

Chemical constituents, larvicidal activity and molluscicidal from fresh leaves of *Alpinia zerumbet* (Pers.) and *Cymbopogon citratus* (DC.) Stapf

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SUMMARY

Introduction: This study evaluated the chemical characterization, larvicidal activity and molluscicidal activity in front of the snail transmitting schistosomes (*Biomphalaria glabrata*) of the essential oils of *Alpinia zerumbet* and *Cymbopogon citratus* (DC.) Stapf. The essential oils (EOs) were extracted by hydrodistillation, with chemical characterization through Gas Chromatography coupled to mass spectrometry (GC-MS). The physical-chemical parameters were determined according to the Brazilian Pharmacopoeia. The toxicity test followed the bioassay with *Artemia salina* Leach, the EOs approved in this assay followed to evaluate their biological properties. **Methodology:** for molluscicidal activity, the methodology recommended by the WHO was performed, and the LC₅₀ of the EOs was performed for their action in the face of the snail obtained by the Reed&Muench method. Both EOs showed low toxicity, and thus were evaluated for the biological properties larvicidal and molluscicidal. *Alpinia zerumbet* EO showed molluscicidal activity with LC₅₀ of 40.63 mg·L⁻¹ and *Cymbopogon citratus* EO 33.94 mg·L⁻¹. **Results:** Both EOs showed larvicidal and molluscicidal potentials against the organisms tested, showing satisfactory results for their action. The results indicate that the evaluated EOs are composed of substances that promote and encourage their application due to their potential for molluscicidal and larvicidal biological activity.

Keywords: Essential oil, *Alpinia zerumbet*, *Cymbopogon citratus*.

RESUMEN

Constituyentes químicos, actividad larvica y molusquica de hojas frescas de *Alpinia zerumbet* (Pers.) y *Cymbopogon citratus* (DC.) Stapf

Introducción: este estudio evaluó la caracterización química, la actividad larvica y la actividad molusquica frente al caracol que transmite esquistosomas (*Biomphalaria glabrata*) de los aceites esenciales de *Alpinia zerumbet* y *Cymbopogon citratus* (DC.) Stapf. Los aceites esenciales (AE) fueron extraídos por hidrodestilación, con caracterización química a través de cromatografía de gases acoplada a espectrometría de masas (GC-MS). Los parámetros físico-químicos se determinaron de acuerdo con la Farmacopea Brasileña. La prueba de toxicidad siguió al bioensayo con *Artemia salina* Leach, los AE probados en este ensayo se evaluaron a continuación en sus propiedades biológicas. **Metodología:** para la actividad molusquica se empleó la metodología recomendada por la OMS, y la LC₅₀ de las AE se realizó para su acción frente al caracol obtenido por el método Reed&Muench. Ambos AE mostraron baja toxicidad, y por lo tanto fueron evaluados para las propiedades biológicas larvicidas y molusquicas. *Alpinia zerumbet* AE mostró actividad molusquica con LC₅₀ de 40,63 mg·L⁻¹ y *Cymbopogon citratus* EO 33,94 mg·L⁻¹. **Resultados:** ambos AE mostraron potenciales larvicidas y molusquicas contra los organismos probados, mostrando resultados satisfactorios para su acción. Los resultados indican que los AE evaluados están compuestos de sustancias que promueven y fomentan su aplicación debido a su potencial para la actividad biológica molusquica y larvica.

Palabras clave: Aceite esencial, *Alpinia zerumbet*, *Cymbopogon citratus*.

RESUMO

Constituintes químicos, atividade larvica e moluscida de folhas frescas de *Alpinia zerumbet* (Pers.) e *Cymbopogon citratus* (DC.) Stapf

Introdução: este estudo avaliou a caracterização química, atividade larvica e atividade moluscida contra o caramujo transmissor de esquistossomos (*Biomphalaria glabrata*) dos óleos essenciais de *Alpinia zerumbet* e *Cymbopogon citratus* (DC.) Stapf. Os óleos essenciais (AE) foram extraídos por hidrodestilação, com caracterização química por cromatografia gasosa acoplada a espectrometria de massas

(CG-EM). Os parâmetros físico-químicos foram determinados de acordo com a Farmacopéia Brasileira. O teste de toxicidade seguiu o bioensaio com *Artemia salina* Leach, os EA testados neste teste foram então avaliados quanto às suas propriedades biológicas. **Metodologia:** para a atividade moluscicida, foi utilizada a metodologia recomendada pela OMS, e o LC_{50} do EA foi realizado para sua ação contra o caramujo obtido pelo método de Reed & Muench. Ambos os AE apresentaram baixa toxicidade e, portanto, foram avaliados quanto às propriedades biológicas larvicidas e moluscicidas. *Alpinia zerumbet* AE apresentou atividade moluscicida com CL_{50} de $40,63 \text{ mg}\cdot\text{L}^{-1}$ e *Cymbopogon citratus* EO $33,94 \text{ mg}\cdot\text{L}^{-1}$. **Resultados:** ambos os AE apresentaram potencial larvicida e moluscicida contra os organismos testados, apresentando resultados satisfatórios para sua ação. Os resultados indicam que os AE avaliados são compostos por substâncias que promovem e estimulam sua aplicação devido ao seu potencial de atividade biológica moluscicida e larvicida.

Palavras-chave: Óleo essencial, *Alpinia zerumbet*, *Cymbopogon citratus*.

INTRODUCTION

Essential oils (EOs) constitute the volatile elements contained in various organs of different plants and are thus called due to the lipophilic composition they present, chemically different from the glyceride composition of oils and fats [1]. These EOs are obtained from different extraction techniques, such as distillation which includes steam drag distillation [2].

The use of EOs as medicinal agents has been known since remote antiquity. There are pictorial records of six thousand years ago among the Egyptians. Aromatic substances were also popular in ancient China and India, hundreds of years before the Christian era. However, it was only from the Middle Ages, through the distillation process, introduced by Muslim scientists, that the real commercialization of aromatic materials began [3].

