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Chemistry

Chemical profile and biotechnological potential larvicidal of a nanoemulsion (o/w) of the essential oil of *Salvia officinalis* L.

Perfil químico e potencial biotecnológico larvicida de uma nanoemulsão (o/a) do óleo essencial de *Salvia officinalis* L.

Ana Patrícia Matos Pereira[I], Brendha Araújo de Souza[I], Thaylanna Pinto de Lima[I], João Pedro Mesquita Oliveira[I], Cassiano Vasques Frota Guterres[I]; Ana Paula Muniz Serejo[I], Victor Elias Mouchrek Filho[I], Gustavo Oliveira Everton[I]

[I]Universidade Federal do Maranhão, São Luís, MA, Brazil

ABSTRACT

This study aims to evaluate the chemical profile and biotechnological larvicidal potential of the nanoemulsion of the essential oil of *Salvia officinalis* L. The leaves of the plant were collected in São Luís, MA, from January to May 2021. The essential oil was extracted by hydrodistillation at 100°C for 3h. Chemical characterization was obtained by GC-MS. The oil-in-water nanoemulsion was formulated by the low-energy phase inversion method and subjected to thermodynamic stability tests. Antioxidant activity is performed by the spectrophotometric method of scavenging hydroxyl radicals from salicylic acid. For larvicidal activity, *Aedes aegypti* larvae were subjected to EO solutions and nanoemulsions in concentrations (10-100 mg L^{-1}), larval mortality was evaluated, and the LC_{50} was determined by the Probit method. The majority compounds of the EO were: eucalyptol with 65.14%, camphor (30.63%), and α-Terpineol (1.53%). The formulations were characterized as nanoemulsions with a droplet size <200 nm. The PDI was <0.200, indicating a narrow size distribution. The antioxidant activity exhibited EC_{50} of 136.29 mg L^{-1} and 51.59 mg L^{-1}. The nanoemulsion with larvicidal potential showed an LC_{50} of 71.17 mg L^{-1}. The nanoemulsion showed bioactive potential for larvicidal action, which may be related to the presence of its chemical compounds, and its use is encouraged in the fight against *Aedes aegypti*.

Keywords: Larvicide; Nanotechnology; *Salvia officinalis*

RESUMO

Este estudo tem por objetivo avaliar o perfil químico e o potencial biotecnológico larvicida da nanoemulsão do óleo essencial de *Salvia officinalis* L. As folhas do vegetal foram coletadas em São Luís, MA, em janeiro a maio de 2021. O óleo essencial foi extraído por hidrodestilação a 100ºC por

3h. A caracterização química foi obtida por CG-EM. A nanoemulsão (O/A) foi formulada pelo método de baixa energia de inversão de fases, sendo submetida a testes de estabilidade termodinâmica e caracterização do tamanho de gota e índice de polidispersidade (PDI). Atividade antioxidante realizada pelo método espectrofotométrico de sequestro de radicais hidroxila do ácido salicílico. Para atividade larvicida submeteu-se larvas *Aedes aegypti* a soluções do OE e nanoemulsões em concentrações (10-100 mg L^{-1}), avaliou-se a mortalidade das larvas e determinou-se a CL_{50} pelo método de Probit. Os compostos majoritários do OE foram: eucaliptol com 65,14%, cânfora (30,63%) e α-Terpineol (1,53%). As formulações foram caracterizadas como nanoemulsões com tamanho de gota <200 nm. O PDI foi <0,200, indicando uma distribuição de tamanho estreita. A atividade antioxidante apresentou EC_{50} de 136,29 mg L^{-1} e 51,59 mg L^{-1}. A nanoemulsão com potencial larvicida apresentou CL_{50} de 71,17 mg L^{-1}. A nanoemulsão apresentou potencial bioativo para ação larvicida, podendo estar relacionado a presença de seus compostos químicos, sendo seu uso incentivado no combate ao *Aedes aegypti.*

Palavras-chave: Larvicida; Nanotecnologia; *Salvia officinalis*

1 INTRODUCTION

Arboviruses have been presented as an important public health problem, as they face diagnostic difficulties both based on clinical-epidemiological examinations and serological analyzes due to the presentation of cross-reactions (Grandadam, 2007; Lima-Camara, 2016; Kurizky et al., 2020). Among the mosquitoes that act directly in the dissemination of arboviruses is Aedes aegypti, vector of yellow fever, dengue, Zika and Chikungunya viruses, which affect people in tropical and subtropical areas around the world (Castillo-Morales & Duque, 2020; An et al., 2020).

