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Chemistry

Potentiation of the anti-inflammatory effect of *attalea speciosa* (babassu) oil microemulsions through the incorporation of *citrus x aurantium* L. (sour-orange) essential oil

Potencialização do efeito anti-inflamatório de microemulsões do óleo de *attalea speciosa* (babaçu) através da incorporação do óleo essencial de *citrus x aurantium* L. (laranja-azeda)

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ABSTRACT

This study analyzed the anti-inflammatory activity of a microemulsion of *A. speciosa* oil with the incorporation of the essential oil of *Citrus* × *aurantium* (sour orange). The plant material used in this research was collected in the region of São Luís-MA. The essential oil was extracted by hydrodistillation. The chemical constituents were identified by Gas Chromatography coupled with Mass Spectrometry (GC/MS). The microemulsions were formulated with A. speciosa oil, *Citrus* × *aurantium,* and Tween 20. The total phenolic content was determined by the Folin-Ciocalteu spectrophotometric method. The anti-inflammatory activity was evaluated using the protein denaturation method with a calculation of the 50% Efficient Concentration (EC_{50}). The total phenolic content and refractive index were found, respectively, for the oil of *A. speciosa* and for the essential oil of C. aurantium, being 238.01; 1.454; and 232.2; 1,470. It was observed that the oil of *A. speciosa* has greater anti-inflammatory activity than the essential oil of *C. aurantium*, while the formulated microemulsion showed greater anti-inflammatory activity than both tested individual oils. Finally, it is concluded that the microemulsion was effective in the anti-inflammatory process, indicating that the incorporation of the essential oil of *C. aurantium* into the microemulsion of *A. speciosa* increased its anti-inflammatory potential, confirming the possibility of its use for anti-inflammatory action

Keywords: Microemulsion; Anti-inflammatory; Essential oil

RESUMO

Este estudo analisou a atividade anti-inflamatória de uma microemulsão do óleo de *A. speciosa* com incorporação do óleo essencial de *Citrus* × *aurantium* (laranja-azeda). O material vegetal utilizado nesta

pesquisa foi coletado na região de São Luís-MA. O óleo essencial foi extraído por hidrodestilação. Os constituintes químicos foram identificados por Cromatografia Gasosa acoplada a Espectrometria de Massas (CG/EM). As microemulsões foram formuladas com óleo de *A. speciosa*, *Citrus* × *aurantium* e Tween 20. O conteúdo fenólico total foi determinado pelo método espectrofotométrico de Folin Ciocalteu. A atividade anti-inflamatória foi avaliada através do método de desnaturação proteica com cálculo da Concentração Eficiente 50% (CE_{50}). O conteúdo fenólico total e índice de refração foram encontrados, respectivamente, para o óleo de *A. speciosa* e para o óleo essencial de *C. aurantium*, sendo eles 238,01; 1,454 e 232,2; 1,470. Observou-se que o óleo de *A. speciosa* possui maior atividade anti-inflamatória que o óleo essencial de *C. aurantium*, enquanto que a microemulsão formulada apresentou maior atividade anti-inflamatória que ambos os óleos individuais testados. Por fim, conclui-se que a microemulsão apresentou-se eficaz no processo anti-inflamatório indicando que a incorporação do óleo essencial de *C. aurantium* à microemulsão de *A. speciosa* aumentou o seu potencial anti-inflamatório, confirmando a possibilidade de seu uso com ação anti-inflamatória.

Palavras-chave: Microemulsão; Anti-inflamatório; Óleo essencial

1 INTRODUCTION

In several parts of the planet the use of medicinal plants is still an alternative method of relief and treatment of diseases, because of its lower cost and easy accessibility of the population (Badke et al., 2012). In this global context, Brazil is considered the holder of the largest biological diversity on the planet, with a number of more than 350,000 species cataloged, representing only a fraction of the biodiversity of the Brazilian flora (Peres et al., 2011).

Specifically, flora has vast applicability in relation to health, mainly as a direct therapeutic resource for populations, since it is very common to use them as medicinal products (Barata, 2005). Within this, the species *Attalea speciosa* (babassu) has been gaining prominence in scientific research. Babassu is part of a research platform for medicinal plants of the Brazilian flora, with the objective of contributing to the development of herbal production (Barroso et al., 2016).

