**In Vitro and In Vivo Antimalarial Activity of Essential Oils and Chemical Components from Three Medicinal Plants Found in Northeastern Brazil**

**Key words**
- *Vanillosmopsis arborea* (Asteraceae)
- *Lippia sidoides* (Verbenaceae)
- *Croton zehntneri* (Euphorbiaceae)
- estragole
- α-bisabolol
- thymol
- antimalarial activity

**Abstract**

The prophylactic and therapeutic arsenal against malaria is quite restricted and all the antimalarials currently in use have limitations. Thus, there is a need to investigate medicinal plants in the search for phytochemicals which can be developed into drugs. In our investigation, essential oils (EOs) were obtained from *Vanillosmopsis arborea* (Gardner) Baker, *Lippia sidoides* Cham. and *Croton zehntneri* Pax & K. Hoffm., aromatic plants abundant in northeastern Brazil, which are found in the caatinga region and are used in traditional medicine. The chemical composition of these EOs was characterized by GC-MS, and monoterpenes and sesquiterpenes were well represented. We assessed the in vitro activity of these EOs and also individual EO chemical components against the human malaria parasite *Plasmodium falciparum* (K1 strain) and the in vivo activity of EOs in mice infected with *Plasmodium berghei*. The acute toxicity of these oils was assessed in healthy mice and in vitro cytotoxicity was determined at different concentrations against HeLa cells and mice macrophages. The EO of *V. arborea* was partially active only when using the subcutaneous route (inhibited from 33 up to 47%). In relation to the EOs, *L. sidoides* and *C. zehntneri* were active only by the oral route (per gavage) and partially inhibited the growth of *P. berghei* from 43 up to 55% and showed good activity against *P. falciparum* in vitro (IC₅₀ = 7.00, 10.50, and 15.20 µg/mL, respectively). Individual EO constituents α-bisabolol, estragole, and thymol also exhibited good activity against *P. falciparum* (IC₅₀ = 5.00, 30.70, and 4.50 µg/mL, respectively). This is the first study showing evidence for the antimalarial activity of these species from northeastern Brazil and the low toxicity of their EOs.

**Introduction**

Malaria is unquestionably one of the most important infectious diseases in the world along with HIV/AIDS and tuberculosis. Roughly half of the world’s human population lives in regions in which malaria is endemic. There are 500 million new cases and around 2 million deaths per year [1] due to malaria. It is the main cause of economic loss, estimated at more than US$ 12 billion annually [2] with a high morbidity in the endemic tropical and subtropical areas worldwide. Approximately 99.8% of all malaria infections recorded in Brazil occur in the Amazon region [3]. The increase in *Plasmodium falciparum* strains that are multiresistant to available antimalarial drugs is of great concern and is a trend that requires innovative strategies for controlling the disease.

It is estimated that around 80% of the tropical and subtropical populations of the world depend on herbal remedies for the treatment of disease [4]. Medicinal plants are a rich source for the discovery of new drugs against malaria [5] and other infectious diseases. This has already been proven by the development of the antimalarials that are currently in use. Thus, the quinoline ring of quinine (a natural product isolated from *Cinchona* spp. in the 19th century) is the structural basis for many synthetic antimalarials developed last century, such as chloroquine, primaquine, and mefloquine. The active principle of *Artemisia annua*, artemisinin (a sesquiterpene lactone) [6], and its semisynthetic derivatives are effective drugs for treating serious malaria caused by multidrug resistant *P. falciparum* (resistant to chloroquine since 1980). A very important class of antimalarial natural products are lower molecular weight components of essential oils (EOs), such as monoterpenes, ses-
Materials and Methods

Collection of plant materials and extraction of EOs
Plant materials were collected (Table 1) in the months of May, July, and December of 2007. *Vanillosmopsis arborea* and *Croton zehntneri* were collected, respectively, at Chapada do Araripe in the municipality of Crato and at Morro do Chapéu in the municipality of Salitre, Ceará State. *Lippia sidoides* was collected in the medicinal plant nursery of the Natural Product Research Laboratory – LPPN/Universidade Regional do Cariri (URCA), Crato, Ceará State. A voucher specimen of each species was classified and deposited at the Prisco Bezerra Herbarium (*V. arborea*) at the Universidade Federal do Ceará and at the Dárdaro de Andrade Lima Herbarium (*C. zehntneri* and *L. sidoides*) at URCA. The collected plant materials were individually extracted using hydrodistillation for two hours in a Clevenger apparatus to obtain the EO from each species. The EOs were then dried with anhydrous Na_2SO_4 and stored in the refrigerator until the analysis could be performed.

