

Chemistry

Development of a larvicide nanoemulsion (O/W) of *Cinnamomum zeylanicum* essential oil against *Aedes aegypti*

Desenvolvimento de nanoemulsão larvicida (O/A) do óleo essencial de *Cinnamomum zeylanicum* frente *Aedes aegypti*

João Durval Ribeiro Neto¹, Thamires de Jesus Teles Ribeiro¹,
Américo Pinheiro Neto¹, Victor Elias Mouchrek Filho¹,
Gustavo Oliveira Everton¹

¹Universidade Federal do Maranhão, São Luís, MA, Brazil

ABSTRACT

Arboviruses arouse the interest of public authorities because they present different diseases such as Dengue, Zika, Chikungunya and Yellow Fever, and one of the ways to fight them is larvicidal action. Thus, this study aimed to evaluate the chemical profile and larvicidal activity of essential oil and nanoemulsions (O/W) of *Cinnamomum zeylanicum* against *Aedes aegypti*. In the present study, the plant material was obtained in the city of São Luís (MA) and the essential oil was extracted by hydrodistillation in a modified Clevenger extraction system. The nanoemulsions were formulated by the low-energy phase inversion method. The chemical profile was evaluated by the total phenolic content. Antioxidant activity was performed by the hydroxyl radical decolorization method. The anti-inflammatory action was evaluated by the protein albumin denaturation method. The larvicidal activity was evaluated by the lethality bioassay against *Aedes aegypti*. Total phenolics were quantified at 118.66 mg EAT g⁻¹, being important predictors of pharmacological activities. Positive results were observed in the antioxidant activity of essential oil and nanoemulsion, which resulted in EC₅₀ of 80.27 mg L⁻¹ and 166.42 mg L⁻¹, respectively, being classified as active potential. For the anti-inflammatory activity of nanoemulsion and essential oil resulted in EC₅₀ 42.21 mg L⁻¹ and 225.13 mg L⁻¹. The larvicidal activity showed promising results where the LC₅₀ in 22.01 mg L⁻¹ of the essential oil and LC₅₀ in 5.30 mg L⁻¹ of the nanoemulsion, bringing unprecedented and expressive results for the nanoemulsion. Finally, the observed results guarantee the effectiveness of the *Aedes aegypti* arbovirus vector.

Keywords: Arboviruses; Nanoemulsion; Essential oil; *Cinnamomum zeylanicum*

RESUMO

As arboviroses despertam interesse do poder público por apresentarem doenças diversas como a Dengue, Zika, Chikungunya e Febre Amarela e uma das formas de combate é ação larvicida. Desta forma, este estudo teve por objetivo avaliar o perfil químico e atividade larvicida do óleo essencial e nanoemulsões (O/A) de *Cinnamomum zeylanicum* frente *Aedes aegypti*. No presente estudo, o material vegetal foi obtido no município de São Luís (MA) sendo realizada a extração do óleo essencial por hidrodestilação em um sistema extrator Clevenger modificado. As nanoemulsões foram formuladas pelo método de baixa energia de inversão de fases. O perfil químico foi avaliado pelo conteúdo fenólico total. A atividade antioxidante foi realizada pelo método de descoloração de radicais hidroxila. A ação anti-inflamatória foi avaliada pelo método de desnaturação proteica de albumina. A atividade larvicida foi avaliada pelo bioensaio de letalidade frente *Aedes aegypti*. Os fenólicos totais foram quantificados em 118,66 mg EAT g⁻¹, sendo importantes preditores para atividades farmacológicas. Resultados positivos foram observados na atividade antioxidante do óleo essencial e da nanoemulsão que resultaram em CE₅₀ de 80,27 mg L⁻¹ e 166,42 mg L⁻¹, respectivamente, sendo classificadas em potencial ativo. Para a atividade anti-inflamatória da nanoemulsão e do óleo essencial resultaram em CE₅₀ 42,21 mg L⁻¹ e 225,13 mg L⁻¹. A atividade larvicida apresentou resultados promissores onde sendo as CL₅₀ em 22,01 mg L⁻¹ do óleo essencial e CL₅₀ em 5,30 mg L⁻¹ da nanoemulsão, trazendo resultados inéditos para a nanoemulsão e expressivos. Por fim, os resultados observados garantem a eficácia da formulação frente ao vetor de arboviroses *Aedes aegypti*.

Palavras-chave: Arboviroses; Nanoemulsão; Óleo essencial; *Cinnamomum zeylanicum*

1 INTRODUCTION

Nanoscience and nanotechnology refer to the study and technological applications of matter in dimensions between 1 and 200nm. As a result, it is assumed that the current difficulty is the unfamiliarity with this nanometric universe (Melo&Pimenta, 2010). Emulsions are systems made up of two or more immiscible liquids where one is called the dispersed phase and the other the dispersing phase. They are classified as macroemulsions, microemulsions and nanoemulsions.

The pharmaceutical and cosmetic industries are on the podium in the use of emulsions, these materials are vehicles for the transport of encapsulated actives and/or drugs coming from medicinal plants (Morais, 2008). It is estimated that approximately 80% of the world population has already used vegetables to relieve the symptoms of some disease. In the late nineteenth century, the World Health Organization (WHO)

reported that 65% to 80% of the population in underdeveloped countries depended exclusively on medicinal plants for their health system (Veiga Junior et al., 2005).

