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Chemistry

Drying, phytochemical analysis and the fungicide potential of oil-in-water nanoemulsion (O/A) incorporated with *Ocimum citriodurum* L.

Secagem, análise fitoquímica e potencial fungicida da nanoemulsão óleo em água (O/A) incorporada com Ocimum citriodurum L.

Cassiano Vasques Frota Guterres[I], Thayane Lopes De Sousa[I], João Pedro Mesquita Oliveira[I], Larissa Gabrielle Pinheiro Ferreira[I], Éverton Holanda Sales[I], Victor Elias Mouchrek Filho[I], Gustavo Oliveira Everton [I]

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

ABSTRACT

This study aimed to analyze the phytochemical profile and drying with prediction of the leaves of *Ocimum citriodurum* L, quantify the total phenolics and flavonoids and determine the unprecedented potential of the fungicidal activity of the oil-in-water nanoemulsion incorporated with essential oil and hydroalcoholic extract of *O. citriodurum* L. The leaves were carefully selected in the rural area of São Luís, Maranhão, Brazil, and subsequently crushed and stored. The essential oil was obtained by hydrodistillation (100°C / 3h). To obtain the hydroalcoholic extract, maceration in solvent extract ethanol PA 70% (v / v) was used. To determine the antifungal activity, the technique recommended by the International Clinical Laboratory Standard was used through the technique of Dilution in Broth and Sowing and Agar to determine the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (CFM), respectively, front to strains of *Aspergillus niger* (ATCC 6275), *Colletotrichum gloeosporioides* (ATCC 96723), *Penicillium chrysogenum* (ATCC 10106). The results obtained for fungicidal action attested to CIM and strong inhibition for the action of essential oils and hydroalcoholic extracts with a concentration of 250 µg mL^{-1} in front of *A. niger*, *C. gloeosporioides* and *P. chrysogenum*. CFM's of 300, 250 and 500 mL^{-1} were observed in front of *A. niger*, *C. gloeosporioides* and *P. chrysogenum*, respectively. With the results obtained it is stated that a stable nanoemulsion was obtained for both (EX) and (OE) and that they present active compounds in which they favor an excellent antifungal activity.

Keywords: Antimicrobial; Fungicide; Inhibition

RESUMO

Este estudo teve por objetivo fazer a análise do perfil fitoquímico e secagem com predição das folhas de *Ocimumcitriodurum* L, quantificar os fenólicos e flavonóides totais e determinar o potencial de forma inédita a atividade fungicida da nanoemulsão óleo-em-água incorporada com óleo essencial e extrato hidroalcoólico da folha de *O. citriodurum* L.As folhas foram cuidadosamente selecionadas na zona rural de São Luís, Maranhão, Brasil, sendo posteriormente trituradas e armazenadas. O óleo essencial foi obtido por hidrodestilação (100 °C/3h). Para obtenção do extrato hidroalcoólico, utilizou-se a maceração em solvente extrator etanol P.A 70%(v/v). Para determinar a atividade antifúngica, fez-se o uso da técnica preconizada pelo Clinical Laboratory Standard Internacional através da técnica de Diluição em Caldo e Semeadura e Ágar para determinação da Concentração Inibitória Mínima (CIM) e Concentração Fungicida Mínima (CFM), respectivamente, frente a cepas de *Aspergillus níger* (ATCC 6275), *Colletotrichum gloeosporioides* (ATCC 96723), *Penicillium chrysogenum* (ATCC 10106). Os resultados obtidos para ação fungicida atestaram uma CIM uma inibição forte para ação dos óleos essenciais e extratos hidroalcóolicos com uma concentração de 250 µg mL^{-1}frente *A.níger*, *C. gloeosporioides* e *P.chrysogenum*. Foram observadas CFM's de 300, 250 e 500 mL^{-1}frente *A. niger*, *C. gloeosporioides* e *P. chrysogenum*, respectivamente. Com os resultados obtidos afirma-se que se obteve uma nanoemulsão estável tanto para (EX) e (OE) e que apresentam compostos ativos nas quais favorecem uma excelente atividade antifúngica.

Palavras-chave: Antimicrobiano; Fungicida; Inibição

1 INTRODUCTION

Pharmacology in relation to natural products is essential since man realized the need in relation to their anti-inflammatory and bactericidal properties, thus there was a constant diffusion of knowledge both culturally (mainly by indigenous culture based on empirical knowledge) and scientific. The study of plants with medicinal potential, especially those present in our nutritional habit (VARGAS *et al.*, 2019), can offer excellent properties and active herbal ingredients. Within this context, there are several scientific studies that significantly prove its herbal action and its antimicrobial activities (LARIBI *et al.*, 2015).

Based on this principle, a plant that stands out for these characteristics is *Ocimum citriodurum* L. (commercial basil), with an erect and branched stem and relatively small leaves with a peculiar smell, popularly known as *O. citriodurum* L small leaf basil. annual life cycle, predominant in regions that have a subtropical or temperate climate, and can thus be easily found in areas where there are hot and humid variations (SOUSA *et al.*, 2014).

The consumption of basil is linked to numerous health benefits. Despite being used as part of the nutritional habit, this food is constantly used in order to protect the body from bacterial attacks, an excellent stimulant to the nervous system, in addition to bringing great benefits to the skin, vision and stress (MODRO *et al.*, 2015). Commonly passed on through recipes based on popular knowledge the tea of this, as a great detoxifier, helping to combat any type of infection that may arise in the body. It is notorious that many of these practices are common in the regions: north and northeast of Brazil, as they present the abundant cultivation of basil (SHAKERI *et al.*, 2016).

In several studies, plants have several biologically active products, supporting that many constitute models for the synthesis of a significant number of drugs. Researchers marvel at the vast variety of these products, but 19 data show that only 15 to 17% of the plants were studied for their medicinal potential (MARTINS, 2010).