Chemical control consists of the application of synthetic insecticides such as organophosphates, carbamates and pyrethroids are used to control the vector, but despite having good results, their continuous use can cause the development of resistance in mosquitoes and lead to contamination of the environment, imbalance and cause damage to human health (De Souza Wuillda et al., 2019; Baz et al., 2022).

Based on this, the use of natural products stands as an ecological alternative in the formation of natural insecticides, safe for the environment and for man. For, plant species produce several bioactive compounds that often work in synergy, are generally biodegradable and present a lower risk of developing resistance to vectors, and to prove this, toxicity tests are necessary against non-target organisms.

Within this are essential oils, composed of secondary metabolites from the most diverse parts of plants, being natural molecules that counteract even the development of bioresistance to natural pesticides, are a satisfactory alternative source due to favorable factors of biodegradability, selectivity, low harmfulness to non-target organisms and the environment. Among the plants used, we can mention the genus Salvia, belonging to the Lamiaceae family, which reveals to have a good insecticidal action and are reported to produce many useful secondary metabolites (Ali et al., 2015; Zahed et al., 2021).

Among the species of the genus *Salvia*, there is *Salvia officinalis* L., an ornamental plant widely used since antiquity within popular, culinary and aromatic medicine and through the most diverse studies demonstrates several biological potentials, such as antiviral, antimicrobial and spasmolytic properties, and its effectiveness is mainly related to secondary metabolites (Rezai et al., 2018; Vosoughi et al., 2018; Jakovljević et al., 2019).

In this context, nanotechnology has high potential for applications in multiple areas, including medicine and pharmaceuticals, optimizing and serving as carrier systems, providing advantages through nanoemulsions, such as protection of the bioactive compound from degradation and the possibility of modulating the release profile (Pasquoto -Stigliani et al., 2017; Fouda et al., 2020). In view of the above, the study aimed to evaluate the chemical profile and the larvicidal biotechnological potential of the nanoemulsion of the essential oil of *Salvia officinalis* L.

2 MATERIAL AND METHODS

2.1 Plant material

The dried leaves of *Salvia officinalis* used in this research were obtained the municipality of São Luís (MA) from the certified distributor of Produtos Naturais Muniz LTDA from January to May 2021. 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 Obtaining the essential oil

For the extraction of essential oils, the hydrodistillation technique was used with a glass Clevenger extractor coupled to a round-bottom flask placed in an electric blanket as a source of heat, using distilled water as solvent (1:10). Hydrodistillation was conducted at 100°C for 3h, collecting the extracted essential oil, being dried by percolation with anhydrous sodium sulfate (Na_2SO_4) and centrifuged. The samples were stored in amber glass ampoules under refrigeration at 4°C. Subsequently being submitted to analysis.

2.3 Chemical constituents

Essential oils of *Salvia officinalis* were chemically characterized by Gas Chromatography coupled to Mass Spectrometry (GC/MS). Analyzes of essential oils were performed on a Gas Chromatograph (GC-2010) coupled to a Mass Spectrometer (GC-MS QP2010 Plus), both from Shimadzu, using a DB-5MS capillary column (30m x 0.25mm x 0.25µm). Carrier gas flow, Helium at a linear velocity of 39.5 cm/sec and column flow 1.0 mL/min, Split ratio 1/100. The oven setting was 45°C for 4 min with a heating ramp of 10°C/min to 180°C, remaining for 7 min, heating at 250°C, with a ramp of 8°C/min, remaining for 3 min until the end of the race. The injector and ion source temperature of 250°C and 200°C, respectively. Running Time 36.25 min. MS - Acquisition Mode: Full Scan, Scan Interval 0.30sec, Mass Range: 35 – 500 a.m.u, Acquisition Time: 3.5 to 36.25 min.

