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Quimica

Chemical profile, stability and fungicide activity of oil-in-water nanoemulsion (O / A) incorporated with *Ba-har* essential oil

Perfil químico, estabilidade e atividade fungicida da nanoemulsão óleo-em-água (O/A) incorporada com o óleo essencial de Ba-har

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ABSTRACT

The increase in strains resistant to conventional antifungals means that there is a need for studies related to alternative therapeutic practices, such as medicinal plants. This study aims to evaluate the chemical profile and fungicidal activity of the nanoemulsion of the essential oil obtained from *Ba-har* (Syrian pepper). The phytochemical profile of the plant material was performed based on the methodology of Matos (2009). For extraction of essential oil, 100g of plant material was used, with the isolation of essential oil by the hydrodistillation technique conducted at 100 °C / 3h. To quantify the total phenolics present in the OE, the Folin-Ciocalteu method was used. For the total flavonoids, the $AlCl_3$ complexation method was used. The oil-in-water nanoemulsion was formulated by the low-energy method of phase inversion using essential oil, non-ionic surfactant and water, and the obtained nanoemulsion was subjected to thermodynamic stability tests. The essential oils and stable nanoemulsions were subjected to evaluation of the fungicidal action against strains of *Aspergillus niger* (ATCC 6275), *Colletotrichum gloeosporioides* (ATCC 96723) and *Penicilium chrysogenum* (ATCC 10106). The fungicidal activity was performed according to CLSI (2020) using the Broth Dilution method to obtain the Minimum Inhibitory Concentration (MIC) and sowing on agar for Minimum Fungicidal Concentration (CFM). The results obtained for the total phenolic content were quantified at 348.3 mg EAT g^{-1} and 346.21 mg EQ g^{-1} for flavonoids. *Ba-har* essential oil was more efficient in inhibiting *A. niger*, as it presented the lowest MIC (200 μg mL^{-1}), followed by *P. chrysogenum* (250 μg mL^{-1}) and later by *C. gloeosporioides* (300 μg mL^{-1}). The identification of the secondary metabolites present in *Ba-har* was quite considerable, since they are responsible for the biological properties, thus inspiring the continuity of studies related to its biological activities. As for the total phenolic content and flavonoids present in the essential oil, they indicated the important antioxidant potential. The fungicidal potential of *Ba-har* oil showed strong inhibition and mortality, however the nanoemulsion product with the essential oil incorporated showed a more efficient action against the pathogenic fungi tested,

Keywords: Pepper; Fungicide; Nanoemulsion

RESUMO

O aumento de cepas resistentes aos antifúngicos convencionais faz com que aja uma necessidade de estudos relacionados a práticas terapêuticas alternativas, como plantas medicinais. Este estudo tem por objetivo avaliar o perfil químico e a atividade fungicida da nanoemulsão do óleo essencial obtido de *Ba-har* (Pimenta síria). O perfil fitoquímico do material vegetal foi executado com base na metodologia de Matos (2009). Para extração do óleo essencial utilizou-se 100g do material vegetal, sendo o isolamento do óleo essencial pela técnica de hidrodestilação conduzida a 100°C/3h. Para quantificação dos fenólicos totais presentes no OE foi utilizado o método de Folin-Ciocalteu. Para os flavonoides totais utilizou-se o método de complexação com $AlCl_3$. A nanoemulsão óleo-em-água foi formulada pelo método de baixa energia de inversão de fases utilizando o óleo essencial, surfactante não iônico e água, sendo a nanoemulsão obtida submetida a testes de estabilidade termodinâmica. Os óleos essenciais e nanoemulsões estáveis foram submetidas a avaliação da ação fungicida frente cepas de *Aspergillus niger* (ATCC 6275), *Colletotrichum gloeosporioides* (ATCC 96723) e *Penicilium chrysogenum* (ATCC 10106). A atividade fungicida foi realizada segundo o CLSI (2020) pelo método de Diluição em Caldo para obtenção da Concentração Inibitória Mínima (CIM) e semeadura em ágar para Concentração Fungicida Mínima (CFM). Os resultados obtidos para o teor fenólico total foram quantificados em 348,3 mg EAT g^{-1} e 346,21 mg EQ g^{-1}para flavonoides. O óleo essencial *Ba-har* foi mais eficiente ao inibir o *A. niger*, pois apresentou a menor CIM (200 μg mL^{-1}), seguido por *P. chrysogenum* (250 μg mL^{-1}) e posteriormente por *C. gloeosporioides* (300 μg mL^{-1}). A identificação dos metabolitos secundários presentes em *Ba-har* foi bastante considerável, visto que estes são responsáveis pelas propriedades biológicas, inspirando assim a continuidade dos estudos relacionados a suas atividades biológicas. Quanto ao teor fenólico total e flavonoides presentes no óleo essencial indicaram o importante potencial antioxidante. O potencial fungicida do óleo de *Ba-har* apresentou forte inibição e mortalidade, todavia o produto nanoemulsão com o óleo essencial incorporado mostrou ação mais eficiente frente aos fungos patogênicos testados, sendo apontada por este estudo como uma nova alternativa no controle e combate de fungos patogênicos por meio do produto nanoemulsão formulada com o óleo essencial de *Ba-har.*