In several applications in the studies of essential oils are nanoemulsions, which are systems of arrangements of very favorable active compounds that have, in most cases, immiscible liquids often stabilized by surfactants (BRUXEL *et al.*, 2012). Essential oils are classified in the category of secondary metabolites acquired by different parts of the plant, in their composition they present volatile compounds that may be linked to biological properties. Among the constituents present in its composition, they are directly associated with classes such as terpenoids and phenylpropanoids.

Because they have defined characteristics such as: hydrophobic and lipophilic, resulting in the difficulty of being soluble in water (DONSI *et al.*, 2012). Because they predominantly have these characteristics, nanoemulsions present themselves as a viable solution in terms of applicability, precisely because they have the size of the small and adequate droplet is justified because it facilitates the biological activity of the compound, and in this way enlarging the contact surface favoring absorption (SOLÉ *et al.*, 2012; AHMAD. *et al.*, 2014; YUKUYAMA *et al.*, 2015).

It is taken into account that the essential oils of vegetables have a high rate of toxicity, thus it is advisable to apply them in small concentrations in which activities related to their volatility are developed. From this perspective, they are supported by their performance as fungicides and bactericides and insecticides (SODAEIZADEH et al., 2010).

Thus, this study aimed to analyze the phytochemical profile, quantify the total phenolics and flavonoids and determine the potential fungicidal activity of *Ocimum citriodurum* L leaf.

2 METHODOLOGY

2.1 Plant material

The collection of plant material used in this research was carried out from October to December 2020. *O.citriodorum* L leaves were collected in the municipality of São Luís, Brazil. The samples were deposited in the Attic Herbarium Seabra of the Federal University of Maranhão. Aftercollection, the plantspecies were transported to the Laboratory of Research and Application of Essential Oils (LOEPAV/UFMA). The material was sent to the Laboratory of Research and Application of Essential Oils at the Federal University of Maranhão (UFMA) for screening, determination of water content and drying in a digital oven. convective air FANEM 520. In the laboratory, the plant material was analyzed and undamaged leaves were selected for visual aspects. The leaves were cut manually, with caution in standardizing the cuts. The cuts were made radially in parts of at most 4 cm in length and 1 cm in width.

Figure 1 – Leaves in natura *O. citriodurum* L

Source: Authors (2021)

2.2 Drying

To carry out the drying, a digital convective air drying oven FANEM 520 was used, standard air speed at 1 m/s. Drying was carried out on alternate days using a temperature of 45 °C and the relative humidity monitored by a digital thermo-hygrometer (model INS-28 Intrusul).

A mass of 100g was used for drying and about 1 g of the samples were placed on aluminum-coated plates of dimensions 90 x 15 mm, the mass being monitored throughout the process by discontinuous weighing on an analytical scale Shimadzu AUY220 and the weighing scheme following intervals of 5, 10, 20, 30 and 60 min, until the end of the process. Weighings were carried out until mass variations were negligible. Drying was completed when there was no mass variation of 0.0100 g between five successive weighings. To determine the moisture ratio (RU) during the drying of garden leaves for different drying temperatures, Equation 1:

$$\mathbf{RU_{(adm)}} = \frac{\mathbf{Ubs} - \mathbf{U_e}}{\mathbf{Ubs_{inicial}} - \mathbf{Ue}} \quad (1)$$

Where: $RU_{(adm)}$ = moisture ratio, (dimensionless); $Ubs_{initial}$ = initial water content (bs); Ue=equilibrium water content (bs); Ubs = water content at time t (bs).

2.3 Mathematical modeling for predicting drying

The RU values obtained for each drying air temperature were analyzed by six different empirical and semi-empirical equations and non-linear regression, as shown in Table 1. For the adjustment of the mathematical models to the experimental data, a non-linear regression analysis was performed, by the QuasiNewton method, using the computer program Statistica 10.0.

Table 1 – Mathematical nonlinear regression models to predict drying

Template designation	**Model**	**Ref.**
Diffusion approximation	RU=a.exp(-kt)+(1−a)exp(−kbt)	(HACIHAFIZOGLU *et al.*, 2008)
broken	RU=c.exp(-kt)	(SOUSA *et al.*, 2011)
two terms	RU=a.exp(-kt)+b.exp(-kt)	(AKPINAR, 2006)
two-term exponential	RU=a.exp(−kt)+(1−a).exp(−kat)	(FARIA *et al.*, 2012)
Herderson and page	RU = a.exp(−k.t^n)+bt	(SOUSA *et al.*, 2011)
Midili	RU= a.exp(-k.t^n)+bt	(MIDILLI *et al.*, 2002)
Newton	RU=exp(-kt)	(MARTINAZZO *et al.*, 2017)
Page	RU=exp(-k.t^n)	(SALILIK, 2007)
Thompson	RX=exp((-a-(a2+4.bt)$^{0.5}$)/2.b)	(SOUSA *et al.*, 2011)
Wang	RU=1 +at+bt^2	(WANG *et al.*, 2007)

Source: Authors (2021)

The criteria used to determine the best fit of the models to the experimental data was the coefficient of determination (R2) and the root mean square deviation (MDQ) by Equation 7.

$$\mathrm{DQM} = \sqrt{\frac{\Sigma\left(\mathrm{RU_{exp}} - \mathrm{RU_{pre}}\right)^2}{\mathrm{N}}} \qquad (7)$$

2.4 Obtaining essential oil

For the extraction of EO, the hydrodistillation technique was used with a glass Clevenger extractor coupled to a round-bottomed balloon packed in an electric blanket as a heat generating source. 100 g of each plant material was used, adding added water (1:10).

Hydrodistillation was conducted at 100°C for 3 h and extracted EO was collected. Each EO was dried by percolation with anhydrous sodium sulfate (Na_2SO_4) and centrifuged. These operations were performed in triplicates and the samples stored in amber glass ampoules under 4°C refrigeration. Subsequently submitted to the analyses.

