







Chemistry

Chemical profile, thermodynamic stability and fungicidal activity of the nanoemulsion incorporated with essential oil and hydroalcoholic extract of *Syzygium aromaticum* (L.) Merr. & LM.Perry

Perfil químico, estabilidade termodinâmica e atividade fungicida da nanoemulsão incorporada com óleo essencial e extrato hidroalcoólico de *Syzygium aromaticum* (L.) Merr. & LM.Perry

Thaylanna Pinto de Lima¹, Thayane Lopes de Sousa¹,
Joao Pedro Mesquita Oliveira¹, Maria Giullia Alves Carneiro Felizardo¹,
Gustavo Oliveira Everton¹, Victor Elias Mouchrek Filho¹

¹ Universidade Federal do Maranhão, Laboratório de Pesquisa e Aplicação de Óleos Essenciais Universidade, São Luiz, Maranhão, Brazil

ABSTRACT

This work aimed to evaluate the fungicidal activity of oil-in-water (O/W) nanoemulsions of hydroalcoholic extract and essential oil of sprouts of *Syzygium aromaticum*. For the extraction of essential oil, the hydrodistillation technique was used. To obtain the hydroalcoholic extract, the process of maceration with solvent extract ethanol methanol P.A 70% (v / v) was performed. Nanoemulsions were obtained using the low-energy phase inversion method with thermodynamic stability assessed by stress tests. For the fungicidal activity of oil-in-water (O/W) nanoemulsions, the Broth Dilution and Agar Seeding assay was used to determine the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (CFM). The action of the biotechnological product was tested against three strains of fungi *Aspergillus niger*, *Colletotrichum gloeosporioides*, *Penicillium chrysogenum*. For antifungal action of the essential oil nanoemulsion, the MIC obtained was 25 µg mL⁻¹, 25 µg mL⁻¹ and 50 µg mL⁻¹ against *A. niger*, *C. gloeosporioides* and *P. chrysogenum*, respectively, for the hydroalcoholic extract nanoemulsion the result was 50 µg mL⁻¹, 50 µg mL⁻¹ and 100 µg mL⁻¹ for *A. niger*, *C. gloeosporioides* and *P. chrysogenum*, respectively. On the other hand, the CIM of the nanoemulsion of the essential oil showed activity against *A. niger* of 25 µg mL⁻¹ and against *C. gloeosporioides* it showed inhibition in 25 µg mL⁻¹ and in the nanoemulsion of the hydroalcoholic extract it had its best performance in front of *A. niger* of 50 µg mL⁻¹ and in front of *C. gloeosporioides*, which showed inhibition in 50 µg mL⁻¹. Finally, this study points to the nanoemulsion of *S. aromaticum* as an alternative fungicide in the control and combat of pathogenic fungi.

Keywords: Fungicide; *Syzygium aromaticum*; Phytochemistry

RESUMO

Este trabalho teve como objetivo avaliar a atividade fungicida das nanoemulsões óleo-em-água (O/A) do extrato hidroalcoólico e do óleo essencial de brotos de *Syzygium aromaticum*. Para extração do óleo essencial utilizou-se a técnica de hidrodestilação. Para obtenção do extrato hidroalcoólico executou-se o processo de maceração com solvente extrato metanol P.A 70% (v/v). As nanoemulsões foram obtidas pelo método de baixa energia de inversão de fases com a estabilidade termodinâmica avaliada por testes de estresse. Para atividade fungicida das nanoemulsões óleo-em-água (O/A) utilizou-se o ensaio de Diluição em Caldo e Semeadura em Ágar para determinação da Concentração Inibitória Mínima (CIM) e Concentração Fungicida Mínima (CFM). A ação do produto biotecnológico foi testada frente a três cepas de fungos *Aspergillus niger*, *Colletotrichum gloeosporioides*, *Penicillium chrysogenum*. Para ação antifúngica da nanoemulsão do óleo essencial a CIM obtida foi de 25 µg mL⁻¹, 25 µg mL⁻¹ e 50 µg mL⁻¹ frente a *A. niger*, *C. gloeosporioides* e *P. chrysogenum*, respectivamente, já para a nanoemulsão do extrato hidroalcoólico o resultado obtido foi de 50 µg mL⁻¹, 50 µg mL⁻¹ e 100 µg mL⁻¹ para *A. niger*, *C. gloeosporioides* e *P. chrysogenum*, respectivamente. Por outro lado, a CIM da nanoemulsão do óleo essencial apresentou atividade frente a *A. niger* de 25 µg mL⁻¹ e frente ao *C. gloeosporioides* apresentou inibição em 25 µg mL⁻¹ e na nanoemulsão do extrato hidroalcoólico teve a seu melhor desempenho em frente a *A. niger* de 50 µg mL⁻¹ e em frente ao *C. gloeosporioides* que apresentou inibição em 50 µg mL⁻¹. Por fim, este estudo aponta a nanoemulsão de *S. aromaticum* como fungicida alternativo no controle e combate de fungos patogênicos.

