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Chemistry

Larvicidal activity of essential oils and nanoemulsions (o/w) from Eucalyptus globulus Labil and Ocimum africanum Lour

Atividade larvicida dos óleos essenciais e nanoemulsões (o/a) de Eucalyptus globulus Labil e Ocimum africanum Lour

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ABSTRACT

The study aimed to evaluate the chemical profile and larvicidal activity of essential oil (EO) and oil-in-water (O/W) nanoemulsions from the leaves of *Eucalyptus globulus* Labil and *Ocimum africanum* Lour. The plant material was collected in the city of São Luís-MA. For the extraction of essential oils, the hydrodistillation technique was used. The determination of total phenolic compounds was performed by the Folin-Ciocalteu spectrophotometric method. Antioxidant activity was performed by the spectrophotometric method of scavenging hydroxyl radicals. Toxicity was measured using the *Artemia salina Leach* bioassay, and the Lethal concentration 50% (LC_{50}) for each of the essential oils was calculated based on the Reed&Muench method. For larvicidal activity, *Aedes aegypti* larvae were submitted to the EO solution at concentrations of 10-100 mg L^{-1}, where the mortality of the larvae was evaluated and the Lethal concentration of 50% (LC_{50}) was determined. The larvicidal action of the EO of *E. globulus* and *O. africanum* was observed, where LC_{50} of 26.58 mg L^{-1} and LC_{50} of 35.89 mg L^{-1}, respectively, and their nanoemulsions with LC_{50} of 12. 01 mg L^{-1} and 14.75 mg L^{-1}. Through the results obtained, it was proven that the larvicidal and improved action of the nanoemulsions in the tests carried out. Finally, this study points to its efficient larvicidal activity against *Aedes aegypti* larvae, encouraging its application in arbovirus vector control areas.

Keywords: *Eucalyptus globulus*; *Ocimum africanum*; Larvicida

RESUMO

O estudo teve como objetivo avaliar o perfil químico e atividade larvicida do óleo essencial (OE) e nanoemulsões óleo-em-água (O/W) das folhas de *Eucalyptus globulus* Labil e *Ocimum africanum* Lour. O material vegetal foi coletado no município de São Luís-MA. Para extração dos óleos essenciais utilizou-se a técnica de hidrodestilação. A determinação dos compostos fenólicos totais foi realizada pelo método espectrofotométrico de Folin-Ciocalteu. A atividade antioxidante foi feita pelo método espectrofotométrico de eliminação de radicais hidroxila. A toxicidade foi mensurada através do bioensaio de *Artemia salina* Leach, sendo a concentração Letal 50% (LC_{50}) para cada um dos óleos essenciais calculados com base no método de Reed&Muench. Para atividade larvicida submeteu-se larvas *Aedes aegypti* a solução do OE em concentrações de 10-100 mg L^{-1}, onde avaliou-se a mortalidade das larvas e determinou-se a concentração Letal 50% (CL_{50}). Observou-se a ação larvicida do OE de *E. globulus* e *O. africanum* onde foi encontrada a CL_{50} de 26,58 mg L^{-1} e CL_{50} de 35,89 mg L^{-1}, respectivamente, e suas nanoemulsões com CL_{50} de 12,01 mg L^{-1} e 14,75 mg L^{-1}. Através dos resultados obtidos, comprovou-se ação larvicida e melhorada das nanoemulsões nos ensaios realizados. E, por fim, este estudo aponta a atividade larvicida eficiente contra larvas *Aedes aegypti*, sendo incentivada sua aplicação em áreas de controle de vetores de arboviroses.

Palavras-chave: *Eucalyptus globulus*; *Ocimum africanum*; Larvicida

1 INTRODUCTION

Medicinal plants have been used for centuries in traditional medicine due to their therapeutic effect (Gonçalves, 2017). In the current scenario, we can see the use of complementary practices aimed at health with medicinal plants used to alleviate or even cure some diseases (Szerwieski et al., 2017). In recent years, there has been considerable attention to the biological effects of products obtained from plant sources, including essential oils (EOs) obtained from aromatic plants and their components. (Shen et al., 2015).

EOs have biological and pharmacological properties in their compositions that can act as an antioxidant, insecticide, analgesic and anti-inflammatory. This is due to their volatile compounds (acids, aldehydes, flavonoids and terpenes) present in EOs, which are of great importance as they are considered highly bioactive compounds, also called phytoalexins (Almeida, 2015).