Among the products coming from medicinal plants are essential oils, which are groups of volatile compounds coming from plants, coming from the secondary metabolism of plants, varying their concentration for each essential oil and differentiated between major and minor compounds, these being the components that give the phytotherapeutic characteristics of the bioproduct or its chemical and biological properties (Gobbo-Neto, 2007).

The significance of identifying new bioactive compounds lies in their potential activity across various in vitro models, particularly in the context of disease combat and the development of innovative therapies. Recent studies highlight the discovery of phytochemicals in plants such as *Vassobia breviflora*, which have demonstrated antioxidant, antimicrobial, and cytotoxic properties in in vitro assays (Viana et al., 2022; Viana et al., 2025). These compounds, detected using techniques like ESI-TOF-MS, show promising activity against multi-resistant bacteria and exert cytotoxic effects on different cancer cell lines, including melanoma, lung carcinoma, and glioblastoma (Viana et al., 2022).

Furthermore, in vitro evaluations have revealed that these compounds can modulate reactive oxygen species (ROS) production and influence apoptotic signaling pathways, reinforcing their therapeutic potential (Viana et al., 2022; Viana et al., 2025). Therefore, the search for novel bioactive compounds and the assessment of their activity in vitro are crucial steps toward expanding the arsenal of treatment options, ultimately contributing to the development of more effective and safer therapeutic strategies.

Furthermore, these bioproducts, when coupled to nanostructured systems, increase their biological potential in wide application, due to the size of the particles and also reduce their toxicity potential, as the hydrophobicity of essential oils in their contact is facilitated by means of the emulsion. As is the case for the larvicidal activity of *Cinnamomum zeylanicum* essential oil incorporated into the nanoemulsion (Felizardo et al., 2021).

C. zeylanicum belongs to the Lauraceae family, popularly known as cinnamon, being widely used in the food and medicine industry over the years, it has several bioactivities, including analgesic, antiseptic, anti-cancer, anti-spasmodic, coagulant, neuroprotective properties, hepatoprotective, gastroprotective, protection potentials, antimicrobials, and larvicidal activity (Santos, 2020).

Within this, it is important to emphasize the growing importance of studies with medicinal plants for the development of drugs with biologically active properties and without or minimal toxicological responses (Souza, 2010). Thus, this study aimed to evaluate the chemical profile and larvicidal activity of essential oil and nanoemulsions (O/W) of *C. zeylanicum* against *Aedes aegypti*.

2 METHODOLOGY

2.1 Obtaining plant material

The shell samples from *Cinnamomum zeylanicum* (cinnamon) were obtained in the municipality of São Luís - MA. The plant material was transported to the Laboratory for Research and Application of Essential Oils (LOEPAV/UFMA), where they were crushed to extract the essential oil.

2.2 Essential oil extraction

For the extraction of essential oils, the hydrodistillation technique was used with a glass Clevenger extractor coupled to a round-bottomed flask packed in an electric blanket as source of heat. 100g were used, adding distilled water (1:8). The hydrodistillation was carried out at 100°C for 3h collecting the extracted essential oil.

Each 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 vials under refrigeration at 4°C. Subsequently submitted for analysis.

2.3 Total Phenolic Content (CFT)

The determination of the total phenolic compounds of the essential oil and of the microparticles was carried out with adaptation of the Folin-Ciocalteu method (Waterhouse, 2002). 5 mg of the essential oil diluted in 1 ml of ethanol was used. To this solution, 3 mL of distilled water, 500 μ L of Folin-Ciocalteu reagent and 2.0 mL of 20% sodium carbonate were added. After two hours, 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.

2.4 Antioxidant activity

Antioxidant activity was performed by the spectrophotometric method of scavenging hydroxyl radicals from salicylic acid, as described by Smirnff&Cumbes (1989) and Sundararajan et al. (2016).

Nanoemulsion and essential oils in different concentrations from 10-500 mg L^{-1} were dissolved in 0.2% DMSO and distilled water, respectively. At these concentrations, 1 mL of salicylic acid (9 mM), 1 mL of ferrous sulfate (9 mM) and 1 mL of hydrogen peroxide (9mM) 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.

2.5 Preparation of nanoemulsions (O/A)

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 heated separately to 65 ± 5 °C, providing a primary formulation, by the phase inversion method.

Final homogenization was achieved using a magnetic stirrer, in which the formulation remained under constant agitation at 6000 rpm, until reaching a temperature

reduction to $25\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$. Subsequently, they were sonicated in an ultrasound bath for 10min. To prove stability, the formulated emulsion was subjected to different stress tests (Shafiq et al., 2007). Heating-cooling cycle: it was carried out keeping the nanoemulsion formulated at 40 and 4°C , alternating each temperature for 48 hours. The cycle was repeated three times. Freeze-thaw stress: nanoemulsion alternatively at -21 and $25\text{ }^{\circ}\text{C}$ for 48h at each temperature. The cycle was repeated three times. The formulations that passed the thermodynamic stress tests were taken to larvicide action studies.