Palavras-chave: Fungicida; *Syzygium aromaticum*; fitoquímica

1 INTRODUCTION

The use of medicinal plants for the treatment of illnesses has been known since prehistory. In Brazil, the introduction of these plants in healing rituals comes from the indigenous culture, and this empirical knowledge remains today. Approximately 66% of the Brazilian population without access to modern medicine makes use of popular medicines, often representing the only therapeutic resource for many communities and ethnic groups (MAZZARI & PRIETO, 2014). Faced with this scenario, the population has been looking for healthier and lower-cost treatments to treat their illnesses. Although modern medicine is well developed in most parts of the world, WHO recognizes that a large portion of the population in developing countries depends on traditional medicine for their primary care (BRASIL, 2006).

The use of plants for medicinal purposes has aroused great interest in the knowledge of the chemical composition of plants (SIMÕES, 2001). In several studies, plants have several biologically active products, supported by the fact that many

are models for the synthesis of a significant number of drugs. Researchers marvel at the wide variety of these products, however 19 datas show that only 15 to 17% of plants have been studied for their medicinal potential (MARTINS, 2010).

Among the plants featured, is the species *Syzygium aromaticum* (L.) Merr. & L.M.Perry, popularly known as India cloves, has its origins in the Philippines and East Africa (PAHLOW, 2004). The name carnation in Portuguese derives from the Latin word *clavus*, which means "prego", due to its physical appearance. In English the name clove was derived from the French old *clou*, which is related to the verb *unir* -function performed by the nail (ORNELAS, 2000). The clove (*Syzygium aromaticum*) is a tree, of the Myrtaceae family adapted to the African and Brazilian climate (CARDOSO *et al.*, 2007). Its leaves have oval, aromatic characteristics and are 7-11 centimeters long (LORENZI *et al.*, 2002). Other authors describe it as originating in India, being also found in Indonesia, Zanzibar and Ceylon, with an elongated characteristic.

The species is mainly exploited for industrial extraction of essential oil (EO) obtained from flower buds, leaves and other parts. The popular use of the species refers to flower bud tea as a carminative and stimulant of digestive functions (LORENZI & MATOS, 2002).

Essential oils are liquid, volatile, clear, rarely colored, fat-soluble or soluble in organic solvents. They can be synthesized by all the organs of the plant and are the result of their secondary metabolism, being characterized by a strong and marked odor. The essential oil of *S. aromaticum* is used by Ayurvedic medicine, for respiratory treatments and eating disorders. The antiseptic and antibiotic properties are also explored in the preparation of homemade toothpaste and mouthwashes (BANERJEE *et al.*, 2006).

Phytochemical studies of cloves reveal the abundant presence of essential oil, in which eugenol is the major component, accompanied by trans-karyophyllene, eugenyl acetate, humulene and vanillin (PAOLI *et al.*, 2007; PEREIRA *et al.*, 2008) ,

where studies prove that eugenol has excellent antifungal activity (PARK *et al.*, 2007).