Among these highlighted species, with scarce studies on pharmacological properties and bioproducts formulated with essential oils, are *Eucalyptus globulus* Labil and *Ocimum africanum* Lour (Shala & Gururani , 2023).

Due to these justifications, the species *O. africanum* Lour *and E. globulus* Labil were used in *in vitro* toxicity tests against *Artemia salina* , as they have a certain degree of toxicity and can be applied in the future to oncological patients, due to their properties anticarcinogenic and chemoprotective (Rossato Viana et al., 2024).

Furthermore, they represent an alternative treatment method compared to traditional methods, due to the low concentrations and efficiency found in medicinal plants. It is clear that *Artemia salina* has become common in these types of toxicity tests due to its low cost, easy reproducibility and effectiveness in terms of results (Castro & Lima, 2021; Rossato Viana et al., 2023).

It is noteworthy that the species highlighted in the present study, as they present a certain level of toxicity, enable larvicidal and insecticidal actions, therefore, they were used against the *Aedes aegypti mosquito* for its control and combat, this being the vector responsible for numerous arboviruses that affects the population, among the most common are dengue, zika and chikungunya. Such actions are possible through the chemical constituents present in essential oils (Lima et al., 2021; Silva Santos et al., 2022).

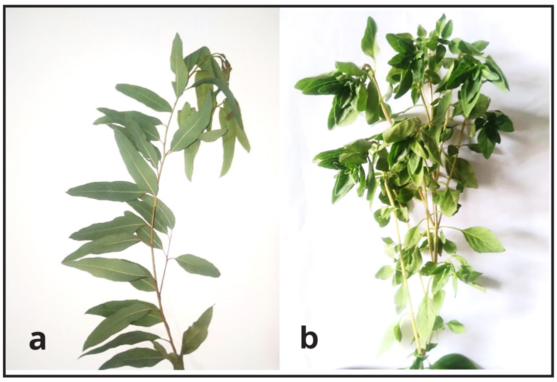
Thus, in order to intensify and stabilize the action of the chemical constituents present in the highlighted plant species, nanoemulsions were developed from the essential oils of the leaf species of *E. globulus* Labil and *O. africanum* Lour, in view of their physical and chemical characteristics. and biological considered unique (Fazis et al., 2023). Therefore, this study aimed to evaluate the chemical profile and toxicity, larvicidal activity and antioxidant activity of essential oils and nanoemulsions from the leaves of *E. globulus* Labil and *O. africanum* Lour.

2 METHODOLOGY

2.1 PLANT MATERIAL

The collection of plant material used in this research was carried out in May 2021. The leaves of *E. globulus* Labil (voucher n°4421) and *O. africanum* Lour (voucher n°4961) (Figure 1) were collected in the morning in the city of São Luis (MA), Brazil.

Figure 1 – Leaves of (a) *E. globulus* Labil and (b) *O. africanum* Lour.



Source: Authors (2023)

After collection, the plant species was transported to the Laboratory for Research and Application of Essential Oils (LOEPAV/UFMA), where the leaves were weighed, crushed and stored for the extraction of essential oil from the plant.

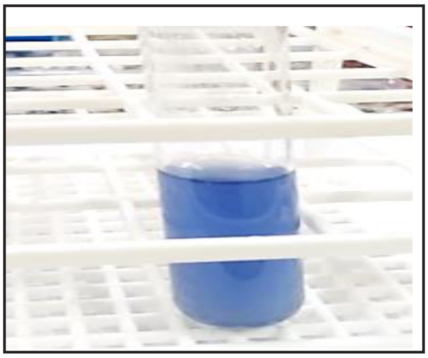
2.2 Essential oil extraction

For essential oil extraction, the hydrodistillation technique was applied with a glass Clevenger extractor coupled to a round-bottom flask placed in an electric blanket as a source of heat. Were used 268g of *E. globulus* Labil and 197g of *O. africanum* Lour from crushed vegetable leaves, with the addition distilled water (1:8). Hydrodistillation was conducted at 100°C for 3 hours and then the extracted essential oil was collected. The essential oil was dried by percolation with anhydrous sodium sulfate (Na_2SO_4) and centrifuged. These operations were performed in triplicate and the samples were stored in amber glass ampoules under refrigeration at 4°C. Subsequently submitted to analysis.