The species is a type of palm tree of the botanical family Arecaceae, found today in several Latin American countries. Its use in the Amazon, in the Atlantic Forest, in the Cerrado and in the Caatinga is quite common, where it can be seen unpretentiously in some states of Brazil (Corrêa et al., 2012). There are some customs exercised by

indigenous peoples in Brazil for the benefit of babassu, such as the Kayapós, which manipulate babassu oil with predominant use in beautification and various rites (Barroso et al., 2016).

Worldwide, added to the value of products obtained from vegetable origin, the essential oils of the *genus Citrus* also stand out, being among the most used. This condition occurs mainly because they are obtained as by-products of the juice industry, such as orange essential oil (Araújo, 2019). These compounds are natural, volatile complexes, characterized by a characteristic odor and consisting of secondary metabolites of aromatic plants (Bakkali et al., 2008).

However, some limitations related to the stability of these substances and their mixtures can be observed in the direct use of a general form (rapid volatilization, oxidation of the chemical constituents of the oil, among others). These limitations can be solved with the use of carrier systems, such as microemulsions (Gonsalves et al., 2009).

Microemulsified systems have been widely researched for their ability to promote the permeation of hydrophilic and lipophilic drugs through the skin, when compared to other pharmaceutical forms, such as aqueous solutions, mycelial solutions, emulsions and liposomes (Kreilgaard, 2002; Yuan et al., 2010).

In vitro and *in vivo* studies involving these systems, with analgesic and anti-inflammatory drugs, demonstrated that there was a greater permeation of these drugs in experiments with rat skin, suggesting that microemulsions are efficient promoters of permeation (Djordjevic et al., 2004; Lee et al., 2005; Yuan et al., 2006; Okur et al., 2011). In principle, microemulsions can be used to administer medicines to patients by various routes, but topical application of microemulsions has gained increasing interest (Peltola et al., 2003).

Thus, the present study aimed to evaluate the chemical profile and potentiation of the anti-inflammatory effect of the microemulsion of *Attalea speciosa* vegetable oil incorporating the essential oil of *Citrus*×*aurantium* L.

2 MATERIAL AND METHODS

2.1 Plant material

The peel samples of *Citrus×aurantium* L. (orange) were collected in the municipality of São Luís – MA, Brazil (-2.49853, – 44.27096). The plant materials were transported to the Laboratory of Research and Application of Essential Oils (LOEPAV / UFMA) of the Federal University of Maranhão (UFMA), where they were dried in a drying oven (FANEM 520). Subsequently, being crushed and having their mass measured for subsequent yield calculations.

2.2 Obtaining essential oil

For essential oil extraction, the hydrodistillation technique was applied with a glass Clevenger extractor coupled to a round bottom balloon packed in an electric blanket as a heat generating source. 100 g of the crushed vegetable peels were used, adding distilled water (1:8) to the plant material contained in the flask. Hydrodistillation was conducted at 100°C for 3h and immediately after the extracted essential oil was collected. The essential oil was dried by percolation with anhydrous sodium sulfate (Na_2SO_4). The samples were stored in amber glass vials under 4°C refrigeration. Subsequently submitted the analyses.

2.3 Obtaining *Attalea speciosa* (babassu) vegetable oil

The oil of *Attalea speciosa* was obtained from the lot produced by the Association of Producers of Sweets and Olive Oil: Flavor of Every Day: Povoado de Todo Dia, Vitória Mearim/MA.

2.4 Chemical constituents

The constituents of the essential oils were identified by gas chromatography coupled to mass spectrometry (GC-MS). The Automated Mass Spectral Deconvolution Mass & Identification System was used to identify the compounds in the sample.