Because they have hydrophobic properties, their application is facilitated through the formulation of nanoemulsions. Recent research reports that the formation of nanoemulsion containing EOs are used as strategies to improve its functionality. Nanoemulsions consist of a very fine dispersion, composed of an oil phase (such as triglycerides or hydrocarbons) and an aqueous phase (water or water) that are presented as drops with a diameter less than 100 nm. They are constituted by a polymeric casing arranged around a nucleus, the active component of interest being able to be dissolved in that nucleus and / or adsorbed to the polymeric wall. The retention of these nuclei is governed by their chemical functionality, solubility, polarity and volatility (GHARSALLAOUI *et al.*, 2007) These systems are believed to have several advantages over conventional emulsions because they are colloidal distribution systems due to their lower particle size. Nanoemulsions appear to have optical transparency, enhanced functionality and physical stability, which would make them very attractive to biotechnology etc. (ASSIS *et al.*, 2012).

Thus, this study aimed to evaluate the phytochemical profile, quantification of total phenolics and flavonoids and evaluation of the fungicidal activity of the essential oil, hydroalcoholic extract and the nanoemulsion formulated with the essential oil and hydroalcoholic extract obtained from the flower buds of *Syzygium aromaticum* (clove).

2 METHODOLOGY

2.1 Plant material

The collection of plant material used in this research was carried out from October to December 2020. *S. aromaticum* were obtained in the municipality of São

Luís, Brazil. After collection, the plant species were transported to the Laboratory for Research and Application of Essential Oils (LOEPAV / UFMA). The material was sent to for sorting, determination of the water content and drying in a digital convection air oven FANEM 520.

2.2 Obtaining essential oil

For the extraction of EO, the hydrodistillation technique was used with Clevenger glass extractor coupled to a round bottom flask wrapped in an electric blanket as a heat generating source. 100g of each plant material were used, plus distilled water (1:10). Hydrodistillation was carried out at 100°C for 3 h and the extracted EO was collected. Each EO was percolated with anhydrous sodium sulfate (Na₂SO₄) and centrifuged. These operations were performed in triplicates and the samples stored in amber glass ampoules under refrigeration at 4°C. Subsequently, they were analyzed.

2.3 Preparation of hydroalcoholic extract

For the preparation of hydroalcoholic extracts, 100g of plant material was used *in natura*. The maceration process with solvent extract methanol PA 70% (v / v) was used following the proportion 1:10. The solution obtained after 7 days was filtered and concentrated on a rotary evaporator under reduced pressure, after the process the extract was dried to remove the residual solvent for further analysis (HARBORNE, 1998). The hydroalcoholic extract was subjected to chemical tests based on the methodology presented by Matos (2009).

2.4 Total phenolics

The phenol content was determined for essential oils and hydroalcoholic extracts by the Folin-Ciocalteu 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 μ L of 10% Folin-Ciocalteu reagent and 2.0 mL of 7.5% sodium carbonate. The formed solution was taken to the water bath at 50°C for 5 min, removed and left to cool; and then the reading was performed on a manual spectrophotometer, at a length of 760 nm. As a reference, an analytical curve with tannic acid was obtained, which provided the line equation for converting the absorbance measured in milligrams equivalent 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 the Folin-Ciocalteu reagent (10% v / v) and 1.2 ml of 20% aqueous Na₂CO₃ 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 line equation for converting the absorbance measured in milligrams equivalent of tannic acid per gram of extract (mg EAT.g⁻¹). The standard curve was expressed in mg L⁻¹ of tannic acid.

2.5 Total flavonoids

To estimate the total flavonoid content, AlCl₃ complexation was used. The content of total flavonoids was estimated spectrophotometrically by reaction with AlCl₃, using quercetin as a standard (DOWLD, 1959; WOISKY & SALATINO, 1998; FREDERICE *et al.*, 2010). The extracts and essential oils were diluted in ethanol to obtain solutions with a concentration of 10 mg mL⁻¹. To a 0.2 ml aliquot of this solution, 4.4 ml of EtOH and 0.4 ml of 2% aqueous AlCl₃ solution were added. 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 line equation for the conversion of absorbance measured in milligrams equivalent of quercetin per gram of extract (mgEQ.g⁻¹).

2.6 Preparation of nanoemulsions

The preparation of the 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) observed in Table 1.

