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Chemistry

Chemical characterization and biotechnological potential of *Salvia rosmarinus* Spenn essential oil nanoemulsions

Caracterização química e potencial biotecnológico de nanoemulsões de óleo essencial de *Salvia rosmarinus* Spenn

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ABSTRACT

This study aimed to determine the total phenolic compounds, evaluate the antioxidant and anti-inflammatory activities of nanoemulsions (O/W) and essential oils (EOs) from Salvia rosmarinus (rosemary). The plant material was obtained in the city of São Luís (MA). The EO was obtained by the hydrodistillation technique in a modified Clevenger extractor, and the NOE's by phase inversion. The chemical constituents of EO were determined by GC-MS. The determination of total phenolic compounds (TPC) was performed by the Folin-Ciocalteu method. The anti-inflammatory activity was performed by the method of protein denaturation, and the antioxidant activity was performed by the spectrophotometric method of scavenging hydroxyl radicals. The GC-MS allowed quantifying 1,8-cineol (30.22%), α-pinene (22.14%), camphor (18.33%), and camphene (10.36%) as major components of the EO. The TPC of the EO was quantified at 26.74 mg EAT g^{-1} and the refractive index at 1.466 nD 25°. In the antioxidant activity test, an EC_{50} of 80.33 mgL^{-1} was obtained for the EO and from 19.56 to 408.85 mg L^{-1} for the nanoemulsions. In the anti-inflammatory activity assay, an EC_{50} of 62.46 mgL^{-1} was obtained for EO and 64.96 to 4220.25 mg L^{-1} for NOE's. Finally, the pharmacological activities tested showed efficient values for EC_{50}, therefore being considered active. This activity is attributed to the chemical compounds present, thus encouraging studies with this species aiming at its potential application in a formulated bioproduct.

Keywords: Rosemary; Anti-inflammatory; Nanoemulsion

RESUMO

Este estudo teve como objetivo determinar os compostos fenólicos totais, avaliar as atividades antioxidante e anti-inflamatória de nanoemulsões (O/A) e óleos essenciais (OEs) de *Salvia rosmarinus* (alecrim). O material vegetal foi obtido na cidade de São Luís (MA). O OE foi obtido pela técnica de hidrodestilação em extrator Clevenger modificado e as NOE´s por inversão de fases. Os constituintes químicos do OE foram determinados por CG-EM. A determinação de compostos fenólicos totais (CFT) foi realizada pelo método de Folin-Ciocalteu. A atividade antiinflamatória foi realizada pelo método de desnaturação de proteínas e a atividade antioxidante pelo método espectrofotométrico de sequestro de radicais hidroxila. A CG-EM pertimitiu quantificar 1,8-cineol (30,22%), α-pineno (22,14%), cânfora (18,33%) e canfeno (10,36%) como componentes majoritários do OE. O CFT do OE foi quantificado em 26,74 mg EAT g^{-1} e o índice de refração em 1,466 nD 25°. No teste de atividade antioxidante, obteve-se EC_{50} de 80,33 mgL^{-1} para o OE e de 19,56 a 408,85 mg L^{-1} para as nanoemulsões. No ensaio de atividade antiinflamatória, obteve-se CE_{50} de 62,46 mgL^{-1} para OE e 64,96 a 4220,25 mg L^{-1} para as NOE´s. Por fim, as atividades farmacológicas testadas apresentaram valores eficientes para CE_{50}, sendo assim consideradas ativas. Essa atividade é atribuída aos seus compostos químicos presentes, incentivando assim estudos com essa espécie visando sua potencial aplicação em um bioproduto formulado

Palavras-chave: Alecrim; Anti-inflamatório; Nanoemulsão

1 INTRODUCTION

Nanoemulsions can be defined as heterogeneous systems, in which a liquid (the internal phase) is dispersed in another (the external phase) in the form of nanometer-sized droplets, in the presence of an emulsifying agent (Fronza et al., 2007). This system has several advantages that increase solubility and provide better physical stability, in addition to better biologic activity at lower concentrations and uniform dispersion of hydrophobic compounds throughout the celular matrix (Noori, Zeynali, & Almasi, 2018). Other advantages include the controlled emissions of an effective formulation and the development of evaporation and loss prevention (Syed, Banerjee, & Sarkar, 2020).

These formulations are characterized by their thermodynamic stability and small droplets, ranging from 20 to 200 nm and have a wide variety of industrial applications (Izquierdo et al., 2002; Tadros et al., 2004; Ostertag et al., 2012).

Nanoemulsions are promising systems for drug delivery with low solubility in water and have already been proposed to be associated with the essential oil of *Salvia* *rosmarinus* Spenn (Duarte et al., 2015). it is popularly known as rosemary, being an aromatic plant with needle-shaped leaves belonging to the Lamiaceae family. It has the characteristics of being a subshrub with green branches, having woody stems and small leaves (Silva et al., 2008; Macedo, 2020).