Palavras-chave: Pimenta; Fungicida; Nanoemulsão

1 INTRODUCTION

Essential oils are complex mixtures of volatile, lipophilic substances, with low molecular weight, usually odorous and liquid. They are formed by molecules of a terpenic nature, constituted of several classes of substances that come from the secondary metabolism of plants (MORAIS, 2009). The chemical composition of these compounds is determined by genetic factors, however other factors can also influence the yield, such as: climate, temperature, microorganism attack, soil composition, water, ultraviolet radiation, nutrients, air pollution, plant age, collection time , organ used for extraction and type of extraction (ANDRÉ et.al., 2018).

The main highlight for essential oils is their high capacity as an active ingredient acting as antioxidants and antimicrobials (XU *et al*., 2019). This function is mainly due to the presence of phenolic compounds, which are increasingly exciting for the industry (MEIRA, 2017). Another attraction to its use is the great biodegradability and biocompatibility, which makes them can be added to other compounds (SILVA *et al*., 2020).

Among the oils with antimicrobial properties, *Ba-har* stands out, popularly known as Syrian Pepper, a condiment used in Syrian Lebanese cuisine and in other regions of Asia. This seasoning is a mixture of spices with potentials widely discussed in isolation in the literature, but with few studies regarding the study of their synergies. *Ba-har* is a synergy of equal proportions on a mass / mass basis of ground grains of *Piper nigrum* L. (black pepper), *Cinnamomum zeylanicum* blume (cinnamon), *Pepper dioica* Lindl. (Allspice), *Syzygium aromaticum* (L.) Merr. (clove), *Illicium verum* Hook.f. (anise) and *Myristica fragrans* Houtt (nutmeg) (ABREU&AGUILERA, 2010; HADJAB, 2014)

Observing the growth of the nanotechnological products market, they have been intensifying significantly in recent years as an area of innovation, mainly for human health, represents a great potential. The potential is huge, whether for final products or process media, since the materials used in nanometer ladders in general acquire new characteristics and improvements, almost impossible on a normal scale. In view of their possibilities, nanotechnological products have been the subject of research in several areas such as: food, pharmaceutical and cosmetics (EFING&ORTIGARA, 2017).

Nanostructured systems, such as nanoemulsions, have the benefits of particle size on a manometric scale, which can increase their performance against microorganisms, due to the hydrophobic properties of essential oils, their aggregation is facilitated through an emulsion, which is characterized by presenting an oil phase, an aqueous phase and a surfactant (AHMAD *et al*., 2014). In addition, essential oils incorporated into nanoemulsions increase the permeability in the bacterium's membrane, and also decrease the concentration necessary to obtain an antimicrobial action (ZIANI *et al*., 2011; SALVIA-TRUJILLO *et al*., 2015).

Since fungi are microorganisms that are related to various pathogens in humans and the discriminated use of antifungals caused the strains to acquire resistance (ARAUJO *et al*., 2018), it is necessary to increase research related to the antimicrobial potential of medicinal plants. 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 and the nanoemulsion formulated with the essential oil obtained from the ground grains of *Ba-har* (synergy of *P. nigrum* L. (black pepper), *C. zeylanicum* Blume (cinnamon), *P. dioica* Lindl. (Jamaica pepper), *S. aromaticum* (L.) Merr. (clove), *Illicium verum* Hook.f. (anise) and *Myristica fragrans* Houtt (nutmeg).

2 METHODOLOGY

2.1 Obtaining plant material

Ba-har grains were purchased from the company Produtos Naturais Muniz, Maranhão and sent to the Laboratory of Research and Application of Essential Oils (LOEPAV/UFMA).

