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Chemical profile and antimicrobial potential of essential oils of *Cymbopogon citratus* (DC.) Stapf, *Ocimum basilicum* Linn and *Aniba rosaeodora* Ducke

Perfil químico e potencial antimicrobiano dos óleos essenciais de *Cymbopogon citratus* (DC.) Stapf, *Ocimum basilicum* Linn e *Aniba rosaeodora* Ducke

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The study evaluated the chemical profile, antimicrobial activity and toxicity of essential oils (EOs) of *C. citratus*, *O. basilicum* and *A. rosaeodora*. The EOs were extracted by hydrodistillation, the chemical profile was determined using the physicochemical parameters and the chemical composition was obtained by gas chromatography coupled to mass spectrometry (GC-MS). The toxicity assay followed the *Artemia salina* Leach bioassay. To perform the antimicrobial activity, the Disc Diffusion technique and the Mueller Hinton Broth Dilution (MH) technique were used. The physicochemical parameters of the EOs showed satisfactory results. The major chemical composition of the EO of *A. rosaeodora* presented linalool (93.60%), the EO of *C. citratus* exhibited geranial (43.96%) and EO of *O. basilicum* found methyl chavicol. All EOs in this study had LC_{50} between 582 mg L^{-1} and 282 mg L^{-1}. The antimicrobial activity of EO *C. citratus* demonstrated inhibition halos for *S. aureus* of 25 mm and 25 mm for *E. coli*, EO of *O. basilicum* exhibited inhibition halo of 18 mm for *E.coli* and 20 mm for *S. aureus* and EO of *A. rosaeodora* presented 11 mm for *E. coli* and 15 for *S. aureus*. As the values of MIC and MBC found, the EO of *O. basilicum* presented a more effective inhibitory action against *S. aureus*. It was concluded that the observed biological potentials encourage the application potentials in the efficiency of the control of pathogenic microorganisms.
Keywords: essential oil; antimicrobial; toxicity.

O estudo avaliou o perfil químico, a atividade antimicrobiana e a toxicidade dos óleos essenciais (OE's) de *C. citratus*, *O. basilicum* e *A. rosaeodora*. Os OE's foram extraídos por hidrodestilação, o perfil químico foi determinado através dos parâmetros físico-químicos e a composição química foi obtida por cromatografia gasosa acoplada à espectrometria de massas (CG-EM). O ensaio de toxicidade seguiu o bioensaio com *Artemia salina* Leach. Para realização da atividade antimicrobiana utilizou-se a técnica de Difusão em Disco e a Diluição em Caldo Mueller Hinton (MH). Os parâmetros físico-químicos os OE's apresentaram resultados satisfatórios. A composição química majoritária do OE de *A. rosaeodora* apresentou o linalol (93,60%), o OE de *C. citratus* exibiu o geranial (43,96%) e o OE de *O. basilicum* constatou o metil chavicol. Todos os OE's deste estudo apresentaram CL_{50} entre 582 mg L^{-1} a 282 mg L^{-1}. A atividade antimicrobiana do OE *C. citratus* demonstrou halos de inibição para *S. aureus* de 25 mm e 25 mm para *E. coli*, o OE de *O. basilicum* exibiu halo de inibição de 18 mm para *E.coli* e 20 mm para *S. aureus* e o OE de *A. rosaeodora* apresentou 11 mm para *E. coli* e 15 para *S. aureus.* Conforme os valores de CIM e CBM constataram, o OE de *O. basilicum* apresentou uma ação inibitória mais eficaz contra *S. aureus.* Conclui-se que os potenciais biológicos observados incentivam os potenciais de aplicação na eficiência do controle de microrganismos patogênicos.
Palavras-chave: óleo essencial; antimicrobiano; toxicidade.

1. INTRODUCTION

In agriculture, diseases affecting plants can be caused by fungi, bacteria and viruses, generating great losses of crop yield. Many strategies have been developed to prevent damage caused by these pathogens, such as avoidance techniques, crop rotation, soil management, plant nutrition and use of resistant varieties. In addition, chemicals are widely applied to plantations. However, especially

in organic farming, it is necessary to replace such products with biological methods of disease control and pests [1].