Table 1 – Nanoemulsion formulations for *Syzygium aromaticum* essential oil

Identification	Essential oil (EO)	Hydroalcoholic extract (EH)	Tween 20	H2O
NEO1	5%	-	5%	90%
NEO 2	5%	-	10%	85%
NEO 3	5%	-	15%	80%
NEH 1	-	5%	5%	90%
NEH 2	-	5%	10%	85%
NEH 3	-	5%	15%	80%

Source: Authors (2021)

The oil concentration E. (5% v / v) and H. Extract (5% v / v) were fixed for the formulation. The required amounts of each constituent of the oil phase (oil + Tween20) and (Extract H. + Tween20) were heated to $65 \pm 5^\circ\text{C}$. The aqueous phase was heated separately to $65 \pm 5^\circ\text{C}$, added gently 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 in constant agitation at 6000 rpm, until the temperature was reduced to $25^\circ\text{C} \pm 2^\circ\text{C}$.

To prove stability, of the formulated nanoemulsions were 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 nanoemulsions at 40 and 4°C ,

alternating each temperature for 48 h. The cycle was repeated three times. This was done to check the stability of the nanoemulsion at variable temperatures. The freeze-thaw stress was carried out by maintaining the nanoemulsions 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 the thermodynamic stress tests were taken for studies of antifungal action.

2.7 Standardization of the microbial inoculum for sensitivity tests

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 seeded into brain and heart infusion broth (BHI) and incubated at 35 ° C until they reached exponential growth phase (4-6 h). After this period, the cultures had their cell density adjusted in sterile 0.85% saline, in order to obtain a turbidity comparable to that of the McFarland 0.5 standard solution, which results in a microbial suspension containing approximately 1.5×10^8 UFC mL⁻¹ according to the standards of the Clinical and Laboratory Standards Institute.

2.8 Minimum Inhibitory Concentration (CIM) and Minimum Concentration (CFM)

This test evaluated the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (CFM) of essential oils and hydroalcoholic extracts. The MIC test was performed using the broth dilution technique, proposed by the Clinical and Laboratory Standards Institute. First, EO solutions were prepared using 2% Tween 20, and serial dilutions were prepared in BHI broth for the fungal assay, resulting in concentrations of 10 to 1000µg mL⁻¹. The nanoemulsions were diluted directly in the culture medium.

To each concentration, fungal suspensions containing 1.5×10^8 CFU mL⁻¹ of the strains were added. The tubes were incubated at 25 ° C for 24-48h for the fungal

strains. Sterility and growth controls were performed for the test performed. After the incubation period, EO MIC was verified, being defined as the lowest concentration that visibly inhibited fungal growth (absence of visible turbidity). Tests performed in triplicate.

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

3 RESULTS AND DISCUSSION

3.1 Phytochemical screening

The phytochemical analysis of *S. aromaticum* obtained was performed according to the methodology of Matos *et al.* (2009). Where, according to the methodology, the following classes of secondary metabolites were detected in Table 2.

Table 2 – Classes of secondary metabolites identified in *Syzygium aromaticum*

Classes	1	2	3	4	5	6	7
<i>S. aromaticum</i>	+	+	+	+	-	-	+

Source: Authors (2021)

Note: 1: Alkaloids; 2: Cardiac Glycosides; 3: Steroids; 4: Flavonoids; 5: Glycosides; 6: Saponins; 7: phenols; (+) positive; (-negative).

Among the secondary metabolites found in this study, as shown in Table 2, the presence of alkaloids, cardiac glycosides, steroids, flavonoids, phenols stands out. Corroborating with previous studies by the author Teles *et al.* (2019). Although the pharmacological activity observed for a given extract is usually the result of the synergistic and conjugated effect, some metabolites present in plant extracts can

account for the majority of biological activity (BESSA *et al.*, 2013). Therefore, it is important to highlight its main biological and pharmacological properties for the classes of metabolites found in this study (COSTA *et al.*, 2018).

The *S. aromaticum* buds are characterized by the presence of about 20% volatile oil rich in eugenol with a concentration ranging from 85 to 95%. This essential oil is used in ethnomedicine and continues to be the object of studies due to its wide diversity of pharmacological and biological activities, already described, such as: analgesic, anesthetic, anti-inflammatory and antibacterial (HEMAISWARYA & DOBLE, 2009).