2.4 Formulation of nanoemulsions

The preparation of nanoemulsions (O/W) was carried out according to the adapted methodologies described by Lima et al. (2020) and Sugumar et al. (2014), through the low energy phase inversion method. Nanoemulsions were formulated with essential oil, non-ionic surfactant and water. The final homogenization was completed by keeping the formulation in constant agitation at 6000 rpm, until the temperature was reduced to 25ºC ± 2 ºC. To prove stability, the formulations were subjected to different stress tests: centrifugation, heating-cooling cycle and freeze-thaw stress, according to the methodology described by Shafiq et al. (2007). The quantifications of the formulations are shown in Table 1.

Tabel 1 – Nanoemulsion formulations for the essential oil of *S. officinalis*

Identification	Essential Oil	Tween 20	H_2O
N E/O1	5%	5%	90%
N E/O 2	5%	10%	85%
N E/O 3	5%	15%	80%

Source: Pereira (2023)

2.5 Characterization of nanoemulsions

The droplet size distribution (volume analysis) and polydispersity index (PDI) of the nanoemulsion formulations were determined using a 90 Plus particle size analyzer (Sugumar et al., 2014).

2.6 Determination of Antioxidant Activity

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

The nanoemulsions in different concentrations of 10-100 mg L^{-1} were dissolved in distilled water. 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.

2.7 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 the methodology of Silva et al. (1995) until reaching the third and fourth stage, age at which the experiments were carried out.

Assays for larvicidal activity were carried out according to the adapted methodology proposed by Silva et al. (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 1.0-90.0 mg L^{-1}. At each concentration, 10 larvae were added at a rate of 1 ml per larva.

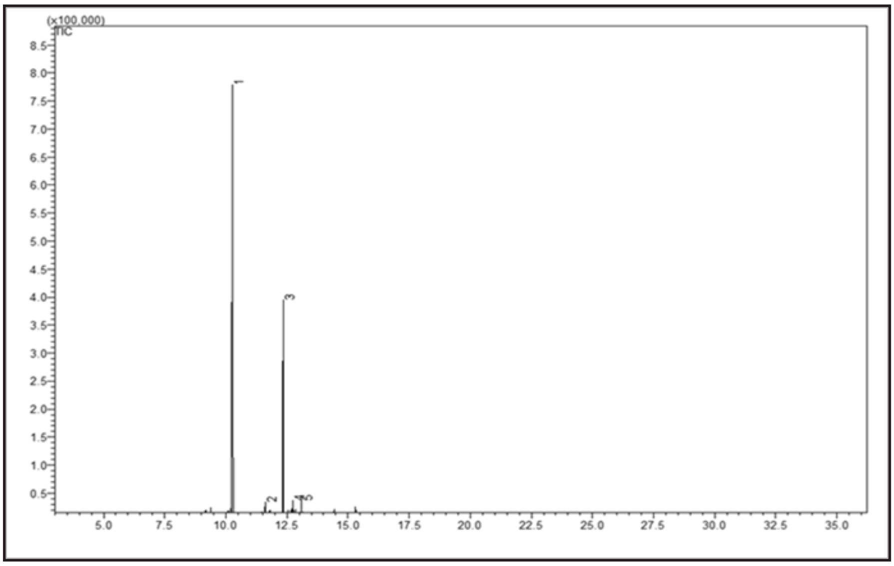
All tests were performed in triplicates and a solution made up of 2% DMSO was used as a negative control, and a 70% v/v ethanol (P.A) solution was used as a positive control. After 24 hours, live and dead were counted, and larvae that did not react to touch 24 hours after the start of the experiment 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) were applied.

3 RESULTS AND DISCUSSION

3.1 Chemical constituents

Through gas chromatography coupled to mass spectrometry (GC/MS) it was possible to separate and identify five constituents of the essential oil, which are shown in Figure 1, following the order of elution.

Figure 1– Chromatogram of the essential oil of *S. officinalis*



Source: Authors (2023)

According to the results obtained, Table 2 presents the chemical compounds identified in the EO of *S. officinalis* through GC/MS. Thus, according to Table 2, 5 components were identified in the EO of *S. officinalis*, the major constituent of the EO being eucalyptol with 65.14%, followed by camphor (30.63%) and α-Terpineol (1, 53%).

Similar results were described by Alcala-Orozco et al., (2019), who reported the presence of 6 constituents characterized and identified by GC/MS, with 23.4% of the sage EO consisting of eucalyptol, followed by cis-thujone (18.9%) and trans-β-caryophyllene (11.0%). Velandia *et al.* (2018, p. 189) also identified eucalyptol (26.6%) as the major compound in the EO of *S. officinalis* collected in Colombia.