2.2 Phytochemical prospection of plant material

For the preparation of hydroalcoholic extracts, 50g of plant material was used. 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 obtained was subjected to chemical tests based on the methodology presented by Matos (2009). The tests performed to identify steroids, alkaloids, flavonoids, glycosides, saponins, phenols, tannins and cardiac glycosides.

2.3 Obtaining essential oil

For the extraction of essential oil, the hydrodistillation technique was performed with a Clevenger glass extractor coupled to a round bottom flask coupled to a heating blanket as a source of heat. 100 g of plant material were used, adding distilled water

(1:10). Hydrodistillation was carried out at 100 °C for 3 h and the extracted essential oil was collected. The essential oil was dried by percolation with anhydrous sodium sulfate (Na_2SO_4) and centrifuged. These operations were carried out in triplicates and the samples stored in amber glass ampoules under 4 °C refrigeration. Subsequently submitted to analysis.

2.4 Total phenolics

The phenol content for essential oils was determined 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 10% Folin-Ciocalteu reagent and 2.0 mL of 7.5% sodium carbonate. The formed solution was taken to the water bath at 50 ºC 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}).

2.5 Total flavonoids

To estimate the total flavonoid content, aluminum chloride complexation was used. The content of total flavonoids was estimated spectrophotometrically by reaction with AlCl3, using quercetin as a standard (DOWLD, 1959; WOISKY and SALATINO, 1998; FREDERICE *et al*., 2010). The 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 $AlCl_3$ 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). The oil-in-water nanoemulsion was formulated with the essential oil obtained, non-ionic surfactant (Tween 20) and water and can be seen in Table 1.

Table 1 – Nanoemulsion formulations for *Ba-har* essential oil.

Identification	Essential oil (*Ba-har*)	Tween 20	H2O
NOE1	5%	5%	90%
NOE 2	5%	10%	85%
NOE 3	5%	15%	80%

Fonte: autores, 2021

The oil concentration (5% v / v) was fixed for the formulation. The required quantities of each constituent of the oil phase (oil + Tween20) were heated to 65 ± 5 °C. The aqueous phase was heated separately to 65 ± 5 °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 ºC ± 2 ºC.

To prove the 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 check the stability of the nanoemulsion at variable temperatures. The freeze-thaw stress was carried out by maintaining 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 the thermodynamic stress tests were taken for studies of antifungal action. Stability was monitored for 30 days.

2.7 Standardization of the microbial inoculum for sensitivity tests

Three strains of fungi were used: *Aspergillus niger* (ATCC 6275), *Colletotrichum gloeosporioides* (ATCC 96723) and *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 x 10^8 UFC mL^{-1} according to the standards of the Clinical and Laboratory Standards Institute (CLSI, 2020)

2.8 Minimum Inhibitory Concentration (MIC) and Minimum Fungicide Concentration (CFM)

This test evaluated the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (CFM) of the essential oil and the nanoemulsion. The MIC test was carried out using the broth dilution technique, proposed by the Clinical and Laboratory Standards Institute (2020). First, essential oil 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 1000 μg mL^{-1}. The nanoemulsions were diluted directly in the culture medium.

To each concentration, fungal suspensions containing 1.5 x 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, MIC of the essential oil 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

Table 2 shows the classes of secondary metabolites that obtained positive results in relation to phytochemical tests.

Table 2 – Secondary metabolites identified in the ground grains of *Ba-har*

Classes	1	2	3	4	5	6	7	8
Ba-har	+	+	+	+	+	+	+	+

Fonte: autores, 2021

note:1: Alkaloids; 2: Steroids; 3: Phenols; 4: Flavonoids; 5: Glycosides; 6: Cardiac Glycosides; 7: Saponins; 8: Tannins; (+):presence; (-):absence;

Table 2 presented a variety of active phytochemical class. This analysis is necessary to be aware of the biological properties of plant species and thus be able to isolate their active ingredients. Plants have a variety of chemicals that can be divided into primary and secondary metabolites. Primary metabolites are those common to all plants and are linked to their development and are responsible for the synthesis of proteins, lipids and nucleic acids, generating energy. Secondary metabolites are specific and perform several important biological activities, thus arousing the interest of the pharmaceutical industry (ROJAS *et al*., 2015; REZENDE *et al*., 2016)

The presence of alkaloids observed is related to antioxidant (FERREIRA, 2020) and antimicrobial (CAMPUSANO *et al*., 2015) activity present in this study. Alkaloids, which are basic substances that react with acid to form salts, have specific biological characteristics such as and are present in morphines, caffeines and nicotines (KLOSS, 2016). In a variety of species, alkaloids are produced and participate in plant defense against cell damage caused by salt stress (SYTAR *et al*., 2018).