The alkaloids identified in phytochemical characterization have diverse and important physiological effects on humans and other animals, are found mainly in plants and are especially common in certain families of flowering plants. They are used as poisons and hallucinogens, known for their action on the central nervous system. The presence of alkaloids can be related to a wide range of investigated biological activities. Many indole alkaloids act on opiate neurotransmitter, GABAergic, muscarinic, serotonergic and dopaminergic cholinergic systems, so they are used as hypotensive, sympatholytic, diuretic, peripheral vasoconstrictors, respiratory stimulants, anesthetics, adrenergic blocking agents, intestinal spasmogens, sedatives, intestinal agents

Cardiac glycosides, on the other hand, are drugs used in protocols for the treatment of CHF (congestive heart failure). Symptomatic in class III patients (asymptomatic at rest and symptomatic in less-than-usual activity) and IV (symptomatic presenting dyspnea, resting fatigue and palpitation), in association with other first-line drugs for treatment. Cardiac glycosides do not promote the regression of the disease however, they act positively in the improvement of symptoms, stabilization of the condition and prevention of worsening (SOCIEDADE BRASILEIRA DE CARDIOLOGIA, 2002).

Steroids that tested positive have anti-inflammatory, antibacterial and analgesic action (OLIVEIRA *et al.*, 2016). Steroids form a class of secondary

metabolites formed by decarboxylations of precursors that originate from triterpenes (RODRIGUES *et al.*, 2010), this can be associated with several pharmacological actions.

In summary, we saw secondary metabolites found in this study and its importance in this way exploration as a biotechnological product is very necessary in view of its vast benefits.

3.2 Quantification of phenolics and total flavonoids

The results of the total phenolic and flavonoid content of the essential oil and the hydroalcoholic extract of *Syzygium aromaticum* are shown in Table 3. The total phenolic content (CPT) of the essential oil and hydroalcoholic extract was expressed as tannic acid equivalents (mg EAT g⁻¹ of plant material) the equation of the line obtained was $y = 0.0586x + 0.06$ ($R^2 = 0.9999$), where y represents the absorbance of the equivalent tannic acid concentration. For the flavonoids, the quercetin standard (mg EQT g⁻¹ of plant material) was used, the equation obtained was $y = 0.0033x + 0.0006$ ($R^2 = 0.9845$), where y represents the absorbance of the quercetin equivalent concentration.

Table 3 – Quantification of phenolics and total flavonoids in essential oil and hydroalcoholic extract of *Syzygium aromaticum*

Phenolics	Essential oil	Hydroalcoholic Extract
Total (mg EAT g ⁻¹)	207,615	143,589
Linear Equation (y = ax + b)	y = 0.0586 + 0.06	y = 0.0586 + 0.06
Correlation Coefficient (R ²)	0.9998	0.9998
Flavonoids	EO	EH
Total (mg EQT g ⁻¹)	125.36	143.36
Linear Equation (y = ax + b)	y = 0.0033 + 0.006	y = 0.0033 + 0.006
Correlation Coefficient (R ²)	0.9845	0.9845

Source: Authors (2021)

According to Table 3, phytochemical studies revealed the presence of phenolic compounds resulting in positive quantity in essential oil 207.6 mg EAT g⁻¹, supporting the following results and scientific studies such as that of Adaramola & Onigbinde (2016) who presented a quantitative of 170.90 mg EAT g⁻¹ and for the extract It can be said that through this test it is possible to predict a good antioxidant activity, already in reference to the study of Teles *et al.* (2019).

The presence of phenolic compounds is related to antioxidant properties, since they are chemical structures that have hydroxyls and aromatic rings, in simple or polymer forms, which give them the antioxidant power. These compounds can be natural or synthetic. When present in vegetables they can be in free forms or complexed with sugars and proteins (ANGELO *et al.*, 2007). Regarding the pharmacological properties, research on the anticarcinogenic or chemopreventive potential, antioxidant, antiplatelet and antithrombotic properties of cloves reinforce the popular use of this species (BANERJEE *et al.*, 2006; KUBATKA *et al* 2017).