2.5 Preparation of hydroalcoholic extract

For the preparation ofhydroalcoholic extracts used 100 g of plant material in natura. The maceration process was used with an ethanol extract solvent PA 70% (v/v) following a 1:10 ratio. The solution obtained after 7 days was filtered and concentrated in a rotary evaporator under reduced pressure, after the process the extract was dried to remove the residual solvent for further analysis (HARBORNE, 1998).

2.6 Phytochemical screening

The hydroalcoholic extract obtained was submitted to chemical tests based on the methodology presented by Matos (2009). Tests performed to identify alkaloids, steroids, phenolics, flavonoids, glycosides, cardiac glycosides, saponins and tannins are described below:

2.6.1 Steroids (Salkowsk test)

About 100 mg of dry extract was dissolved in 2 mL of chloroform. Sulfuric acid was carefully added to form a lower layer. A reddish brown color at the interface indicated the presence of a ringsteroid.

2.6.2 Alkaloids (Mayer's test)

1.36 mg of mercury chloride were dissolved in 60 mL and 5 mg of potassium iodide dissolved in 10 mL of distilled water, respectively. These two solvents were mixed and diluted to 100 mL using distilled water. A few drops of the previously prepared reagent were added to 1 mL of the acidic aqueous solution of the samples. The formation of white or pale precipitation showed the presence of alkaloids.

2.6.3 Flavonoids

In a test tube containing 0.5 mL of alcoholic extract from the samples, 5 to 10 drops of diluted HCl were added and a small amount of Zn or Mg were added to the solution, which was then boiled for a few minutes. The appearance of a reddish pink or dark brown color indicated the presence of flavonoids.

2.6.4 glycosides

A small amount of alcoholic extract from samples was dissolved in 1 mL of water and then aqueous sodium hydroxide was added. The formation of a yellow color indicated the presence of carbohydrates.

2.6.5 Cardiac glycosides [Keller killiani test]

About 100 mg of extract was dissolved in 1 mL of glacial acetic acid containing one drop of ferric chloride solution and 1 mL of concentrated sulfuric acid was added. A brown ring obtained at the interface indicated the presence of an oxy sugar characteristic of cardenolides.

2.6.6 saponins

One drop of baking soda was added to a test tube containing about 50 mL of an aqueous extract of the sample. The mixture was shaken vigorously and held for 3 min. A honey-foam comb was formed and showed the presence of saponins.

2.6.7 Phenols [Deronic Chloride Test]

For 1 mL of alcoholic sample solution, 2 mL of distilled water was added followed by a few drops of 10% aqueous ferric chloride solution. The formation of a blue or green color indicated the presence of phenols.

2.6.8 Tannins [Lead Acetate Test]

In a test tube containing about 5 mL of an aqueous extract, a few drops of 1% lead acetate solution were added. The formation of a yellow or red precipitate indicated the presence of tannins.

2.7 Total Phenolics

The phenol content was determined for essential oils and hydroalcoholic extracts by the Folin-Ciocalteau spectrophotometric method (LUGASI *et al.*, 1998; OLIVEIRA *et al.*, 2009)

5 mg of the essential oil diluted in 1 mL of ethanol was used. To this solution was added 7 mL of distilled water, 800 µL of the Folin-Ciocalteu 10% reagent and 2.0 mL of 7.5% sodium carbonate. The solution formed was placed in a 50°C water bath for 5 min, removed and left to cool; and then reading was performed in a manual spectrophotometer, at a length of 760 nm. As a reference, an analytical curve with tannic acid was obtained, which provided the equation of the line for the conversion of the measured absorbance in equivalent milligrams of tannic acid per gram of extract (mg EAT.g^{-1}).

The hydroalcoholic extract was diluted in ethanol to obtain solutions with a concentration of 10 mg mL^{-1}. To an aliquot of 0.1 mL of each solution, 7.0 mL of water, 0.8 mL of Folin-Ciocalteau reagent (10% v/v) and 1.2 mL of 20% aqueous Na_2CO_3 solution were added. After 2 hours, the absorbances of the samples were measured at 760 nm. As a reference, an analytical curve with tannic acid was obtained, which provided the equation of the line for the conversion of the measured absorbance in equivalent milligrams of tannic acid per gram of extract (mg EAT.g^{-1}). The standard curve was expressed in terms of values. mg L^{-1} of tannic acid.

2.8 Total flavonoids

To estimate the content of Total flavonoids were used and complexation with $AlCl_3$ The total flavonoid content was estimated spectrophotometrically by reaction with $AlCl_3$, using quercetin as standard (DOWLD, 1959; WOISKYESALATINO, 1998; FREDERICE, *et al.*, 2010) The extracts and essential oils were diluted in ethanol to obtain solutions with a concentration of 10 mgmL^{-1}. To an aliquot of 0.2 mL of this solution were added 4.4 mL of EtOH and 0.4 mL of 2% aqueous $AlCl_3$ solution. After 30 minutes, the absorbances of the samples were measured at 425 nm. As a reference, an analytical curve was obtained with quercetin, which provided the

equation of the line for the conversion of the measured absorbance in equivalent milligrams of quercetin per gram of extract ($mgEATg^{-1}$).

2.9 Preparation of nanoemulsions

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 the obtained essential oil, non-ionic surfactant (Tween 20) and water and can be seen in Table 1.

The oil concentration (5% v/v) was fixed for the formulation. 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, gently added and mixed with the oil phase, 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 °C ± 2 °C.

Table 2 – Nanoemulsion formulations for *Ocimum citriodurum* L essential oil

Identification	**Essential oil (EO)**	**Hydroalcoholic Extract (EH)**	**Tween 20**	**H2O**
NOE1	5%	-	5%	90%
NOE2	5%	-	10%	85%
NOE3	5%	-	15%	80%
NEH1	-	5%	5%	90%
NEH2	-	5%	10%	85%
NEH3	-	5%	15%	80%

Source: Authors (2021)

To prove stability, the formulated nanoemulsion was subjected to different stress tests according to the methodology described by Shafiq *et al.*, (2007). They were evaluated for phase separation by centrifugation. The heating-cooling cycle was carried out keeping the formulated nanoemulsion at 40 and 4 °C, alternating each temperature for 48 h. The cycle was repeated three times. This was done to verify the stability of the nanoemulsion at varying temperatures. Freeze-thaw stress was performed by keeping the nanoemulsion alternatively at -21 and 25 °C for 48 h at each temperature. The cycle was repeated twice. The experiment was carried out in triplicate. The formulations approved in thermodynamic stress tests were taken to antifungal action studies.

