkaloids and eight flavonoids.

The spectrophotometric quantification of compounds demonstrated that the aerial parts extract possesses a high concentration of flavonoids and the roots extract is rich in other phenolic compounds. The roots extract also showed larvicidal activity against *Ae. aegypti*, with the potential of it being used in effective and economically viable preparations that cater towards domestic uses in the controlling of the vector insect of severe diseases, such as dengue and Zika.

Authors' contributions

MDLF (PhD student), JBLA, YMR, MFVS carried out the phytochemical study and an identification of the compounds. MFVS supervised the work, YCFT and EML performed the spectrophotometric analysis, MFA collected and identified the plant material, and FCN and DAF carried out the biological assay.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgment

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.bjp.2019.05.008.

References

Agati, G., Azzarello, E., Pollastri, S., Tattini, M., 2012. Flavonoids as antioxidants in plants: location and functional significance. Plant Sci. 196, 67–76.

Alakurtti, S., Bergström, P., Sacerdoti-Sierra, N., Jaffe, C.L., Yli-Kauhaluoma, J., 2010. Anti-leishmanial activity of betulin derivatives. J. Antibiot. 63, 123–126.

Almeida, J.G.L., Silveira, E.R., Pessoa, O.D.L., 2008. NMR spectral assignments of a new [C—O—C] isoflavone dimer from *Andira surinamensis*. Magn. Reson. Chem. 46, 103–106.

APG IV, 2016. Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Bot. J. Linn. Soc. 181, 1–20.

Barbosa, E., Calzada, F., Campos, R., 2007. *In vivo* antigiardial activity of three flavonoids isolated of some medicinal plants used in Mexican traditional medicine for the treatment of diarrhea. J. Ethnopharmacol. 109, 552–554.

Caridade, T.N.S., Rusceli, D.A., Oliveira, A.N.A., Souza, T.S.A., Ferreira, N.C.F., Avelar, D.S., Teles, Y.C.F., Silveira, E.R., Araújo, R.M., 2018. Chemical composition of four different species of the *Waltheria* genus. Biochem. Syst. Ecol. 80, 81–83.

Castillo, R.M., Stasehnko, E., Duque, J.E., 2017. Insecticidal and repellent activity of several plant-derived essential oils against *Aedes aegypti*. J. Am. Mosq. Control Assoc. 33, 25–35.

Chaves, O.S., Teles, Y.C.F., Monteiro, M.M.O., Mendes-Junior, L.G., Agra, M.F., Braga, V.A., Silva, T.M.S., Souza, M.F.V., 2017. Alkaloids and phenolic compounds from *Sida rhombifolia* L. (Malvaceae) and vasorelaxant activity of two indoquinoline alkaloids. Molecules 22, http://dx.doi.org/10.3390/molecules22010094.

Corrêa, M.P., 1974. Dicionario de Plantas uteis do Brasil e das Exoticas Cultivadas. Di Giorgio, Rio de Janeiro, pp. 44.

Costa, D.A., Matias, W.N., Lima, I.O., Xavier, A.L., Costa, V.B.M., Melo, M.F.F., Agra, M.F., Batista, L.M., Souza, M.F.V., 2009. First secondary metabolites from *Herissantia crispa L* (Brizicky) and the toxicity activity against *Artemia salina* Leach. Quim. Nova 32, 48–50.

Costa, F.J., Bandeira, P.N., Albuquerque, M.R.J.R., Pessoa, O.D.L., Silveira, E.R., Braz-Filho, R., 2008. Constituintes químicos de *Vernonia chalybaea* mart. Quim. Nova 31, 1691–1695.

Cretton, S., Breant, L., Pourrez, L., Ambuehl, C., Marcourt, L., Ebrahimi, S.N., Hamburger, M., Perozzo, R., Karimou, S., Kaiser, M., Cuendet, M., Christen, P., 2014. Antitrypanosomal quinoline alkaloids from the roots of *Waltheria indica*. J. Nat. Prod. 77, 2304–2311.

Cretton, S., Bréant, L., Pourrez, L., Ambuehl, C., Perozzo, R., Marcourt, L., Kaiser, M., Cuendet, M., Christen, P., 2015. Chemical constituents from *Waltheria indica* exert *in vitro* activity against *Trypanosoma brucei* and *T. Cruz*. Fitoterapia 105, 55–60.

Cretton, S., Dorsaz, S., Azzollini, A., Favre-Godal, Q., Marcourt, L., Ebrahimi, S.N., Voinesco, F., Michellod, M., Sanglard, D., Gindro, K., Wolfender, J., Cuendet, M., Christen, P., 2016. Antifungal quinoline alkaloids from Waltheria indica. J. Nat. Prod. 79, 300–307.