This is a medicinal plant native to the Mediterranean region and cultivated all over the world. In addition to being commonly used as a condiment and food preservative, it is widely used in cosmetics and flavoring agents, it has antibacterial, cytotoxic, antimutagenic, antioxidant, anti-inflammatory and chemopreventive properties (De Oliveira, 2019; Hussain et al., 2010).

The EO of *S. rosmarinus* is characterized by being EO with high concentrations of some monoterpenes, such as α-pinene, limonene, 1,8-cineol, borneol and camphor, which are known to inhibit seed germination, growth and seedling survival of many plant species (De Martino et al., 2012; Maccioni et al., 2019).

Salvia species EO was shown to inhibit the 5-LOX enzyme (IC_{50} = 36.15 ± 1.27 mg/L), which may be due to the presence of 1,8-cineole (22.22%), myrcene (0.96%), β-caryophyllene (0.27). %), alpha-pinene (1.71%), beta-pinene (1.29%), camphene (4.88%), borneol (2.64%) have been found to be involved in the treatment of some infections and effectively inhibit the inflammation called enzymes (El Euch et al., 2019; El Jery et al., 2020).

S. rosmarinus contains an abundance of properties attributed to its secondary metabolites, but few reports relate these activities to its nanoemulsions, thus resulting in the relevant interest in exploring the particularities of its biological activities. Thus, this study aimed to evaluate the chemical constituents and biotechnological potential of nanoemulsions (O/W) of *Salvia rosmarinus* EO formulated by phase inversion.

2 MATERIAL AND METHODS

2.1 Plant material

Leaves of *S. rosmarinus* were acquired in the municipality of São Luís (MA) from the certified distributor of Produtos Naturais Muniz LTDA. The sample was sent to the Laboratory for Research and Application of Essential Oils (LOEPAV/UFMA) for extraction of the EO.

2.2 Obtaining the essential oil

For extraction of the EO, the technique of hydrodistillation – Farmacopeia Brasileira, with a glass Clevenger extractor coupled to a round bottom flask placed in an electric blanket as a source of heat. 200 g of dried and crushed leaves of *S. rosmarinus* were used, adding distilled water (1:8) to the plant material contained in the flask. Hydrodistillation was conducted at 100°C for 3h and then the extracted EO was collected. The EO was dried by percolation with anhydrous sodium sulfate (Na_2SO_4). The sample was stored in an amber glass vial under refrigeration at 4°C.

2.3 Chemical constituents

The EO constituents were identified by gas chromatography coupled to mass spectrometry (GC-MS). 1.0 mg of the sample was dissolved in 1000 µL of dichloromethane (99.9% purity).

The analysis conditions were as follows: Method: Adams. M; Injected volume: 0.3µL; Column: Capillary HP-5MS (5% diphenyl, 95% dimethyl polysiloxane) (equivalent DB-5MS or CP-Sil 8CB LB/MS), in dimensions (30m x 0.25 mm x 0.25 µm); Carrier gas: He (99.9995); 1.0 $mL.min^{-1}$; Injector: 280ºC, Split mode (1:10); Oven: 40ºC (5.0 min.) to 240ºC at a rate of 4ºC min^{-1}, from 240ºC to 300ºC (7.5 min) at a rate of 8ºC.min^{-1}); tT=60.0 min; Detector : EM; IS (70 eV); Scan mode (0.5 sec scan-1); Mass range: 40–500 daltons(one); Line transfer: 280º C.; Filament: off 0.0 to 4.0 min; Linear quadrupole type mass spectrometer. For the identification of the compounds in the sample, the program AMDIS (Automated Mass spectral Deconvolution Mass & Identification System) was used.

2.4 Formulation of nanoemulsions

The preparation of nanoemulsions was carried out according to the adapted methodologies described by Lima et al. (2020), Sugumar et al. (2014), Kubitschek et al. (2014) and Rodrigues et al. (2014). The oil-in-water nanoemulsion was formulated with essential oil, non-ionic surfactant (Tween 20 and Tween 80) and water. The required amounts of each oil phase constituent (oil+surfactant) were heated to 65 ± 5°C. The aqueous phase was separately heated to 65 ± 5°C, providing a primary formulation, by the phase inversion method.

To prove the stability, the formulated emulsion was submitted to different stress tests (Shafiq et al., 2007). Heating-cooling cycle: it was carried out by keeping the formulated nanoemulsions at 40 and 4°C, alternating each temperature for 48h. The cycle was repeated three times. Freeze-thaw stress: nanoemulsion alternately at -21 and 25°C for 48h at each temperature. The cycle was repeated three times. Formulations that passed thermodynamic stress tests were carried over to further studies.