Several studies claim that the use of medicinal plants is related to popular culture which is transmitted from generation to generation in traditional communities [4]. According to Santos *et al.* [5], empirical knowledge is derived from many of the current knowledge of the effects of known plant species. However, it is emphasized that several plants have toxic effects, and that the false idea that everything that is natural is innocuous, needs to be reviewed and made aware [6]. Among several plant species composed of EOs in which these properties can be found are *Alpinia zerumbet*, and *Cymbopogon citratus* (DC.) Stapf.

The species *C. citratus*, also known as lemongrass, belongs to the Gramineae family and is characterized as a perennial herb, with narrow leaves and high commercial value. It has been widely studied as it exhibits antifungal activity [7], antibacterial [8], anthelmintic [9], insecticide [10], diuretic [11] and anticarcinogenic [12], these properties are attributed to volatile oils α -citral, β -citral and mycene [13].

On the other hand, the species *Alpinia zerumbet* (Pers.) is a plant originating in Asia and belongs to the Zingiberaceae family [14]. Among the proven pharmacological properties for *A. zerumbet*, we highlight the hypotensive and diuretic effects obtained through leaf tea, which were confirmed by studies of Mendonça *et al.* [15]. Its classes of chemical constituents, alkaloids, flavonoids, and as main components of the EO are monoterpenes with a higher concentration of 1,8-cineol and terpene-4-ol, with studies proving their antimicrobial activity [16].

Considering that the use of EOs may represent an alternative and innovative way in the control of Neglected Tropical Diseases (NTD), afflicting more than one billion people in 149 tropical and subtropical conditions [17], coma researchers around the world have been studying natural alternatives to synthetic products, since natural products are an option with less toxicity. Thus, this study aimed to determine the chemical constituents, larvicidal activity, molluscicidal and toxicity of the EOs of *A. zerumbet* and *C. citratus*.

MATERIAL AND METHODS

Plant material

The collection of plant material used in this research was carried out in October to December 2019. The leaves of *C. citratus* were collected in the Attic Herbarium Seabra do Maranhão of the Federal University of Maranhão and the leaves of *A. zerumbet* were collected in the municipality of São José de Ribamar, São Luís, Brazil. The samples were deposited in the Attic Seabra Herbarium of the Federal University of Maranhão. After collection, the plant species were transported to the Laboratory of Research and Application of Essential Oils (LOEPAV/Ufma).

Obtaining the EO

For extraction of EOs, the hydrodistillation technique was used with a glass Clevenger extractor coupled to a round bottom balloon packed in an electric blanket as a heat generating source, according to figure 1. 120 g of each plant material were used, adding distilled water (1:10).

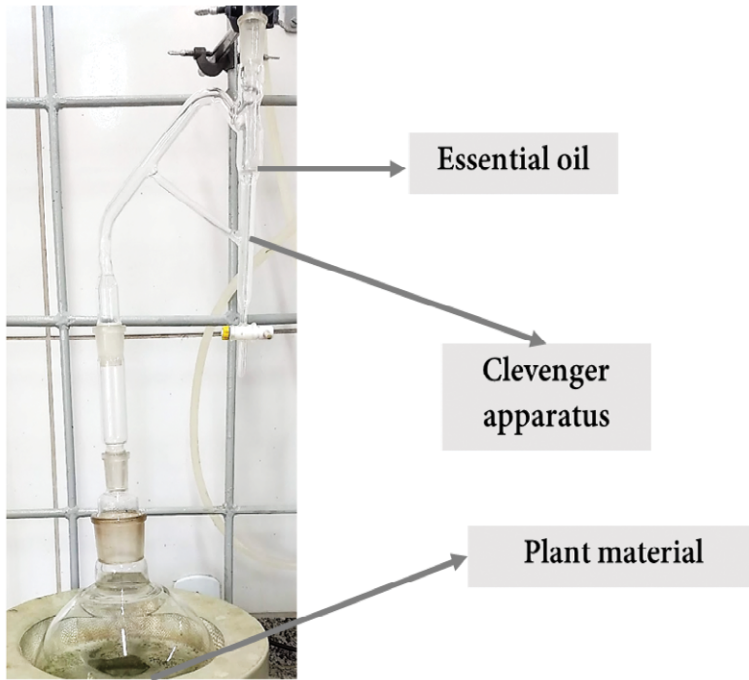


Figure 1. Experimental scheme to obtain the EO.

Hydrodistillation was conducted at 100 °C for 3 h by collecting the extracted EO. Each EO was dried by percolation with anhydrous sodium sulfate (Na_2SO_4) and centrifuged. These operations were performed in triplicates and the samples were stored in amber glass ampoules under 4 °C refrigeration. Subsequently submitted the analyses.

Analyses of chemical constituents

The constituents of the EOs were identified by gas chromatography coupled to mass spectrometry (CG-MS). 1.0 mg of the sample was dissolved in 1000 μL dichloromethane (purity 99.9%). The conditions of analysis were as follows: Method: Adams. M; Volume injected: 0.3 μL ; Column : HP-5MS capillary (5 % diphenyl, 95 % dimethyl polysiloxane) (DB-5MS equivalent or CP-Sil 8CB LB/MS), in dimensions (30 mm x 0.25 mm x 0.25 μm); Drag gas : He (99.9995); 1.0 $\text{mL}\cdot\text{min}^{-1}$; Injector: 280 °C, Split mode (1:10); Oven: 40 °C (5.0 min.) up to 240 °C at a rate of 4 °C $\cdot\text{min}^{-1}$, from 240 °C to 300 °C (7.5 min) at a rate of 8 °C $\cdot\text{min}^{-1}$; $t\text{T}$ = 60.0 min; Detector : IN; EI (70 eV); Scan mode (0.5 sec scan $^{-1}$); Mass range: 40-500 daltons (one); Transfer line: 280 °C.; Filament: off 0.0 to 4.0 min; Linear quadrupole mass spectrometer. The Automated Mass spectral Deconvolution Mass & Identification System (AMDIS) program was used to identify the compounds in the sample.

