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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 LC_{50} 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 LC_{50} 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 larvicida y molusquicida de hojas frescas de *Alpinia zerumbet* (Pers.) y *Cymbopogon citratus* (DC.) Stapf

Introducción: este estudio evaluó la caracterización química, la actividad larvicida y la actividad molusquicida 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 molusquicida se empleó la metodología recomendada por la OMS, y la LC_{50} 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 molusquicidas. *Alpinia zerumbet* AE mostró actividad molusquicida con LC_{50} de 40,63 mg·L^{-1} y *Cymbopogon citratus* EO 33,94 mg·L^{-1}. **Resultados:** ambos AE mostraron potenciales larvicidas y molusquicidas 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 molusquicida y larvicida.

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

Resumo

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

Introdução: este estudo avaliou a caracterização química, atividade larvicida e atividade moluscicida 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 CL50 de 40,63 mg·L^{-1} e *Cymbopogon citratus* EO 33,94 mg·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 a-citral, b-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).

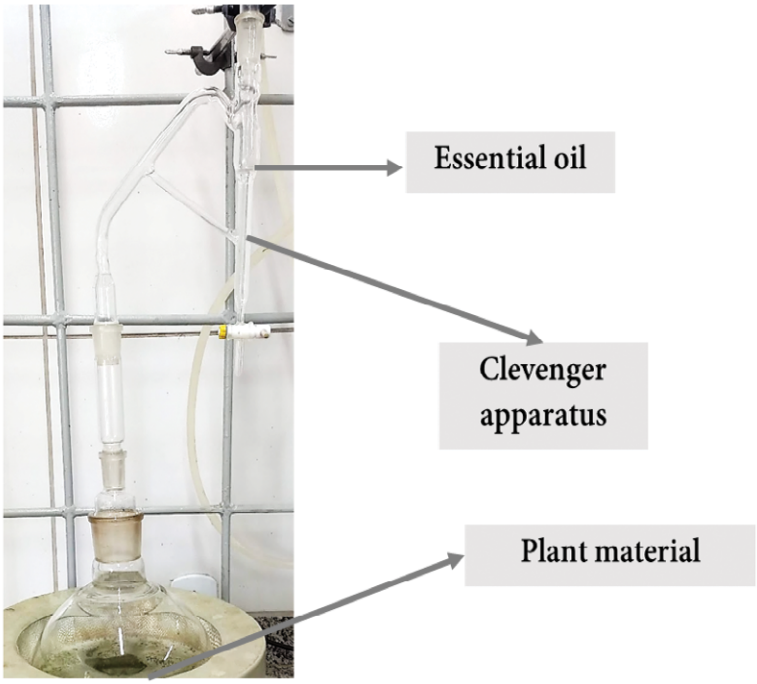

Figure 1. Experimental scheme to obtain the EO.

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 mL·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·min^{-1}, from 240 °C to 300 °C (7.5 min) at a rate of 8 °C·min^{-1}); tT = 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 $mg{\cdot}L^{-1}$ 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 $mg{\cdot}L^{-1}$. 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 Fundation (Funasa) for larvicidal vector control, in addition to novaluron (±-1-[3-chlorine-4-(1-3-trifluro-2-trifluoromethoxyethoxy) phenyl-3-(2,6-difluro-benzoyl) urea at 0.02 $mg{\cdot}L^{-1}$, a dose adopted by the Ministry of Health, which indicates by the WHO in the range of 0.01 to 0.05 $mg{\cdot}L^{-1}$. 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 *Schistossoma 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 fences 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 *Schistossoma 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 (LC_{50}), 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 LC_{90} 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 LC_{50} 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 mg·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 $LC_{50} \leq 80$ mg·L^{-1}, moderately toxic to 80 mg·$L^{-1} \leq LC_{50} \leq 250$ mg·L^{-1} and mildly toxic or nontoxic when $LC_{50} \geq 250$ mg·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 terpene-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 mg·L^{-1} are considered non-active, those who obtain LC_{50} less than 100 mg·L^{-1} are considered active and those who obtain LC_{50} below 50 mg·L^{-1} are highly active. Thus, as observed in table 1, the EO of *A. zerumbet* presented the LC_{50} of 37.96 mg·L^{-1}, potentially active [43] and LC_{90} of 65.61 mg·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 mg·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 LC_{50} 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	χ2
C. citratus	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					
A. zerumbet	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 LC_{50} of 40.14 mg·L^{-1} and LC_{90} 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 LC_{50} of 63.89 mg·L^{-1} and LC_{90} 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 ($mg{\cdot}L^{-1}$)	% Mortality	LC_{50}	LC_{90}	R^2	σ	χ2
C. citratus	150	90	40.63	85.55	0.923	0.406	0.880
	100	90					
	50	50					
	30	20					
	10	10					
A. zerumbet	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 schistossomosis, since the WHO [49], the molluscs ive activity is considered significant when LC_{90} is less than 100 $mg{\cdot}L^{-1}$ [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. LC_{50} for EO action front *Artemia salina* L.

EO	LC_{50}	R^2
C. citratus	315.12 ± 6.10 mg·L^{-1}	0.9942
A. zerumbet	284.15 ± 4.28 mg·L^{-1}	0.9843

According to table 3, the EO of *C. citratus* presented LC_{50} equivalent to 315.12 mg·L^{-1}, being classified as nontoxic according to the criterion of [22] that standardizes $LC_{50} \geq$ 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 LC_{50} equivalent to 704.67 ± 31.44 µg·mL^{-1}, classifying the methanolic extract of the leaves of *C. citratus* as nontoxic. Divergentresults 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 (LD_{50}) 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 LC_{50} 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 LC_{50} 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 LC_{50} 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.

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