Chemical composition of EOs using GC-MS
Chemical composition analyses on the EOs obtained in the above procedure were performed using a GC-MS system (Shimadzu CG-17A gas chromatograph/MS-QP5050A spectrometer); DB-5HT capillary column (30 m × 0.251 mm); carrier gas: helium = 1.0 mL/min; column pressure = 72.3 kPa; linear velocity = 37.2 cm/sec; total flow = 85 mL/min; carrier flow = 85 mL/min; injector temperature = 280°C; detector temperature = 280°C; column temperature = 60 (2 min) – 180°C (1 min) at 4°C/min; then 180–260°C at 10°C/min (10 min), operating under ionization energy of 70 eV. Standard hydrocarbon reference samples were injected and Kovats indices were corrected by a linear equation. Component identification was based on spectral fragmentation, using a computer library (Wiley 229), on retention indices and comparison with literature data [12].

Chromatographic separation of the EO of *V. arborea* and isolation of α-bisabolol
Column chromatography (CC) was performed using a glass column (10 × 5 cm) having a 0.3 mm glass thickness. The column was packed with 20.0 g of silica gel 60 (Vetec Química Fina Ltda.). The EO of *V. arborea* (2.0 g) was loaded onto the column head and the column was eluted with petroleum ether, pure dichloromethane, and chloroform, or in binary combinations of these solvents. All the solvents used were purchased from Labsynth Prod. Lab. Ltda. Fractions measuring 5.0 mL were collected in test tubes. Monitoring by thin-layer chromatography (TLC) was used to compare and combine the fractions obtained. Combined fractions 48–80 yielded 0.78 g of a colorless liquid, which was shown to be pure by TLC.

Monoterpenes and phenylpropanoid substances
As described above, pure α-bisabolol was isolated from the EO of *V. arborea*. Samples of pure thymol and estragole were kindly donated by Kaapi. The purity of these samples based on GC-MS analysis was > 99.9%.

Animals and ethics committee approval
Adult Swiss albino mice were used for the acute toxicity and antimalarial assays. The animals were housed in standard cages and received water and food *ad libitum*. The use of the animals was approved by the Ethics Committee of the Universidade Federal do Rio Grande do Norte (CEUA 043/2010).

In vivo acute toxicity assay
The acute toxicity of the EOs was determined in healthy mice using a modified version of the procedure described by Lorke [11]. Briefly, this involved gavage administration of different doses of the EO. This route was chosen because it is the same administration route used in antimalarial tests. Groups of three mice (fe-
males) weighing 20 ± 2 g were given oral doses of 0.15, 0.31, 0.6, 0.12, 0.25, 0.5, and 10 g/kg. The EOs were diluted in a 2% Tween-20 solution in distilled water, and administered in a single 200 µL dose. The negative control group received 2% Tween-20 in distilled water. The mortality, weight, and overall aspects of the animals were monitored for eight consecutive days. Mortality was expressed in accumulated percentage per group and median lethal doses (LD50) were established [12]. The surviving animals were reutilized in the toxicity assay and received 200 µL oral doses of EO suspension per animal for four consecutive days. The mortality rate and overall aspects of these animals were monitored daily for twenty consecutive days.

**In vitro cytotoxicity assays**

HeLa cells (human cervical carcinoma) and mice macrophages were maintained in continuous culture in DMEM (Dulbecco’s modified Eagle medium) supplemented with 10% bovine fetal serum (BFS) in a low oxygen atmosphere (5% CO2, 2% O2, and N2 balance) at 37°C. The cytotoxicity of the plant EOs was determined using the methylthiazoletetrazolium colorimetric assay (MTT) [13]. For the assays, the cells were trypsinized, washed, suspended in DMEM, and distributed into 72 wells per plate (5 × 10³ cells per well) then incubated for 18 h at 37°C. The EO from the stems of V. arborea, the leaves of L. sidoides, and the leaves of C. zehntneri were separately diluted in DMEM and tested in duplicate at the following concentrations: 10, 50, 75, 125, 250, and 500 µg/mL. The positive control group used DMEM with 10% BFS and a negative control group used DMEM without BFS. After 24 and 48 h of incubation at 37°C, 100 µL of MTT (1 mg/mL DMEM) was added to each well. After 4 h of incubation at 37°C, the supernatant was removed and 100 µL of isopropyl alcohol was added to 0.04 M hydrochloric acid in each well. The absorbance of each well was obtained from a spectrophotometric reading at 562 nm. The minimum lethal doses that inhibited 50% of cell growth were obtained from the drug concentration-response curves. Results are expressed in mean ± standard deviation.