2.3 Total phenolic content (CFT)

The determination of the total phenolic compounds of the essential oil was carried out with an adaptation of the Folin-Ciocalteu method (Waterhouse, 2002). 5 mg of essential oil diluted in 1 mL of ethanol were used. To this solution, 7 mL of distilled water, 800 µL of Folin-Ciocalteu reagent and 2.0 mL of 20% sodium carbonate were added. After two hours, as shown in Figure 2, the reading was performed in a UV-VIS spectrophotometer at a length of 760 nm. The standard curve was expressed in mg L^{-1} of tannic acid.

Figure 2 – Determination of phenolic compounds



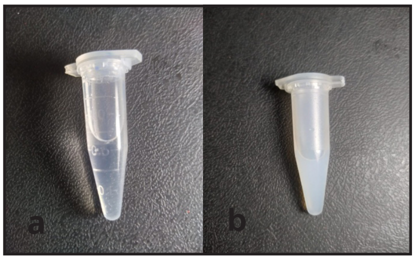
Source: Santos (2023)

2.4 Preparation of nanoemulsions (O/W)

The preparation of nanoemulsions was carried out according to the adapted methodologies described by Lima et al. (2020), Sugumar et al. (2014), Kubitschek et al. (2014) and Rodrigues et al. (2014). The oil-in-water nanoemulsion was formulated with each oil, nonionic surfactant (tween 20) and water. The required amounts of each oil phase constituent (oil+Tween20) were heated to 65 ± 5°C. The aqueous phase was separately heated to 65 ± 5°C, providing a primary formulation, by the phase inversion method.

To prove stability, the formulated emulsion was subjected to different stress tests (Shafiq et al., 2007). Heating-cooling cycle: it was carried out by keeping the formulated nanoemulsion at 40 and 4°C, alternating each temperature for 48 h. The cycle was repeated three times. Freeze-thaw stress: nanoemulsion alternatively at – 21 and 25 °C for 48 h at each temperature. The cycle was repeated three times. The formulations that passed the thermodynamic stress tests were taken for larvicidal action studies, as shown in Figure 3.

Figure 3 – Nanoemulsion (O/W) (a) *E. globulus* Labil and (b) *O. africanum* Lour.



Source: Santos (2023)

2.5 Antioxidant activity

Antioxidant activity was determined by the spectrophotometric method of elimination of hydroxyl radicals from salicylic acid, according to the methods described by Smirnoff & Cumbes (1989) and Sundarajan et al. (2016).

The nanoemulsion and the essential oils in different concentrations of 10-500 mg L^{-1} were dissolved in Dimethylsulfoxide (DMSO) 0.2% and distilled water, respectively. To these concentrations, 1 mL of salicylic acid (9 mM), 1 mL of ferrous sulfate (9 mM) and 1 mL of hydrogen peroxide (9 mM) were added. Ascorbic acid was used as a positive standard. The reaction mixture was incubated for 60 min at 37°C in a water bath; after incubation, the absorbance of the mixtures was measured at 510 nm using a UV/VIS spectrophotometer and the EC_{50} calculated.

2.6 Larvicidal activity

The eggs were collected in São Luís/MA, through traps called ovitraps. These consist of brown polyethylene buckets (500 mL) with 1 mL of brewer’s yeast and 300 mL of running water, and two Eucatex straws are inserted for mosquito oviposition.

The traps were inspected weekly to replace the straws and collect the eggs and sent to the Laboratory of Research and Application of Essential Oils (LOEPAV/UFMA) of the Technological Pavilion of the Federal University of Maranhão – UFMA.

Initially, *Aedes aegypti* eggs were placed to hatch at room temperature in a circular glass aquarium containing mineral water. Species identification followed the methodology proposed by Forattini (1962). The obtained larvae are fed with cat food according to methodology Silva et al. (1995) until they reach the third and fourth instar, age at which the experiments were carried out.

Assays for larvicidal activity were carried out according to the adapted methodology proposed by Silva (2006). Initially, a mother solution of 100 mg L^{-1} of each of the essential oils was prepared, being diluted in a 2% DMSO solution and nanoemulsions (without dilution). From this solution, five dilutions were prepared at concentrations 10-90 mg L^{-1}. At each concentration, 10 larvae were added at a rate of 1 mL per larva.

All tests were performed in triplicates and a solution made up of 2% DMSO was used as a negative control, and a 70% v/v ethanol (PA) solution was used as a positive control. After 24 hours, live and dead were counted, and larvae that did not react to touch 24 hours after the start of the experiment were considered dead. To quantify the efficiency of essential oils and nanoemulsions, the Probit statistical test (Finney, 1952) and the action classification by Cheng et al. (2003).