2.10 Microbial inoculum standardization for sensitivity testing

Three strains of fungi were used: *Aspergillus niger* (ATCC 6275), *Colletotrichum gloeosporioides* (ATCC 96723), *Penicillium chrysogenum* (ATCC 10106). These were previously identified and confirmed by biochemical tests. Pure cultures maintained on TSA agar were subcultured to brain heart infusion broth (BHI) and incubated at 35 °C until reaching exponential growth phase (4-6 h). After this period, the cultures had their cell density adjusted in sterile 0.85% saline solution, in order to obtain a turbidity comparable to the standard McFarland 0.5 solution, which results in a microbial suspension containing approximately 1.5 x 10^8 CFU mL^{-1} in accordance with the Clinical and Laboratory Standards Institute (2020).

2.11 Minimum Inhibitory Concentration (MIC) and Minimum Concentration (CFM)

This trial evaluated the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of essential oils and hydroalcoholic

extracts. The MIC assay was performed using the broth dilution technique proposed by the Clinical and Laboratory Standards Institute (2020). First, EO solutions were prepared using 2% Tween 20, and serial dilutions were prepared in BHI broth for the fungal assay, resulting in concentrations from 10 to 1000 µg mL^{-1}. Nanoemulsions were diluted directly into the culture medium.

At each concentration, fungal suspensions containing 1.5 x108 CFU mL^{-1} of the strains were added. Tubes were incubated at 25°C for 24-48h for fungal strains. Sterility and growth controls were performed for the assay performed. After the incubation period, MIC of the EO was verified, being defined as the lowest concentration that visibly inhibited fungal growth (absence of visible turbidity). Trials performed in triplicate.

For the CFM assay of the BHI broth dilutions that visibly inhibited fungal growth. Aliquots were inoculated in Sabourad Dextrose Agar (ASD) with subsequent incubation at 35°C for 24h. CFM was determined as the lowest concentration that visually in the CIM assay showed growth inhibition and that in the cultures for the fungicide assays also did not show visible growth.

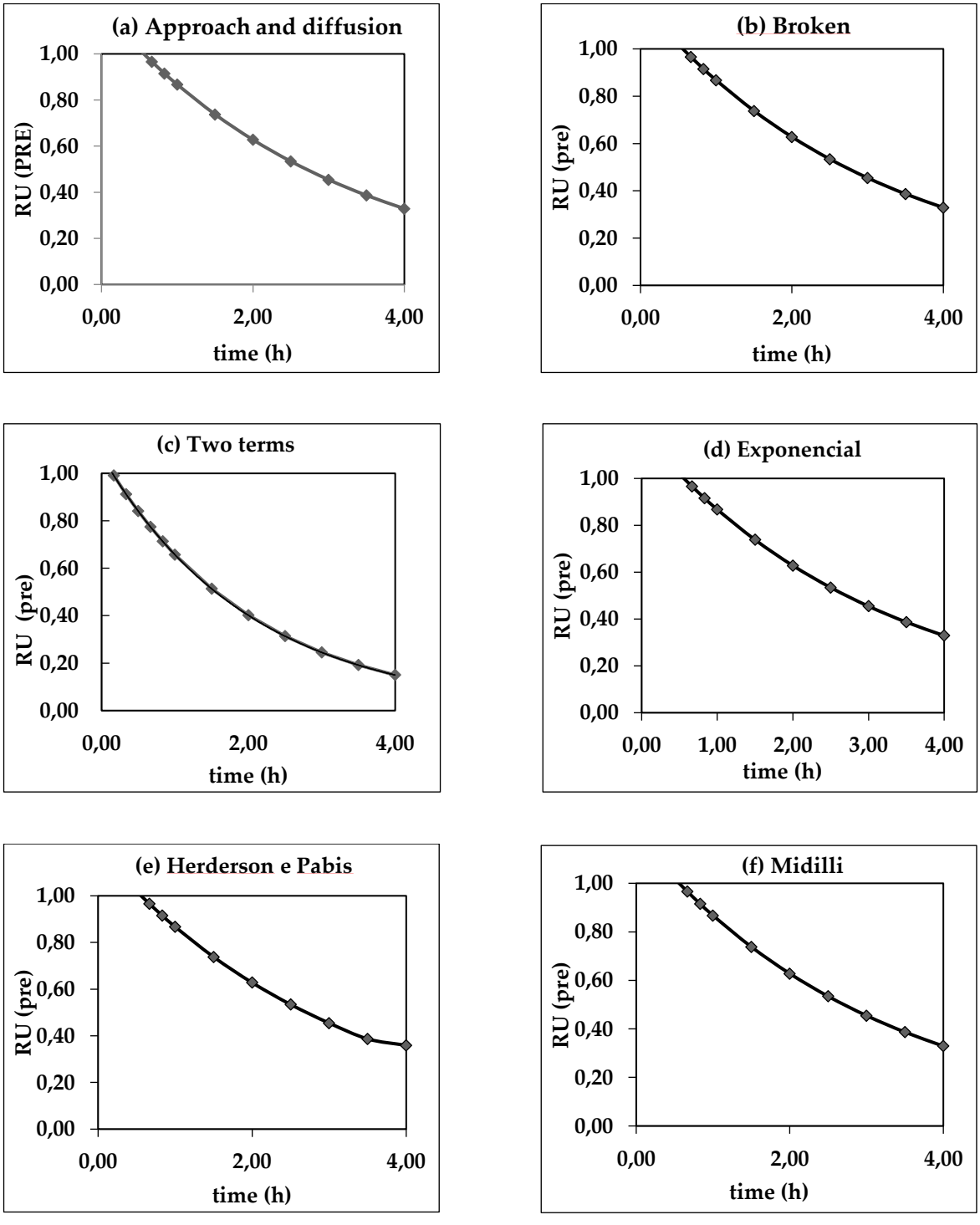
3 RESULTS AND DISCUSSION

3.1 Drying

In this process, several models were used as parameters to predict the drying kinetics, the behavior of the models to the adjustments made through the software is shown in Figure 2.