Larvicidal activity

The eggs of *Aedes aegypti* were collected at the Federal University of Maranhão, Bacanga Campus in São Luís/MA, through traps called ovitraps.

These consist of brown buckets (500 mL), polyethylene, with 1 mL of brewer's yeast and 300 mL of running water and inserted two eucatex reeds for mosquito egg position. The traps were inspected weekly for the replacement of reeds and egg collection and forwarded to the Laboratory of Research and Application of Essential Oils (PCQA-Ufma) of the Technological Pavilion of the Federal University of Maranhão (Ufma).

The tests for larvicidal activity were carried out according to the adapted methodology proposed by Silva [18]. Initially, a 100 mg·L⁻¹ mother solution of EO diluted in 2% dimethylsulfoxide solution (DMSO). Five dilutions were prepared from this solution at concentrations 10, 20, 50, 70 and 100 mg·L⁻¹. At each concentration, 10 larvae were added in the proportion 1 mL/larva. All tests were performed in triplicates and as negative control was used a solution formed of DMSO 2%, and as a positive control, a solution of temephos (O,O,O',O'-tetramethyl O,O'-thiodi-p-phenylene bis (phosphothiothioate) at 100 ppm, equivalent to the concentration used by the National Health Foundation (Funasa) for larvicidal vector control, in addition to novaluron (\pm -1-[3-chlorine-4-(1-3-trifluoro-2-trifluoromethoxyethoxy) phenyl-3-(2,6-difluorobenzoyl) urea at 0.02 mg·L⁻¹, a dose adopted by the Ministry of Health, which indicates by the WHO in the range of 0.01 to 0.05 mg·L⁻¹. After 24 hours, the live and dead were found, and the larvae that did not react to the touch after 24 hours of the beginning of the experiment were carried out. To quantify the efficiency of the EO, the Statistical Probit Test was applied [19].

Obtaining and cultivating snails

Samples of snails of the species *Biomphalaria glabrata* were captured in rainy periods, in areas with low sanitation in the neighborhood Sá Viana, São Luís-MA. The collection technique was performed according to a proposal from Brazil (2007) performing a scan with a shell in the submerged areas and the captured snails were collected in a glass container with lid, with water from the breeding site itself. Their search was carried out at various points in each breeding site, and then sent to the molluscum of the Laboratory of Research and Application of Essential Oils (LOEPAV/Ufma).

The snails were kept in the laboratory for 30 days and analyzed every 7 days to confirm the absence of infection by *Schistosoma mansoni*. For this, 5 snails were placed in transparent glass containers with 25 mL of dechlorinated water, that is, 5 mL/snail, exposed to light (60 W lamps) for one hour with a distance of 30 cm to stimulate the release of the feces and taken to be analyzed, through visualization with the aid of a

stereoscopic magnifying glass (8x), those that were parasitized (positive) were labeled and separated for future individual analysis and those who showed no signs of trematoid infection in the period of 30 days were selected for the molluscicidal activity test.

Evaluation of molluscicide activity

For the evaluation of molluscicide activity, the technique recommended by the World Health Organization [20] was used, where two tests were performed in triplicate. In the first, called a pilot test, a solution of the oil under study was prepared in a volume of 500 mL at a concentration of 100 mg·L⁻¹ and 0.15 mL of Tween 80 (active tense), where 10 adult snails were placed, negative for *Schistosoma mansoni*, obtaining at the end a ratio of 50 mL/snail and feeding them with hydroponic lettuce.

They were exposed in the solution for 24 h, at room temperature, removed from the solution, washed twice with dechlorinated water, placed in a glass container containing 500 mL of dechlorinated water, feeding them with hydroponic lettuce and observed to every 24 hours for 4 days to assess mortality.

In the second test, called lethal concentration (LC₅₀), solutions of each oil were prepared in a volume of 500 mL at concentrations 100, 75, 62.5, 50, 20, 10, 5 and 2 mg·L⁻¹ and 0.15 mL of Tween 80 (surfactant), using the same methodology of the pilot test. For the negative control, two tests were also used, in the first we placed 500 mL of dechlorinated water and 10 snails in a glass container and in the second 10 snails immersed in a solution with 0.15 mL of Tween 80 in 500 mL of distilled water, feeding both with hydroponic lettuce and the analysis also performed in the previous tests.

The lethal concentration LC₉₀ of the bioassay was determined by linear regression, obtaining the concentration versus mortality ratio of molluscs. Mortality rates were obtained by averaging dead individuals as a function of the logarithm of the tested dose. The statistical analysis of the data for the LC₅₀ was performed according to the Probit [19].

Toxicity

For the evaluation of the lethality of *Artemia salina* Leach, the methodology described by Meyer *et al.* [21]. *Artemia salina* solution stock of each EO was prepared at the concentration of 10 000 mg·L⁻¹ and 0.02 mg of Tween 80 (active tense). Aliquots of 5, 50 and 500 µL of this were transferred to test tubes and supplemented with saline solution previously prepared up to 5 mL, obtaining at the end concentrations of 10, 100 and 1000 mg·L⁻¹, respectively. All tests were performed in triplicates, where ten larvae in the nauplium phase were transferred to each of the test tubes.

For white, 5 mL of saline solution was used for positive control $K_2Cr_2O_7$ and for negative control 5 mL of a $4\text{ mg}\cdot\text{L}^{-1}$ solution of Tween 80. After 24 hours of exposure, the live larvae were counted, considering dead those that did not move during the observation or with the slight agitation of the vial.

The criterion established by Dolabela [22] for classification of the toxicity of EOs, being considered highly toxic when $LC_{50} \leq 80\text{ mg}\cdot\text{L}^{-1}$, moderately toxic to $80\text{ mg}\cdot\text{L}^{-1} \leq LC_{50} \leq 250\text{ mg}\cdot\text{L}^{-1}$ and mildly toxic or nontoxic when $LC_{50} \geq 250\text{ mg}\cdot\text{L}^{-1}$.