2.6 Anti-inflammatory activity

The anti-inflammatory activity was evaluated by the protein denaturation method (Padamanabhan & Jangle, 2012). The reaction mixture (5 mL) consisted of 2mL of different concentrations of the essential oil ($100\text{-}500\text{ mg L}^{-1}$) and 2 ml of a 10% solution of albumin diluted in PBS and incubated at $(37\pm 1)\text{ }^{\circ}\text{C}$ for 15 minutes.

Denaturation was induced by keeping the reaction mixture at 60°C in a water bath for 10 minutes. after the cooling, absorbance was measured at 660 nm. The inhibition of protein denaturation was expressed in percentage and the Efficient Concentration 50% ($\text{EC}_{50}/\text{IC}_{50}$) capable of inhibiting 50% of the denaturation was expressed in mg L^{-1} .

2.7 Larvicide activity

Eggs were collected in São Luís/MA, using traps called ovitraps. These consist of brown (500 ml) polyethylene buckets with 1 ml of brewer's yeast and 300 ml of running water and two Eucatex straws are inserted for the mosquito to lay eggs. The traps were inspected weekly to replace the straws and collect eggs and were sent to the Laboratory for 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. The identification of the species followed the methodology proposed by Forattini (1962). The larvae obtained are fed with cat

food according to Silva's methodology (1995) until they reach the third and fourth instars, the age at which the experiments were carried out.

The tests for larvicidal activity were carried out according to the adapted methodology proposed by Silva (2006). Initially, a 100 mg L⁻¹ stock solution of each of the essential oils was prepared and diluted in a 2% DMSO solution and nanoemulsions (free of dilution). From this solution, five dilutions were prepared at concentrations 1.0-90.0 mg L⁻¹. At each concentration, 10 larvae were added at the rate of 1 ml per larva.

All tests were performed in triplicate and a 2% DMSO solution was used as a negative control, and a 70% v/v ethanol (PA) solution was used as a positive control.

After 24 hours the count of live and dead was carried out, with larvae that do not react to touch being considered dead after 24 hours from the beginning of the experiment. To quantify the efficiency of essential oils and nanoemulsions, the statistical test of Probit (Finney, 1952) and classification of the action by Cheng et al. (2003).

3 RESULTS AND DISCUSSION

3.1 Total Phenolic Content

The total phenolic content (TFC) of the essential oil of *C. zeylanicum* is shown in Table 1.

Table 1 – Total Phenolic Content (CFT) for *C. zeylanicum* essential oil

	CFT	Equation	R ²
EO	118.66 mg EAT g ⁻¹	y = 0.058x + 0.0600	0.9998

Note: EO - essential oil

Source: Autorship (2025)

A similar result was presented by Behrooz et al. (2020) which quantified the phenolics present in the EO of *C. zeylanicum* at 106.19 mg GAE/g dry essential oil.

Furthermore, the quantification of phenolics in 53.74 mg of gallic acid equivalent/mL in an EO of *C. zeylanicum* presented by Valizadeh et al. (2015) comparatively lower than that presented in this study.

The total phenolic content (CFT) observed for the essential oil of *C. zeylanicum* they are often associated with antioxidant activities, anti-inflammatory properties and decreased cardiovascular risks (Singh et al., 2012). Still, generally associated with food because they inhibit lipid oxidation, in addition to acting in processes such as color, astringency and aroma (Soares, 2002).

The differences observed between the studies are due to the different parts of plants or even climate and geographic location resulting in quantitative changes in chemical compounds, in the case of the essential oil of *C. zeylanicum*, it is observed that the oil extracted from the leaves is the major component. eugenol and when the essential oil is extracted from the bark it is cinnamaldehyde (Andrade et al., 2012).

3.2 Antioxidant activity

The results obtained for the antioxidant action of the essential oil and the nanoemulsion of *C. zeylanicum* are shown in Table 2.

Table 2 – Antioxidant capacity of *C. zeylanicum* quantified by EC₅₀

	EC ₅₀	Equation	R ²
EO	80.27 mg L ⁻¹	y = 99.681x - 139.85	0.9994
NEO	166.42 mg L ⁻¹	y = 62.586x - 89.016	0.9815

Note: EO - essential oil; NEO - essential oil nanoemulsion; CE - Efficient Concentration

Source: Autorship (2025)

The results observed in Table 2 confirm the effective action of the essential oil according to the criteria used by Campos et al. (2003) which classifies natural products with CE₅₀ less than 500 mg L⁻¹ as potentially active and promising for antioxidant purposes.

The observed activity is affirmed by Ruberto & Baratta (2000) after performing two model systems for the antioxidant efficiency, and as a conclusion it was proved

that phenols are responsible for this activity, constituents that were observed in the previous item of this study.

The antioxidant action for the analyzed essential oil is also proven by Jayaprakasha et al. (2003) that proved the antioxidant action of the essential oil of *C. zeylanicum* when performing the antioxidant activity test by the phosphomolybdenum method and proved that it has a radical reduction action of up to 66% at a concentration of 200 mg L⁻¹. Schmidt et al. (2006) also confirm the efficiency of *C. zeylanicum* essential oil as an antioxidant due to its inhibition of lipid peroxidation, using the evaluation of antioxidant activity in the linoleic acid model system.

Results that confirm the antioxidant capacity of *C. zeylanicum*, was also observed in alcoholic extracts both in leaves and in bark in the study carried out by Abeysekera et al. (2013).