Thus, compounds derived from plants with antimicrobial activity are increasingly explored for use in the preservation and improvement of food quality [2]. Among the natural products, the essential oils (EOs) stand out as a complex natural mixture of varied chemical composition. Being extracted from aromatic plants, their use as antimicrobial has been highly researched because it is safer substitutes for food conservation in relation to chemical additives [3]. The antimicrobial activity of EOs is usually associated with compounds such as eugenol, allicin, thymol and carvracol, linalool, among others. These active ingredients, due to their hydrophobic characteristics, act by breaking the microbial cell wall, resulting in the loss of its functionality [4-6].

EOs have been used for several decades in the pharmaceutical, cosmetic and, more recently, industries as flavoring, flavorings and natural preservatives by the food industries [7]. In their condition, natural antimicrobials have the potential to be used in the control of microorganisms, reducing the need for additives, controlling contamination in food and improving shelf life extension technologies to eliminate undesirable pathogens and slow down the deterioration of products [8].

Among the plants that have EOs stand out *Cymbopogon citratus* (DC.) Stapf, *Ocimum basilicum* Linn and *Aniba rosaeodora* Ducke. *Cymbopogon citratus*, known as lemongrass, belonging to the Poaceae family, an herbaceous plant originating in India, is widely distributed in several tropical countries, including Brazil. Popular medicine uses its tea or muffled, prepared from its leaves such as soothing, analgesic, antipyretic, antirheumatic, diuretic and digestive disorders. Through studies it is possible to attribute antimicrobial activity to the geranial and neral major compounds present in their EO [9].

Aniba rosaeodora, known as pink wood, belongs to the Lauraceae family, was discovered in Brazil in 1925, is a species native to the Amazon. In its composition the majority compound present in its EO is linalool, in which it was found in other plants such as Ho-Sho (*Cinnamomum camphora* L.), a substance responsible for inhibiting the growth of microorganisms. In this sense, EO *A. rosaeodora* is promising as a source of linalool and it is possible to attribute its antimicrobial activity to its majority compound [10].

Ocimum basilicum, known as basil is a medicinal plant which belongs to the Lamiaceae family, has become important in society for presenting cosmetic use, food and due to its therapeutic properties in the pharmaceutical industry. The antimicrobial activity of EO *O. basilicum* is associated with its methyl chavicol and linalool constituents, thus widely used in folk medicine in combating bacterial infections [11]. In view of the importance of EOs and studies on their activities, this study determined the chemical profile, antimicrobial activity and toxicity of *C. citratus, O. basilicum* and *A. rosaeodora*.

2. MATERIALS AND METHODS

2.1. Botanical material

The leaves of *C. citratus* and *O. basilicum* were collected at the Herbário Ático Seabra of the Universidade Federal do Maranhão, São Luís – MA, Brazil, in July 2018. Samples from the stem of *A. rosaeodora* were collected in the Ducke Forest Reserve of the National Institute of Amazonian Research, located at Km 26 of the AM-010 Highway (Manaus-Itacoatiara) in the municipality of Manaus, Amazonas. All species were identified by the Herbarium Attic Seabra (UFMA) and a sample of each deposited species. The plant materials were transported to the Laboratory of Research and Application of Essential Oils (LOEPAV/UFMA) of the Federal University of Maranhão (UFMA), kiln dry FANEM 520 at 45°C/24h. Subsequently, they were crushed in an electric mill and their mass measured for yield calculations.

2.2. Extraction of EOs

For extraction of EOs, the hydrodistillation technique was used with a glass Clevenger extractor coupled to a round-bottomed balloon packed in an electric blanket as a heat generating source. 122g of *C. citratus* dry leaves, 100g of *O. basilicum* and 30g of *A. rosaeodora* were used adding distilled

water (1:10). Hydrodistillation was conducted at 100°C for 3h collecting the extracted EO. EO was dried with sodium sulfate (Na_2SO_4). These operations were performed in triplicates and samples were stored in amber glass ampoules under 4°C refrigeration to avoid possible losses of volatile constituents.