2.5 Spectrophotometric determination of Total Phenolic Content (TPC)

The determination of the total phenolic compounds of the EO and of the nanoemulsions was carried out with an adaptation of the Folin-Ciocalteu method (Waterhouse, 2006). 5 mg of diluted EO or nanoemulsions were used in 1 mL of 70% ethanol. To this solution, 7 mL of distilled water, 800 μL of Folin-Ciocalteu reagent and 2.0 mL of 20% sodium carbonate were added. After two hours, the reading was performed in a UV-VIS spectrophotometer at a length of 760 nm. The standard curve was expressed in mg L^{-1} of tannic acid.

2.6 Refractive index determinations

The refractive index of the EO and the formulations was determined with the aid of the Abbé Refractometer device, initially calibrated according to the refractive index of distilled water at 25ºC, informed by the manufacturer, of 1.332.

2.7 Determination of Antioxidant Activity

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

EO and nanoemulsions in different concentrations of 10-100 mg L^{-1} were dissolved in 0.2% DMSO and distilled water, respectively. To these concentrations, 1 mL of salicylic acid (9 mM), 1 mL of ferrous sulfate (9 mM) and 1 mL of hydrogen peroxide (9 mM) were added. Ascorbic acid was used as a positive standard. The reaction mixture was incubated for 60 min at 37°C in a water bath; after incubation, the absorbance of the mixtures was measured at 510 nm using a UV/VIS spectrophotometer and the EC_{50} calculated.

2.8 Determination of Anti-Inflammatory Activity

The 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 EO, as well as for the formulations (50-500 mg L^{-1}) and 2 mL of a 10% albumin solution 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, the absorbance was measured at 660nm. Inhibition of protein denaturation was expressed as a percentage and the 50% Efficient Concentration (EC_{50}/IC_{50}) capable of inhibiting 50% of denaturation was expressed in mg L^{-1}.

3 RESULTS AND DISCUSSION

3.1 Chemical constituents

The Table 1 lists the components found in the sample with their respective retention times. The 12 compounds were identified reaching a total of 100% of the composition, in this total are the major compounds such as 1,8-cineol (30.22%), α-pinene (22.14%), camphor (18.33%) and camphene (10.36%).

Table 1 - Identification of chemical constituents in the essential oil sample of *Salvia rosmarinus*

Peak	Retention Time (min)	Compounds NIST08	Content (%)
1	6.01	α-pinene	22.14
2	6.41	camphene	10.36
3	7.22	β-pinene	5.98
4	8.26	*P*-Cymene	1.08
5	8.37	limonene	1.88
6	8.51	1,8-Cineole	30.22
7	9.16	Linalool	0.75
8	10.28	Isopulegol 2	1.55
9	11.88	Camphor	18.33
10	13.19	α-Terpineol	3.01
11	13.60	Verbenone	2.01
12	19.70	β-caryophyllene	2.69

Source: Autorship (2024)

Chemical composition like this one was identified by Zaouali et al. (2010), who found, among the 25 identified components, 1,8-cineol (40.0%), camphor (17.9%), α-pinene (10.3%), and camphene (6.3%) as major components.

Similarly, Bouyahya et al. (2017) also presented as the main components of the EO of *S. rosmarinus*, α-pinene (14.07%), 1,8-Cineol (23.67%) and camphor (18.74%) among the 29 components identified.

In another study proposed by Sales and Sales (2020), 19 compounds were identified in the EO of *S. rosmarinus*, corresponding to 96.57% of the EO. The major components were 1.8 cineole (21.8%), α-pinene (18.7%), camphor (14.6%), linalool (13.4%) and camphene (7.2%). Maia et al. (2014, p. 330) identified the following composition:

1,8-cineole (44.39%), camphor (19.75%), α-pinene (12%) and β-caryophyllene (4.53%).

So far, about 150 chemical compounds have been identified in *S. rosmarinus* EO samples, the most frequently reported molecules being the monoterpenes 1,8-cineole, α-pinene and camphor (Borges et al., 2019). The presence of monoterpenes in EOs is widely reported in several studies as the main metabolites. They are responsible for several biological activities, including the allelopathic effect (Filho et al., 2009; Sawi et al*.,* 2019; Assaeed et al., 2020).

Comparing the results for the EO of *S. rosmarinus* studied with those available in the reference literature, there were similarities between the values found, with differences being observed only in their concentration levels and in the number of compounds found. According to Zaouali et al. (2010), these differences may depend on factors such as the time and stage of harvesting the plant, plant age, soil type, climate and EO extraction method, since these are effective in the effective constituents of the plant.