The antioxidant action observed is the result of compounds that supply electrons or hydrogen and contribute to the formation of stable intermediate radicals during the reaction with free radicals and are characterized as molecules that have a benzene ring linked to one or more hydroxyl groups. Phenolic compounds are related to the amount of plant antioxidants, so their quantification is important (Silva et al., 2010) and is presented in this study.

The antioxidant characteristics of the studied essential oil are focused on the search for natural antioxidants due to the fact that synthetic antioxidants have been associated with toxic effects in recent years, based on this natural antioxidants are gaining fame for their responsibility to be free radical scavengers, interrupting the chain reaction, in addition, act in the oxidative process catalyzed by metals both in vivo and in vitro (Del Ré, 2012)

The results presented in Table 2 are promising since the main mechanisms involved in the pathogenesis and progression of chronic diseases such as cancer, cardiovascular and inflammatory diseases are related to oxidative stress that are solved with antioxidant agents (Krishnaiah et al., 2011).

3.3 Anti-inflammatory Activity

Table 3 shows the results obtained regarding the anti-inflammatory activity of the *C. zeylanicum* nanoemulsion and its respective essential oil.

Table 3 – Quantification of the anti-inflammatory capacity of *C. zeylanicum*

	EC ₅₀	Equation	R ²
NEO	42.21 mg L ⁻¹	y = 43.857x - 21.286	0.9986
EO	225.13 mg L ⁻¹	y = 143.94x - 288.61	0.9437

Note: EO - essential oil; NEO - essential oil nanoemulsion; CE - Efficient Concentration

Source: Autorship (2025)

After performing the experiments, the 50% Efficient Concentration assay quantified an EC₅₀ at 225.13 mg L⁻¹ for the action of the essential oil to inhibit albumin denaturation by thermal degradation. For the activity of the nanoemulsion incorporated into the essential oil of *C. zeylanicum*, an EC₅₀ of 42.21 mg L⁻¹ was obtained.

This study refers to the nanoemulsion of *C. zeylanicum* with anti-inflammatory action is unprecedented. However, other nanoemulsions were used and proven for such activity in the literature as in the study by Elshamy et al. (2020), injecting carrageenan in the paw of the rat and, as a consequence, edema, it was observed anti-inflammatory activity of the nanoemulsion of *Araucaria heterophylla* resin, reducing edema by 32.0%, 30.3% and 39.0%. Another study carried out by Borges et al. (2018) also using the carrageenan-induced rat paw edema assay evaluated the nanoemulsion potential in models of inflammation, administering 498 µg kg⁻¹ inhibited the peak edema peak by 46%.

According to Borges et al. (2019) inflammation is a response of the immune system due to injury, stress or infection in the body's tissues and anti-inflammatory drugs are intended to prevent or alleviate this inflammation reaction and its failure to resolve it leads to pathological conditions.

The essential oil of *C. zeylanicum* showed satisfactory in vitro anti-inflammatory activity, being justified by the presence of phenolics in their composition, as they have

hydroxyl and aromatic rings in their chemical structures, which guarantees the anti-inflammatory property (Soares et al., 2015).

3.4 Larvicide activity

Table 4 presents the results regarding larval mortality *Aedes aegypti* against the action of essential oil and in an unprecedented way for the nanoemulsion of *C. zeylanicum*.

Table 4 – Larvicidal activity against *Aedes aegypti* for the essential oil of *C. zeylanicum*

	Concentration	%Mortality	CL ₅₀ mg L ⁻¹	δ	X ²	R2
EO	100	93.30	22.01 (12.70-38.16)	0.691	0.994	0.845
	75	73.30				
	50	53.30				
	25	50.00				
	10	36.70				
	5	20.0				
NEO	100	100.0	5.30 (1.92-14.65)	0.913	0.983	0.816
	75	92.2				
	50	71.2				
	25	59.9				
	10	57.8				
	5	33.0				
	Positive control				All active larvae	
	Negative Control				All inactive larvae	
	White				All inactive larvae	

Source: Autorship (2025)

As shown in Table 4, larvae mortality was observed. *Aedes aegypti* at concentrations of up to 5 mg L⁻¹ for the essential oil and *C. zeylanicum* nanoemulsion.

The CL₅₀ evaluated for essential oil and nanoemulsion were below 50 mg L⁻¹, being classified by Cheng et al. (2003) as a very active action of the essential oil, its potential use and application being encouraged. It is important to emphasize that the nanoemulsion bioproduct had a LC₅₀ relatively lower than that of the essential oil.

It is important to emphasize that the study presents the nanoemulsion of *C. zeylanicum* by low energy phase inversion method targeting the biological activities tested in this study.

Similar results are pointed out by Porto (2016) when presenting the larvicidal action of the essential oil of *C. zeylanicum* against *Aedes aegypti* also obtaining a mortality of 100% after 24h of application of essential oil at concentrations of 10 to 20mg L⁻¹. Warikoo et al. (2011) also observed larvicidal activity of *C. zeylanicum* essential oil at commercial concentrations of 0.63 mg mL⁻¹ against Indian strains of *Aedes aegypti* mosquito larvae, thus ensuring biological potential.

Pereira et al. (2016) also observed 100% mortality of larvae *Aedes aegypti* when in contact for 24h, but at a concentration of 125 ppm of the essential oil from the leaves of *C. zeylanicum*, and its LC₅₀ was 55.94 mg L⁻¹. This result highlights the use of the bark of the species, since the mortality associated with the larvae was much higher at lower concentrations.