2.3. Chemical analysis

The physicochemical parameters of the EOs were determined: density, solubility, color and appearance according to the Farmacopeia Brasileira [12] and the EO constituents were identified by gas chromatography coupled to mass spectrometry (GC-MS). 1.0 mg of the sample was dissolved in 1000 μL of dichloromethane (purity 99.9%). The conditions of analysis were as follows: Method: Adams. M; Injected volume: 0.3 μL; Column: Capillary HP-5MS (5% diphenyl, 95% dimethyl polysiloxane) (Equivalent DB-5MS or CP-Sil 8CB LB/MS), in dimensions (30 m x 0.25 mm x 0.25 μm); Drag gas: He (99.9995); 1.0 mL/min; Injector: 280°C, Split mode (1:10); Oven: 40°C (5.0 min.) up to 240°C at a rate of 4 oC .min-1, from 240°C to 300°C (7.5 min) at a rate of 8 oC.min-1); tT = 60.0 min; Detector: EM1; EI (70 eV); Scan mode (0.5 sec/scan); Mass range: 40 - 500 daltons (one); Line transfer: 280°C; Filament: off 0.0 to 4.0 min; Linear quadrupole mass spectrometer. The AMDIS (Automated Mass spectral Deconvolution Mass & Identification System) program was used to identify the compounds in the sample. Chromatographic peaks were identified by comparing the respective mass spectra with the data of the spectrothecae (1) WILEY 139; (2) NIST107; (3) NIST21.

2.4. Toxicity

This test was performed according to the methodology described by Meyer et al. (1982) [13]. In a rectangular container, with a partition containing holes of approximately 0.02 cm thickness spaced by 0.5 cm and evenly distributed, artificial saline solution (60 gL^{-1} of distilled water) were added (60 g of sea salt/1L of distilled water). The container was placed inside an incubator illuminated by a fluorescent lamp, with aeration. On one side of this container, about 64 mg of *Artemia salina* cysts were added, taking care that they did not cross the partition. The part of the system containing *Artemia salina* cysts was covered with aluminium foil, so that the organisms, at birth, were attracted by light on the other side of the system, forcing them to cross the partition. This procedure aims at homogenizing the physical conditions of the test organisms. Incubation was performed for a period of 48 hours. Throughout the test the temperature was monitored.

For the evaluation of the lethality of *Artemia salina* Leach, a saline solution stock of each EO was prepared in the concentration of 10.000 mgL^{-1} and 0.02 mg of Tween 80 (active tense). Rates 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 mgL^{-1}, respectively. All tests were carried out in triplicates, where ten larvae in the nauplium phase were transferred to each of the test tubes.

For white, 5 mL of the saline solution was used for positive control $K_2Cr_2O_7$ and for negative control 5 mL of a 4 mgL^{-1} solution of Tween 80. After 24 hours of exposure, the count of the live larvae was performed, considering dead those that did not move during observation or with the slight agitation of the vial. The criterion established by Dolabela (1997) [14] was adopted for classification of EOs toxicity, being considered highly toxic when $CL_{50} \leq 80$ mgL^{-1}, moderately toxic to 80 $mgL^{-1} \leq LC_{50} \geq 250$ mgL^{-1} and slightly toxic or nontoxic when $LC_{50} \geq 250$ mgL^{-1}. Statistical analysis of the data for the toxicity test was performed according to the Reed and Muench (1938) [15] and the confidence interval according to Pizzi (1950) [16].

2.5. Antimicrobial activity

Two strains of bacteria were used: *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923). These were previously identified and confirmed by biochemical tests.

Pure microbial cultures maintained in Agar TSA were peaked for Brain and Heart Infusion Broth (BHI) and incubated at 35ºC until they reached exponential growth phase (4-6h). After this period, the cultures had their cell density adjusted in 0.85% sterile saline solution, in order to obtain

turbidity comparable to that of the standard McFarland solution 0.5, which results in a microbial suspension containing approximately $1.5x10^8$ $CFUmL^{-1}$.

Antimicrobial activity was performed according to the disc diffusion technique of the Clinical and Laboratory Standards Institute (2015) [17] that standardizes antimicrobial sensitivity tests by disc-diffusion, using standardized suspensions of *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) strains distributed in plates containing Agar Mueller Hinton (AMH) culture medium plus discs containing 50 μL of EO. Gentamycin (30 μg) was used as positive control. The plates were incubated in a bacteriological greenhouse at 35 °C, 24 h. Inhibition halo diameters were measured, including disc diameter. These trials were done in triplicate.