**Parasite culture and in vitro antimalarial tests**

The parasite used for the in vitro tests was the chloroquine, pyrimethamine, and cycloguanil-resistant Plasmodium falciparum K1 strain which was acquired from MR4 (Malaria Research and Reference Reagent Resource Center). Parasites were maintained in continuous culture in human erythrocytes (blood group A+, using RPMI 1640 medium supplemented with 10% human serum). The antiparasitic effects of the EOs were measured by the percent inhibition of parasite growth in relation to the negative control (parasites cultivated in drug-free medium). Briefly, the samples tested were diluted in RPMI 1640 culture medium (with 0.02% Tween-20). These stock solutions were further diluted in complete medium (RPMI 1640 plus 10% human serum) to give each of the test concentrations used (seven dilutions from 100 to 0.13 µg/mL). The cultures used in the tests exhibited trophozoites in sorbitol-synchronized blood [14] at 1 to 1.5% parasitemia and 2.5% hematocrit, and were incubated with extracts, fractions, or isolated compounds for a total of 48 h at 37°C. A positive control containing the reference antimalarial drug chloroquine diphosphate, 97% (Sigma-Aldrich), and a negative control with medium and the Tween-20 solution were used in each experiment. The 50% inhibitory concentrations (IC50) as compared to the drug-free control responses were estimated by linear interpolation using Microcal Origin® software. Each experiment was performed in triplicate and each experiment was repeated three times. Blood smears were prepared from each test well and read in a double-blind manner.

**In vivo antimalarial assays**

The traditional suppression test [15] with modifications [16] was used. Briefly, adult Swiss albino mice weighing 20 ± 2 g were infected intraperitoneally with infected blood containing 1 × 10⁵ Plasmodium berghei NK65. The mice were randomly allocated to groups of three to five animals per cage. The EOs were tested in different experiments using the gavage technique and, when necessary, by subcutaneous administration. For oral treatment, the EOs of the stem of V. arborea, the leaves of L. sidoides, and the leaves of C. zehntneri were diluted in a Tween-20 solution with distilled water (final concentration of 2% Tween-20), and 200 µL of the concentration of test solution was administrated orally to each animal in doses of 100, 500, and 1000 mg/kg/day. Chloroquine diphosphate, 97% (Sigma-Aldrich), was dissolved in distilled water and was administered orally as an antimalarial control reference at a dose of 10 mg/kg/day. A negative control group consisted of a 2% Tween-20 solution in distilled water that was orally administered to each animal. On the 5th and 7th day after parasite inoculation, blood smears from all the mice were prepared, fixed with methanol, stained with Giemsa, and examined microscopically (1000× magnification).

For subcutaneous treatment, the EO from the stem of V. arborea was suspended in saline phosphate buffer (SPB) solution, and 200 µL was administrated to each animal in doses of 25, 50, 100, and 500 mg/kg/day. Chloroquine diphosphate was dissolved in SPB at a dose of 10 mg/kg/body weight, and administered subcutaneously. The negative control group consisted of the subcutaneous administration of 200 µL of SPB to each animal. Blood smears were obtained from all the mice on the 5th and 7th day after parasite inoculation as shown above. The parasitemia of all the experiments was determined by counting the number of parasitized erythrocytes out of 1000–3000 in random fields of the microscope. Parasite growth inhibition in the drug-treated groups was defined as the parasitemia in the non-treated control group minus the parasitemia in the treated control group, divided by the parasitemia in the non-treated control group, expressed in percentages. The EOs were considered partially active if parasitemia decreased by 30% or more [16]. Mortality was monitored in all the groups for four weeks after inoculation.