2.7 Toxicity

To assess the lethality of *Artemia salina* Leach, the methodology described by Meyer et al. (1982). Initially, a stock saline solution of each essential oil and nanoemulsions was prepared at a concentration of 10,000 mg L^{-1} and 0.02 mg of Tween 80 (active surfactant). Aliquots of 5, 50 and 500 µL were transferred to test tubes and completed with previously prepared saline solution up to 5 mL, finally obtaining concentrations of 10, 100 and 1000 mg L^{-1}, respectively. All tests were performed in triplicates, where ten larvae in the nauplius stage were transferred to each of the test tubes.

For the blank, 5 mL of saline solution was used, for the positive control $K_2Cr_2O_7$ and for the negative control, 5 mL of a 4 mg L^{-1} solution of Tween 80. After 24 hours of exposure, the counting of live larvae, considering dead those that do not move during observation or with slight agitation of the flask. The 50% Lethal Concentration (LC_{50}) for each essential oil and nanoemulsion was calculated based on the method by Reed & Muench (1938), with classification of toxicity according to the criterion by Dolabela (1997).

RESULTS AND DISCUSSION

3.1 Total Phenolic Content

The results of the total phenolic content of the essential oils of *E. globulus* and *O. africanum* are presented in Table 1. The total phenolic content (TFC) was expressed as tannic acid equivalents, where it represents the absorbance and the equivalent concentration of tannic acid.

Comparing the data observed in Table 1, the result observed by Cidres (2018), when analyzing the volatile compounds of the hydroalcoholic extract of the leaves of *E. globulus,* it was possible to conclude an inferiority quantity in the analyzed work at the concentration of 136.50 ± 14.54 mg EAG g^{-1}. It was also observed by Haida et al. (2011) superior results of 465.82 mg EAT g^{-1} for the hydroalcoholic extract of *E. globulus*.

Table 1 – Total phenolic content obtained from *E. globulus* and *O. africanum.*

EO	TPC (mg EAT g^{-1})	Equation	R^2
EO *E. globulus*	93.05	y = 0.05857x + 0.06000	0.9998
NEO *E. globulus*	13.32		
EO *O. africanum*	198.97		
NEO *O. africanum*	9.56		

Where; EO – essential oil; NEO – essential oil nanoemulsion

Source: Santos (2023)

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Scarce studies are correlated with works analyzing the species of *O. africanum*. However, comparing with the data in Table 2, significant results were observed by Carriço et al. (2018) when analyzing the volatile compounds of the leaves of *O. basilicum* by extraction in infusion, quantifying a total of 295.9 ± 19.6 μg EAG mL^{-1}, a higher result for the species similar to this study.

Significant results were also observed by Vasconcelos et al. (2021) when analyzing the essential oils of *Ocimum gratissimum* and *Ocimum basilicum* quantified, respectively, the values of 17.90 ± 0.75 mg EAG g^{-1} and 16.44 ± 0.69 mg EAG g^{-1}. Pitaro et al. (2012) analyzed the extract of *Ocimum basilicum* in natura and after drying and also observed similar values, respectively, 184 mg EAG g^{-1} and 135.6 mg EAG g^{-1}.

According to Table 1, the essential oils used in the present study showed a significant number of phenolic compounds, which is of great relevance since phenolics are often associated with several positive health effects, responsible for their antioxidant and anti-inflammatory action (Dalmarco, 2012).

3.2 Antioxidant activity

Table 2 presents the Efficient Concentration (EC_{50}), equation of the straight line and linear regression for the antioxidant capacity of the EO of *O. africanum* and *E. globulus* for the employed method.

Table 2 – Antioxidant capacity of *E. globulus* and *O. africanum*

	EC_{50} (mg L^{-1})	**Equation**	**R^2**
EO *E. globulus*	65.42	y=18.917x+15.652	0.9177
NEO *E. globulus*	45.81	y=38.404x-13.787	0.9919
EO *O. africanum*	62.30	y=41.537x-24.538	0.9845
NEO *O. africanum*	20.12	y=29,788x+11,165	0.9759

Note: EO – essential oil; NEO – essential oil nanoemulsion

Source: Santos (2023)

According to Campos et al. (2003), the oils evaluated are classified as active with antioxidant action because the quantified EC_{50} was less than 500 mg L^{-1}.

Inferior results were observed by Alarcón et al. (2019) when evaluating the antioxidant activity of *E. globulus* essential oil, the authors obtained an EC_{50} of 505.0 mg L^{-1} by the DPPH radical decolorization method and an EC_{50} of 100.30 mg L^{-1} by the method ABTS radical discoloration.