RESULTS AND DISCUSSION

Chemical constituents

The chemical constituents were obtained through GC/MS, in the EO samples of the in natura leaves of *C. citratus* and *A. zerumbet*. They were identified in the EO of *C. citratus*, obtained by hydrodistillation, as major constituents: geranial (41.96%) and neral (33.71%). Similar results were found by Antonioni [23] identifying geranial (41.8%) and neral (25.6%). Costa *et al.* [24] also identified geranial geratus (49.98%) in the EO of *C. citratus* and neral (37.78%). Gonçalves *et al.* [25] reported the presence of the major components of the EO of *C. citratus* being geranial (46.32%) and neral (31.28%), equivalent to 77.6% citral. Franz *et al.* [26] observed similar geranial values (47.56%) and neral (31.50%). Sacchetti *et al.* [27] identified in the chemical composition of the EO of this species about 65 to 86% of citral present in the EO, Andrade *et al.* [28] also identified 30.1% of neral and 39.9% of geranial leaves in the EO of *C. citratus* leaves cultivated in northern Brazil. However, Negrelle *et al.* [29] stated that regardless of the origin of lemongrass, The EO has 30 to 93.74% citral, with generally the predominance of geranial.

Thus, it is possible to affirm that citral (neral and geranial) is the major compound for the EO of *C. citratus*, corroborating the results obtained in this study. Studies of the chemical composition of the EO of *C. citratus* in different localities characterize citral as the main chemical constituent of EO. According to Pinto *et al.* [30] citral is a mixture that is a mixture of isomers, geranial (α -citral) and neral (β -citral).

Through CG/MS, the major compounds of the EO of the in natura leaves of *A. zerumbet* were identified as p-cymene (40.15%) and 1.8-cineol (26.70%). Similar results were reported by Castro *et al.* [31] when observing that the EO of the leaves of *A. zerumbet* presented the p-cymene (32.72%), 1.8-cineol (24.05%) and 4-terpineol (20.23%) as the majority, corroborating the analyses of this study. The volatile constituents of the EO of *A. zerumbet* have been the subject of research from several studies,

such as Lahlou *et al.* [32] in which the chemical compounds were identified by the CG-MS method, among the major chemical constituents of the EO, terpinen-4-ol, 1,8-cineol and γ -terpineine stood out. In the study by Barcelos *et al.* [33] terpinen-4-ol monoterpene (37.45 %) was identified and followed by sesquiterpene caryoene oxide (7.56 %) and sabine transhydrate monoterpenes (6.61 %) and 1,8-cineol (4.02 %). Ali *et al.* [34] also detected terpinen-4-ol, 1,8-cineol and β -pineno as the major components of *A. zerumbet* EO.

The major compounds present in the EO of *A. zerumbet* are responsible for several biological effects. Its classes of chemical constituents, alkaloids, flavonoids, and as main components of essential oil are monoterpenes with higher concentration of 1,8-cineol and terpinen-4-ol, with studies proving its antimicrobial activity [35]. The gardener also has anxiolytic, anesthetic action [36], antimicrobial, hypotensive and sedative [37]. It presents anti-inflammatory action was proven by [38]. These effects are fully associated with the majority compounds present in the EO.

Leaf maturation, seasonality, place and time of collection, drying process and storage are factors that influence the quality and composition of EOs [39, 40], which could explain the difference in chemical composition observed in this work with the previously described data. The differences observed in quantity and chemical composition of the EO of plants of the same species in different regions can be caused by microclimatic, phytogeographic, genotypic and geographical and agronomic factors, conditions, mainly in the soil. However, as a general rule, the main components remain the same, varying only their concentration levels [41].

Larvicidal activity

Table 1 presents the results obtained in the lethality assay for the action of EOs in the face of larvae of *Aedes aegypti*.

According to Dias *et al.* [42], larvicidal potential is classified according to criteria based on lethal concentration (LC), EOs that obtain LC_{50} greater than $100 \text{ mg}\cdot\text{L}^{-1}$ are considered non-active, those who obtain LC_{50} less than $100 \text{ mg}\cdot\text{L}^{-1}$ are considered active and those who obtain LC_{50} below $50 \text{ mg}\cdot\text{L}^{-1}$ are highly active. Thus, as observed in table 1, the EO of *A. zerumbet* presented the LC_{50} of $37.96 \text{ mg}\cdot\text{L}^{-1}$, potentially active [43] and LC_{90} of $65.61 \text{ mg}\cdot\text{L}^{-1}$ against the larvae *Aedes aegypti*, this result stimulates the potential for applicability of this EO, since Cavalcanti *et al.* [44] when verifying the larvicidal activity of the EO of the leaves and branches of *A. zerumbet* against *Aedes aegypti* found LC_{50} equivalent to $313 \text{ mg}\cdot\text{L}^{-1}$, a value much higher than the LC_{50} of this study [45] when analyzing the larvicidal activity of the EO of the seeds of *A. zerumbet*

front *Aedes aegypti* found LC₅₀ of 125 µg·mL⁻¹, a value also higher than those observed in this study.

Table 1. LC for EOs action against larvae of 4 instar of *Aedes aegypti*.

| EO | Concentration (mg·L ⁻¹) | % Mortality | LC ₅₀ | LC ₉₀ | R ² | σ | χ ² |
|--------------------|-------------------------------------|-------------|---------------------|------------------|----------------|-------|----------------|
| <i>C. citratus</i> | 70 | 75.00 | 40.14 | 71.55 | 0.9600 | 0.632 | 0.967 |
| | 50 | 45.00 | | | | | |
| | 30 | 35.00 | | | | | |
| | 20 | 10.00 | | | | | |
| | 10 | 0.00 | | | | | |
| <i>A. zerumbet</i> | 70 | 75.00 | 37.96 | 65.61 | 0.982 | 0.447 | 0.997 |
| | 50 | 55.00 | | | | | |
| | 30 | 45.00 | | | | | |
| | 20 | 25.00 | | | | | |
| | 10 | 10.00 | | | | | |
| Positive control | | | All inactive larvae | | | | |
| Negative control | | | All active larvae | | | | |
| White | | | All active larvae | | | | |

As also observed in table 1, the EO of *C. citratus* showed a LC₅₀ of 40.14 mg·L⁻¹ and LC₉₀ of 71.55 mg·L⁻¹ in front of the *Aedes aegypti* larvae, also presenting great potential in their larvicidal activity [43]. Higher concentrations were observed in the study by [46] presenting a LC₅₀ of 63.89 mg·L⁻¹ and LC₉₀ of 112.21 mg·L⁻¹ also for the EO of this species and in other studies the same EO demonstrated relevant results in relation to insecticide activity [47]. The biological activity of *C. citratus* is conventionally attributed to citral, its main component [48].