Table 2 – Chemical constituents in the *S. officinalis* EO sample

Peak	Retention time (min)	Area	Content%	Compounds
1	10.268	1312912	65.14	Eucalyptol
2	11.617	24822	1.23	Thujone
3	12.352	617523	30.63	Camphor
4	12.753	29549	1.47	Cyclobutyl ester
5	13.092	30939	1.53	α-Terpineol

Source: Pereira (2023)

According to El Hadri et al., (2010), the composition of the EO of *S. officinalis* can vary according to the place of collection of the plant, genetic factors, stage of development, extraction methods, and other climatic, seasonal and environmental factors. Thus, in the study by Hamidpour et al. (2014), the composition and quantification of sage EO extracted from different locations varied significantly, with eucalyptol responsible for 55 to 62% of the extracted EO's composition and camphor with a variation of 8 to 10% of the content. Rguez et al., (2019), obtained variations in the yield of compounds according to the time of harvest, with camphor with 22.4 to 18.8%, followed by α-thujone (21.4 to 21.6 %) and eucalyptol (15.1 to 7.4%).

3.2 Thermodynamic stability

Table 3 displays the thermodynamic stability of nanoemulsion formulations with *S. officinalis* EO.

According to Table 3, the N O/W 1 and N O/W 2 formulations showed stability in the tests used and were used for the other tests, however, the N O/W 3 formulation proved to be unstable with phase separation after heating and was not used in the following tests. According to Walker et al. (2015), the stability of nanoemulsions may depend on factors such as initial droplet size, surfactant concentration and production method. In this study, a larger droplet size was observed for the unstable formulation compared to the other formulations, which may explain its instability. It should be noted that in addition to the fact that there are no specific studies on the incorporation of sage EO in nanosystems, stability has not yet been studied, which configures the importance of this development proposal.

Table 3 – Study of thermodynamic stability of nanoemulsion formulations with *Salvia officinalis* essential oil

Identification	SF	AQ	CG	DCG	Ultimate Stability
N O/W 1	-	-	-	-	+
N O/W 2	-	-	-	-	+
N O/W 3	-	+	-	-	-

Where; SF-phase separation or creaming at room temperature; AQ- phase separation after heating; GC- phase separation or creaming after freezing; DCG- phase separation or creaming after thawing; + positive; - negative.

Source: Pereira (2023)

Similar results were described by Bolzan et al., (2015) in the study on the nanoemulsion of the EO of *Origanum vulgare* L., where the formulation was unstable when subjected to high temperatures, between 75ºC and 100ºC. Borges et al. (2018), observed that no sample of the EO nanoemulsion of *Rosmarinus officinalis* L., exhibited signs of instability such as creaming and phase separation. The formulation was prepared with the same concentration of compounds used in this study, which showed stability.

3.3 Characterization of nanoemulsions

Table 4 shows the average size of formulations containing Tween 20 surfactant, *S. officinalis* EO and H_2O, as well as the polydispersity index over time from 0 to 60 days.

According to the concept that nanoemulsions are systems that have droplets in the size range between 50 and 200 nm (Jaiswal et al., 2015) the formulations produced were characterized as nanoemulsions because they had an average droplet size < 200 nm after 0 to 60 days of handling, indicating small droplet size. The polydispersity index for all NEs during the evaluated period was low and was between 0.169 and 0.187, indicating a narrow size distribution (PDI= < 0.200) (Table 4). Thus, NE's were used for the larvicidal bioassay against *Ae. aegypti* and other biological tests.

Table 4 – Variation of Z mean (nm) and polydispersity index (PDI) for the nanoemulsion over time (T0-T60 days)

	Time (days)	**Average Z (nm)**			**PDI**		
NO/W 1	0	106.47	±	1.66	0.187	±	0.010
	T15	102.52	±	0.83	0.184	±	0.005
	T30	96.09	±	0.58	0.181	±	0.009
	T45	93.03	±	1.67	0.178	±	0.010
	T60	90.71	±	0.52	0.182	±	0.002
	Time (days)	**Average Z (nm)**			**PDI**		
NO/W 2	0	101.14	±	1.58	0.177	±	0.009
	T15	97.40	±	0.79	0.175	±	0.005
	T30	91.29	±	0.55	0.172	±	0.008
	T45	88.38	±	1.58	0.169	±	0.009
	T60	86.18	±	0.49	0.173	±	0.002

Source: Pereira (2023)

According to Moraes-Lovison et al. (2017), the method used to obtain nanoemulsions can influence their characterization. Low energy methods are more efficient, as they require less energy to produce nanoemulsions with smaller droplet sizes compared to NE's obtained with the high energy methodology (Solans & Solé, 2012).