Figure 2 – Representation of the behavior of the drying prediction of Ocimum citriodurum L. leaf to the fit of the mathematical models (a) Approximation and infusion(b) Broken(c) Two Terms(d) Exponential(e) Herderson and Pabis(f) Midili(g) Newton(g) Page(h) Thompson(i) Wang

To be continued...



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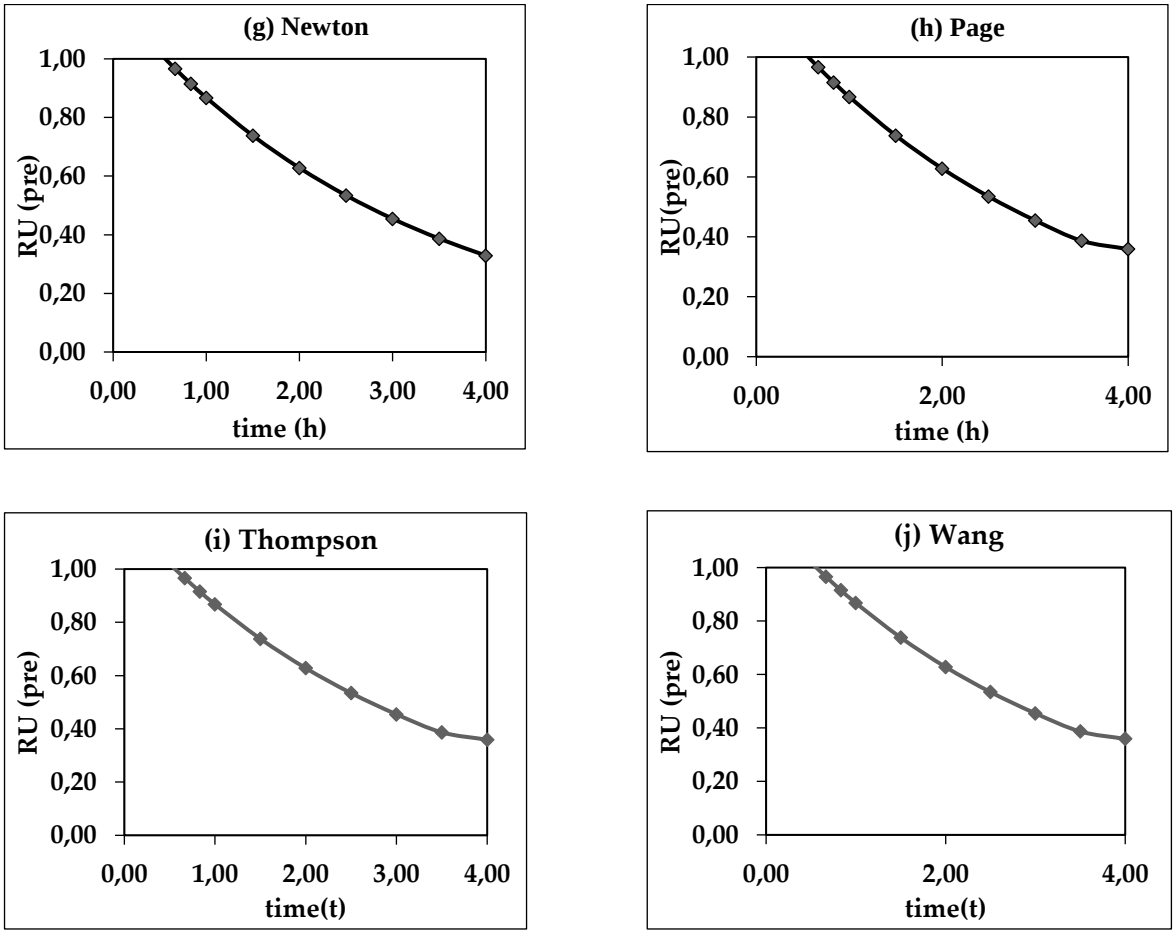
To be continued...



Source: Authors (2021)

The parameters in relation to the mean models with the corresponding coefficients of determination and mean squared deviation (MDQ) and the mean relative error (P) obtained are presented in Table 3.

According to Table 3, All analyzes performed using the drying kinetic models demonstrated an adequate fit (R2>0.90). The model that stood out in relation to the adjustment was the two-term model in favor of drying kinetics, as it presented

the highest coefficient of determination (R2≥0.98) and a lower mean square deviation value (<10%), thus, it is stated that in this case it is the most susceptible model to predict the behavior of the drying kinetics of *O.citriodurum* L leaf under the conditions studied.

Table 3 – Parameters of the identified models, correlation coefficient and relative mean square deviation (DQM) for convective drying of sheets of *Ocimum citriodorum* L.