Cuong, L.C., Trang, T., Cuc, N.T., Nhiem, N.X., Yen, P.H., Anh, H.L.T., Uong, L.T., Minh, C.V., Kiem, P.V., 2015. Flavonoid glycosides from *Antidesma ghaesembilla*. Vietnam J. Chem. 53, 94–97.

Dixit, P., Khan, M.P., Swarnkar, G., Chattopadhyay, N., Maurya, R., 2011. Osteogenic constituents from *Pterospermum acerifolium* Willd. Flowers. Bioorg. Med. Chem. Lett. 21, 4617–4621.

Esteves, G., 2015. *Waltheria* in Lista de Espécies da Flora do Brasil. Rio de Janeiro, Jardim Botânico do Rio de Janeiro, Available at:http://floradobrasil.jbrj.gov.br/2012/FB009270Accessed on 10 December 2018.

Fernandes, D.A., Souza, M.S.R., Teles, Y.C.F., Oliveira, L.H.G., Lima, J.B., Conceição, A.S., Nunes, F.C., Silva, T.M.S., Souza, M.F.V., 2018. New sulphated flavonoids and larvicidal activity of *Helicteres velutina* K. Schum (Sterculiaceae). Molecules 23, http://dx.doi.org/10.3390/molecules23112784.

Fernandes, D.A., Souza, M.S.R., Oliveira, L.H.G., Ferreira, M.D.L., Barros, R.P.C., Oliveira, M.S., Lima, J.B., Nunes, F.C., Scotti, M.T., Conceição, A.S., Souza, M.F.V., 2018b. Phytochemical study of *Helicteres velutina* K. Schum (Sterculiaceae) biomonitoring by the tests against *Aedes aegypti* L. (Diptera: culicidae). In: Anais eletrônicos, DDNDIC, Available at: http://ddndic.com/DDNDIC_2018_Book_of_abstracts.pdf. Accessed on 23 May 2019.

Gomes, R.A., Ramirez, R.R.A., Maciel, J.K.S., Agra, M.F., Souza, M.F.V., Falcão-Silva, V.S., Siqueira-Junior, J.P., 2011. Phenolic compounds from *Sidastrum micranthum* (A. St.-Hil.) Fryxell and evaluation of acacetin and 7,4′-Di-*O*-methylisoscutellarein as motulator of bacterial drug resistence. Quim. Nova 34, 1385–1388.

Govindarajan, M., 2010. Larvicidal and repellent activities of *Sida acuta*Burm. F. (Family: Malvaceae) against three important vector mosquitões. Asian Pac. J. Trop. Med. 3, 691–695.

Govindarajan, M., Jebanesan, A., Pushpanathan, T., Samidurai, K., 2008a. Studies on effect of *Acalypha indica* L. (Euphorbiaceae) leaf extracts on the malarial vector, *Anopheles stephensi* Liston (Diptera: culicidae). Parasitol. Res. 103, 691–695.

Govindarajan, M., Jebanesan, A., Pushpanathan, T., 2008b. Larvicidal and ovicidal activity of *Cassia fistula* Linn. Leaf extract against filarial and malarial vector mosquitoes. Parasitol. Res. 102, 289–292.

Govindarajan, M., Jebanesan, A., Reetha, D., Amsath, R., Pushpanathan, T., Samidurai, K., 2008c. Antibacterial activity of *Acalypha indica* L. Eur. Riv. Eur. Sci. Med. Farmacol. 2, 299–302.

Gressler, V., Stuker, C.Z., Dias, G.O.C., 2008. Quinolone alkaloids from *Waltheria douradinha*. Phytochemistry 69, 994–999.

Guarda, C., Lutinski, J.A., Roman-Junior, W.A., Busato, M.A., 2016. Atividade larvicida de produtos naturais e avaliação da susceptibilidade ao inseticida temefós no controle do *Aedes aegypti* (Diptera: culicidae). Interciência 41, 243–247.

Gulcin, I., Sat, G., Beydemir, S., Elmastas, M., Kufrevioglu, O.I., 2004. Comparison of antioxidant activity of alove (*Eugenia caryophylata* Thunb) buds and lavender (*Lavandula stoechas* L.). Food Chem. 87, 393–400.

Harini, R., Sundaresan, A., Pugalendi, K.V., 2012. Antihyperlipidemic effect of biochanin A on streptozotocin induced diabetic rats. J. Pharm. Res. 5, 707–710.

Havaux, M., Kloppstech, K., 2001. The protective functions of carotenoid and flavonoid pigments against excess visible radiation at chilling temperature investigated in Arabidopsis npq and tt mutants. Planta 213, 953–966.