Steroids have cardiotonic functions, activating anabolism, antimicrobial activity, anti-inflammatory action, vitamin D precursors and contraceptives (BESSA *et al*., 2013; ROBBERS *et al*., 1997; COELHO *et al*., 2003).

Phenolic compounds are substances with at least one aromatic ring in which at least one hydrogen has been replaced by a hydroxyl group and are found in high amounts in fruits and vegetables and are known mainly for their antioxidant action. In addition, they have antimicrobial, anti-inflammatory, cardioprotective and vasodilator activity, and for this reason they have gained the attention of the pharmaceutical, cosmetic and food industry (REZENDE *et al*., 2016).

Flavonoids are abundant in plants, soluble in water and act in the protection of plant tissues from the mutagenic action of ultraviolet rays and contribute to the attraction of pollinators and seed dispersers. Its main property is the antioxidant action (CIDRES, 2018).

Glycosides are soluble in water and are stored and transported within plant tissues, where they participate in plant signaling, growth and development, are responsible for the plant's defense against pathogens and herbivores and are still part of the allelopathy phenomenon (When a plant inhibits the growth of the other (BARTNIK, 2017).

Cardiac glycosides are complex molecules of triterpenes and have several biological effects on living beings. Despite being quite toxic, it can be used medicinally when applied in regulated quantities. In humans, they block the sodium and potassium pumps of cardiac cells, which results in the strengthening and slowing of heart beats (BARBOSA *et al*., 2017).

Saponins form a group of heterosides. Its name stems from the ability to form a lot of foam when stirred with water, similar to soap, which emulsifies the oil in the water and has a hemolytic effect. Saponin plants have mucolytic and diuretic action and also favor the action of the other active ingredients of the plant (ARAÚJO *et al*., 2014).

Tannins that are phenolic compounds that react with proteins forming a protective layer, inhibiting the action of microbial enzymes, thus containing the growth of microorganisms, also have antioxidant activity and antiseptic action (PEREIRA *et al*., 2015; SOARES *et al*., 2016).

Due to the presence of metabolites that are responsible for various biological activities such as: antioxidant, antiseptic, antimutagenic, antimicrobial, anti-inflammatory and other actions, it is recommended to continue studies to verify these biological activities, as well as to formulate nanotechnological products for application of the same activities.

3.2 Quantification of phenolics and total flavonoids

Table 3 presents the values that were quantified for phenolics expressed in tannic acid equivalents, in which they obtained a straight line equation of $y = 0.0586x + 0.06$ ($R^2 = 0.99998$) and total flavonoids expressed in quercetin equivalents , the equation of the obtained line was $y = 0.0033x + 0.0006$ ($R^2 = 0.9845$) in *Ba-har* essential oil, where x corresponds to absorbance and y corresponds to equivalent concentration.

Table 3 – Total phenolic and flavonoids present in *Ba-har* essential oil

	Total Content	**Linear**	**R2**
Phenolics	348.3 mg EAT g^{-1}	$y = 0.0586x + 0.06$	0.9998
Flavonoids	346.21 mg EQ g^{-1}	$y = 0.0033x + 0.0006$	0.9845

Fonte: autores, 2021

According to Table 3, the total phenolic content was expressed in tannic acid equivalents and was 348.3 mg EAT g^{-1} and 346.21 mg EQ g^{-1} for flavonoids present in the essential oil that was quantified, being able to confirm a number of phenolic compounds and flavonoids, which has great prominence, since phenolic compounds have an important antioxidant action.

There are few studies related to total phenolics and flavonoids of a mixture of species rich in essential oils, as well as *Ba-har* essential oil, and thus the importance of spreading this study is emphasized. The imbalance between oxidative systems and the production of free radicals has the consequence of oxidative stress, which is linked to various diseases such as cancer, cardiovascular diseases and inflammatory disorders (SILVA *et al*., 2010).