3.2 Refractive Index and Total Phenolic Content (TPC)

The results for the refractive index and total phenolic content of the EO and O/W of the plant material are shown in Table 2.

The *S. rosmarinus* EO sample had a refractive index of 1.466. According to Santos et al., (2005), Brazilian *S. rosmarinus* oils had an average refractive index of 1.468, an average optical rotation of +11.82. Carreiro et al. (2020) found similar values for commercial samples and a cultivated sample of *R. officinalis*, namely 1.466, 1.469 and 1.471 respectively.

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According to the Farmacopeia Brasileira (2019), *S. rosmarinus* EOs have a refractive index in the range of 1.460 - 1.476. Similarly, the specifications recommended by the International Standard Organization (ISO) establish, for *S. rosmarinus*, a refractive index in the range of 1.464 - 1.472 according to ISO 1342:2012.

Table 2 – Refractive index and total phenolic content of essential oil and stable nanoemulsions from *S. rosmarinus*

ID	Refractive index (nD 25°)	TPC (mg EAT g^{-1})	Equation	R^2
EO	1,466	26.74		
NEO1	1,341	7.32		
NEO2	1,343	6.59		
NEO3	1,344	7.44	y=0.3151x-0.1813	0.998
NEO4	1,338	6.56		
NEO5	1,344	6.73		
NEO6	1,342	7.05		

where; EO- essential oil; NEO– Stable essential oil nanoemulsion formulated by phase inversion; 1 – Nanoemulsion composition 2.5% essential oil, 2.5% Tween 80 and 95% H_{2O}; 2 – Nanoemulsion composition 2.5% essential oil, 5.0% Tween 80 and 92.50% H_2O; 3 – Nanoemulsion composition 2.5% essential oil, 7.5% Tween 80 and 90.0% H_2O; 4 – Nanoemulsion with 2.5% essential oil, 2.5% Tween 20 and 90.0% H_2O; 5 – Nanoemulsion with 2.5% essential oil, 5.0% Tween 20 and 92.50% H_2O; 6 – Nanoemulsion with 2.5% essential oil, 7.5% Tween 20 and 92.50% H_2O. Autorship (2024)

This physical-chemical parameter is used to identify and determine the purity of compounds and to analyze the composition of homogeneous binary mixtures of known constituents. This property is used to control the purity of EOs, which have a characteristic refractive index (Bandoni, 2000). Observing the values for the EO studied in this work and those in the literature, it can be seen that there are similarities between them, with the refractive index of the sample within the standards established by the Farmacopeia Brasileira (2019).

Table 2 also presents the results of nanoemulsions (O/W) in different concentrations, these values are lower, having the EO as a reference, but still close results. According to Instituto Adolfo Lutz (1985), the refractive index is characteristic for each type of oil, within certain limits.

This is related to the degree of bond saturation but is affected by other factors such as free fatty acid content, oxidation and heat treatment. Nanoemulsions, being a nanometric material, which have a reduced band size, have particular physicochemical properties in relation to materials structured on a macroscopic scale (Duran, 2006).

The total phenolic content (TPC) of the EO, as well as the O/W, were shown in Table 2. The total phenolic content (TPC) was expressed as tannic acid equivalent (mg EAT/g of plant material) the equation of the straight line obtained was y = 0.3151x – 0.1813 (R^2 = 0.998), y absorbance being x the equivalent concentration of tannic acid.

The TPC result for the EO of *S. rosmarinus* in this study showed a value of phenolic compounds of 26 mg EAT g^{-1}. Silva *et al.* (2011) reported a similar result, through a study with aqueous extract of *S. rosmarinus*, on an equivalent basis in grams of gallic acid, an expressive number of phenolic compounds with an average of 30.70 mg g^{-1}. The total phenolic content for the EO of the species under study has few references available in the literature, thus highlighting the importance of this study.

The TPC content of *S. rosmarinus* is also confirmed by Wang et al. (2018) and Moczkowska et al. (2020). In a study carried out by. Wang et al. (2018) reported the amount of TPC in the range of 10.30 to 160.70 mg EAG g^{-1} of extract.

*S. rosmarinus*is recognized as a potential source of phenolic compounds, highlighting three major ones: phenolic acids, flavonoids and phenolic diterpenes (Silva, 2012). The biological activities of this plant have been related to its phenolic compounds and its volatile constituents, such as α-pinene, bornyl acetate, camphor and 1,8-cineol present in the essential oil of this plant (Babovic et al., 2010).

The presence of phenolic compounds has been constantly related to antioxidant potential and other biological activities, such as anti-inflammatory (Bastianetto et al., 2000; Silva et al., 2008; Liu et al., 2014; Lou et al., 2014).

3.3 Antioxidant activity

Table 3 shows the antioxidant activity of the EO and the stable formulations.