He, M., Min, J.-W., Kong, W.-L., He, X.-H., Li, J.-X., Peng, B.-W., 2016. A review on the pharmacological effects of vitexin and isovitexin. Fitoterapia 115, 74–85.

Herrera-Calderon, O., Enciso-Roca, E., Pari-Olarte, B., Arroyo-Acevedo, J., 2016. Phytochemical screening, antioxidant activity and analgesic effect of *Waltheria ovata* Cav. Roots in mice. Asian Pac. J. Trop. Dis. 6, 1000–1003.

Hoelzel, S.C.S.M., Vieira, E.R., Giacomelli, S.R., Dalcol, I.I., Zanatta, N., Morel, A.F., 2005. An unusual quinolinone alkaloid from *Waltheria douradinha*. Phytochemistry. 66, 1163–1167.

Hou, Z., Hub, Y., Yangc, X., Chen, W., 2017. Antihypertensive effects of tartary buckwheat flavonoids by improvement of vascular insulin sensitivity in spontaneously hypertensive rats. Food Funct. 7, 01–32.

Kwon, H.A., Cha, J.W., Park, J., Chun, Y.S., Moodley, N., Maharaj, V.J., Youn, S.H., Chung, S., Yang, H.O., 2011. Rapid identification of bioactive compounds reducing the production of amyloid β-peptide (Aβ) from South African plants using an automated HPLC/SPE/HPLC coupling system. Biomol. Ther. 19, 90–96.

Lakshmi, K.V., Sudhikumar, A.V., Aneesh, E.M., 2018. Larvicidal activity of phytoextracts against dengue fever vector, *Aedes aegypti*—a review. Plant Sci. Today 5, 167–174.

Li, T., Hou, Y., Cao, W., Yan, C.X., Chen, T., Li, S.B., 2012. Role of dopamine D3 receptors in basal nociception regulation and in morphine-induced tolerance and withdrawal. Brain Res. 1433, 80–84.

Lima, M.M.C., López, J.A., David, J.M., Silva, E.P., Giulietti, A.M., Queiroz, L.P., David, J.P., 2009. Acetylcholinesterase activity of alkaloids from the leaves of *Waltheria brachypetala*. Planta Med. 75, 335–337.

Maciel, J.K.S., Chaves, O.S., Filho, S.G.B., Teles, Y.C.F., Fernandes, M.G., Assis, T.S., Andrade, A.P., Felix, L.P., Silva, T.M.S., Ramos, N.S.M., Silva, G.R., Souza, M.F.V., 2016. New alcamide and antioxidant activity of *Pilosocereus gounellei* A. Weber ex K. Schum. Bly. Ex. Rowl. (Cactaceae). Molecules 21, http://dx.doi.org/10.3390/molecules21010011.

Monteillier, A., Cretton, S., Ciclet, O., Marcourt, L., Ebrahimi, S.N., Christen, P., Cuendet, M., 2017. Cancer chemopreventive activity of compounds isolated from *Waltheria indica*. J. Ethnopharmacol. 203, 214–222.

Mundo, J., Villeda-Hernandez, J., Herrera-Ruiz, M., Gutierrez, M.C., Arellano-Garcia, J., Leon-Rivera, I., Perea-Arango, I., 2017. Neuropharmacological and neuroprotective activities of some metabolites produced by 102 cell suspension culture of *Waltheria americana* Linn. Biomed. Pharmacother. 94, 129–139.

Muqarrabun, L.M.R.A., Ahmat, N., 2015. Medicinal uses, phytochemistry and pharmacology of Family Sterculiaceae: a review. Eur. J. Med. Chem. 92, 514–530.

Murugan, K., Murugan, P., Noortheen, A., 2007. Larvicidal and repellent potential of *Albizzia amara* Boivin and *Ocimum basilicum* Linn against dengue vector, *Aedes aegypti* (Insecta: Diptera: Culicidae). Bioresour. Technol. 98, 198–201.

Niraimathi, S., Balaji, N., Venkataramanan, N., Govindarajan, M., 2010. Larvicidal activity of alkaloid from *Sida acuta* against *Anopheles subpictus* and *Culex tritaeniorhynchus*. Int. Curr. Res. 11, 034–038.

Nour, A.H., Sandanasamy, J.D., Nour, A.H., 2012. Larvicidal activity of extracts from diferent parts of neem (*Azadirachta indica*) against *Aedes aegypti* mosquitoes larvae. Sci. Res. Essays. 7, 2810–2815.