In the study by Andrade *et al*. (2015), the authors reported that the extraction of *P. nigrum* in Soxhlet with ethyl acetate had a content of total phenolic compounds of 55 mg

EAG mg g^{-1}, a value lower than that obtained in this study. Ahmad *et al*. (2015) found values of total phenolic and flavonoids in the methanolic extract of *P. nigrum* of (1.73 ± 0.04) mg g^{-1} and (1.09 ± 0.01) μg g^{-1} respectively. These results showed that *P. nigrum* extract contains a significant amount of phenolic and flavonoid compounds.

In the work carried out by Ribeiro-Santos *et al*. (2015), highlighted that the essential oil of the leaves of C. *zeylanicum*, another component of the plant under study, had a content of total phenolic compounds of 912 mg EAG g^{-1} and a content of total flavonoids of 371 (mg EQ g^{-1}).

Radunz (2017) presented a content of phenolic compounds present in the essential oil of *S. aromaticum* of 9.07 EAG mg g^{-1} of sample. In the study by El-Maati *et al*. (2016) when analyzing the best solvents for extracting flavonoids from *S. aromaticum* obtained the following results: Water was the best solvent for extracting flavonoids from cloves (17.50 mg EQ g^{-1} extract), followed by ethanol (12.0 mg EQ g^{-1} extract) and ethyl acetate (4.70 mg EQ g^{-1} extract).

In the work carried out by Neves *et al*. (2016), cited the important antioxidant role of beta-carotene, a compound found in *M. fragrans* Houtt, and encouraged further studies on the antioxidants present in the species. Piaru *et al*. (2012) reported a significant antioxidant activity of nutmeg oil with IC_{50} = 136 μg mL^{-1}, which is half of the maximum inhibitory concentration.

Aly *et al*. (2016), when determining the content of total phenolics (CFT) and flavonoids (CFT) of *I. verum* (star anise) show that the CFT of ethanol and methanol extracts registered 112.40 ± 0.95 and 96.30 ± 0.51 mg GAE g g^{-1} DW, respectively. While the results for flavonoids revealed that the star anise methanol extract exhibited high CFT at 65.90 ± 0.90 mg ECAT g^{-1}, while the ethanol extract showed lower CFT 46.80 ± 0.64 mg ECAT g^{-1}.

Thus, the results obtained with this work encourage further studies with the species, since the mixture of the species that make up *Ba-har* provides a higher value of phenolics and flavonoids than observed in individual species. The amount of phenolics observed is of enormous importance, since phenolic compounds have been the subject of several studies, as they are often associated with positive health effects, due to their antioxidant action. It is believed that this is due to its reducing properties present in the adsorption and neutralization of free radicals (SOBRAL, 2012). While, flavonoids make up the largest class of phenolic compounds, are of low molecular weight and act in the protection against UV-B radiation, among its properties the ability to sequester free radicals and prevent oxidation stands out (CIDRES, 2018).

3.3 Thermodynamic stability and antifungal activity of nanoemulsion

Table 4 is related to the results of the thermodynamic stress tests, in order to identify the nanoemulsion formulation that has stability for the antifungal action tests.

Table 4 – Study of the thermodynamic stability of nanoemulsion formulations with *Ba-har* essential oil

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

Fonte: autores, 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, the formulation of NOE 1 did not become viable, since there was a phase separation after thawing, thus failing the thermodynamic stability test. NOE 3, suffered a phase separation shortly after heating, thus becoming unstable. However, the formulation of NOE 2, remained stable throughout the thermodynamic stress test, and thus proving to be viable for antifungal action tests.

Table 5 shows the results related to the antifungal action of *Ba-har* essential oil and nanoemulsion against strains of *Aspergillus niger*, *Colletotrichum gloeosporioides* and *Penicilium chrysogenum*.

Table 5 – Fungicidal activity of the essential oil of *Ba-har* and the formulated nanoemulsion NOE2 against strains of pathogenic fungi

	Essential oil *Ba-har*		**NOE2**	
	CIM (μg mL^{-1})	**CFM (μg mL^{-1})**	**CIM (μg mL^{-1})**	**CFM (μg mL^{-1})**
A. niger	200	500	50	100
C. gloeosporioides	300	600	100	200
P. chrysogenum	250	450	50	200

Fonte: autores, 2021

note: CIM, Minimum Inhibitory Concentration; CFM, Minimum Fungicide Concentration.