The active potential of EOs and their compounds against *Aedes aegypti* may vary significantly according to intrinsic and extrinsic factors, plant species, plant part, manufacturing age, chemotypes and geographical conditions (such as occurrence season, precipitation, moisture percentage, temperature, sunlight, and altitude), in which the plant was collected, the source of larvae, and the methods generally used to induce different larval responses [42].

Molluscicidal activity

Table 2 presents the results obtained in the lethality assay for the action of the EOs in the face of adult snails of *Biomphalaria glabrata*.

Table 2. LC para ação do EO frente aos caramujos adultos de *Biomphalaria glabrata*.

| EO | Concentration (mg·L ⁻¹) | % Mortality | LC ₅₀ | LC ₉₀ | R ² | σ | χ ² |
|--------------------|-------------------------------------|-------------|---------------------|------------------|----------------|-------|----------------|
| <i>C. citratus</i> | 150 | 90 | 40.63 | 85.55 | 0.923 | 0.406 | 0.880 |
| | 100 | 90 | | | | | |
| | 50 | 50 | | | | | |
| | 30 | 20 | | | | | |
| | 10 | 10 | | | | | |
| <i>A. zerumbet</i> | 150 | 90 | 33.94 | 55.60 | 0.926 | 0.530 | 0.934 |
| | 100 | 80 | | | | | |
| | 50 | 70 | | | | | |
| | 30 | 30 | | | | | |
| | 10 | 20 | | | | | |
| Positive control | | | All inactive larvae | | | | |
| Negative control | | | All active larvae | | | | |
| White | | | All active larvae | | | | |

By verifying table 2 we perceived the effectiveness of the species *C. citratus* and *A. zerumbet* in the face of the snail transmitting schistosomosis, since the WHO [49], the molluscicide activity is considered significant when LC₉₀ is less than 100 mg·L⁻¹ [50-53]. In order to be considered molluscicidal the substance must eliminate the snail at all stages of its life cycle and in its natural habitat, have low concentrations, low cost, be stable in storage under tropical conditions; easy to carry and apply; have selective lethal action to snails, be harmless to man, domestic animals, fish and plants, do not suffer decomposition in water and soil and be stable in conditions of temperature and solar irradiation [53].

Studies with EO of *Cymbopogon citratus* leaves also show its effectiveness with the aqueous and alcoholic extract, showing significant results against *Biomphalaria* [54]. The species also exhibits an excellent bactericidal activity in the face of many pathogens, such as *Malassezia* [55]. The EOs do not yet have many studies published in scientific journals with the species or with the extracted oils, showing the relevance of studies with such species. As seen, for the EO of *Cymbopogon citratus*, some studies are reported in relation to other biological activities in scientific journals. The results found in the present work demonstrate that the volatile constituents obtained from plants present molluscicidal activity.

Toxicity

Table 3 presents the results obtained in the lethality assay for the action of the EOs in the face of larvae of *Artemia salina*.

Table 3. LC₅₀ for EO action front *Artemia salina* L.

| EO | LC ₅₀ | R ² |
|--------------------|----------------------------------|----------------|
| <i>C. citratus</i> | 315.12 ± 6.10 mg·L ⁻¹ | 0.9942 |
| <i>A. zerumbet</i> | 284.15 ± 4.28 mg·L ⁻¹ | 0.9843 |

According to table 3, the EO of *C. citratus* presented LC₅₀ equivalent to 315.12 mg·L⁻¹, being classified as nontoxic according to the criterion of [22] that standardizes LC₅₀ ≥ 250 mg·L⁻¹ of the EO as nontoxic. Lima *et al.* [56] evaluated the toxicity of the methanol extract of medicinal plants according to the *A. salina* toxicity bioassay, found LC₅₀ equivalent to 704.67 ± 31.44 µg·mL⁻¹, classifying the methanolic extract of the leaves of *C. citratus* as nontoxic. Divergent results were found by Ribeir *et al.* [57] when analyzing the toxicity of EO *C. citratus* against *A. salina* in the form of a lethal dose (LD₅₀) quantified in 18.85 (µg·mL⁻¹), containing variations in the limits of 13.71 to 26 (µg·mL⁻¹).

Also, according to table 3, EO of *A. zerumbet* presented LC₅₀ of 284.15 mg·L⁻¹ in front of *Artemia salina* larvae, being considered nontoxic by the criterion established by [22]. Similar results were found by dos Santos *et al.* [58] when evaluating the EO toxicity of the leaves of *A. zerumbet* front *A. salina* found the LC₅₀ equal to 280.2 mg·L⁻¹, classifying the EO as nontoxic [59] when analyzing the ethanol extract of leaves and flowers of *A. zerumbet* found a LC₅₀ equal to 740 ppm, being considered toxic by the criteria used by the authors. The extract of *A. zerumbet* showed considerable toxicity at concentrations higher than 800 ppm, with mortality percentage > 63.3% and promoting 100% at concentrations above 1400 ppm. Considering that a plant is toxic when its extract is lethal to at least 50% of *Artemia salina* larvae at a concentration of less than 1000 ppm, it is plausible to state that the ethanol extract of leaves and flowers of *A. zerumbet* presents relevant toxicity, thus showing the EO as an alternative for its application.