This fact can be justified, since the larvicidal activity is guaranteed by the majority component of the essential oil, in the case of *C. zeylanicum* we have Eugenol as the majority when the essential oil is extracted from the leaves, corroborated by Lima et al. (2005) who identified the presence of 60% eugenol in leaves compared to branches (3%). However, when this essential oil is extracted from the barks Silva et al. (2018) describe cinnamaldehyde as the major component of the essential oil of *C. zeylanicum* extracted from the bark, presenting approximately 60 to 75% of its constituents.

The larvicide action for nanoemulsions of *C. zeylanicum* is described for other disease vectors. Firoozian et al. (2021) report that nanoformulation by phase inversion was able to eliminate 100% of the larvae at concentrations of 37 mg L⁻¹, ensuring the efficiency of the larvicidal activity of nanoemulsion associated with the essential oil of *C. zeylanicum*, generating reliability to the expected result in the present study.

Other nanoemulsions are described in the literature for larvicidal action, such as Saavedra et al. (2018), in their present study on the larvicidal activity of *P. fragrantissimum* nanoemulsion, showed that with 150 mg L⁻¹ they were able to achieve 100% mortality of *Aedes aegypti* larvae. Also, Brazil (2017) describes the larvicidal activity of the nanoemulsion associated with *Pterocaulon polystachyum* essential oil using a concentration of 750 mg L⁻¹ obtained 80% mortality of *A. aegypti* larvae.

4 CONCLUSIONS

Finally, this study demonstrates that the EO of *C. zeylanicum* has bioactive characteristics, with the larvicidal activity of the nanoemulsion being classified as very active and unprecedented against *Aedes aegypti* larvae and brings expressive and important results for the control and combat of arboviruses.

REFERENCES

- Abeysekera, W.P.K.M., Premakumara, G.A.S., & Ratnasooriya, W.D. (2013). In vitro antioxidant properties of leaf and bark extracts of ceylon cinnamon (*Cinnamomum zeylanicum* Blume). http://www.pgia.pdn.ac.lk/files/Annual_congress/journal/v24_2/Papers/2-_23_-W.P.K.M.pdf
- Alizadeh Behbahani, B., Falah, F., Lavi Arab, F., Vasiee, M., & Tabatabaee Yazdi, F. (2020). Chemical composition and antioxidant, antimicrobial, and antiproliferative activities of *Cinnamomum zeylanicum* bark essential oil. *Evidence-based component and alternative medicine*, 2020. doi: <https://doi.org/10.1155/2020/5190603>
- Andrade, M.A., Cardoso, M.D.G., Batista, L.R., Mallet, A.C.T., & Machado, S.M.F. (2012). Essential oils of *Cymbopogon nardus*, *Cinnamomum zeylanicum* and *Zingiber officinale*: composition, antioxidant and antibacterial activities. *Revista Ciência Agronômica*, 43, 399-408. doi: <https://doi.org/10.1590/S1806-66902012000200025>
- Borges, R.S., Lima, E.S., Keita, H., Ferreira, I.M., Fernandes, C.P., Cruz, R.A.S., ... & Carvalho, J.C.T. (2018). Anti-inflammatory and antialgic actions of a nanoemulsion of *Rosmarinus officinalis* L. essential oil and a molecular docking study of its major chemical constituents. *Inflammopharmacology*, 26(1), 183-195. doi: <https://doi.org/10.1007/s10787-017-0374-8>
- Borges, R.S., Ortiz, B.L.S., Pereira, A.C.M., Keita, H., & Carvalho, J.C.T. (2019). *Rosmarinus officinalis* essential oil: A review of its phytochemistry, anti-inflammatory activity, and mechanisms of action involved. *Journal of ethnopharmacology*, 229, 29-45. doi: <https://doi.org/10.1016/j.jep.2018.09.038>
- Brazil, N.T. (2017). Larvicidal activity of a nanoemulsion containing *Pterocaulon polystachyum* essential oil against *Aedes aegypti* larvae. <http://hdl.handle.net/10183/217526>
- Cheng, S.S., Chang, H.T., Chang, S.T., Tsai, K.H., & Chen, W.J. (2003). Bioactivity of selected plant essential oils against the yellow fever mosquito *Aedes aegypti* larvae. *Bioresource Technology*, 89(1), 99-102. doi: [https://doi.org/10.1016/S0960-8524\(03\)00008-7](https://doi.org/10.1016/S0960-8524(03)00008-7)
- Del Ré, P.V., & Jorge, N. (2012). Spices as natural antioxidants: applications in food and implications for health. *Brazilian Journal of Medicinal Plants*, 14, 389-399. doi: <https://doi.org/10.1590/S1516-05722012000200021>