Cidres (2018) also observed low antioxidant activity for the hydroalcoholic extract of *E. globulus* leaves with an EC_{50} of 510 mg L^{-1} by the DPPH radical discoloration method. Furthermore, Brum (2012), when quantifying the antioxidant activity through the method of discoloration of DPPH radicals from the ethyl acetate extract of leaves of the Eucalyptus genus, quantified the EC_{50} at 162 μg mL^{-1}. It is noteworthy, according to Rossato Viana (2023) that in concentrations considered very high they can cause a pro-oxidant effect, which can lead to oxidative stresses, already being directed to other types of therapies such as cancer instead of the process of slowing cellular aging.

According to the results observed by Silva (2011), effective antioxidant activity was found in the essential oil of the genus Ocimum with an EC_{50} of 364 mg L^{-1} by the DPPH radical discoloration method. It was also observed by Gontijo et al. (2014), the antioxidant activity of the aqueous extract of the leaves of the species *Ocimum gratissimum* with IC_{50} of 83.00 ± 1.76 μg mL^{-1} by the DPPH radical decolorization method.

According to the results observed by Mafra et al. (2020), the antioxidant activity of the genus Ocimum was also confirmed with an EC_{50} of 39.96 mg L^{-1} by the ABTS radical decolorization method and an EC_{50} of 25.35 mg L^{-1} by the radical decolorization method. DPPH.

It is important to highlight that the formulated nanoemulsions showed potential for improvement in relation to the EC_{50} of essential oils in vitro. According to Table 2, the results obtained from essential oils show an important capacity for antioxidant activity that stabilizes free radicals and inhibits the initiation chain or interrupts the chain of propagation of oxidative reactions by radicals (Silva et al., 2010).

3.3 Toxicity

Table 3 presents the 50% Lethal Concentrations referring to the action of essential oils against *Artemia salina* and its subsequent classification.

Table 3 – LC_{50} for toxicity of *E. globulus* and *O. africanum* against *Artemia salina* L.

EO	LC_{50} (mg L^{-1})	Classification
E. globulus	120.22	Moderately toxic
O. africanum	446.68	Non-toxic
Positive control		All inactive larvae
Negative control		All active larvae
White		All active larvae

where; EO – essential oil

Source: Santos (2023)

According to Table 3, essential oils from *E. globulus* and *O. africanum* were classified, respectively, as moderately toxic and non-toxic according to the method by Dolabela (1997).

According to the result observed by Rosa et al. (2020), a lower toxicity of *E. globulus essential oil* was observed, quantifying a LC_{50} of 595.2 ± 2.12 mg L^{-1} against *Artemia salina*, when compared to this study presented by Atmani-Merabet et al. (2018), who quantified the toxicity of *E. globulus essential oil* extracted in Algeria with a LC_{50} of 67.55 µg mL^{-1} against *Artemia salina*.

According to the result observed by Atmani-Merabet et al. (2020), toxicity of the *genus Eucalyptus* was also reported when extracted in Algeria with a total LC_{50} value of 116.06 µg mL^{-1} against *Artemia salina*.

Results for the toxicity of the genus *Ocimum* are described by Silva et al. (2010) with a LC_{50} of 233.8 mg L^{-1} against *Artemia salina*, allowing the essential oil obtained by the authors to be classified as moderately toxic. According to the result observed by Everton et al. (2020), obtaining a LC_{50} result of 582 mg L^{-1} for the species of the genus *Ocimum*, classifying it as non-toxic. It was also observed by Silva et al. (2010), the toxicity of essential oil from inflorescences of the genus *Ocimum* quantified a CL_{50} of 233.8 µg mL^{-1} with a 95% confidence interval against *Artemia salina*.

3.4 Larvicidal activity

Table 4 presents the 50% Lethal Concentrations referring to the action of essential oils and nanoemulsions against *Aedes aegypti*.

According to Cheng et al. (2003) larvicidal tests performed with essential oils that obtain $LC_{50} < 50$ mg L^{-1} are highly efficient. According to Table 4, it is important to emphasize that both essential oils were active, as well as their nanoemulsions, which also showed improved larvicidal potential.

The larvicidal action for *E. globulus* was observed by Calvaca et al. (2010), who obtained a larvicidal activity of the *Eucalyptus* genus essential oil with a LC_{50} of 270 mg L^{-1} against *Aedes aegypti* larvae. Similar results were observed by Simom et al. (2016), an EC_{50} of 45.92 µg mL^{-1} was found for the essential oil genus *Eucalyptus* against *Aedes aegypti*. The larvicidal action against other species was observed by Macedo et al. (2009), who obtained a LC_{50} of 6.92 mg mL^{-1} against *Haemonchus contortus* larvae, determining a good potential for use in the control of gastrointestinal nematodes in sheep and goats.