**Statistical analyses**

ANOVA and the Tukey test were used for parasitemia analysis in the antimalarial assays, and the Student’s t-test was used in the analysis of the reduction in parasitemias between the groups, and cytotoxicity in the assays with HeLa cells and mice macrophages. The statistics were significant when p ≤ 0.05.

**Results**

This is the first report on the antimalarial properties of EOs from V. arborea, L. sidoides, and C. zehntneri. In this study, chemical composition, acute toxicity, cytotoxicity, and the antimalarial activity of the EOs of these plant species were investigated. Results of chemical composition analysis of the EOs are summarized in Table 2. The EOs contained a variety of monoterpenes and sesquiterpenes. The hexane-chloroform fractions were obtained from successive column chromatographic analyses. The
The main component of the EO from the stem of *V. arborea* was the sesquiterpene α-bisabolol. Using GC-MS analysis, it was established that this component was present in ca. 80.4% of the fraction [10]. Analysis of the EO obtained from the leaves of *L. sidoides* revealed a chemical composition consisting of monoterpenoids and sesquiterpenoids, and thymol as the main component (84.9%). Analysis of the EO from the leaves of *C. zehntneri* revealed the presence of monoterpenoids and sesquiterpenoids, and estragole as the main component (76.80%).

The EOs of all three medicinal plants exhibited significant inhibition of the human malaria parasite *in vitro* as well as individual major chemical components which are found in these EOs (Table 3). The antimalarial activity of EOs and individual compounds against the *P. falciparum* K1 strain were similar based on *IC*₅₀ values, which were 7.00 to 15.20 µg/mL for EOs and 4.50 to 30.70 µg/mL for isolated compounds. The sensitivity of the *P. falciparum* strain toward the EOs tested was similar and reproducible in assays on duplicate on separate occasions. The *in vitro* cytotoxicity assay results for EOs and estragole, thymol, and α-bisabolol are shown in Table 3. The *in vitro* cytotoxicity of the EOs from these plants to the HeLa cell line and mice macrophage was evaluated in three different experiments. The minimum lethal dose that inhibits 50% of cell growth for the EOs exhibited values ranging from 340–500 µg/mL.

**Table 3** *In vitro* biological activity of the essential oils from the stem of *V. arborea*, and from the leaves of *L. sidoides* and *C. zehntneri*.

<table>
<thead>
<tr>
<th>EOs/compounds</th>
<th>Biological assay</th>
<th>Inhibition of <em>P. falciparum</em></th>
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<tr>
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<tr>
<td><strong>HeLa</strong></td>
<td><strong>Macrophages</strong></td>
<td><strong>IC₅₀ (µg/mL)</strong></td>
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<tr>
<td><strong>HeLa</strong></td>
<td></td>
<td><strong>IC₅₀ (µg/mL)</strong></td>
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<tr>
<td><strong>Macrophages</strong></td>
<td></td>
<td><strong>IC₅₀ (µg/mL)</strong></td>
</tr>
<tr>
<td><strong>24 h</strong></td>
<td><strong>48 h</strong></td>
<td><strong>24 h</strong></td>
</tr>
<tr>
<td><em>V. arborea</em> EO</td>
<td>≥ 500</td>
<td>≥ 500</td>
</tr>
<tr>
<td><em>L. sidoides</em> EO</td>
<td>480 ± 23</td>
<td>340 ± 34</td>
</tr>
<tr>
<td><em>C. zehntneri</em> EO</td>
<td>≥ 500</td>
<td>≥ 500</td>
</tr>
<tr>
<td>Estragole</td>
<td>≥ 500</td>
<td>–</td>
</tr>
<tr>
<td>Thymol</td>
<td>≥ 500</td>
<td>–</td>
</tr>
<tr>
<td>α-Bisabolol</td>
<td>≥ 500</td>
<td>–</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard error of the mean (SEM). Chloroquine diphosphate, 97%, was tested in parallel (control) and showed an IC₅₀ = 0.30 ± 0.04 µg/mL. in all the experiments conducted, the control drug chloroquine diphosphate, 97%, assayed in parallel, reduced parasitemia by 98 to 100%, and no mortality was observed in the groups that received chloroquine diphosphate after 30 days of observation. When the EO from the stem of *V. arborea* was administered orally at doses of 500 and 1000 mg/kg, it did not significantly reduce parasitemia compared to the untreated control. When administered subcutaneously for eight days, the EO from the stem of *V. arborea* at a dose of 100 mg/kg reduced parasitemia by 47 and 42%, on the 5th and 7th day after infection, respectively. At a dose of 500 mg/kg, the reduction was higher on the 7th day. The mean survival of the mice was 30 and 29 days at doses of 100 and 500 mg/kg, respectively, and 26 days for the untreated control.
In the experiments conducted with the EO from the leaves of *C. zehntneri*, only the dose of 500 mg/kg/day was effective and significant in all the biological assays. This dose reduced parasitemia by 53.5% on the 5th day after infection. On the 7th day, inhibition was 43%. The other doses used were inactive (≤30%). The mean survival of the mice was 23 days, quite similar to that of the untreated control.