2.5. Total Phenolic Content (TPC) spectrophotometric determination

The determination of the total phenolic compounds of the essential oil was performed with adaptation of the Folin-Ciocalteu method (Waterhouse, 2002). 5 mg of the essential oil diluted in 1 mL of ethanol was used. To this solution was added 7 mL of distilled water, 800 µL of the reagent Folin-Ciocalteu and 2.0 mL of sodium carbonate at 20%. After two hours, uv-vis spectrophotometer was read in length of 760 nm. The standard curve was expressed in mg $L^{-1}$of tanic acid.

2.6 Formulation of microemulsions

For the formulation of microemulsions, the methodology adapted from Carnicel (2014) was used. Initially, different systems were developed, using distilled water, Tween 20 (EHL=16.7) and babassu vegetable oil. In the stable formulations, 2% of the essential oil was used. In each system, 36 formulations were prepared varying the concentrations between 10 and 80% of oil phase, aqueous phase and surfactant.

Each formulation was prepared individually, measuring the volume in milliliters of each component, then homogenizing using an electric magnetic stirrer at intermediate speed for approximately 10 minutes. The table containing the proportions of components used in the 36 formulations is shown in Table 1.

Table 1 – Table of proportions of the components of the formulations

(to be continued)

Point	Oil (%)	Tween 20 (%)	Water (%)	Point	Oil (%)	Tween 20(%)	Water (%)
1	10	80	10	19	30	30	40
2	20	70	10	20	20	30	50
3	10	70	20	21	10	30	60
4	30	60	10	22	70	20	10
5	20	60	20	23	60	20	20
6	10	60	30	24	50	20	30
7	40	50	10	25	40	20	40
8	30	50	20	26	30	20	50

Table 1 – Table of proportions of the components of the formulations

(conclusion)

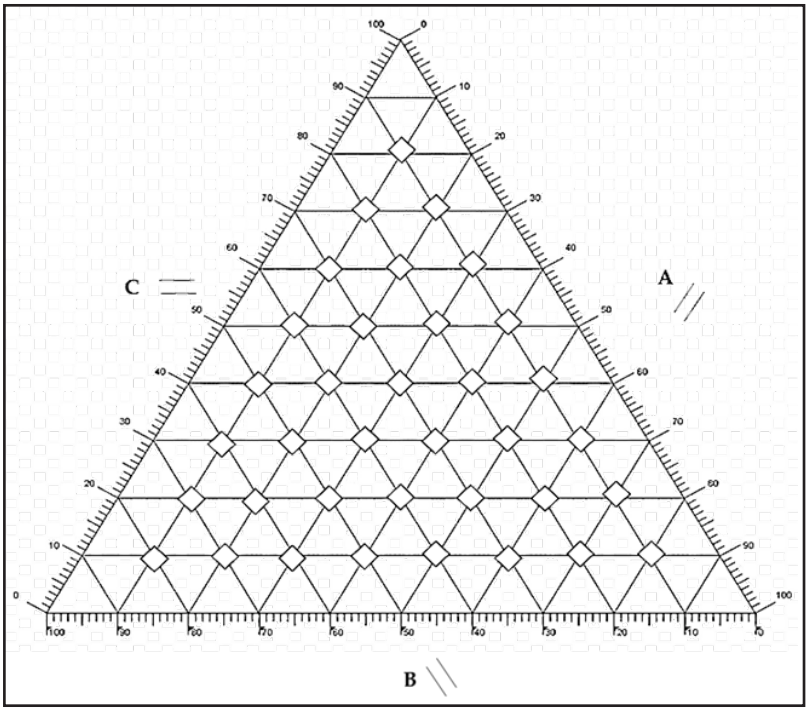
Point	Oil (%)	Tween 20 (%)	Water (%)	Point	Oil (%)	Tween 20 (%)	Water (%)
9	20	50	30	27	20	20	60
10	10	50	40	28	10	20	70
11	50	40	10	29	80	10	10
12	40	40	20	30	70	10	20
13	30	40	30	31	60	10	30
14	20	40	40	32	50	10	40
15	10	40	50	33	40	10	50
16	60	30	10	34	30	10	60
17	50	30	20	35	20	10	70
18	40	30	30	36	10	10	80

Source: Bógea (2024)

The formulations made from Table 1 were kept at rest at room temperature for 24 hours. After this period, the visual classification of the stitches was performed, according to their physical aspect, in liquid emulsion (LE), gel emulsion (GE), liquid microemulsion (LME), gel microemulsion (GME), transition or phase separation (TPS). Being classified as LE the homogeneous formulations of white color and fluid consistency, GE those that presented homogeneous mixtures of white color and gel consistency, LME the homogeneous mixtures translucent and fluid, GME the gel consistency formulations of translucent aspect. The points that presented cloudy aspect and TPS were classified as transition, those that did not mix homogeneously. For this study, only formulations classified as LME and GME were used.