Flavonoids reveal an abundant presence. Corroborating with studies by Adaramola (2016), where the content of the Hydroalcoholic extract was higher 143,36 mg EAT g⁻¹ that of essential oil 125.36 mg EAT g⁻¹ for having 70% methanol in its composition that has a low polarity favoring its greater yield.

The flavonoid compounds quantified previously, are known to have several biological activities, act as antioxidants in the inactivation of free radicals, and stand out in terms of the ability to act on inflammation, on the immune system, anticancer, antitumor, cardioprotection, antiAlzheimer, neuroprotective providing enormous pharmacological potential. Plants containing flavonoids have significant antimicrobial activity, which may be associated with their solubility and the assignment of hydroxyl phenolic groups, which have an affinity for proteins, acting as inhibitors of bacterial enzymes, as well as intervening in their synthesis pathways (COSTA *et al.* , 2018).

The variations in the concentrations of total phenolics and total flavonoids occur due to different factors, such as flora ecology (PARK *et al.*, 2002), by the period of collection (SANTOS *et al.*, 2003).

3.3 Thermodynamic stability

Table 4 shows a study of the thermodynamic stability of nanoemulsion formulations with the essential oil and hydroalcoholic extract of *Syzygium aromaticum*.

Table 4 – Study of the thermodynamic stability of nanoemulsion formulations with the essential oil of *Syzygium aromaticum*

Identification	SF	AQ	CG	DCG	Stability Final
NEO1	-	-	-	+	-
NEO 2	-	-	-	-	+
NEO 3	-	+	-	-	-
NEH 1	-	-	-	+	-
NEH 2	-	-	-	-	+
NEH 3	-	+	-	-	-

Source: Authors (2021)

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.

According to Table 4, it was determined as a result by means of the analyzes, that the best surfactant was exactly Tween 20, presenting a higher sensitivity when it comes to fungicidal activity. Through the work of Pontes & Sylvania (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 at

achieving more formulations stable. In the case of the formulations used, they did not present phase separations, however when they reached higher and lower temperatures, only the formulation NEO2 and NEH2 were stable for terms of applicability. Precisely because these nanoemulsions are described as stable systems.

3.4 Antifungal activity

Table 5 presents the antifungal activity of the essential oil, hydroalcoholic extract and nanoemulsions of *Syzygium aromaticum*

Table 5 - Antifungal activity of *Syzygium aromaticum*

	<i>Aspergillus niger</i>		<i>Colletotrichum gloeosporioides</i>		<i>Penicillium chrysogenum</i>	
	CIM $\mu\text{g mL}^{-1}$	CFM $\mu\text{g mL}^{-1}$	CIM $\mu\text{g mL}^{-1}$	CFM $\mu\text{g mL}^{-1}$	CIM $\mu\text{g mL}^{-1}$	CFM $\mu\text{g mL}^{-1}$
EO	100	250	100	250	250	300
EH	100	250	250	300	250	300
NEO2	25	50	25	100	50	100
NEH2	50	100	50	100	100	250

Source: Authors (2021)

Note: EO, essential oil; EH, hydroalcoholic extract; NEO2, nanoemulsion formulated with essential oil; CIM, Minimum Inhibitory Concentration; CFM, Minimum Fungicide Concentration.

According to Table 5, the Minimum Inhibitory Concentration test showed the result of inhibition of the microbial growth of *A. niger* from $100 \mu\text{g mL}^{-1}$, *C. gloeosporioides* from $100 \mu\text{g mL}^{-1}$ and *P. chrysogenum* from $250 \mu\text{g mL}^{-1}$ for essential oil. For the hydroalcoholic extract, the Minimum Inhibitory Concentration test showed the result of inhibition of the microbial growth of *A. niger* from $100 \mu\text{g mL}^{-1}$, of *C. gloeosporioides* from $250 \mu\text{g mL}^{-1}$ and of *P. chrysogenum* a from $250 \mu\text{g mL}^{-1}$ with the best performance in the fungus *A. niger*. For the essential oil nanoemulsion,

the MIC was obtained of 25 $\mu\text{g mL}^{-1}$, 25 $\mu\text{g mL}^{-1}$ and 50 $\mu\text{g mL}^{-1}$ for *A. niger*, *C. gloeosporioides* and *P. chrysogenum*, respectively and for the hydroalcoholic extract nano, the result obtained was 50 $\mu\text{g mL}^{-1}$, 50 $\mu\text{g mL}^{-1}$ and 100 $\mu\text{g mL}^{-1}$ for *A. niger*, *C. gloeosporioides* and *P. chrysogenum*, respectively.