To determine the Minimum Inhibitory Concentration (MIC), the dilution technique was used in broth. With serial EO dilutions in Broth Mueller Hinton (BMH), resulting in concentrations of 1000, 500, 250, 100, 50, 25, 10 and 5 μgmL^{-1}, performing sterility controls and incubation at 35ºC for 24 h. After the incubation period, MIC was verified, being defined as the lowest concentration that visibly inhibited bacterial growth (absence of visible cloudiness). Trials were carried out in triplicate. The Minimum Bactericidal Concentration (MBC) was measured from the inoculation of 10 μL of the tubes resulting from dilution in BMH, performed in a plate count after 24 h, where plaques that did not grow colonies were classified as bactericidal concentrations for the action of the EO.

3. RESULTS AND DISCUSSION

3.1. Physicochemical parameters

Physicochemical determinations of density, refractive index and solubility are presented in Table 1. According to the results expressed in Table 1, a yield of 2.1% was obtained for the EO of *C. citratus*, for the OE extracted from the leaves of *O. basilicum* 0.38% and for *A. rosaeodora* 1.87%.

Table 1: Physicochemical parameters of the Eos.

EO	Density	Refractive index	Solubility EtOH 70%	Color	Yield
C. citratus	0.8560 ± 0,0070 ($g\ mL^{-1}$)	1.5200 ± 0,0035 (nD 25°)	1:2 (v/v)	Yellow	2.10 % ± 0,15
O. basilicum	0.9396 ± 0,0031 ($g\ mL^{-1}$)	1.4980 ± 0,0177 (nD 25°)	1:2 (v/v)	Yellow	0.38 % ± 0,03
A. rosaeodora	0.8660 ± 0,0087 ($g\ mL^{-1}$)	1.4660 ± 0,0554 (nD 25°)	1:2 (v/v)	Yellow	1.87 % ± 0,18

These physicochemical parameters are essential factors to ensure the quality and purity of the EOs studied, being extremely relevant for applications to observe the individual yield of each EO. In the study conducted by Pinto et al. (2015) [18] the EO yield of *C. citratus* varied between 1.8% and 2.6%, since this result is affected by factors such as the crop region and seasonality [19], and the yield of this EO was reported in this study was within the reported range by the author. Studies report that when this plant is obtained in the periods of the year with higher rainfall index and high temperature, they present higher OE yield, when compared to other seasons of the year [20].

Previous studies show a significant variation in EO production of *O. basilicum*. According to the study by Al-Maskria et al. (2011) [21], the yield of this EO ranged from 0.1% to 0.3% according to the season. Beatović et al. (2015) [22] reported EO yields between 0.65% and 1.9%, justifying the large variation in levels to the detriment of agroclimatic differences and cultivation techniques. Pravuschi et al. (2010) [23] observed that there was an increase in the yield of this EO from the third harvest of plant material, since the cut of inflorescences can stimulate and increase the concentration of EOs in the remaining leaves of the plant. The low EO yield of this study can be attributed to the climatic conditions in which the plant in question was submitted, since the high temperatures of the state of Maranhão, during the period in which it was collected, benefited the early evaporation of volatile compounds from the plant. This phenomenon was observed in the

study by Hussain et al. (2008) [24], where EO yield was significantly lower in the Pakistani summer, ranging from 30 to 40°C.

The value obtained for the EO yield of *A. rosaeodora* in this research was higher than the result found by May et al. (2004) [25] and Amazonas (2012) [26] where they report that EO yield can range from 1 to 1.2%. However, according to Takeda (2008) [27], the yield of EO *A. rosaeodora* ranges from 2.24% for branches and 3.37% for leaves in five-year planting, making them potential for industrial production. In the present study, the EO was extracted from the stem and presented a considerable income, which encourages its extraction and application.

3.2. Chemical constituents

Table 2 presents the constituents identified in the EO of *C. citratus*. They were identified in the EO as geranial majority compounds (α–citral) with 43.96%, followed by neral (β-citral) with 35.71% and oxidized epoxy-linalool oxide with 8.61%.

Table 2: Chemical constituents identified in the EO of Cymbopogon citratus.