Oliveira, A.M.F., Pinheiro, L.S., Pereira, C.K.S., Matias, W.N., Gomes, R.A., Chaves, O.S., Souza, M.F.V., Almeida, R.N., Assis, T.S., 2012. Total phenolic content and antioxidant activity of some Malvaceae family species. Antioxidants 1, 33–43.

Oliveira, G.L., Cardoso, S.C., Júnior, C.R.L., Vieira, T.M., Guimarães, E.F., Figueiredo, L.S., Martins, E.R., Moreira, D.L., Kaplan, M.A.C., 2013. Chemical study and larvicidal activity against *Aedes aegypti* of essential oil of *Piper aduncum* L. (Piperaceae). An. Acad. Bras. Cienc. 85, 1227–1234.

Oliveira, P.V., Ferreira-Jr, J.C., Moura, F.S., Lima, G.S., Oliveira, F.M., Oliveira, P.E.S., Conserva, L.M., Giulietti, A.M., Lemos, R.P.L., 2010. Larvicidal activity of 94 extracts from ten plant species of northeastern of Brazil against *Aedes aegypti* L. (Diptera: Culicidae). Parasitol. Res. 107, 403–407.

Orhan, N., Aslan, M., Hosbas, S., Deliorman, O.D., 2009. Antidiabetic effect and antioxidant potential of *Rosa canina* fruits. Phcog. Mag. 5, 309–315.

Pushpalatha, E., Muthukrishnan, J., 1999. Efficacy of two tropical plant extracts for the control of mosquitoes. J. Appl. Ent. 123, 258–262.

Ravikumar, S.M., Ali, S., Beula, J.M., 2011. Mosquito larvicidal efficacy of seaweed extracts against dengue vector of *Aedes aegypti*. Asian Pac. J. Trop. Biomed. 1, 143–146.

Rice-Evans, C.A., Miller, N.J., Paganga, G., 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic. Biol. Med. 20, 933–956.

Santiago, G.M.P., Viana, A.F., Pessoa, O.D.L., Pouliquen, Y.B.M., Arriaga, A.M.C., Andrade-Neto, M., Braz-Filho, R., 2005. Avaliação da atividade larvicida de saponinas triterpênicas isoladas de *Pentaclethra macroloba* (Willd.) Kuntze (Fabaceae) e *Cordia piauhiensis* Fresen (Boraginaceae) sobre *Aedes aegypti*. Rev. Bras. Farmacogn. 15, 187–190.

Santos, D.B., 2015. Atividade larvicida da *Copaifera langsdorffii* (Leguminosae), evidenciada pelas alterações morfohistológicas em *Aedes aegypti* (Diptera, Culicidae). Dissertação (Mestrado), Pós-graduação em Biologia da Relação Parasito-Hospedeiro, Universidade Federal de Goiás, Goiânia, Brasil, pp. 73.

Santos, E.A., Carvalho, C.M., Costa, A.L.S., Conceição, A.S., Moura, F.B.P., Santana, A.E.G., 2012. Bioactivity evaluation of plants extracts used in indigenous medicine against the snail, *Biomphalaria glabrata*, and the larvae of *Aedes aegypti*. Evid. Based. Complement. Alternat. Med., http://dx.doi.org/10.1155/2012/846583.

Silva, D.A., Silva, T.M.S., Lins, A.C.S., Costa, D.A., Cavalcante, J.M.S.C., Matias, W.N.M., Souza, M.F.V., Braz-Filho, R., 2006a. Constituintes químicos e atividade antioxidante de *Sida galheirensis* Ulbr. (Malvaceae). Quim. Nova 26, 1250–1254.

Silva, S.L.C., Gualberto, S.A., Carvalho, K.S., Fries, D.D., 2014. Avaliação da atividade larvicida de extratos obtidos do caule de *Croton linearifolius*Mull. Arg. (Euphorbiaceae) sobre larvas de *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae). Biotemas 27, 79–85.

Silveira-Júnior, C.E.A., Lima, L.C.L., Saba, M.D., 2017. Pollen morphology of *Waltheria* L. (Malvaceae-Byttnerioideae) from Bahia. Brazil. Acta. Bot. Bras. 31, 597–612.

Simões, C.M.O., Schenkel, E.P., Gosmann, G., Mello, J.C.P., Mentz, L.A., Petrovick, P.R., 2010. Farmacognosia da Planta ao Medicamento, 6a ed. UFRGS/UFSC, Porto Alegre/Florianópolis, Brasil, pp. 1104.

Singh, P.A., Brindavanam, N.B., Kimothi, G.P., Aeri, V., 2016. Evaluation of *in vivo* anti-inflammatory and analgesic activity of *Dillenia indica* f. *elongata* (Miq.) and *Shorea robusta* stem bark extracts. Asian Pac. J. Trop. Dis. 6, 75–81.