- Dolabela, M.F. (1997). In vitro screening for antitumor and anti-T. cruzi of plant extracts, natural and synthetic products (Doctoral dissertation, master's thesis, Belo Horizonte Universidade Federal de Minas Gerais).
- dos Santos, A.B.D.S., Everton, G.O., Júnior, R.G.D.O.C., Rosa, P.V.S., Pereira, A.P.M., dos Santos Souza, L., ... & Mouchrek Filho, V.E. (2020). *Cinnamomum zeylanicum* Blume and *Plectranthus amboinicus* (lour.) Spreng essential oils as larvicidal agents against *Aedes aegypti* larvae. *Brazilian Journal of Development*, 6(4), 22355-22369. doi: <https://doi.org/10.34117/bjdv6n4-413>
- Felizardo, M.G.A.C., Pereira, A.P.M., de Lima, T.P., de Sousa, T.L., Guterres, C.V.F., Oliveira, J.P.M., ... & Everton, G.O. (2021). Chemical profile, stability and fungicide activity of oil-in-water nanoemulsion (O/A) incorporated with Ba-har essential oil. *Science and Nature*, 43, 57. doi: <https://doi.org/10.5902/2179460X63881>
- Finney, D.J. (1952). Probit analysis: a statistical treatment of the sigmoid response curve. *Cambridge university press, Cambridge*. <https://psycnet.apa.org/record/1953-01596-000>
- Firoozian, S., Amani, A., Osanloo, M., Moosa-Kazemi, S.H., Basseri, H.R., Hajipirloo, H.M., ... & Sedaghat, M.M. (2021). Preparation of nanoemulsion of *Cinnamomum zeylanicum* oil and evaluation of its larvicidal activity against a main malaria vector *Anopheles stephensi*. *Journal of Environmental Health Science and Engineering*, 1-10. doi: <https://doi.org/10.1007/s40201-021-00667-0>
- Gobbo-Neto, L., & Lopes, N.P. (2007). Medicinal plants: factors influencing the content of secondary metabolites. *New Chemistry*, 30(2), 374-381. doi: <https://doi.org/10.1590/S0100-40422007000200026>
- Gomes, P.R.B., Mouchrek Filho, V.E., Rabêlo, W.F., do Nascimento, A.A., Louzeiro, H.C., da Silva Lyra, W., & Fontenele, M.A. (2018). Chemical characterization and cytotoxicity of clove (*Syzygium aromaticum*) essential oil. *Revista Colombiana de Ciencias Químico-Farmacéuticas*, 47(1), 37-52. doi: 10.15446/rcciquifa.v47n1.70657
- Jayaprakasha, G.K., Jagan Mohan Rao, L., & Sakariah, K.K. (2003). Volatile constituents from *Cinnamomum zeylanicum* fruit stalks and their antioxidant activities. *Journal of agricultural and food chemistry*, 51(15), 4344-4348. doi: <https://doi.org/10.1021/jf034169i>
- Kubitschek-K.M., A.R.J., & Zero, J.M. (2014). Development of jojoba oil (*Simmondsia chinensis* (Link) CK Schneid.) based nanoemulsions. *Lat. Am. J. Pharm*, 33(3), 459-63. <http://www2.unifap.br/quimica/files/2020/02/Development-of-Jojoba-Oil-Simmondsia-chinensis-Link-C.K.-Schneid.-Based.pdf>
- Lima, M.D.P., Zoghbi, M.D.G.B., Andrade, E.H.A., Silva, T.M.D., & Fernandes, C.S. (2005). Volatile constituents of leaves and branches of *Cinnamomum zeylanicum* Blume (Lauraceae). *Amazon Act*, 35(3), 363-366. doi: <https://doi.org/10.1590/S0044-59672005000300009>
- Lima, T.C.P., de Almeida, A.F., de Oliveira, E.C.P., Carrera Silva Júnior, J.O., Ribeiro Costa, R.M., Pena Matos, A., & Fonseca Gomes, M.R. (2020). Development of nanogels from *copaifera reticulata* on muscle injury in rats using phonophoresis. *Health and Research*, 13(1). doi: <https://doi.org/10.17765/2176-9206.2020v13n1p181-192>