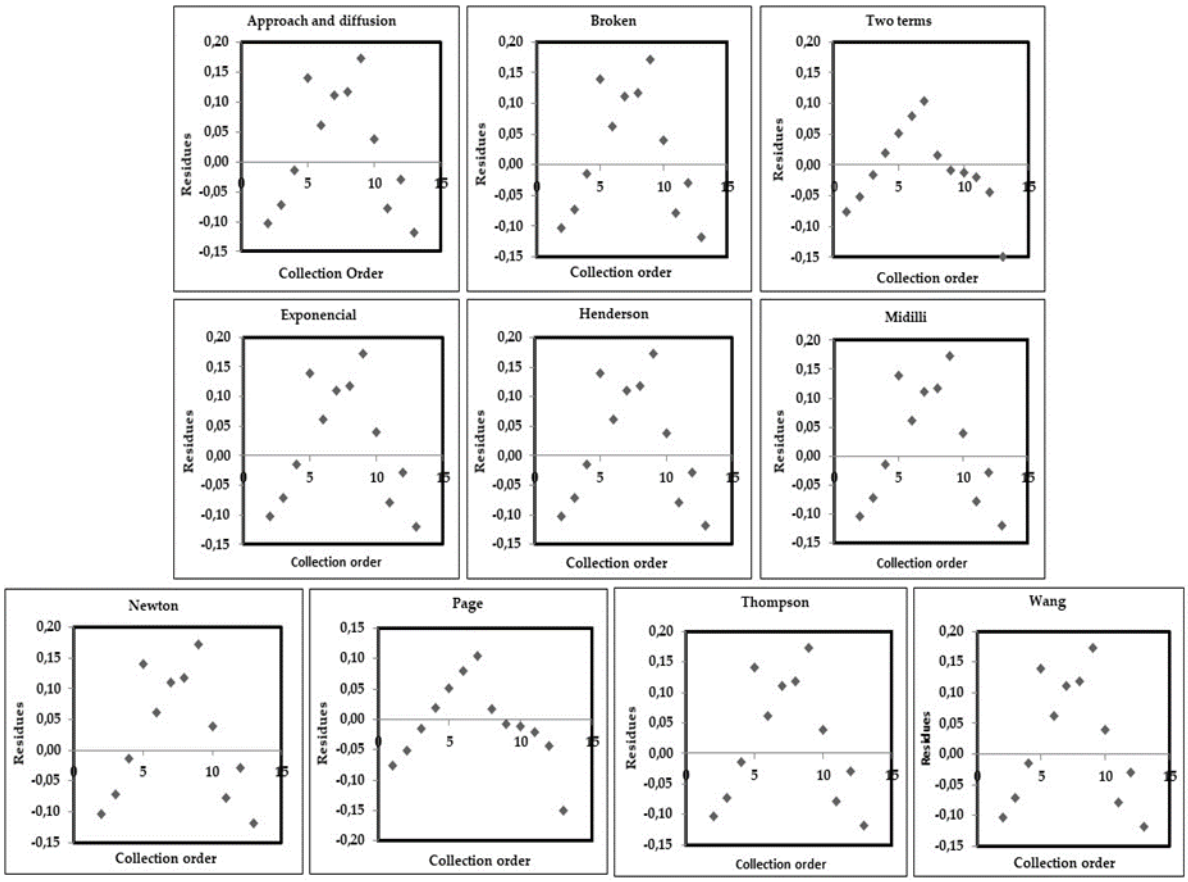
Model	R^2	DQM	P	a	k	b	c
Approximation and diffusion	0.9786	0.0769	0.0782	-10.6474	0.4765	9.7689	-
Broken	0.9543	0.0847	1.2487	-	0.5673	-	-1.2706
Two Terms	0.9879	0.0645	0.0432	-8.6574	0.4930	11.7226	-
Exponential	0.9786	0.0748	0.0781	0.7491	0.6752	-	-
Herderson and Parbis	0.9546	0.0759	1.2534	-3.4563	0.7689	-5.9364	-
Militti	0.9764	0.0776	0.0756	0.1818	0.4574	-	-
Newton	0.9784	0.0668	0.0862	-	0.8978	-	-
Page	0.9867	0.0687	0.0482	-	0.9675	-	-
Thompson	0.9589	0.0878	1.2587	1.8745	0.5478	3.4478	-
Wang	0.9674	0.0723	1.1274	1.4376	0.8791	4.7645	-

Source: Authors (2021)

It was noted that through Figure 3 the behavior through the waste distribution data through the drying models. It was attested that the two models that stood out were Two Terms and Page. In this way, with a greater random distribution of the residues at a temperature of 45º C, in relation to the other models studied, the residues became more biased. It is used as a parameter to designate that a model is random if the data are close to the horizontal axis around zero and if they do not form defined figures, not establishing any trend around the

results. If there is a biased distribution, the model is identified as inadequate to represent a reliable drying process (GONELI, 2011).

Figure 3 – Graphic representation of the residues described for the models: (a) Approximation and diffusion; (b) Broken; (c) Two Terms; (d) Exponential; (e) Herderson and Pabis; (f) Midilli; (g) Newton; (h) Page; (i) Thompson; (j) Wang



Source: Authors (2021)

3.2. Phytochemical profile, quantification of total phenolics and flavonoids

Table 4 presents the metabolites secondaries identified in the leaf of *O. citriodurum* L.

As shown in Table 4, the presence of alkaloids was observed, with the positive result determining the presence of a nitrogenous product in the composition of the *O.citriodurum* L leaf. Alkaloids are cyclic organic compounds that have at least a

single nitrogen atom in their ring, being synthesized by secondary metabolism. They are widely used as poisons and hallucinogens because they have a relevant effect on the nervous system (VIZZOTTO *et al.*, 2010). These compounds are structurally diverse and have a wide use in pharmacopoeia, highlighting their anesthetic, antitumor and antimicrobiological activities (SANTOS *et al.*, 2018).

Table 4 – Secondary metabolites identified in the leaf of *O.citriodurum* L.

Classes	1	2	3	4	5	6	7	8	9
Ocimum citriodurumL	+	-	+	+	+	-	+	-	-

Note:1:Alkaloids;2:Steroids;3:Phenolics;4:Phenols;5:Flavonoids;6:Glycosides; 7: Cardiac glycosides; 8: Saponins; 9: Tannins; (+) presence; (-) absence
Source: Authors (2021)

It was also noted the presence of cardiotonic glycosides, it was found that the reactions were positive, also determining through the work of Rosa Dornelis (2015) being positive for the leaf variety of *O. citriodurum* L, determining that this secondary metabolite is common for that variety.