Order	RT	Components	%
2	4.350	β-myrcene	2.71
5	6.043	linalool	0.90
8	8.367	neral	35.71
10	8.875	geranial	43.96
11	9.100	epoxy-linalool oxide	8.61
12	10.174	isoamyl geranate	0.56
14	19.053	methyl geranate	0.77
19	20.090	cyclopropanemethanol	0.31
Other			6.47

Chemical studies of *C. citratus* in different habitats around the world identified citral as the main volatile constituent, which is a mixture of isomers, geranial (α-citral), and neral (β-citral) [18]. The chemical composition for the EO of *C. citratus* is in accordance with that observed in several studies. Sacchetti et al. (2005) [28] reported the presence of 65 to 86% citral in the EO of this species. In more recent studies, Lucena et al. (2015) [29] found levels of neral 43.69% and 34.05% geranial, and Gonçalves et al. (2015) [9] reported the presence of geranial (46.32%) and neral (31.28%) values similar to those found in OE *C. citratus* analysis in this study.

It is noteworthy that both fresh and dry leaves of *C. citratus* are used for the purpose of obtaining EO, where the yield of the EO is 0.28 to 0.50% of fresh mass. The authors recommend that the harvest of *C. citratus* be performed at 08:00 to 13:00 h when a higher citral concentration is observed, because high temperatures influence the quality of EO [30]. The differences observed in the quantity and chemical composition of the EOs of plants of the same species in different regions can be caused by microclimatic, phytogeographic, genotypic and geographical and agronomic conditions, mainly in the soil. However, as a general rule, the main components remain the same, varying only their concentration levels [22].

Table 3 presents the constituents identified in EO *O. basilicum*. In the OE was identified as the majority constituent the methyl chavicol with 62.39% followed by linalool (25.88%) and α-farnesene (6.14%).

Table 3: Chemical composition of EO Ocimum basilicum.

Order	RT	Components	%
1	15.66	Eucalyptol	3.48
2	18.50	Linalool	25.88
3	21.32	α-terpineol	1.43
4	22.16	methyl chavicol	62.39
5	20.12	(E,E)-α-farnesene	6.14
6	36.21	α-cadinol	0.67

For EO *O. basilicum*, similar results were described by Joshi (2014) [32], who reported that of the 25 compounds characterized and identified by CG/MS, 38.3% of the EO obtained was composed of methyl chavicol, followed by 39.9% methyl eugenol. In the study by Beatović et al. (2015) [22], EO *O. basilicum* of the genotype Siam Queen presented methyl chavicol in 83.6% of its composition. The methyl chavicol also presented itself as majoritarian in the OE of Iranian *O. basilicum* reported by Sajjadi (2006) [33].

However, the linalool content of the EO of the present study was lower than those found by Carović-Stanko et al. (2010) [34] and Al-Abbasy et al. (2015) [35] where the percentages were 66.40% and 69.86%, respectively. This result can be explained by seasonality and plant genotype collected in this study. The third majoritarian compound, α-farnesene (6.14%) was not found in any variation of *O. basilicum* of the study of Carović-Stanko et al. (2010) [34] and in any of the articles mentioned above. In the work of Labra et al. (2004) [36], the farnesene content ranged from 6.97 to 13.53% to the detriment of environmental and genetic factors. In the work of Calín-Sánchez et al. (2012) [37], the concentrations of α-farnesene were affected by convective drying temperatures, presenting higher levels in the extraction of the fresh plant.

Table 4 presents the constituents identified in the OE of *A. rosaeodora*. In the OE of *A. rosaeodora*, 3 chemical components were identified as shown in Table 4, and its majority constituent linalool was identified, with a content of 93.60%.

Table 4: Chemical constituents identified in EO Aniba rosaeodora.

Order	RT	Components	%
1	8.361	α-terpinolene	3.37
2	8.702	cis-linalool oxide (furanoid)	3.03
3	9.177	Linalool	93.60

The linalool content found in the EO of *A. rosaeodora* was higher than that found in the EO of the study by Cansian et al. (2010) [10] when analyzing *Cinnamomum camphora* and observing a percentage of 91.98% linalool. In this sense, EO *A. rosaeodora* is promising as a source of linalool.