Thus, this method used in this study allowed obtaining NE's with reduced droplet size and low polydispersity. Results by Ferreira et al. (2020), on the *Lippia alba* EO nanoemulsion produced with a low energy method, indicated an average particle size of 116.2±0.3 nm and a polydispersion index of 0.205±0.001, corroborating this study.

3.4 Antioxidant activity

Table 5 presents the EC_{50}, straight line equation and linear regression for the antioxidant capacity of *S. officinalis* EO for the employed methods.

Table 5 – Antioxidant capacity of *S. officinalis* nanoemulsions

Identification	EC_{50} (mg L^{-1})	y=ax-b	R^2
N O/W 1	136.29	y=55.26x-67.963	0.9589
N O/W 2	51.59	y=90.021x-104.17	0.9941

Source: Pereira (2023)

According to Campos et al. (2003), vegetable products with a concentration lower than 500 mg L^{-1} are classified as active. Thus, the formulations of EO from *S. officinalis* were promising and active for antioxidant activity. The lower the EC_{50} value, the greater the antioxidant action of the natural compound, as a lower concentration of the natural product (nanoemulsion) is required to reduce the H_2O_2 radical by 50% (Sousa et al., 2007). Thus, when evaluating Table 5, it was verified that the EO nanoemulsion of S*. officinalis* presented better antioxidant activity for the N O/W 2 formulation (EC_{50}= 51.59 mg L^{-1}), when compared to EC_{50} of 136.29 mg L^{-1} of the N O/W 1 formulation. In the literature, it was not possible to find studies on the antioxidant activity of the O/W nanoemulsion of *S. officinalis* EO, which emphasizes the importance of this research.

Results similar to those obtained in this study were reported by Melo (2016), where an EC_{50} of 48,800 EAA/g of the dry sage plant was observed. On the other hand, Ugalde et al. (2016), reported only 19.70% of antioxidant action of EO from *S. officinalis*, at a concentration of 1000 µL mL^{-1}.

The antioxidant potential of plant species may be related to the presence of phytoantioxidants found in medicinal plants such as flavonoids, tannins, anthocyanins, ascorbic acid, including phenolics present in this study. Therefore, several authors point out the relationship between the antioxidant potential and the phenolic content of natural products. In addition, the production of these substances also depends on environmental conditions, such as high temperatures, radiation, pathogen attack or even mineral imbalance (Wilmes et al., 2011).

3.5 Larvicidal activity

Table 6 shows the mortality of *Aedes aegypti* larvae in the concentrations of nanoemulsions tested with calculation of LC_{50} and statistical parameters by the Probit method.

Table 6 – *Aedes aegypti* mortality due to the action of *S. officinalis* essential oil nanoemulsions

Identification	LC_{50}	$\chi 2$	σ	R^2
NO/W 1	71.17	0.976	0.462	0.925
NO/W 2	> 100 mg L^{-1}	-	-	-

Source: Pereira (2023)

As shown in Table 6, the LC_{50} for NO/W 1 formulated regarding the estimated mortality of 50% of *Aedes aegypti* larvae was below 100 mg L^{-1}, being classified by Dias&Moraes (2014) as active action (LC_{50} of 71.17 mg L^{-1}) for larvicidal activity, and its potential for use and application was encouraged, while nanoemulsion 2 (O/W) did not obtain active potential (LC_{50} of 214.32 mg L^{-1}).

According to the nanometric characteristics of the oil-in-water (O/W) formulations, they can be identified as good larvicidal agents as they increase the biological action of the essential oil (Oliveira et al., 2016), as observed in this study. However, investigations on the larvicidal potential of the nanoemulsion of the EO of S*. officinalis* were not found in the literature, which is a study of great relevance regarding the results obtained, therefore, the results will be compared to studies with the EO of *S. officinalis*.