- Melo, C.P.D., & Pimenta, M. (2010). Nanosciences and nanotechnology. *Strategic partnerships*, 9(18), 09-22. <https://www.redalyc.org/pdf/4675/467546317001.pdf>
- Morais, J.M.D. (2008). Development and evaluation of the process for obtaining multiple W/O/W emulsions in a single step using canola oil and nonionic surfactant derived from castor oil (Doctoral dissertation, Universidade de São Paulo). doi: <https://doi.org/10.11606/T.60.2008.tde-23072008-152433>
- Moreira, L. A. O. (2013). Side evaluation of toxic activity in artemia salina leach. Of extracts from two species of the melastomataceae family. <https://www.ifg.edu.br/attachments/article/1704/TCC%20-%20Layssa%20Aparecida%20de%20Oliveira%20Moreira.pdf>
- Padmanabhan, P., & Jangle, S.N. (2012). Evaluation of in-vitro anti-inflammatory activity of herbal preparation, a combination of four medicinal plants. *International journal of basic and applied medical sciences*, 2(1), 109-116. <http://cibtech.org/J-MEDICAL-SCIENCES/PUBLICATIONS/2012/JMS-02-01-PDF/22%20JMS%2043%20Preeti%20Padmanabhan.pdf>
- Pereira, E.C.T., & Santiago, A.D.S. (2016). Analysis of the Larvicidal Action of Essential Oils of Cinnamomum zeylanicum (BIJDR, 1825) and Melaleuca quinquenervia (Queensland, 1958) against Malaria and Dengue Vectors. In *Annals of the VINPA-CONIC Initiation Congress*. <https://repositorio.inpa.gov.br/bitstream/1/37248/1/PEREIRA%20Elga%20Cristina%20Torres.pdf>
- Porto, B.C.Z. (2016). Ovicidal and larvicidal activity of essential oils on Sarconesia chlorogaster (diptera: calliphoridae). <http://repositorio.ufsm.br/handle/1/17447>
- Reis, J.B. (2012). Analytical study, evaluation of the toxicity and molluscicidal activity of the essential oil Cinnamomum zeylanicum Blume (cinnamon) against the snail Biomphalaria glabrata (say, 1818). doi: <https://doi.org/10.15446/rcciquifa.v48n1.80069>
- Rodrigues, E.D.C., Ferreira, A.M., Vilhena, J.C., Almeida, F.B., Cruz, R.A., Florentino, A.C., ... & Fernandes, C.P. (2014). Development of a larvicidal nanoemulsion with Copaiba (Copaifera duckei) oleoresin. *Brazilian Journal of Pharmacognosy*, 24, 699-705. doi: <https://doi.org/10.1016/j.bjp.2014.10.013>
- Ruberto, G., & Baratta, M.T. (2000). Antioxidant activity of selected essential oil components in two lipid model systems. *Food chemistry*, 69(2), 167-174. doi: [https://doi.org/10.1016/S0308-8146\(99\)00247-2](https://doi.org/10.1016/S0308-8146(99)00247-2)
- Saavedra, M.G. (2018). Evaluation of larvicidal activity of the essential oil nanoemulsion from the roots of Philodendron fragrantissimum (Hook) G. Don (Araceae) against Aedes aegypti (Linnaeus 1762) (Diptera: Culicidae). <http://www2.unifap.br/ppcs/files/2018/10/Disserta%3%A7%C3%A3o-AVALIA%3%87%C3%83O-DA-ATIVIDADE-LARVICIDA-DA-NANOEMULS%3%83O-DO-%3%93LEO-ESSENCIAL-DAS-RA%3%8DZES-DE-Philodendron-fragrantissimum.pdf>
- Schmidt, E., Jirovetz, L., Buchbauer, G., Eller, G.A., Stoilova, I., Krastanov, A., ... & Geissler, M. (2006). Composition and antioxidant activities of the essential oil of cinnamon (Cinnamomum zeylanicum Blume) leaves from Sri Lanka. *Journal of essential oil bearing plants*, 9(2), 170-182. doi: <https://doi.org/10.1080/0972060X.2006.10643490>

- Shafiq, S., Shakeel, F., Talegaonkar, S., Ahmad, F.J., Khar, R.K., & Ali, M. (2007). Development and bioavailability assessment of ramipril nanoemulsion formulation. *European journal of pharmaceutics and biopharmaceutics*, 66(2), 227-243. doi: <https://doi.org/10.1016/j.ejpb.2006.10.014>
- Silva, C.S., Figueiredo, S., de Oliveira, P.V., de Silva, W.F., Saminez, R.M.D., Rodrigues, J.F.S., ... & Grisotto, M.A.G. (2018). Cinnamon essential oil (Cinamaldehyde) and its biological applications. *Journal of Biomedical Research*, 9(2), 192-197. https://www.researchgate.net/profile/Luis-Claudio-Nascimento-Da-Silva/publication/325459376_Oleo_essencial_da_Canela_Cinamaldeido_e_suas_aplicacoes_biologicas/links/5b90f864a6fdcce8a4c954ca/Oleo-essencial-da-Canela-Cinamaldeido-e-suas-aplicacoes-biologicas.pdf?_sg%5B0%5D=started_experiment_milestone&origin=journalDetail&_rtd=e30%3D
- Silva, H.H.G.D., Silva, I.G.D., Elias, C.N., Lemos, S.P.S., & Rocha, A.P. (1995). Physiological age of eggs of aedes (stegomyia) aegypti (Linnaeus, 1762)(diptera, culicidae). doi: <https://doi.org/10.5216/rpt.v24i2.19696>
- Silva, M.L.C., Costa, R.S., dos Santos Santana, A., & Koblitz, M.G.B. (2010). Phenolic compounds, carotenoids and antioxidant activity in plant products. *Semina: Agricultural Sciences*, 31(3), 669-681. <https://www.redalyc.org/pdf/4457/445744097017.pdf>
- Silva, W.J.D. (2006). Larvicidal activity of essential oil from plants existing in the state of Sergipe against Aedes aegypti Linn. doi: <https://doi.org/10.1590/S1519-566X2005000500018>
- Singh, H.P., Kaur, S., Negi, K., Kumari, S., Saini, V., Batish, D.R., & Kohli, R.K. (2012). Assessment of in vitro antioxidant activity of essential oil of Eucalyptus citriodora (lemon-scented Eucalypt; Myrtaceae) and its major constituents. *LWT-Food science and Technology*, 48(2), 237-241. doi: <https://doi.org/10.1016/j.lwt.2012.03.019>
- Smirnoff, N., & Cumbes, Q.J. (1989). Hydroxyl radical scavenging activity of compatible solutes. *Phytochemistry*, 28(4), 1057-1060. doi: [https://doi.org/10.1016/0031-9422\(89\)80182-7](https://doi.org/10.1016/0031-9422(89)80182-7)
- Soares, D.J., de Moura Neto, L.G., Damaceno, M.N., de Souza, P.A., Braga, R.C., & Brasil, I.M. (2015). Anti-inflammatory activity of fruits. *Saúde em Revista*, 15(39), 33-45. doi: <http://dx.doi.org/10.15600/2238-1244/sr.v15n39p33-45>
- Soares, S.E. (2002). Phenolic acids as antioxidants. *Journal of nutrition*, 15, 71-81. doi: <https://doi.org/10.1590/S1415-52732002000100008>
- Sugumar, S., Clarke, S.K., Nirmala, M.J., Tyagi, B.K., Mukherjee, A., & Chandrasekaran, N. (2014). Nanoemulsion of eucalyptus oil and its larvicidal activity against Culex quinquefasciatus. *Bulletin of entomological research*, 104(3), 393-402. doi: <https://doi.org/10.1017/S0007485313000710>
- Sundararajan, R., & Koduru, R. (2016). In vitro antioxidant activity on roots of Limnophila heterophylla. *Free Radicals and Antioxidants*, 6(2), 178-185. doi: <https://doi.org/10.5530/fra.2016.2.8>