When analyzing Table 5, it is noted that the essential oil was more efficient in inhibiting *A. niger*, as it had the lowest MIC, followed by *P. chrysogenum* and later by *C. gloeosporioides*. The results obtained allow us to classify sensitive fungi against the tested essential oil according to the classification of Aligiannis *et al*. (2001) for the results of the Minimum Inhibitory Concentration in antimicrobial assays, with strong inhibition when MIC is up to 500 μg mL^{-1}; moderate inhibition: MIC between 600 and 1000 μg mL^{-1}; and weak inhibition: MIC above 1000 μg mL^{-1}.

The test for Minimum Fungicide Concentration presented showed better results for the essential oil against *P. chrysogenum*, with a CFM of 450 μg mL^{-1} and for the nanoemulsion the best CFM was 100 μg mL^{-1} compared to *Aspergillus niger*.

Looking at Table 5, we can also see that the nanoemulsion had an amplified action by inhibiting the studied fungi and using the Aligiannis *et al*. (2001), this is due to the size of its particles being nanometrically smaller, thus improving its disposition and stability in a given area and improving its properties of asset deliveries (YUKUYAMA *et al*., 2016) thus facilitating the access of essential oils to fungicidal cells.

In a study by Ramos *et al*. (2016) the authors reported similar activity when analyzing the fungus *C. gloeosporioides* in which it obtained a MIC and CFM of 6.25% compared to essential oil of clove and a MIC of 3.2% and CFM of 6.25% compared to oil essential oil of *C. zeylanicum*, thus confirming the sensitivity of the fungus, a result similar to that presented in the present study.

In another research carried out by Leal *et al*. (2017), the aqueous extract of Pepper dioica at concentrations of 25% and 50% proved to be efficient in inhibiting the mycelial growth of *Aspergillus niger*, with the concentration of 50% totally inhibiting the sporulation of the pathogen. According to Rozwalka *et al*. (2008) the essential oil of *S. aromaticum* (L.) Merr. it also totally inhibited the pathogen *Colletotrichum gloeosporioides*. Dhiman&Aggarwal (2019) when analyzing an extract of *Illicium verum* Hook.f. showed a MIC value of 30 μg mL^{-1} and 20 μg mL^{-1} for A. flavus and *Penicillium chrysogenum*.

The results obtained can be justified due to the chemical constituents present in the components of *Ba-har*, such as eugenol, present in *S. aromaticum* (L.) Merr. (GUIMARÃES *et al*., 2017), *C. zeylanicum* Blume (MILLEZI *et al*., 2019) and *P. dioica* Lindl. (EVERTON *et al*., 2020) in which it has several pharmacological effects, among which we can highlight: antimicrobial (RAMOS *et al*., 2016), anti-inflammatory (TSAI *et al*., 2017), antioxidant (SEDIGUI *et al*., 2018). The main constituent found in the essential oil of *Illicium verum* Hook.f. is trans-anethole, responsible for its antimicrobial action, for being hydrophobic, it interacts with the microbial plasma membrane causing an imbalance of intracellular ions, leading to lysis of the fungus or bacteria (NETO *et al*., 2019).

According to Nascimento (2017) when analyzing the chemical constituent eugenol, which has antimicrobial action, but has high volatilization and low solubility in water, he observed that the use of nanoemulsion would be a promising possibility. The results of this research corroborate with other studies that showed that essential oil nanoemulsions are more effective in their antimicrobial activity (MOGHIMI *et al*., 2016).

The results obtained by Lyra (2019) in relation to the antimicrobial activity of the essential oil of palmarosa and geraniol, demonstrate that the oils were more effective when they integrated nanoemulsions. In a study by Seibert (2015), the essential oil and nanoemulsion of *Cymbopogon densiflorus* leaves showed a remarkable inhibition of microbial multiplication for *E. coli, P. aeruginosa* and *S. aureus* and for the fungus *C. albicans* and also observed that the concentration of essential oil contained in the nanoemulsion to cause a 50% reduction in the initial amount of DPPH free radicals was four times lower when compared to the oil in its free form.

4 CONCLUSION

Finally, the identification of the secondary metabolites presents in *Ba-har* had been quite significant, since they infer the presence of several biological properties, thus stimulating future studies related to its biological activities. As for the total phenolic content and flavonoids present in the essential oil, they indicated the important antioxidant potential present in *Ba-har*. The fungicidal potential of the essential oil of *Ba-har* showed strong inhibition and mortality, however the nanoemulsion product with the incorporated essential oil showed a more efficient action against the pathogenic fungi tested even in low concentrations, being indicated by this study as a new alternative in the control and combating pathogenic fungi through the nanoemulsion product formulated with the essential oil of *Ba-har*.

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