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CONFLICT OF INTEREST

The authors state that there is not conflict of interest.

REFERENCES

1. A. Siani, A. Sampaio, M. Sousa, M. Henriques, M. Ramos, Óleos essenciais: potencial anti-inflamatório, *Biotecnologia: Ciências e Desenvolvimento*, **16**, 38-46 (2006).
2. F. Solórzano-Santos, M. G. Miranda, Essential oils from aromatic herbs as antimicrobial agentes, *Curret Opinion in Biotechnology*, **23**(2), 136-141 (2012). S.P.D. Cantanhede, A.d.M. Marques, N. Silva-Souza, A.L. Valverde, Atividade molusquicida de plantas: uma alternativa profiática, *Revista Brasileira de Farmacognosia*, **20**(2), 282-288 (2010).
3. M.H. Tyrrell, Evolution of natural flavor development with the assistance of modern technologies, *Food Technology* (Chicago), **44**(1), 68-72 (1990).
4. E.R. Oliveira, L. Menini Neto, Levantamento etnobotânico de plantas medicinais utilizadas pelos moradores do povoado de Manejo, Lima Duarte-MG, *Revista Brasileira de Plantas Mediciniais*, **14**(2), 311-320 (2012).
5. G.G. Santos, R.C. Trindade, J.A.B. Alves, P.O. Santos, P.B. Alves, A.F. Blank, L.M. Carvalho, L.C. Aquino, Atividade antimicrobiana dos óleos essenciais de erva cidreira e manjeriço frente a bactérias de carnes bovinas, *Alimentos e Nutrição Araraquara*, **21**(21), 529-535 (2010).
6. V. O. Bednarczuk, M. C. S. Verdam, M. D. Miguel, O. G. Miguel, Testes *in vitro* e *in vivo* utilizados na triagem toxicológica de produtos naturais, *Visão Acadêmica*, **11**(2), 43-50 (2010).
7. V.J. Schuck, M. Fratini, C.S. Rauber, A. Henriques, E.E. Schapoval, Avaliação da atividade antimicrobiana de *Cymbopogon citratus*, *Revista Brasileira de Ciências Farmacéuticas*, **37**(1), 45-49 (2001).
8. K. Cimanga, K. Kambu, L. Tona, S. Apers, T. De Bruyne, N. Hermans, A.J. Vlietinck, Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo, *Journal of Ethnopharmacology*, **79**(2), 213-220 (2002).
9. M.D. Almeida, M.B. Botura, M.M. Santos, G.N. Almeida, L.F. Domingues, S.L. Costa, M.J.M. Batatinha, Efeitos dos extratos aquosos de folhas de *Cymbopogon citratus* (DC.) Stapf (capimsanto) e de *Digitaria insularis* (L.) Fedde (Capim-açu) sobre cultivos de larvas de nematóides gastrintestinais de caprinos, *Revista Brasileira de Parasitologia Veterinaria*, **12**(3), 125-129 (2003).

10. R. Rajapakase, H.F. Vanenden, Potential of four vegetable oils and ten botanicals' powers for reducing infestation of cowpeas by *Callosobruchus maculatus*, *C. chinensis* and *C. rhodesianus*, *Journal of Stored Products Research*, **33**(1), 59-68 (1997).
11. J.L. Hernández-Gálvez, I. Paredes-Torres, O.E. Alfonso-Aguilar, M. Llanuch-Lara, Estudio del efecto diurético de la hoja de *Cymbopogon cytratus* en modelo de ratas, *Revista Cubana de Plantas Medicinales*, **3**(2), 79-82 (1998).
12. R. Puatanachokchai, H. Kishida, A. Denda, N. Murata, Y. Konishi, U. Vinitketkumnun, Inhibitory effects of lemon grass (*Cymbopogon citratus*, Stapf) extract on the early phase of hepatocarcinogenesis after initiation with diethylnitrosamine in male Fisher 344 rats, *Cancer Letters*, **183**(1), 9-15 (2002).
13. C. Carriconde, D. Mores, M. Von Fritschen, E.L. Cardozo Junior, *Plantas medicinais e alimentícias*, Olinda: Centro Nordestino de Medicina Popular, Universidade Federal Rural de Pernambuco, 1996, p. 45-47.
14. H. Lorenzi, H.M. Souza, *Plantas ornamentais no Brasil: arbustivas, herbáceas e trepadeiras*, Nova Odessa/SP, Instituto Plantarum, 2001.
15. V.L.M. Mendonça, C.L.A. Oliveira, A.A. Craveiro, V.S. Rao, M.L. Fonteneles, Pharmacological and toxicological evaluation of *Alpinia speciosa*, *Memórias do Instituto Oswaldo Cruz*, **86**, 93-97 (1991).
16. C.P. Victorio, D.S. Alviano, C.S. Alviano, C.L. Lage, Chemical composition of the fractions of leaf oil of *Alpinia zerumbet* (Pers.) BL Buertt & RM Sm. and antimicrobial activity, *Revista Brasileira de Farmacognosia*, **19**(3), 697-701 (2009).
17. World Health Organization, *Investing to Overcome the Global Impact of Neglected Tropical Diseases: Third – Report on Neglected Tropical Diseases*, WHO library Cataloguing, Geneva, Switzerland, 2015.
18. W. Silva, *Atividade larvicida do óleo essencial de plantas existentes no Estado de Sergipe contra Aedes aegypti Linn*, Tese de Mestrado, Universidade Federal do Sergipe, São Cristóvão-SE, Brasil, 2006.
19. D.J. Finney, *Probit analysis: a statistical treatment of the sigmoid response curve*, Cambridge University Press, Cambridge, 1952.
20. World Health Organization, *Report of the Scientific working Group on Plant Molluscicide & Guidelines for evaluation of plant molluscicides*, TDR/SC, Geneva, 1983.