The pseudo-ternary diagram was constructed using the data, according to the example in Figure 1. Stable microemulsions were selected for continuity of work.

Figure 1 – Pseudo-ternary diagram for classification of formulations in the proportions established



Source: Authors (2024)

2.7 Stability study of microemulsions

The formulations were submitted to 3600 rpm for 30 minutes and classified again as microemulsion (ME) or phase separation (PS), and PS was excluded from the following experiments.

The microemulsions that remained stable after centrifugation were subjected to alternating exposure in a refrigerator at 5º C + 1º C and a greenhouse at 40ºC, every 24 hours for 14 days. After the established period, the samples that separate the phases were excluded from the study and those that remained stable were submitted to the following tests.

All stable formulations were repeated with the addition of the essential oil being used at the concentration of 2% (m/m), and the required amount was solubilized in absolute and homogenized ethanol using an electric magnetic stirrer at intermediate speed for approximately 10 minutes.

2.8 Determinations of the refraction index

The refractive index of *Attalea speciosa* oil (babassu) and *Citrus* × *aurantium* L. (sour orange) was determined with the aid of the Abbé Refractometer apparatus, initially calibrated according to the refractive index of distilled water, at 25ºC, informed by the manufacturer, of 1.332.

2.9 Anti-inflammatory activity *in vitro* by Protein Denaturation

In this assay, all stable formulations with and without the addition of essential oil were evaluated. Anti-inflammatory activity was evaluated by the protein denaturation method (Padmanabhan & Jangle, 2012). The reaction mixture (4 mL) consisted of 2 mL of different concentrations of essential oil (100-500 mg L^{-1}) and 2 mL of a 10% solution of albumin diluted in PBS and incubated at (37±1) °C for 15 minutes.

Denaturation was induced by keeping the reaction mixture at 60°C in a water bath for 10 minutes. After cooling, absorbance was measured at 660 nm. Inhibition of protein denaturation was expressed as a percentage and The Efficient Concentration 50% (EC_{50}/IC_{50}) capable of inhibiting 50% of denaturation was expressed in mg L^{-1}.

3 RESULTS AND DISCUSSIONS

3.1 Stability of Microemulsions

After the 14-day period, it was possible to identify the formation of only 2 stable emulsions among the others, among them only a liquid microemulsion was stable containing 10% of the oil, 80% of tenso active and 10% of water was used in this study.

3.2 Total phenolics

Table 2 presents the total phenolic content (mg EAT g^{-1}) for *Attalea speciosa* oil and *Citrus* × *aurantium* L. essential oil.

Table 2 – Total phenolic content (mg EAT g^{-1}) for *Attalea speciosa* (babassu) and *Citrus* × *aurantium* L. (sour orange)

Species	Total phenolic content (mg EAT g^{-1})	Line equation	R^2
Citrus × *aurantium*	232,2	y=0,0586x+0,06	0,9998
Attalea speciosa	238,01		

Source: Bogéa (2024)

According to Table 2, the vegetable oil *of Attalea speciosa* (babassu) presented a higher number of phenolic compounds than *Citrus* × *aurantium* essential oil*.* However, both showed similarity in the quantity of total phenolics.

The presence of total phenolics in *Attalea speciosa* oil is also pointed out by Amorim (2020) when identifying a large amount of total phenolics in babassu oil, and its range ranges from 191.8 mg EAG 100 g^{-1}.