Table 4 – Larvicidal activity against *Aedes aegypti.*

	Conc. (mg L^{-1})	% Mortality	LC_{50} mg L^{-1}	δ	X^2	R^2
EO *E.globulus*	100	100.0	26.58 (16.75-42.19)	0.522	0.982	0.941
	75	80.0				
	50	70.0				
	25	50.0				
	10	20.0				
NEO *E.globulus*	50	80.0	12.01 (6.36-22.68)	0.733	0.942	0.933
	25	60.0				
	10	50.0				
	5	40.0				
	2	10.0				
EO *O. africanum*	100	90.0	35.89 (24.19-53.27)	0.440	0.979	0.951
	75	75.0				
	50	54.0				
	25	31.0				
	10	13.6				
NEO *O. africanum*	50	70.0	14.75 (7.13-30.55)	0.854	0.944	0.900
	25	70.0				
	10	40.0				
	5	20.0				
	2	20.0				
Positive Control				All active larvae		
Negative Control				All inactive larvae		
White				All inactive larvae		

Source: Santos (2023)

According to Cheng et al. (2003) larvicidal tests performed with essential oils that obtain $LC_{50} < 50$ mg L^{-1} are highly efficient. According to Table 4, it is important to emphasize that both essential oils were active, as well as their nanoemulsions, which also showed improved larvicidal potential.

The larvicidal action for *E. globulus* was observed by Calvaca et al. (2010), who obtained a larvicidal activity of the *Eucalyptus* genus essential oil with a

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According to the result observed by Sousa et al. (2021), larvicidal activity of the essential oil of the *genus Ocimum* was observed with a LC_{50} of 196.5 ppm. According to the study observed by Veloso et al. (2015), larvicidal activity of the essential oil of the *genus Ocimum* with a LC_{50} of 67 mg mL^{-1} against *Aedes aegypti* larvae, with a larvicidal activity lower than that obtained in this work, which obtained a LC_{50} of 35.89 mg L^{-1} for *O. africanum* essential oil. According to the study observed by Mafra et al. (2020), the larvicidal activity of the *genus Ocimum* with a LC_{50} of 43.84 mg L^{-1} against *Aedes aegypti* larvae.

According to the result observed by Santos et al. (2018), the larvicidal activity of the nanoemulsion of the *genus Ocimum* is observed with a LC_{50} of 42.15 mg L^{-1} against *Aedes aegypti* larvae, compared to the value obtained in this work with a LC_{50} of 14.75 mg L^{-1} for the *O. africanum* nanoemulsion notifying a greater larvicidal activity for the species under study in this work.

Other nanoemulsions in the literature were also tested for this purpose, but showed a lower action potential, emphasizing the need and promising results of this study. According to the results obtained by Saavedra et al. (2018), the larvicidal activity of the nanoemulsion of essential oil from the roots of *Pilodendron fragrantissimum* was observed with a LC_{50} of 55.07 ppm against *Aedes aegypti* larvae. Silva (2017) also showed the larvicidal activity of a nanoemulsion, but of the essential oil of *Hyptis suaveolens* with a LC_{50} of 202.66 ppm against *Aedes aegypti* larvae.

The use of *E. globulus* essential oils and *O. africanum* in the formulation of larval control products can be advantageous over other control methods, due to its easy production, residual effectiveness and safety to organisms and the environment.

3 CONCLUSIONS

In view of the analyzes carried out in the present study, the essential oils incorporated into their respective nanoemulsions from the leaves of *E. globulus* Labil and *O. africanum* Lour appear to be non-toxic products against *Artemia salina*. These findings suggest that these oils may serve as viable and future alternatives for patients with oncological diseases. Moreover, the study demonstrated a relatively low LC_{50} against Aedes aegypti due to larvicidal activity, indicating that these bioformulations are capable of being applied to the benefit of the population to combat and control the mosquito vector *A. aegypti*. Furthermore, the study also showed formidable results for antioxidant activity, classifying it as active, such values are consolidated by the high amounts of total phenolic content, categorizing it as an excellent antioxidant when compared to synthetics available on the market. In short, these products based on plant materials have a wide spectrum of biological applications that could significantly benefit the population.

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