21. B.N. Meyer, N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.J. Nichols, J.L. McLaughlin, Brine shrimp: a convenient general bioassay for active plant constituents, *Planta Medica*, **45**(5), 31-34 (1982).
22. M. Dolabela, *Triagem in vitro para atividade antitumoral e anti Trypanossoma cruzi de extratos vegetais, produtos naturais e substâncias sintéticas*, Tese de Mestrado, Universidade Federal de Minas Gerais, Belo Horizonte, Brasil, 1997.
23. G. Antonioli, *Desenvolvimento de nanocápsulas de poli (ácido láctico) contendo óleo essencial de capim-limão e avaliação contra fungos fitopatogênico*, Tese de Mestrado, Universidade de Caxias do Sul, Caxias do Sul, Brasil, 2020.
24. A.V. Costa, P.F. Rondelli, V.T. de Queiroz, A.C. Tuler, K.B. Britto, D. Pratissolli, *Cymbopogon citratus* (Poaceae) essential oil on *Frankliniella schultzei* (Thysanoptera: Thripidae) and *Myzus persicae* (Hemiptera: Aphididae), *Bioscience Journal*, **29**(6), 1840-1847 (2013).
25. A.H. Gonçalves, A.S. Pereira, G.R.S. Santos, L.G.L. Guimarães, Atividade fungitóxica *in vitro* dos óleos essenciais de *Lippia sidoides* Cham., *Cymbopogon citratus* (DC.) Stapf. e de seus constituintes majoritários no controle de *Rhizoctonia solani* e *Sclerotium rolfsii*, *Revista Brasileira de Plantas Mediciniais*, **17**(4), 1007-1015 (2015).
26. A.R. Franz, N. Knaak, L.M. Fiuza, Toxic effects of essential plant oils in adult *Sitophilus oryzae* (Linnaeus) (Coleoptera, Curculionidae), *Revista Brasileira de Entomologia*, **55**(1), 116-120 (2011).
27. G. Sacchetti, S. Maietti, M. Muzzoli, M. Scaglianti, S. Manfredini, M. Radice, R. Bruni, Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods, *Food Chemistry*, **91**(4), 621-632 (2005).
28. E.H.A. Andrade, M.D.G.B. Zoghbi, M.D.P Lima, Chemical composition of the essential oils of *Cymbopogon citratus* (DC.) Stapf cultivated in North of Brazil, *Journal of Essential Oil-Bearing Plants*, **12**(1), 41-45 (2009).
29. R.R.B. Negrelle, E.C. Gomes, *Cymbopogon citratus* (DC.) Stapf: chemical composition and biological activities, *Revista Brasileira de Plantas Mediciniais*, **9**, 80-92 (2007).
30. Z.T. Pinto, F.F. Sánchez, A.R.D. Santos, A.C.F. Amaral, J.L.P. Ferreira, J.C. Escalona-Arranz, M.M.D.C. Queiroz, Chemical composition and insecticidal acti-

- vity of *Cymbopogon citratus* essential oil from Cuba and Brazil against housefly, *Revista Brasileira de Parasitologia Veterinaria*, **24**(1), 36-44 (2015).
31. K.N.D.C. Castro, D.F. Lima, L.C. Vasconcelos, R.C. Santos, A.M.L. Pereira, F.H.D.S. Fogaça, R.M. Calvet, Composição química e eficácia do óleo essencial e do extrato etanólico de *Alpinia zerumbet* sobre *Staphylococcus aureus*, *Arquivos do Instituto Biológico*, **83**, e0192014 (2016).
 32. S. Lahlou, L.F.L. Interaminense, J.H. Leal-Cardoso, G.P. Duarte, Antihypertensive effects of the essential oil of *Alpinia zerumbet* and its main constituent, terpinen-4-ol, in DOCA-salt hypertensive conscious rats, *Fundamental & Clinical Pharmacology*, **17**(3), 323-330 (2003).
 33. F.F. Barcelos, M.L. Oliveira, N.P.B. Giovaninni, T.P. Lins, C.A. Filomeno, S.Z. Schneider, T.U. Andrade, Estudo químico e da atividade biológica cardiovascular do óleo essencial de folhas de *Alpinia zerumbet* (Pers.) BL Burtt & RM Sm. em ratos, *Revista Brasileira de Plantas Mediciniais*, **12**(1), 48-56 (2010).
 34. S. Ali, S. Sotheeswaran, M. Tuiwawa, R.M. Smith, Comparison of the composition of the essential oils of *Alpinia* and *Hedychium* species essential oils of Fijian plants, part 1, *Journal of Essential Oil Research*, **14**(6), 409-411 (2002).
 35. C.P. Victório, D.S. Alviano, C.S. Alviano, C.L. Lage, Composição química das frações do óleo foliar de *Alpinia zerumbet* (Pers.) BL Burtt & RM Sm. e atividade antimicrobiana, *Revista Brasileira de Farmacognosia*, **19**(3), 697-701 (2009).
 36. E.S.B. Albuquerque, L.J. Neves, Anatomia foliar de *Alpinia zerumbet* (Pers.) Burtt & Smith (Zingiberaceae), *Acta Botanica Brasileira*, **18**(1), 109-121 (2004).
 37. A.J.C. Correa, C.E. Lima, M.C.C.D. Costa, *Alpinia zerumbet* (Pers.) BL Burtt & RM Sm. (Zingiberaceae): levantamento de publicações nas áreas farmacológica e química para o período de 1987 a 2008, *Revista Brasileira de Plantas Mediciniais*, **12**(1), 113-119 (2010).
 38. C. Kriek, T. Finatto, T. S. Müller, M. P. Guerra, A. I. Orth, Biologia reprodutiva de *Alpinia zerumbet* (Pers.) BL Burtt & RM Sm. (Zingiberaceae) em Florianópolis, Santa Catarina, *Revista Brasileira de Plantas Mediciniais*, **10**(2), 103-110 (2008).
 39. E.D. Oliveira, O.A. Lameira, M.G.B. Zoghbi, Identificação da época de coleta do óleo-resina de copaíba (*Copaifera* spp.) no município de Moju, PA, *Revista Brasileira de Plantas Mediciniais*, **8**(3), 14-23 (2006).