**Discussion**

Terpene compounds are responsible for the antimalarial activity of many plants [17, 18]. Lopes et al. [19] isolated the sesquiterpene nerolidol and demonstrated that it exhibited antimalarial activity. In the Katzin group at the University of São Paulo, it has been shown that the terpenoid compounds farnesol, nerolidol, limonene, and linalool inhibited dolichol biosynthesis in the trophozoite and schizont stages of *P. falciparum* *in vitro*, while farnesol, nerolidol, and linalool exhibited stronger inhibitory activity on the biosynthesis of the isoprenic side chain of the benzoquinone ring of ubiquinones in the schizont stage.

The main component, α-bisabolol, is an unsaturated hydroxy sesquiterpene that is present at high levels in the EO from the stem of *V. arborea* [20]. The monoterpene thymol is present in the essential oil from the leaves of *Lippia sidoides* [21]. In this work, all EOs and individual components were considered to be active according to the criteria established by Andrade-Neto et al. (IC50 < 50 µg/mL) [22]. Although the compounds tested are less active than chloroquine diphosphate *in vitro*, the data reported here provide some rational evidence to support studies for their improvement. This fact should be assessed in terms of the balance between the ineffectiveness of antimalarial drugs available against *P. falciparum*-resistant strains and prototypes of new drugs, as well as the risks of drug toxicity and the benefits of the pharmacological action. The EO from the stem of *V. arborea* showed effective antimalarial activity only when administered subcutaneously, which may limit its usefulness as an antimalarial substance. This may be the result of slow uptake or rapid elimination of the active metabolites due to intracellular compartmentalization, or deactivation of the compound in vivo as has been shown for other molecules [23]. Factors such as absorption indices and bioavailability are likely acting to make the subcutaneous route the most effective route of administration of the EO of *V. arborea*, where the speed of disintegration and dissolution of the drug occurred more rapidly [24–26]. This outcome means that the animal organism absorbed a larger amount of the drug, while plasma levels remained stable, thus generating the expected effect. However, by the oral route, there were likely losses in bioavailability, consequently interfering with distribution [27]. It was observed that, in almost all the doses of EOs tested, there is little evidence of a dose-response relationship, a fact often observed when using crude extracts/fractions and outbred mice. These factors are likely related to the time elapsed for the drug to reach its action site, a time period of around five to seven days. In our study, the sample population consisted of genetically variable animals, which may have been a factor in the activity of the drug. This occurs in the human population, where the phenomena of latency and duration of drug activity are altered by individual variations [27]. The EO from the leaves of *L. sidoides* showed effective antimalarial activity in all the experiments, but with variations in parasitemia reduction among the days analyzed. This fact, which was not statistically significant, is likely related to the individual variation of animals as a response to the treatment performed, as well as to oral administration, which may be associated with the absorption and metabolism of active components. In just one experiment (data not shown), only the higher doses showed borderline

### Table 4  Antimalarial activity of the essential oil from the stem of *Vanillosmopsis arborea* administered orally (per gavage) and subcutaneously.