From the equations of the line, the respective values of the 50% effective concentration (EC_{50}) responsible for the inhibition of 50% of hydroxyl radicals were calculated.

It can be seen in the results of Table 3, the EC_{50} values of the samples under study, as well as the respective equations of the lines obtained from the ascorbic acid standard. The EC_{50} value for the *S. rosmarinus* EO was 80.33 mg L^{-1}, its nanoemulsions showed superior results, except for the NEO6 formulation (nanoemulsion with 2.5% EO and 7.5% Tween 20) with the value of 19.56 mg L^{-1}.

Table 3 – EO antioxidant activity and stable formulations

ID	EC_{50} (mg L^{-1})	Equation	R^2
EO	80.33	y=144.22x-224.72	0.9882
NEO1	408.85	y= 26.733x-19.815	0.9929
NEO2	157.41	y= 31.528x-19.268	0.9919
NEO3	82.51	y=36.125x+19.234	0.9998
NEO4	110.51	y=59,963x-72,529	0.991
NEO5	109.03	y=35.808x-22.961	0.9995
NEO6	19.56	y= 39.916x+1.5459	0.9907

where; EO- essential oil; NEO– Stable essential oil nanoemulsion formulated by phase inversion; 1 – Nanoemulsion composition 2.5% essential oil, 2.5% Tween 80 and 95% H_2O; 2 – Nanoemulsion composition 2.5% essential oil, 5.0% Tween 80 and 92.50% H_2O; 3 – Nanoemulsion composition 2.5% essential oil, 7.5% Tween 80 and 90.0% H_2O; 4 – Nanoemulsion with 2.5% essential oil, 2.5% Tween 20 and 90.0% H_2O; 5 – Nanoemulsion with 2.5% essential oil, 5.0% Tween 20 and 92.50% H_2O; 6 – Nanoemulsion with 2.5% essential oil, 7.5% Tween 20 and 92.50% H_2O; Autorship (2024)

The studied samples showed effective concentrations lower than 500 mg L^{-1}, according to Campos et al. (2003), natural products with this level of concentrations have antioxidant activity.

According to Sousa et al. (2007), the lower the EC_{50} value, the greater the antioxidant activity of the plant compound, since a lower concentration of essential oil is required to reduce the hydroxyl radical by 50%. Thus, highlighting the NEO6 formulation with EC_{50} of 19.56 mg L^{-1}, which is the best result for antioxidant activity.

Wanderley (2016) presented EC_{50} in different samples of this EO by the DPPH free radical scavenging method, concentrations ranging from 33.44 to 36.43 mg mL^{-1} were observed. It is known that the antioxidant activity of an EO is primarily related to the genetics of the plant and the climatic conditions of its growth, and as a second parameter the process of extracting the EO.

Antioxidant activity is also one of the biological activities that have been reported for both EO and its isolated compounds. Scientific evidence concluded that the EO of *S. rosmarinus* has greater antioxidant activity due to its major compounds, namely 1,8-cineole, α-pinene and camphor (Wang et al., 2018).

According to Takayama et al. (2016) monoterpenes are among those responsible for the antioxidant action of *S. rosmarinus* EO. The literature demonstrates that *S. rosmarinus*, in the form of its EO, has antioxidant capacity. However, the composition of the oil must be taken into account, considering that environmental and cultural factors can alter the antioxidant response (Tiuzzi & Furlan, 2016).

3.4 Anti-inflammatory activity

Table 4 presents the results of the anti-inflammatory activity of the EO and nanoemulsions of *S. rosmarinus*, these values are the Inhibitory Efficient concentrations (EC_{50}) resulting from the processes of protein denaturation.

The result obtained for the EO of *S. rosmarinus*, compared to those presented by the formulations, was lower, having an EC_{50} of 62.46 mg L^{-1}, so the EO has a greater anti-inflammatory activity.

The evaluated nanoemulsions showed responses of higher concentrations, however related to protein denaturation, with the exception of NEO1 which differs from the others by presenting inhibition of the denaturation process, with an EC_{50} of 64.96 mg L^{-1}, this result being the best anti-aging activity. inflammatory response to nanoemulsions. The NEO3 nanoemulsion did not show satisfactory or conclusive results.