Ríos et al. (2017) analyzed the larvicidal potential of the EO of *S. officinalis* obtained in Colombia and obtained an LC_{50} of 76.43 mg/mL, being classified as active. Castillo-Morales & Duque (2020), using the same methodology, found the same value for 50% mortality of *Aedes aegypti* larvae. On the other hand, Ali et al. (2015), also found active larvicidal action against *Ae. aegypti*, with an LC_{50} of 56.9 ppm. These results corroborate with this study that shows the larvicidal potential of *S. officinalis*.

The biological potential of an essential oil may be related to different concentrations and combinations of its chemical compounds. Abdellaoui et al. (2017) chemically characterized the EO of *S. officinalis* and found that the main chemical compounds were: β-thujone (20.1%), eucalyptol (15.91%) and camphor (14.79%). In the same study, significant larvicidal activity was observed, showing that such potential can be attributed to the chemical substances of the plant. In a study by Castillo-Morales et al. (2019), they verified the active biological potential of the EO of *S. officinalis* against *Ae. aegypti* larvae, which presented eucalyptol as the major compound.

It is important to highlight that the EO under study has great biological action, so the nanoemulsion obtained from this bioproduct has high market potential, being a viable alternative in the fight and control of *Aedes aegypti*.

4 CONCLUSIONS

We identified the chemical constituents eucalyptol (65.14%), camphor (30.63%), α-Terpineol (1.53%), cyclobutyl ester (1.47%) and thujone (1.23%) through Gas Chromatography coupled to Mass Spectrometry. It was possible to quantify the antioxidant action by the decolorization method of hydroxyl radicals of the formulated nanoemulsions. The nanoemulsions presented concentrations below 500 mg L^{-1}, being classified as active according to the established criteria. It was possible to obtain a smaller droplet size for the NO/W 1 and NO/W 2 formulations, with an average droplet <200 nm, characterizing them as nanoemulsions. The formulation NO/W 1 showed active potential for larvicidal activity, being encouraged as a new alternative for the control and combat of *Aedes aegypti* cases.

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Authorship contributions

1 - Ana Patrícia Matos Pereira

Universidade Federal do Maranhão - Mestre em Saúde e Ambiente
https://orcid.org/0000-0003-4478-4209 - anna.maattoos@gmail.com

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

2 - Brendha Araújo de Sousa

Universidade Federal do Maranhão - Graduada em Química
https://orcid.org/0000-0003-4504-4341 - asbrendha@gmail.com
Contribution: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Visualization, Writing – original draft, Writing – review editing.

3 - Thaylanna Pinto de Lima

Universidade Federal do Maranhão - Graduanda em Química Industrial
https://orcid.org/0000-0003-1172-3004 - thaylanna.lima@discente.ufma.br
Contribution: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Visualization, Writing – original draft, Writing – review editing.

4 - João Pedro Mesquita Oliveira

Universidade Federal do Maranhão - Graduando em Química Industrial
https://orcid.org/0000-0003-1833-9814 - joao-p01@live.com
Contribution: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Visualization, Writing – original draft, Writing – review editing.

5 - Cassiano Vasques Frota Guterres

Universidade Federal do Maranhão - Graduando em Química
https://orcid.org/0000-0003-2725-9429 - cassiano.guterres@discente.ufma.br
Contribution: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Visualization, Writing – original draft, Writing – review editing.

6 - Ana Paula Serejo Muniz

Universidade Federal do Maranhão - Doutorado em Biotecnologia
https://orcid.org/0000-0002-4376-4364 - apsmuniz@gmail.com
Contribution: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Visualization, Writing – original draft, Writing – review editing.

7 - Victor Elias Mouchrek Filho

Universidade Federal do Maranhão - Doutorado em Química
https://orcid.org/0000-0003-2855-7292 - gustavo.oliveira@discente.ufma.br
Contribution: Conceptualization, Formal Analysis, Visualization.

8 - Gustavo Oliveira Everton

Universidade Federal do Maranhão - Doutorando em Química
https://orcid.org/0000-0002-0457-914X - gustavooliveiraeverton@gmail.com
Contribution: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing-original draft, writing- review & editing, Obtaining Financing.

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