<table>
<thead>
<tr>
<th>Administration route</th>
<th>Dose (mg/kg/day)</th>
<th>% Parasitemia on day 5 or 7 ± SEM* (% inhibition of parasite growth)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>1.83 ± 0.27 (20)</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>3.48 ± 1.56 (16)</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>2.17 ± 1.16 (40)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.93 ± 1.60 (47)</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>3.50 ± 1.30 (33)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>4.20 ± 1.50 (29)</td>
</tr>
</tbody>
</table>

* Mean ± standard error of the mean (four independent experiments). Parasitemia reduction compared to untreated control mice. ** EO dose that reduced parasitemia by ≥30% is considered active. Chloroquine diphosphate, 97% (CQ), administered in parallel (10 mg/kg/day) reduced parasitemia by 98–100%

### Table 5  Antimalarial activity of the essential oils from the leaves of *Lippia sidoides* and *Croton zehntneri* by the oral route (per gavage).

<table>
<thead>
<tr>
<th>EOs</th>
<th>Dose (mg/kg/day)</th>
<th>% Parasitemia on day 5 or 7 ± SEM* (% inhibition of parasite growth)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. sidoides</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0.81 ± 0.23 (48)</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.60 ± 0.14 (47)</td>
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<tr>
<td></td>
<td>100</td>
<td>0.41 ± 0.10 (55)</td>
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<tr>
<td></td>
<td>50</td>
<td>0.68 ± 0.09 (25)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>0.56 ± 0.25 (28)</td>
</tr>
<tr>
<td>C. zehntneri</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>0.12 ± 0.08 (53.50)</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.43 ± 0.53 (20)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.77 ± 0.23 (43)</td>
</tr>
</tbody>
</table>

* Mean ± standard error of the mean (four independent experiments). Parasitemia reduction compared to untreated group control. ** EO dose that reduced parasitemia by ≥30% is considered active. Chloroquine phosphate, 97% (CQ), administered in parallel (10 mg/kg/day) reduced parasitemia by 98–100%
antimalarial activity on the 5th day after infection, and on the 7th day its activity increased significantly, which would suggest a slower pharmacological effect. The toxicity was considered low for *V. arborea* and *C. zehntneri*, and moderate for *L. sidoides*, at the highest concentrations tested (up to 500 μg/mL) since it was nearly 20–30 times higher than the dose inhibiting 50% of *P. falciparum* growth (16.4 μg/mL). The isolated compounds showed cytotoxicity similar to the EOs.

**In vivo** acute toxicity assays on the EOs under study showed low or moderate toxicity [28]. The EO from the stem of *V. arborea*, when administered only once at different doses (0.15–10 g/kg), produced mainly ruffled fur in the mice, resulting in the death of some of the animals. The lethal dose for 50% of the animals (LD₅₀) was 7 g/kg (no acute toxicity). When the EO was administered for four consecutive days, the signs of toxicity observed were: weight loss at the start of the experiment, a loss that was recovered after dose suspension, and ruffled fur in all the animals. However, no deaths occurred in any of the groups. It was not possible to assess the toxicity of the doses higher than 0.12 g/kg (highest dose tested in this experiment) owing to the small amount of material available. The EO from the leaves of *L. sidoides*, when administered in a single dose (0.6–10 g/kg), produced toxic effects such as tachycardia, weight loss, and ruffled fur at all the doses tested, and death at doses between 0.25 and 10 g/kg. The LD₅₀ determined was 7 g/kg (no acute toxicity). When administered for four consecutive days, the following toxic aspects were observed: weight loss, ruffled fur, and death. The LD₅₀ was 1.8 g/kg [low acute toxicity].

Mendonça et al. [29], in preclinical acute toxicological assays, demonstrated the low toxicity of the hydrosoluble components carried by water vapor during the extraction of the EO from the leaves of *L. sidoides*. The contact reaction test, performed with the external application of 1% EO in animals, showed no hypersensitive reactions. In acute toxicity assays with mice, it was demonstrated that the EOs of *L. sidoides* and *C. zehntneri* showed no toxic effects up to 3 g/kg [30]. This study shows, for the first time, partial antimalarial activity in *P. berghei*-infected mice for the EOs of *V. arborea*, *L. sidoides*, and *C. zehntneri*, as well as important activity against *P. falciparum* in *vitro* for its active components. In the present study, preliminary in vivo acute toxicity and *in vitro* cytotoxicity assays on the EOs enabled a more thorough analysis of the toxic effects, suggesting no or low acute toxicity.

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**Conflict of Interest**

The authors have no conflicts of interest concerning the work reported in this paper.

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