According to Table 5, the quantification of total phenolics and flavonoids in the essential oil of *O.citriodurum* L is defined. For total phenolics, their respective relative values were determined using the equation y=0.0598x+0.0600 (R^2=0.9994), where 0.0600(independent factor) applies to the absorbance value and 0.0598x (dependent factor) applies to the tannic acid value. The total flavonoids were determined using the quercetin standard (mgEATg^{-1}), which was determined using the equation y=0.033x+0.0600(R^2=0.9845), taking into account that (0.060) applies to the value of the absorbance and (0.033x) the concentration of quercetin. The values of total phenolics and flavonoids obtained were satisfactory, this is due to the fact that the major constituents present in the *O.citriodrum* L as linalool is an important antioxidant(PINHEIRO *et al.*, 2017).

In this context, work carried out in relation to plant *O.citriodrum* L. the quantity of total phenolics determined were respectively (OE) and (EH) 145.56 and

91.00 mg EAT g^{-1}, resulting in a positive quantitative, supporting the following results and scientific studies such as that of Pitaro *et al.* (2012), which presented a quantity of 52.71 mg EAT g^{-1}. It can be said that through this test it is possible to predict a good antioxidant activity, already in reference to the study by Bobiano (2020) the determined quantity of 142.07 mg EAT g^{-1} for and hydroalcoholic extract of *O citriodurum* L., through the amounts discussed, it was noted that the value (EH) in this specific variety *O.citriodurum* L has a substantial amount compared to its essential oil.

Table 5 – Quantification of total phenolics and flavonoids in essential oil and hydroalcoholic extract of *Ocimumcitriodorum* L.

Phenolics	**Essential oil**	**Hydroalcoholic Extract**
Total (mg EAT g^{-1})	145.56	91.00
Linear Equation (y = ax+ b)	y=0.05857x+0.06	y=0.05857x+0.06
Correlation Coefficient (R^2)	0.9994	0.9994
+	Essential oil	Hydroalcoholic Extract
Total (mg EQT g^{-1})	69.69	69.69
Linear Equation(y = ax+ b)	y=0.033x+0.06	y=0.033x+0.06
Correlation Coefficient (R^2)	0.9845	0.9845

Source: Authors (2021)

Total flavonoids, and their presence, determine the antioxidant ability dependent on the potential for chelation with metals, which is intensely dependent on the arrangement of hydroxyl and carboxyl groups around the molecule, on the presence of hydrogen or electron donors substitutes capable of reducing free radicals and finally, the ability of the flavonoid to displace the unpaired electron leading to the synthesis of a stable phenoxy radical (GÜLÇIN, 2012). The potential for use and study of *O.citriodurum* L. is quite vast. Given its various chemical characteristics, *O.citriodurum* L. has great ability to add to the preparation of products with functional characteristics determining the antioxidant character.

MAJDI *et al.* (2020), determined values of total phenolics for ethanolic extract for the same species of the present work (*O.citriodurum* L) of (1.41 ± 0.031) $mg.g^{-1}$, this work emphasizes that for this species in particular, presenting a significant content of total phenolics for its hydroalcoholic extract.

The highlight in the quantification of phenolics and Total flavonoids as antioxidant activity predictors is noted, since the search for natural antioxidants has become significant in the last 20 years, for applications in food and pharmacological sectors, presenting as a priority objective in the area of industrial research in relation to antioxidants, in order to replace with synthetic antioxidants. Taking into account that they present a degree of rejection according to the toxicities they may present, thus it is necessary to search for these products through natural sources (SILVA *et al.*, 2010).

3.3. Thermodynamic stability

Table 6 presents the results obtained for thermodynamic stability.

Table 6 – Study of thermodynamic stability of nanoemulsion formulations with *O.citriodurum* L essential oil

Identification	SF	AQ	CG	DCG	Stability Final
NOE1	-	-	-	+	-
NOE 2	-	-	-	-	+
NOE 3	-	+	-	-	-
NEH 1	-	-	-	+	-
NEH 2	-	-	-	-	+
NEH 3	-	+	-	-	-

Note: SF - phase separation or creaming at room temperature; AQ- phase separation after heating; CG - phase separation or creaming after freezing; DCG - phase separation or creaming after thawing; + positive; - negative
Source: Authors (2021)

According to Table 6, as a result of the analysis, it was determined that the best surfactant was exactly Tween 20, presenting a greater sensitivity when it comes to fungicidal activity. Through the work of Pontes and Silvania (2013), the selection parameter for the best concentration of essential oil and surfactant for the synthesis of nanoemulsions was the elimination of concentrations that presented phase separations (instability) of the nanoemulsions, aiming to achieve more stable formulations. In the case of the formulations used, there were no phase separations, however when they reached higher and lower temperatures, only the formulation NOE2 and NEH2 were stable for applicability terms. Precisely because these nanoemulsions are described as stable systems, this characteristic is justified by its translucent appearance, the reduced size of its droplets, which ensure bioavailability and intensification for certain positive activities in the health area, such as antioxidant and antimicrobial activity (GADIOLI, 2017).

3.4. Antifungal activity

According to Table7, the results are described for the determination of the antifungal activity of the essential oil and nanoemulsion.