Teles et al. (2018) [38] identified 9 chemical components present in the EO of *A. rosaeodora* by CG-MS, they are: linalool (89.34%), α-terpinolene (3.06%) and cis-linalool oxide (1.94%), elements also found in this EO, but with higher percentages. Cunha et al. (2011) [39] verified the seasonal influence of linalool present in the leaves and branches of EO *A. rosaeodora*. Based on the analyses performed through CG and CG-MS, it was possible to identify 76.69% linalool present in the EO even with the variations of harvest and climate time. These results were reaffirmed by Pimentel et al. (2018) [40] with GC-MS analysis of EO extracted from leaves and branches of *A. rosaeodora* Ducke collected in rainy and dry seasons showed quantitative and qualitative differences in chemical compositions. A total of 15 compounds were found in the EO of the leaves during the rainy season, while the EOs of the branches contained 11 compounds. Confirming the presence of a large number of chemical constituents in this OE.

Based on the chemical compositions of the EO of *A. rosaeodora*, it is possible to realize that linalool is the majority compound of this EO with a variation of 71 to 84%. However, in this study, a percentage of 93.60% was found, presenting as the highest in the literature.

3.3. Antimicrobial activity

Table 5 presents the results obtained for antimicrobial assays.

By the classification of Moreira et al. (2005) [41], the bacteria tested in this study were sensitive to the action of the EOs when they present inhibition halos greater than 9 mm. Thus, it was possible to verify that *E. coli* and *S. aureus* present sensitivity to the action of all EOs tested.

The two strains tested were sensitive to the action of EO *C. citratus*, presenting the largest inhibition halos (25 mm for *E. coli* and 25 mm for *S. aureus*) compared to other tested EOs. According to Table 5, the OE *C. citratus* presented lower MIC for *S. aureus*, compared to *E. coli*, making it a potential for the control of these pathogenic microorganisms [42].

Studies reveal that EOs possessing as majority components, terpenes and monoterpenes, such as geranial, methyl chavicol and linalool, can destroy the cellular integrity of microorganisms by inhibiting the cell breathing process microbial membrane [43] and the disruption of the cytoplasmic membrane of these [44].

Table 5: Diameter of inhibition halos (mm), MIC (μg mL^{-1}) and MBC (μg mL^{-1}) for action of EOs against tested microorganisms.

EO	Microorganisms	DIH (mm)	MIC (ug mL^{-1})	MBC (ug mL^{-1})
C. citratus	***E. coli***	25 ± 2,20	50 (40-60)	290 (270-310)
	S. aureus	25 ± 1,30	25 (23-27)	200 (180-220)
O. basilicum	***E. coli***	18 ± 1,70	100 (90-110)	320 (300-340)
	S. aureus	20 ± 2,50	50 (45-55)	200 (175-225)
A. rosaeodora	***E. coli***	11 ± 3,20	200 (150-250)	450 (410-490)
	S. aureus	15 ± 3,10	150 (140-160)	300 (290-310)

The data regarding MIC and MBC obtained in the current study demonstrated that EO *O. basilicum* bears similarity to those reported by Trajan et al. (2009) [45]. According to the data contained in Table 5, it is possible to conclude that EO *O. basilicum* had more effective inhibitory action against *S. aureus*, since it presented smaller MIC and MBC than the *E. coli* front test. In addition, the results of the MIC and MBC were in accordance with the literature [46, 47].

According to the results shown in Table 5, it was possible to observe that The EO of *A. rosaeodora* presented the smallest inhibition halos against the two bacteria and the largest MIC and MBC, compared to the other EOs. However, as much as it has been the least effective compared to other EOs, its antimicrobial profile meets the inhibition criteria of natural products [41].

The low toxicity observed in the EOs in this study is directly related to the great potential to use these products as flavoring agents in food, beverages, confectionery products, among others, as possible natural agents for the conservation of food [42]. The factors that influence the toxicity of EOs are related to the chemical composition plant, the dose used and the form of use [48].

3.4. Toxicity

Figure 1 presents the dead and living accumulated curve of *Artemia salina* in front of the action of the EO of *C. citratus*, where it was possible to observe the intersection of curves at 2.75 and LC_{50} in 582 mg L^{-1} ± 3.41 mg L^{-1}, by[14] is classified as nontoxic.