Results observed are also proven by Oliveira (2018) who evaluated the content of total phenolic compounds in the mesocarp of babassu *(Orbignya phalerata,* Mart) finding values ranging from 185.53 to 1257.25 mg GAE 100 g^{-1} extract, showing a high value of compounds. Vieira et al. (2011), identified the total phenolic content of 98.3 mg EAG 100 g^{-1} in the mesocarp of babassu. Compared to these authors, the value found in the present study for babassu oil presented higher phenolic content, 232.2 mg EAT g^{-1}.

For the essential oil of *Citrus* × *sinensis*, the phenolic content found was close to that observed in the study by Costa et al. (2016) by quantifying 0,0990 µg EAG $µg^{-1}$, species belonging to the same family as the present study. While the results found by Ussevane (2014) found a phenolic content in the houses of *Citrus sinensis* of only 31.76 mg EAT g^{-1}.

The differences in the quantities of phenolic compounds when compared to the literature are probably due to the different extractions used as well as the patterns

used to determine the total content of phenolic compounds (Ussevane, 2014), or the agricultural conditions in which the plants were submitted, as well as storage and processing conditions thereof (Silveira, 2013).

These phenolic compounds are defined as substances that have an aromatic ring with one or more hydroxyl substitutes, including their functional groups (Malacrida, 2005). Its important quantification in this study is because they are found widely in fruits and vegetables, such as in citrus fruit husks and seeds, besides being responsible for several biological potentials such as antioxidant and anti-inflammatory (Faller, 2009).

3.3 Chemical constituents of *Citrus×aurantium* l essential oil.

Through Gas Chromatography coupled to Mass Spectrometry (GC/MS) the major compound of the essential oil of *Citrus×aurantium* L. bark was (-)-Terpinen-4-ol (32.56%), followed by caryophylene oxide (23.52%).

Assunção (2013) identified in total, 15 different constituents with concentrations from 0.06 to 89.55%, corresponding to 100% of the oil composition. The main constituents present in the essential oil of *Citrus sinensis* L. Osbeck bark were monoterpene: d-Limoneno (89.55%) as the majority constituent, followed by monoterpene oxygenated with Linalool (3.85%) and beta-myrcene monoterpene (1.90%).

Leão (2015) identified a total of 10 compounds from the essential oils of the dried and fresh shells of *Citrus sinensis,* among them α-terpineol (14.45%), β-citral (13.35%), linalool (12.12%), octanal (8.01%) and α-terpineno (6.07%), as majority constituents. The chemical constitution of the essential oil is quite varied and can be formed from terpenoid derivatives or phenylpropanoids. In which terpenoids represent approximately 90% of the chemical composition of essential oil (Bakkali et al., 2008).

3.4 Determination of the refraction index

According to the literature, the refraction index of *Attalea speciosa* oil ranges

from 1.448 to 1.455 (Viana, 2022). In the study conducted by Viana et al. (2006) at temperatures of 28°C and 34°C, the refraction indices for *Attalea speciosa* oil were 1.448 and 1.450, respectively. While the value found in the studies of Araújo et al. (2016) was 1.467 at 27 °C. According to Queiroga Júnior (2016), vegetable oils with refraction rates in the range of 1,466 – 1,477 are suitable for consumption (Araújo et al., 2016).

Table 3 – shows the refraction index found for the vegetable oil of *Attalea speciosa* and *Citrus* × *aurantium* L. (sour orange) vegetable oil

	Refractive index (nD 25°)
Essential oil: *Citrus* × *aurantium*	1.470
Vegetable oil: *Attalea speciosa*	1.454

Source: Bogéa (2024)

Analyzing the work carried out by Silvia et al. (2019) for *Attalea speciosa* oil at 40ºC, the refraction index 1.447 was found. Being like the index determined in this study. In addition, the physicochemical parameters of essential oils are important not only for determining quality, but also for controlling their purity (Assunção, 2013).

In an analysis of the essential oil of *Citrus sinensis*, Leão (2015) found the values of refraction index at 20 °C of 1.471 and 1.476 for the dried and fresh peels of the fruit, respectively. Assunção (2013) in his study for *Citrus*×*aurantium* essential oil at 25ºC presented refraction index 1.476. Compared to another fruit of the *Citrus* species*, Citrus limon* Lineo, the study of physical-chemical parameters of essential oils made by Boukhennoufa (2011) obtained a refraction index of 1.457.