40. M.B. Stefanini, L.C. Ming, M.O.M. Marques, M.A.A. Meireles, L.S. Moura, J.A. Marchese, Seed productivity, yield, and composition of the essential oil of fennel *Foeniculum vulgare* var. *dulcis* in the season of the year, *Revista Brasileira de Plantas Mediciniais*, **8**, 86-90 (2006).
41. D. Beatović, D. Krstic-Milosevic, S. Trifunovic, J. Siljegovic, J. Glamoclija, M. Ristic, S. Jelacic, Chemical composition, antioxidant, and antimicrobial activities of the essential oils of twelve *Ocimum basilicum* L. cultivars grown in Serbia, *Records of Natural Products*, **9**(1), 62-75 (2015).
42. C.N. Dias, D.F.C. Moraes, Essential oils and their compounds as *Aedes aegypti* L. (Diptera: Culicidae) larvicides: review, *Parasitology Research*, **113**(2), 565-592 (2014).
43. S.S. Cheng, G.G. Huang, Y.J. Chen, J.J. Yu, W.J. Chen, S.T. Chang, Chemical compositions and larvicidal activities of leaf essential oils from two eucalyptus species, *Bioresource Technology*, **100**(1), 452-456 (2009).
44. E.S.B. Cavalcanti, S.M.D. Morais, M.A.A. Lima, E.W.P. Santana, Larvicidal activity of essential oils from Brazilian plants against *Aedes aegypti* L., *Memórias do Instituto Oswaldo Cruz*, **99**(5), 541-544 (2004).
45. E.W. Chan, E.W. Chiang, S.K. Wong, H.T. Chan, *Alpinia zerumbet*, uma planta de gengibre com inúmeras propriedades medicinais: uma atualização sobre os resultados de sua pesquisa, *Chinese Pharmacy*, **26**(11), 775-788 (2017).
46. R.F. Furtado, M.G. de Lima, M. Andrade-Neto, J.N. Bezerra, M.G.D.V. Silva, Atividade larvicida de óleos essenciais contra *Aedes aegypti* L. (Diptera: Culicidae), *Neotropical Entomology*, **34**(5), 843-847 (2005).
47. Z.T. Pinto, *Caracterização química e atividade inseticida dos óleos essenciais de plantas aromáticas procedentes do Brasil e de Cuba sobre o desenvolvimento pós-embriônico de dípteros muscoides*, Ph.D. thesis, Universidade Federal Rural do Rio de Janeiro, 2015.
48. S.Y. Yang, L. Yao, Antimicrobial activity of *Cymbopogon citratus* against utilized bacteria and fungus, *Journal of Shanghai Jiaotong University (Agricultural Science)*, **4**, 374-376 (2005).
49. Organização Mundial da Saúde, *O controle da esquistossomose: segundo relatório do Comitê de Especialistas da OMS*, Fiocruz, 1994.

50. N. Kumar, P. Bhandari, B. Singh, S.S. Bari, Antioxidant activity and ultra-performance LC-electrospray ionization-quadrupole time-of-flight mass spectrometry for phenolics-based fingerprinting of Rose species: *Rosa damascena*, *Rosa bourboniana* and *Rosa brunonii*, *Food and Chemical Toxicology*, **47**(2), 361-367 (2009).
51. G. Yang, L. Kong, W. Zhao, X. Wan, Y. Zhai, L.C. Chen, J.P. Koplan, Emergence of chronic non-communicable diseases in China, *The Lancet*, **372**(9650), 1697-1705 (2008).
52. M. Cuenot, Classificazione internazionale del funzionamento, della disabilità e della salute, *EMC-Medicina Riabilitativa*, **25**(1), 1-6 (2008).
53. B. Otarigho, O.A. Morenikeji, Molluscicidal effects of aqueous and ethanolic extracts of Lemongrass (*Cymbopogon citratus*) leaf against the different developmental stages of *Biomphalaria pfeifferi*, *New York Science Journal*, **5**(8), 70-77 (2012).
54. World Health Organization, *Control de la esquistosomiasis: informe de un Comité de Expertos de la OMS*, TDR/SC, Geneva, 1985.
55. S. Prakash, R. Ramasubburayan, V.S. Ramkumar, E. Kannapiran, A. Palavesam, G. Immanuel, *in vitro* Scientific evaluation on antimicrobial, antioxidant, cytotoxic properties and phytochemical constituents of traditional coastal medicinal plants, *Biomedicine & Pharmacotherapy*, **83**, 648-657 (2016).
56. A.B.S.d. Lima, I.M.B.N. Queiroga, G.M.d.S. Silva, J.S. da Costa, J.P.d.S. Guedes, C.d.O. Dantas, M.T. Cavalcanti, Characterization and application of *Lippia alba* (Mill) and *Cymbopogon citratus* D.C. Stapf. essential oils as natural sanitizers in coriander, *Food Science and Technology*, Campinas, **39**(4), 993-998 (2019).
57. M.D.S. Ribeir, G.O. Everton, P.V. Serra-Rosa, E.R.N. Pimenta, A.P.d. Araújo-Neto, A.A.S. Dias, A.P. Matos-Pereira, D.P. do Nascimento, A.M.A.S. Carvalho, L.d.S. Silveira, I.T.P. de Sousa, V.E. Mouchrek-Filho, Total phenolics, toxicity and antimicrobial activity of the essential oil of the leaves of *Alpinia zerumbet* (Pers.) BL Burt & RM Sm, *Research Society and Development*, **9**(7), e953975253 (2020).
58. C.P. dos Santos, R.P. de Lira-Lemos, A.F. Santos, Avaliação da toxicidade das espécies medicinais *Alpinia zerumbet* (Pers.) e *Sambucus australis* Cham. & Schltdl. frente *Artemia salina* Leach, *Revista Ambientale*, **2**(2), 65-76 (2010).

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