- Valizadeh, S., Katiraei, F., Mahmoudi, R., Fakheri, T., & Mardani, K. (2015). Biological properties of *Cinnamomum zeylanicum* essential oil: phytochemical component, antioxidant and antimicrobial activities. *International Journal of Food Safety Nutrition and Public Health*, 6(3), 174-184. <https://api.semanticscholar.org/CorpusID:101186186>
- Veiga Junior, V.F., Pinto, A.C., & Maciel, M.A.M. (2005). Medicinal plants: a safe cure?. *New Chemistry*, 28, 519-528. doi: <http://dx.doi.org/10.1590/S0100-40422005000300026>
- Viana, A. R., Bianchin Bottari, N., Santos, D., Serafin, M. B., Rossato, B. G., & Moresco, R. N. (2022). Insights of ethyl acetate fraction from *Vassobia breviflora* in multidrug-resistant bacteria and cancer cells: from biological to therapeutic. *Journal of Toxicology and Environmental Health, Part A*, 85(23), 972-987. doi: <https://doi.org/10.1080/15287394.2022.2130844>
- Viana, A. R., Can Aytar, E., Pippi, N., Santos, D., Rhoden, C. R. B., & Vizzotto, B. S. (2025). Chemical composition, in vitro and in silico activity of the methanolic extract derived from *Vassobia breviflora* against clinically relevant bacteria. *Journal of Toxicology and Environmental Health, Part A*, 88(11), 463-478. doi: <https://doi.org/10.1080/15287394.2025.2453858>
- Warikoo, R., Wahab, N., & Kumar, S. (2011). Larvicidal potential of commercially available pine (*Pinus longifolia*) and cinnamon (*Cinnamomum zeylanicum*) oils against an Indian strain of dengue fever mosquito, *Aedes aegypti* L. (Diptera: Culicidae). *Acta Entomol Sin*, 54, 793-9. <http://www.insect.org.cn/EN/Y2011/V54/I7/793>
- Waterhouse, A.L. (2002). Determination of total phenolics. *Current protocols in food analytical chemistry*, 6(1), 11. https://www.researchgate.net/profile/Roberto-Molteni/post/Does_Folin-Ciocalteu_total_phenolic_compound_analysis_include_tannins/attachment/5eaaefff155db0001f88a68/AS%3A886026180521987%401588256511570/download/totalphenolsmethods.pdf

Authorship contributions

1 – João Durval Ribeiro Neto

Bachelor's degree in Industrial Chemistry (UFMA).

<https://orcid.org/0000-0002-0457-914X> • durval.joao@discente.ufma.br

Contribution: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Visualization, Writing– original draft, Writing– review editing.

2 – Thamires de Jesus Teles Ribeiro

Bachelor's degree in Chemistry (UFMA).

<https://orcid.org/0000-0003-1495-9282> • thamiresbelle@gmail.com

Contribution: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Visualization, Writing– original draft, Writing– review editing.

3 – Américo Pinheiro Neto

Bachelor's degree in Industrial Chemistry (UFMA).

<https://orcid.org/0009-0005-6095-0336> • americo.pn@discente.ufma.br

Contribution: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Visualization, Writing– original draft, Writing– review editing.

4 – Victor Elias Mouchrek Filho

Full Professor (UFMA).

<https://orcid.org/0000-0003-2855-7292> • gustavo.oliveira@discente.ufma.br

Contribution: Funding acquisition, Project administration.

5 – Gustavo Oliveira Everton

PhD student in Chemistry (UFMA).

<https://orcid.org/0000-0002-0457-914X> • gustavooliveiraevertton@gmail.com

Contribution: Conceptualization, Data, Curation, Formal Analysis, Funding, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review editing.

How to quote this article

Ribeiro Neto, J. D., Ribeiro, T. de J. T., Pinheiro Neto, A., Mouchrek Filho, V. E. & Everton, G. O. (2026). Development of a larvicide nanoemulsion (O/W) of *Cinnamomum zeylanicum* essential oil against *Aedes aegypti*. *Ciência e Natura*, Santa Maria, 48, e88049. DOI: <https://doi.org/10.5902/2179460x88049>.