Table 4 – Anti-inflammatory activity of EO and stable nanoemulsions

ID	EC_{50} (mg L^{-1})	Equation	R^2
EO	62.46	y=6.782x+37.822	0.9986
NEO1	64.96	y=24.196x+ 6.1419	0.9977
NEO2	624.66	y=111.62x-262.05	0.9998
NEO3	-	-	-
NEO4	4220.25	y=32.117x-66.435	0.9997
NEO5	477.71	y=110.71x+246.61	0.9981
NEO6	101.57	y=56,881x+64,148	0.9997

where; EO- essential oil; NEO– Stable essential oil nanoemulsion formulated by phase inversion; 1 – Nanoemulsion composition 2.5% essential oil, 2.5% Tween 80 and 95% H_2O; 2 – Nanoemulsion composition 2.5% essential oil, 5.0% Tween 80 and 92.50% H_2O; 3 – Nanoemulsion composition 2.5% essential oil, 7.5% Tween 80 and 90.0% H_2O; 4 – Nanoemulsion with 2.5% essential oil, 2.5% Tween 20 and 90.0% H_2O; 5 – Nanoemulsion with 2.5% essential oil, 5.0% Tween 20 and 92.50% H_2O; 6 – Nanoemulsion with 2.5% essential oil, 7.5% Tween 20 and 92.50% H_2O; Autorship (2024)

In the study developed by Borges et al. (2018) evaluated the anti-inflammatory potential of nanoemulsions containing EO from S. rosmarinus in vitro and in vivo, and observed the ability of nanoemulsions to enhance the anti-inflammatory action of EO.

While studies carried out by Altinier et al. (2007) of anti-inflammatory activity resulted in a weak effect for *S. rosmarinus* extracts.

This result for the nanoemulsions can be justified by the majority composition found in the sample evaluated in this work, mainly the monoterpenes, in the disposition of the formulations or even in the phenotype of the plant. Protein denaturation is one of the main problems documented in inflammatory conditions (Padmanabhan & Jangle, 2012). However, studies that evaluated the anti-inflammatory activity by protein denaturation were not found for EO and *S. rosmarinus* formulations, this being an unprecedented study.

4 CONCLUSIONS

This study deals with the chemical and biotechnological potential of nanoemulsions containing *Salvia rosmarinus* essential oil. Through the results obtained in this study, the EO of *Salvia rosmarinus* (rosemary) is within established norms and standards, considering its physical-chemical parameters and the majority composition of its chemical constituents. The formulations of nanoemulsions were also evaluated, the refractive index and total phenolics presented satisfactory values very similar to their EO and being in agreement with the references found in the literature of other studies. The pharmacological activities, such as anti-inflammatory, showed low values for $EC_{50,}$ thus being considered efficient. The antioxidant activity showed potential and satisfactory result, showing one of the nanoemulsions with a better result than the EO. As this is a relatively new study, few references are available for *S. rosmarinus* nanoemulsions. Therefore, this study is important for demonstrating potential effects and serving as a basis for future studies regarding the potential use of nanoemulsions containing rosemary essential oil.

REFERENCES

Altinier, G. et al. (2007). Characterization of topical antiinflammatory compounds in Rosmarinus officinalis L. *Journal of agricultural and food chemistry*, 55(5), 1718-1723. doi: https://doi.org/10.1021/jf062610+.

Assaeed, A. et al. (2020). Sesquiterpenes-rich essential oil from above ground parts of Pulicaria somalensis exhibited antioxidant activity and allelopathic effect on weeds. *Agronomy*, 10(3), 399. https://doi.org/10.3390/agronomy10030399. doi: https://doi.org/10.3390/agronomy10030399.

Atti-Santos, A. et al. (2005). Physico-chemical evaluation of Rosmarinus officinalis L. essential oils. *Brazilian archives of biology and technology*, 48(6), 1035-1039. doi: https://doi.org/10.1590/S1516-89132005000800020.

Babovic, N. et al. (2010). Supercritical carbon dioxide extraction of antioxidant fractions from selected Lamiaceae herbs and their antioxidant capacity. *Innovative Food Science & Emerging Technologies*, 11(1), 98-107. doi: https://doi.org/10.1016/j.ifset.2009.08.013.

Bandoni, A. Los recursos vegetales aromáticos en Latinoamérica, su aprovechamiento industrial para la producción de aromas y sabores. 2. ed. Buenos Aires (Argentina): Editorial de la Universidad Nacional de La Plata, 2003.

Bastianetto, S. et al. (2000). Neuroprotective abilities of resveratrol and other red wine constituents against nitric oxide-related toxicity in cultured hippocampal neurons. *British journal of pharmacology*, 131(4), 711-720. doi: https://doi.org/10.1038/sj.bjp.0703626.

Borges, R. et al. (2018). Anti-inflammatory activity of nanoemulsions of essential oil from Rosmarinus officinalis L.: in vitro and in zebrafish studies. *Inflammopharmacology*, 26(4), 1057-1080. doi: https://doi.org/10.1007/s10787-017-0438-9.

Bouyahya, A. et al. (2017). Chemical composition of Mentha pulegium and Rosmarinus officinalis essential oils and their antileishmanial, antibacterial and antioxidant activities. *Microbial pathogenesis*, 111(2017), 41-49. doi: https://doi.org/10.1016/j.micpath.2017.08.015.