Table 7 – Antifungal activity of essential oil, hydroalcoholic extract and *O. citriodorum* L

	Aspergillus niger		***Colletotrichum gloeosporioids***		***Penicillium chrysogenum***	
	MIC mg/L	**CFM mg/L**	**MIC mg/L**	**CFM mg/L**	**MIC mg/L**	**CFM mg/L**
OE	250	300	250	400	250	300
EH	250	500	250	500	250	500
NOE2	120	250	110	300	130	250
NEH2	120	250	110	300	130	250

Source: Authors (2021)

According to Table 7, in favor of the activity carried out with the leaf of the *O.citriodurum* L plant, it is stated that an excellent result was attested to the strains used, highlighting a more effective inhibitory action against *A. niger*, finding a very reasonable MIC and low of 250 mgL^{-1} for essential oil and hydroalcoholic extract, in relation to nanoemulsions (NOE2) and (NEH2) presented a MIC of 110 mgL^{-1}, showing a more effective action. According to the data mentioned, it was possible to identify the fungal strains with greater inhibition following the selection pattern of Aligiannis *et al.* (2001), which employs the following criteria for the minimum inhibitory concentration (MIC) for antimicrobial activities, with strong inhibition for MIC up to a quantitative 500 µg mL^{-1}, moderate inhibition:

Regarding the Minimum Inhibitory Concentration (MFC) test, Table 7 shows that the most satisfactory results for the essential oil nanoemulsion and the hydroalcoholic extract against *A. niger* and *P. chrysogenum* strains, with 250 mgL^{-1} CFM compared to *C. gloeosporioides*. Through the data cited on CFM, we can state that the results involving nanoemulsions potentiated the action against the microorganisms studied through the method of Aligiannis *et al.* (2001), this is justified due to the fact that the particles involved are at a nanometric scale, which exactly characterizes the nanoemulsion, thus facilitating the action of the essential oil and hydroalcoholic extract in relation to the morphology of pathogenic fungi (ATHANASSIOU *et al.*, 2018).

In front of the work of Cardoso, *et al.* (2017), the essential oil of basil proved to be effective in favor of antifungal activity, as it inhibited the formation of ergosterol, pigmentation, attenuating the capsule size of Cryptococcus neoformans by 16.94 %, knowing that the capsule is the factor that highlights the characteristic of virulence and this ends up changing the susceptibility of drugs to combating these microorganisms.

In view of the study by Walker (2017) and Mohr (2017), how basil is part of the family of Lamiaceae, and these plants have an excellent antifungal activity compared to conventional antifungal agents, among them the following stand

out: ketoconazole, fluconazole and amphoterecin. Basil essential oil in relation to *A. fumigates*, this fungus commonly known in crops for causing a series of problems to fruits and vegetables, in addition to causing respiratory disease in humans called arpergillosis (AL-MASKRI *et al.*, 2011).

It is worth noting that the mechanism that establishes the action capacity of the essential oil in relation to the strains studied is directly linked to one of the secondary metabolites very common in relation to (OE) which are terpenes, such as limonene, found in the composition of basil leaf of the small leaf, ensuring the ability to have toxic effects in relation to the cell membrane structure (MAIA *et al.*, 2015). It can be taken into account that the same essential oil can act against a substantial amount of strains distinct, however the inhibitory concentration becomes variable.

According to the work of (MEDONÇA; FRANCIELLE, 2018) for the variety *O. basilicum* L, very close to the species studied by this assay, a quantity of 62.5 mgL^{-1} was determined, verifying that the varieties of basil have stable and nanoemulsions efficient when dealing with applications of antimicrobial activities.

In view of the studies obtained by Cavalcanti *et al.* (2012) the authors determined positive antifungal activity for basil extract against *Candida albicans strains* (ATCC289065), *C. tropicalis* (ATCC40042) and *C.krusei* (ATCC40147), thus the basil extract shows considerable sensitivity to these microorganisms.

4 CONCLUSIONS

Through the work carried out, it can be seen that the secondary metabolites present both in the essential oil and in the hydroalcoholic extract of the *O. citriodurum* L proved to be relevant, presenting favorable characteristics that can cause significant biological changes, sharpening this product for future biological activities. In relation to its antioxidant properties, its amounts of total

phenolics and flavonoids for essential oil and the hydroalcoholic extract of the *O.citriodurum* L proved promising and thus characterizing it as a potential antioxidant product. The fungicidal activity in relation (OE) and (EH) of *O.citriodurum* L applied with the nanoemulsion, inhibition was verified at very low concentrations, identifying that the product under study is suitable for application of antifungal activities, formulated through nanoemulsion and synthesized through (OE) and (EX) of *O. citriodurum* L.

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Authorship contributions

1 – Cassiano Vasques Frota Guterres (Corresponding author)
Laboratory for Research and Application of Essential Oils; Bachelor's Degree in Chemistry
https://orcid.org/0000-0003-2725-9429 • cassianovasques447@gmail.com
Contributions: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology.

2 – Thayane Lopes De Sousa
Laboratory for Research and Application of Essential Oils; Bachelor's Degree in Chemistry
http://orcid.org/0000-0001-5383-9350 • Thayane.lopes@discente.ufma.br
Contributions: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology.

3 – João Pedro Mesquita Oliveira
Chemical Researcher of Natural Products; Bachelor's Degree in Chemistry
http://orcid.org/0000-0003-1833-9814 • Jpm.oliveira@discente.ufma.br
Contributions: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology.

4 – Larissa Gabrielle Pinheiro Ferreira
Laboratory for Research and Application of Essential Oils; Bachelor's Degree in Biological Sciences
https://orcid.org/0000-0001-5788-3359 • igabriellepinheiro@gmail.com
Contributions: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology.

5 – Éverton Holanda Sales
Laboratory for Research and Application of Essential Oils; Degree in Chemical Engineering
https://orcid.org/0000-0002-9497-9103 • Eh.sales@discente.ufma.br
Contributions: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology.

6 – Victor Elias Mouchrek Filho
Full Professor at the Department of Chemical Technology at UFMA; PhD in Chemistry
http://orcid.org/0000-0003-2855-7292 • victor.mouchrek@ufma.br
Contributions: Funding acquisition, Project administration.

7 – Gustavo Oliveira Everton
Chemical Engineer; PhD in Chemistry
http://orcid.org/0000-0002-0457-914X • gustavooliveiraeverton@gmail.com
Contributions: Conceptualization, Data curation, Formal Analysis,Funding acquisition, Investigation, Methodology, Project administration

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