According to the standards for essential oils adopted by the European Pharmacopoeia and ISO, the values of the refraction index for *Citrus* essential oil should be between1,464 and 1.474 (Martins et al., 2011). Thus, the essential oil of this study is in accordance with the standards established.

The determination of this index has great utility in the control of hydrogenation processes, not only for oils, but also for fats, whose indicated temperature is 40° C (Lima et al., 1998). The refraction index is characteristic for each type of oil, within certain limits. It is related to the degree of saturation of the bonds but is affected by other factors such as: free fatty acid content, oxidation, and heat treatment. When the light changes from the medium of propagation, it usually suffers a change in the direction of this propagation, calling this phenomenon of refraction. Oils and fats have different powers of regency according to their nature. They can deflect the light rays with greater or lesser intensity. Thus, the refraction index is used to determine the degree of purity of substances, being a simple and fast method (Kobori & Jorge, 2005).

3.5 Anti-inflammatory activity

Table 4 presents the results of the Efficient Injunction Concentrations (EC_{50}) resulting from the protein denaturation processes, thus evaluating the anti-inflammatory activities of *Attalea speciosa* vegetable oil, *Citrus* × *aurantium* L. and formulated microemulsion.

When we observe Table 4, it can be noticed that the vegetable oil of *Attalea speciosa* presents the EC_{50} lower than that of the essential oil of *Citrus* × *aurantium* L., thus the *Attalea speciosa* oil presents higher anti-inflammatory activity. While the formulated microemulsion presented the lowest inhibitory concentration among the others, demonstrating that the incorporation of *Citrus* × *aurantium* L. essential oil enhanced the anti-inflammatory effect, making the anti-inflammatory activity of the microemulsion better than that of *Attalea speciosa* oil.

The inflammatory activity observed in Table 4 is described by Dos Santos (2019), conducting an *in vivo* study with induced ear-induced edemas evaluating the anti-inflammatory activity of babassu and licuri oils. In the edema induced by aquidonic acid it was possible to observe that the dose of 10 µL of *Attalea speciosa* oil, *Syagrus coronata*

and the basilic acid showed anti-inflammatory activity, once, that there was a reduction in edema formation when compared with negative control. The preclinical evaluation of the therapeutic effects of babassu demonstrates that this product has antitumor, anti-inflammatory properties and antimicrobial properties (Barroqueiro et al., 2011).

Table 4 – Anti-inflammatory activity for *Attalea speciosa,* Citrus × *aurantium* L. essential oil and formulated microemulsion

	EC_{50} (µg mL^{-1})	**Straight Equation**	**R^2**
Essential oil: *Citrus × aurantium*	82,42	y= 40,442x – 27,488	0,9934
Vegetable oil: *Attalea speciosa*	31,59	y= 34,164x – 1,2286	0,9944
Microemulsion: *Attalea speciosa* + *Citrus × aurantium*	2,26	y= 17,003x – 43,982	0,9900

Source: Bogéa (2024)

Different anti-inflammatory drugs can be used in the treatment of inflammatory diseases, in which it acts by inhibiting the expression of the phospholipase Enzyme A_2 with a release of AA, as well as anti-inflammatory drugs that block only the action of COX, such as indomethacin (Santos, 2015; Saraiva et al., 2011). Thus, it can be observed that possibly the anti-inflammatory action attributed to babassu oil, licuri and uric acid is related to the blockade of COX enzymes (Santos, 2019).

The study conducted by Reis et al. (2017) through edema in the ears of PMA-induced mice, evaluated the anti-inflammatory activity of *Attalea speciosa* oil and it was observed that babassu oil administered topically (3 and 10 L/ear) was able to inhibit PMA – induced ear edema in 19.1 (<0.05) and 54.1% (<0,001), respectively. The uric acid (4mg/ear), the main fatty acid of babassu oil, showed 90.3% inhibition (<0,001). When administered orally, babassu oil (100, 300 and 1000 mg/kg) showed 23.5 (<0.05), 39.7 and 51.9% inhibition of edema (<0,001), evidencing the anti-inflammatory activity of this oil.