In a study by Meneze *et al.* (2009) on the antifungal activity of *Syzygium aromaticum* the authors reported that MIC from the concentration of 100 $\mu\text{g mL}^{-1}$ is considered a good antifungal activity and from 101 to 500 $\mu\text{g mL}^{-1}$ Moderate antifungal activity as we can see in Table 5 our results range from good antifungal activity to moderate, so we can consider *Syzygium aromaticum* to be a good inhibitor. Corroborating with previous studies by the authors Neto *et al.* (2017) and Ascensão & Mouchrek Filho (2013).

The test for Minimum Fungicide Concentration showed good efficacy. For EO, the best result was in front of *A. niger* and in front of *C. gloeosporioides*, with fungicidal action from 100 $\mu\text{g mL}^{-1}$ already in the hydroalcoholic extract the best result was in front of *A. niger* with fungicidal action from 100 $\mu\text{g mL}^{-1}$, as the essential oil nanoemulsion had a highlight in its performance in front of *Aspergillus niger* and in front of *Colletotrichum gloeosporioides* that showed inhibition in 25 $\mu\text{g mL}^{-1}$ of both and the nanoemulsion of the hydroalcoholic extract had its best performance against *Aspergillus niger* and against *Colletotrichum gloeosporioides* which showed inhibition in 50 $\mu\text{g mL}^{-1}$ both. In the study by Neto *et al.* (2017) and Costa *et al.* (2011) corroborate the results presented in this work, proving the efficiency of *Syzygium aromaticum*.

In short, nanoemulsions in this work had the most efficient performance when compared to essential oil and hydroalcoholic extract. Several recent researches have reported the formation of nanoemulsions as a strategy to improve the functionality and chemical property of its constituents, one of the efficiencies incorporated in a nanoemulsion is because it has smaller details that increases the permeability of the fungus membrane. Thus improving its antifungal capacity.

4 CONCLUSION

In this work it was possible to prove the secondary metabolisms present in the buttons of *S. aromaticum* fact that influences the inhibition of microorganisms, pharmacological potential, biological and chemical properties. It was possible to demonstrate the levels of phenolics and total flavonoids being significant. The use of *S. aromaticum* for medicinal purposes it is very significant due to its wide applicability and its efficiency in the biotechnological product nanoemulsion used in this study against pathogenic fungi, showing to be an alternative to the current market for the combat and control of pathogenic fungi.

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Authorship contributions

1 - Thaylanna Pinto de Lima

Graduated in Chemistry

<http://orcid.org/0000-0003-1172-3004> – thaylanna.lima@discente.ufma.br

Contributions: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology

2 - Thayane Lopes de Sousa

Graduated In Industrial Chemistry

<http://orcid.org/0000-0001-5383-9350> – thayane.lopes@discente.ufma.br

Contributions: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology

3 - Joao Pedro Mesquita Oliveira

Graduated In Industrial Chemistry

<http://orcid.org/0000-0003-1833-9814> – jpm.oliveira@discente.ufma.br

Contributions: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology

4 - Maria Giullia Alves Carneiro Felizardo

Graduated In Industrial Chemistry

<http://orcid.org/0000-0002-1921-9067> - giullia.73@hotmail.com

Contributions: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology

5 - Gustavo Oliveira Everton

Master in Health and Environment

<http://orcid.org/0000-0002-0457-914X> – gustavooliveiraeverton@gmail.com

Contributions: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration

6 - Victor Elias Mouchrek Filho

Professor

<http://orcid.org/0000-0003-2855-7292> - victor.mouchrek@ufma.br

Contributions: Funding Acquisition, Project Administration

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