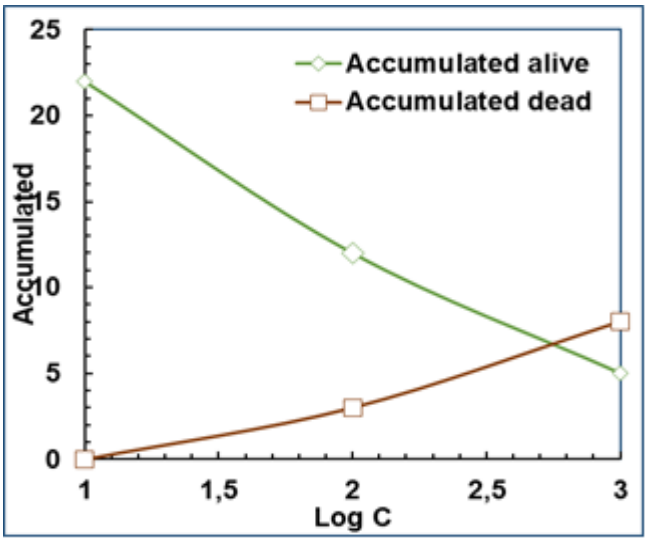

Figure 1: Accumulated curve of dead and alive Artemia salina versus EO Cymbopogon citratus concentration log.

Similar results regarding EO *C. citratus* toxicity were reported by Prakash et al. (2016) [49] evaluating the toxicity of the methanolic extract of six medicinal plants by the bioassay of *Artemia salina*. The authors obtained CL_{50} 704.67 ± 31.44 μg mL^{-1} for *C. citratus* extract being thus classified as nontoxic. De Lima et al. (2017) [50] investigated the EO toxicity of *C. citratus* by determining the Lethal Dose 50% (DL_{50}) in mice, it was observed that when receiving orally doses between 2000 and 4000 mg kg^{-1} of the EO of *C. citratus*, the value of the LD_{50} corresponded to 3000 ± 91.32 mg kg^{-1} of body mass, classifying the EO as a substance of low toxicity [51]. Discordant results were reported by Füller (2013) [52] who observed high EO toxicity of *C. citratus* obtaining a LC_{50} of 20 ug mL^{-1}.

Thus, it is possible to observe that the toxicity test is an indispensable tool for assessing or predicting the effects of toxic substances on biological systems and ascertaining the relative toxicity of substances that are predominant in environmental assessment [53]. In this sense this assay has been used to verify the toxicity of several plants [54, 55].

Figure 2 presents the dead and living accumulated curve of *Artemia salina* in front of the action of the EO of *O. basilicum*, where it was possible to observe the intersection of curves at 2.55 and LC_{50} in 355 mg L^{-1} ± 3.25 mg L^{-1}, by[14] it is classified as nontoxic.

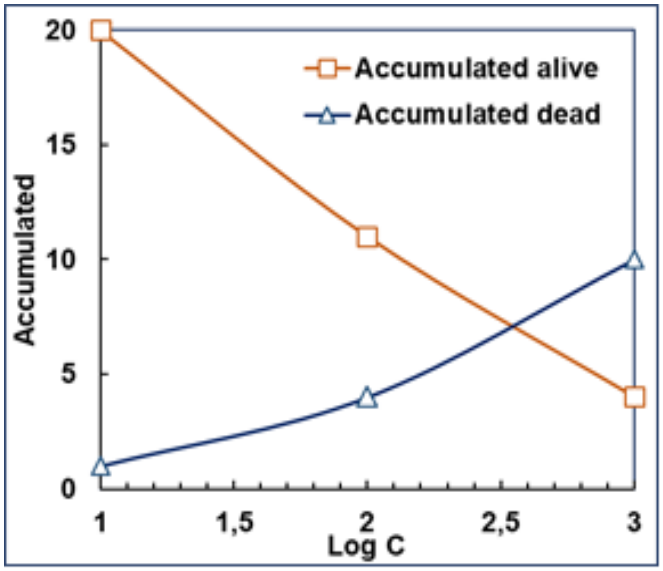

Figure 2: Accumulated curve of dead and alive Artemia salina versus EO Ocimum basilicum concentration log.