Santos et al. (2020) conducted *in vitro* and *in vivo* studies using babassu oil in wound healing and concluded that the oil stimulates fibroblasts to migration and modulates the LPS-induced inflammatory response in peritoneal macrophages of mice. *In vivo,* babassu oil was able to accelerate the healing process in a wound model with full thickness splint due to an increase in the number of fibroblasts, blood vessels and collagen deposit positioned in the wounds. Babassu oil also increased the recruitment of inflammatory cells to the wound site and showed an anti-inflammatory effect on a chronic ear edema.

No studies of babassu oil were found to evaluate anti-inflammatory activity by protein denaturation.

On the other hand, *Citrus×aurantium* essential oil also exerts anti-inflammatory action (Sarrou et al., 2013). *Citrus* flavonoids contain compounds with anti-inflammatory activity, due to the presence of regulatory enzymes (protein kinase C, phosphodiesterase, phospholipase, lipoxyromase and cyclooxygenase) that control the formation of biological mediators, responsible for the activation of endothelial cells and specialized cells involved in inflammation (Tripoli et al., 2007).

Wei (2010) in his study on the inhibiting activity and antioxidants of essential oils found linalool and linalila acetate as the main constituents of *Citrus×aurantium* essential oil, these have anti-inflammatory actives, in this same study *Citrus×aurantium* oil showed slight inhibiting lipoxygenase activity at 5 µg/mL. Because of their involvement in lipid oxidation and inflammation, lipoxygenases have been implicated in the development of inflammatory vascular diseases such as atherosclerosis and diabetes (Miguel, 2010).

The study by Yang et al. (2010) with *Citrus sunki* essential oil in skin pathogens, the anti-inflammatory activity of this oil whose limonene predominated in its composition was identified, and the inhibition of nitric oxide induced by inflammatory mediators was identified, stimulating the production of RAW 264.7 cells in a dose-dependent manner, indicating that they had an anti-inflammatory character.

In this study it was possible to identify the anti-inflammatory activity of

Citrus×*aurantium* essential oil. Compared to studies conducted by Janakiraman (2014) who used the same protein denaturation method to measure the anti-inflammatory activity of *Coleus amboinicus* Lour leaf (coarse mint), it was concluded that the essential oil of *Citrus* × *aurantium* (orange-sour) used in this study presented EC_{50} of 82.42 µg/mL lower than that of *Coleus amboinicus* Lour of 98.28 µg mL^{-1}. Thus, *Citrus*×*aurantium* essential oil exhibits higher anti-inflammatory activity than coarse mint.

Inflammation is a complex response of the body, always with the intention of eliminating the cause of tissue lesions or areas of necrosis. Thus, inflammation has protective function against the action of harmful agents (e. g. microorganisms, toxins, radiations) to eliminate them. Thus, without this response, infections could continue, and healing would not exist (Patil & Patil, 2017).

Drugs with anti-inflammatory properties are used for the treatment and cure of acute and chronic inflammatory diseases (Hilário et al., 2006). Another class of drugs of interest to the pharmaceutical industry with anti-inflammatory properties are herbal medicines (Ghasemian et al., 2016). Different medicinal plants have been a promising source of bioactive molecules with pharmacological properties to produce new drugs (Veiga Junior et al., 2005), emphasizing the innovation of this study by bringing a new formulation with broad application potential.

4. CONCLUSION

Throughout the study, it was possible to conclude that the essential oil of *Citrusxaurantium* obtained is within the legislative standard in view of the physical-chemical parameters and their majority composition. A stable microemulsion was observed, containing 10% of the oil, 80% of tenso active and 10% of water. The total phenolic content and refraction index of both oils presented satisfactory values and according to the literature and other studies. After the incorporation of the essential oil in the formulated microemulsion, it was possible to observe the increase of its

anti-inflammatory potential. Thus, it can be concluded that the essential oil of *Citrus x aurantium* increases the ability to fight inflammations of a microemulsion containing *Attalea speciosa* oil.

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