Campos, M. G. et al. (2003). Age-induced diminution of free radical scavenging capacity in bee pollens and the contribution of constituent flavonoids. *Journal of agricultural and food chemistry*, 51(3), 742-745. doi: https://doi.org/10.1021/jf0206466.

De Martino, L. et al. (2012). Chemistry and antigerminative activity of essential oils and monoterpenoids from Mediterranean Plants. *Current Bioactive Compounds*, 8(1), 13-49. doi: https://doi.org/10.2174/157340712799828179.

De Oliveira Carreiro, G. et al. (2020). Determinação dos índices físico-químicos dos óleos essenciais de Rosmarinus officinalis, Cymbopogon citratus e Cymbopogon winterianus. *Research, Society and Development*, 9(11), e4359119959-e4359119959.

De Oliveira, J. R., Camargo, S. E. A., & De Oliveira, L. D. (2019). Rosmarinus officinalis L.(rosemary) as therapeutic and prophylactic agent. *Journal of biomedical science*, 26(1), 1-22. https://jbiomedsci.biomedcentral.com/articles/10.1186/s12929-019-0499-8?fbclid=IwAR2AMZyJKZPpkkeDHe4IPAeNpZtTwYsPzK2DOF4Eydt3RwQn4bNJLGxbskY. doi: https://doi.org/10.1186/s12929-019-0499-8.

Donsì, F., & Ferrar, G. (2016). Nanoemulsões de óleo essencial como agentes antimicrobianos em alimentos. *J. Biotechnol*, 233, 106-120.

Duarte, J. L. et al. (2015). Evaluation of larvicidal activity of a nanoemulsion of Rosmarinus officinalis essential oil. *Revista Brasileira de Farmacognosia*, 25(2), 189-192. doi: https://doi.org/10.1016/j.bjp.2015.02.010.

Dúran, N. (2006). Nanotecnologia: introdução, preparação e caracterização de nanomateriais e exemplos de aplicação. 1.ed. São Paulo (Brasil): Editorial Artliber, 2006.

El Euch, S. K., Hassine, D. B., Cazaux, S., Bouzouita, N., & Bouajila, J. (2019). Salvia officinalis essential oil: Chemical analysis and evaluation of anti-enzymatic and antioxidant bioactivities. *South African Journal of Botany*, *120*, 253-260.

El Jery, A., Hasan, M., Rashid, M. M., Al Mesfer, M. K., Danish, M., & Rebah, F. B. (2020). Phytochemical characterization, and antioxidant and antimicrobial activities of essential oil from leaves of the common sage L. from Abha, Saudi Arabia. *Asian Biomedicine*, *14*(6), 261-270.

El Sawi, S. A. et al. (2019). Allelopathic potential of essential oils isolated from peels of three citrus species. *Annals of Agricultural Sciences*, 64(1), 89-94. doi: https://doi.org/10.1016/j.aoas.2019.04.003.

Farmacopeia brasileira. (2019). Agência Nacional de Vigilância Sanitária. Brasília: Anvisa, 6 ed., Brasília (Brasil): Editorial Anvisa.

Fronza, T. et al. Nanocosméticos: Em direção ao estabelecimento de Marcos Regulatórios. 1 ed., Rio Grande do Sul (Brasil): Editorial UFRGS, 2007. http://repositorio2.unb.br/jspui/handle/10482/19398 https://doi.org/10.11606/T.9.2012.tde-13032013-105302

Hussain, A. I. et al. (2010). Rosmarinus officinalis essential oil: antiproliferative, antioxidant and antibacterial activities. Brazilian Journal of Microbiology, 41(4), 1070-1078. doi: https://doi.org/10.1590/S1517-83822010000400027.

Instituto Adolfo Lutz. (1985). Normas analíticas do Instituto Adolfo Lutz. v.1.:Métodos Químicos e Físicos para Análise de Alimentos. 3. ed. São Paulo (Brasil): Editorial IMESP.

Izquierdo, P. et al. (2002). Formation and stability of nano-emulsions prepared using the phase inversion temperature method. Langmuir, 18(1), 26-30. doi: https://doi.org/10.1021/la010808c.

Jafari-Sales, A., & Pashazadeh, M. (2020). Study of chemical composition and antimicrobial properties of Rosemary (Rosmarinus officinalis) essential oil on Staphylococcus aureus and Escherichia coli in vitro. International Journal of Life Sciences and Biotechnology, 3(1), 62-69. doi: https://doi.org/10.38001/ijlsb.693371.

Kubitschek-KM, A. R. J. et al. (2014). Development of jojoba oil (Simmondsia chinensis (Link) CK Schneid.) based nanoemulsions. Lat. Am. J. Pharm, 33(3), 459-63.