The reported results for EO *O. basilicum* toxicity was consistent with those reported by Sharopov et al. (2016) [56], where EO presented active lethality against *Artemia salina*. Hamidi et al. (2014) [57] and Silva et al. (2010) [58] find a LC_{50} of 9.92 mg mL^{-1} and 233.8 (200.7-272.0) μg mL^{-1}, respectively, stating moderate EO toxicity. Parra et al. (2001) [59] also reported high toxicity of *O. basilicum* extract (LC50 9.92 μg mL^{-1}). The high toxicity of the previously mentioned studies was attributed to the synergistic effects among their chemical components. In this study, EO showed little toxic due to its singular chemical profile, where lower linalool values and mostly methyl-chavicol were found. It is believed that the low concentration of other possibly toxic chemical assets in the EO of this study has caused its nontoxicity.

Figure 3 presents the dead and living accumulated curve of *Artemia salina* in front of the action of the EO of *A. rosaeodora*, where it was possible to observe the intersection of curves at 2.45 and LC_{50} in 282 mg L^{-1} ± 2.95 mg L^{-1}, by[14] is classified as nontoxic.

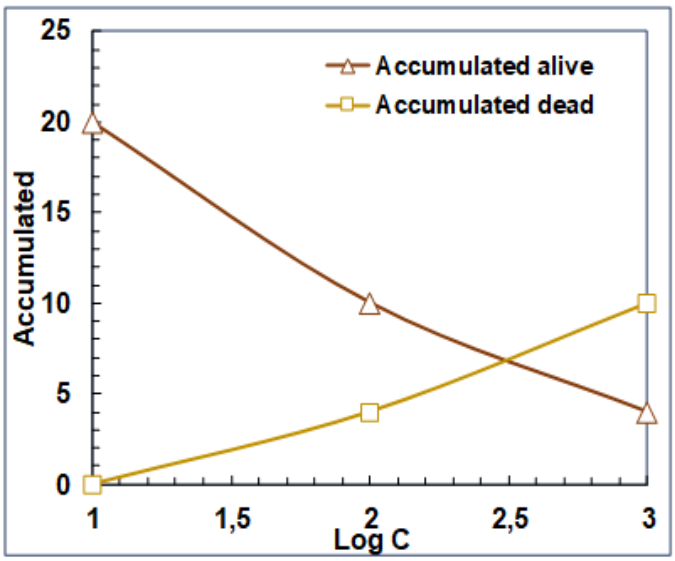

Figure 3: Accumulated curve of dead and alive Artemia salina versus OE Aniba rosaeodora concentration log.

Studies in the literature on toxicity by the bioassay of *Artemia salina* Leach in front of the EO of *A. rosaeodora* are still scarce and poorly disclosed. Therefore, the results regarding toxicity were compared to studies with linalool as a major component. Ramos et al. (2017) [60] used gas chromatography coupled to mass spectrometry (GC/MS) and identified linalool (51.8%) as a major component of EO *Mentha piperita* L. and in the evaluation of toxicity by *Artemia salina* bioassay obtained LC_{50} 414.6 μg mL^{-1} classifying EO as nontoxic.

The chemical composition correlates the majority of linalool compound, as nontoxic being used in the medical area, justifying the result found of the classification of the same. Fujiwara et al. (2017) [61] verified the toxicity of linalool by the bioassay of primary *in vitro* toxicity of *Artemia salina* obtaining the LC_{50} 275.2 μg mL^{-1} classifying the linalool compound as nontoxic. Similar results were also observed by Brasil et al. (2009) [62] when analyzing the EO of *Croton palanostigma* trunk bark, whose linalool was the majority component and through the bioassay of *Artemia salina* verified a LC_{50} 371 μg mL^{-1}, confirming EO toxicity used. Goel et al. (2019) [63] state that linalool is nontoxic, thus confirming applicability as a tool for manipulation in cancer cells, because it has a cytostatic effect [64]. It is concluded then that nontoxic EOs may also have a relative efficiency in antimicrobial properties in contrast to what was stated by MacBae et al. (1988) [65], where the authors report that the higher the toxicity the better the properties antimicrobials of the OE.

4. CONCLUSIONS

The EOs showed satisfactory results against the microorganisms tested, revealing their efficiency in the fight and control of pathogenic microorganisms.

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