Soares, F.P., Ronconi, C.A.V., Cunha, E.V.L., Barbosa-Filho, J.M., Silva, M.S., Braz-Filho, R., 1998. Four known triterpenoids isolated from three Brazilian plants: ^{1}H and ^{13}C chemical shift assignments. Magn. Reson. Chem. 36, 608–614.

Sumroiphon, S., Yuwaree, C., Arunlertaree, C., Komalamisra, N., Rongsriyam, Y., 2006. Bioactivity of *Citrus* seed for mosquitoborne diseases larval control. Southeast. Asian. J. Trop. Med. Public. Health. 37, 123–127.

Tao, X., Sun, X., Xu, L., Yin, L., Han, X., Qi, Y., Xu, Y., Zhao, Y., Wang, C., Peng, J., 2016. Total flavonoids from *Rosa laevigata* Michx fruit ameliorates hepatic ischemia/reperfusion injury through inhibition of oxidative stress and inflammation in rats. Nutrients 8, http://dx.doi.org/10.3390/nu8070418.

Teles, Y.C.F., Horta, C.C.R., Agra, M.F., Siheri, W., Boyd, M., Igoli, J.O., Gray, A.I., Souza, M.F.V., 2015a. New sulphated flavonoids from *Wissadula periplocifolia* (L.) C. Presl (Malvaceae). Molecules, http://dx.doi.org/10.3390/molecules201119685.

Teles, Y.C.F., Ribeiro-Filho, J., Bozza, P.T., Agra, M.F., Siheri, W., Igoli, J.O., Gray, A.I., Souza, M.F.V., 2015b. Phenolic constituents from *Wissadula periplocifolia* (L.) C. Presl. and anti-inflammatory activity of 7,4′-di-*O*-methylisoscutellarein. Nat. Prod. Res. 7, 1–5.

Tennyson, S., Ravindran, K.J., Arivoli, S., 2012. Bioefficacy of botanical insecticides against the dengue and chikungunya vector *Aedes aegypti* (L.) (Diptera:Culicidae). Asian Pac. J. Trop. Med. 3, 1842–1844.

Vasques, C.A.R., Côrtes, S.F., Silva, S.M., Medeiros, I.A., 1999. Muscarinic agonist properties of the hydrobutanol extract from aerial parts of *Waltheria viscosissima* St. Hil. (Sterculiaceae) in rats. Phytother. Res. 13, 312–317.

Veeramani, P., Alagumanivasagam, G., 2016. *In-vitro* antioxidant activities of ethanolic extract of whole plant of *Waltheria indica* (Linn.). Der. Pharm. Lett. 8, 299–302.

Veni, T., Pushpanathan, T., Mohanraj, J., 2016. Larvicidal and ovicidal activity of *Terminalia chebula* Retz. (Family: Combretaceae) medicinal plant extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. J. Parasit. Dis. 41, 693–702.

Xiao, P., Zheng, B., Sun, J., Yang, J., 2017. Biochanin A induces anticancer effects in SK Mel 28 human malignant melanoma cells via induction of apoptosis, inhibition of cell invasion and modulation of NF κB and MAPK signaling pathways. Oncol. Lett. 14, 5989–5993.

Yang, S.J., Liu, M.C., Zhao, Q., Hu, D.Y., Xue, W., Yang, S., 2015. Synthesis and biological evaluation of betulonic acid derivatives as antitumor agents. Eur. J. Med. Chem. 96, 58–65.

Yougbare-Ziebrou, M.N., Lompo, M., Ouedraogo, N., Yaro, B., Guissoun, I.C., 2016. Antioxidant, analgesic and anti-inflammatory activities of the leafy stems of *Waltheria indica* L. (Sterculiaceae). Int. J. Appl. Pharm. Sci. Res. 6, 124–129.

Zhang, Z., Li, G., Szeto, S.S., Chong, C., Quan, Q., Huang, C., Cui, W., Guo, B., Wang, Y., Han, Y., Michael Siu, K.W., Lee, S.M., Chu, I.K., 2015. Flavonoid glycosides from *Rubus chingii* Hu fruits display anti-inflammatory activity through suppressing MAPKs activation in macrophages. Free Rad. Biol. Med. 84, 331–343.

Zongo, F., Ribuot, C., Boumendjel, A., Guissou, I., 2013. Botany, traditional uses, phytochemistry and pharmacology of *Waltheria indica* L. (syn. *Waltheria americana*): a review. J. Ethnopharmacol. 148, 14–26.