Lima, T. C. P. et al. (2020). DESENVOLVIMENTO DE NANOGEL DE Copaifera reticulata SOBRE A LESÃO MUSCULAR EM RATOS USANDO FONOFORESE. Saúde e Pesquisa, 13(1), 181-192. doi: https://doi.org/10.17765/2176-9206.2020v13n1p181-192.

Liu, P. et al. (2014). Flavonol glycosides and other phenolic compounds in buds and leaves of different varieties of black currant (Ribes nigrum L.) and changes during growing season. Food chemistry, 160(1), 180-189. doi: https://doi.org/10.1016/j.foodchem.2014.03.056.

Lou, S. N. et al. (2014). Soluble and insoluble phenolic compounds and antioxidant activity of immature calamondin affected by solvents and heat treatment. Food chemistry, 161(1), 246-253. doi: https://doi.org/10.1016/j.foodchem.2014.04.009.

Maccioni, A. et al. (2019). Inhibitory effect of rosemary essential oil, loaded in liposomes, on seed germination of Acacia saligna, an invasive species in Mediterranean ecosystems. Botany, 97(5), 283-291. doi: https://doi.org/10.1139/cjb-2018-0212.

Macedo, L. M. et al. (2020). Rosemary (Rosmarinus officinalis L., syn Salvia rosmarinus Spenn.) and It's topical applications: a review. Plants, 9(5), 1-12. doi: https://doi.org/10.3390/plants9050651.

Maia, A. J. et al. (2014). Óleo essencial de alecrim no controle de doenças e na indução de resistência em videira. Pesquisa Agropecuária Brasileira, 49(5), 330-339. doi: https://doi.org/10.1590/S0100-204X2014000500002.

Moczkowska, M. et al. (2020). Effect of rosemary extract addition on oxidative stability and quality of hemp seed oil. Food and Bioproducts Processing, 124(1), 33-47. doi: https://doi.org/10.1016/j.fbp.2020.08.002.

Noori, Soheila, Zeynali, Fariba, & Almasi, Hadi. (2018). Antimicrobial and antioxidant efficiency of nanoemulsion-based edible coating containing ginger (Zingiber officinale) essential oil and its effect on safety and quality attributes of chicken breast fillets. Food control, 84, 312-320.

Ostertag, F. et al. (2012). Low-energy formation of edible nanoemulsions: factors influencing droplet size produced by emulsion phase inversion. Journal of colloid and interface science, 388(1), 95-102. doi: https://doi.org/10.1016/j.jcis.2012.07.089.

Padmanabhan, P., & Jangle, S. N. (2012). Evaluation of in-vitro anti-inflammatory activity of herbal preparation, a combination of four medicinal plants. International journal of basic and applied medical sciences, 2(1), 109-116.

Rodrigues, E. D. C. et al. (2014). Development of a larvicidal nanoemulsion with Copaiba (Copaifera duckei) oleoresin. Revista Brasileira de Farmacognosia, 24(6), 699-705. doi: https://doi.org/10.1016/j.bjp.2014.10.013.

Shafiq, S. et al. (2007). Development and bioavailability assessment of ramipril nanoemulsion formulation. European journal of pharmaceutics and biopharmaceutics, 66(2), 227-243. doi: https://doi.org/10.1016/j.ejpb.2006.10.014.

Silva, A. (2011). Efeito do extrato aquoso de alecrim (Rosmarinus officinalis L.) sobre o estresse oxidativo em ratos diabéticos. Revista de Nutrição, 24(1), 121-130. doi: https://doi.org/10.1590/S1415-52732011000100012.

Silva, A. M. D. O. (2012). Efeito dos compostos fenólicos do alecrim (Rosmarinus officinalis L.) na inflamação aguda e sobre os marcadores de estresse oxidativo de ratos diabéticos. [Tese Doutorado em Nutrição experimental, Faculdade de Ciências Farmacêuticas da Universidade de São Paulo]. Repositório Institucional da USP.

Silva, M. D. S. A. et al. (2008). Atividade antimicrobiana e antiaderente in vitro do extrato de Rosmarinus officinalis Linn. sobre bactérias orais planctônicas. Revista Brasileira de Farmacognosia, 18(2), 236-240. doi: https://doi.org/10.1590/S0102-695X2008000200017.

Smirnoff, N., & Cumbes, Q. J. (1989). Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry, 28(4), 1057-1060. doi: https://doi.org/10.1016/0031-9422(89)80182-7.

Soares, S. E. (2002). Ácidos fenólicos como antioxidantes. Revista de nutrição, 15(1), 71-81. doi: https://doi.org/10.1590/S1415-52732002000100008.

Sousa, C. M. D. M. et al. (2007). Total phenolics and antioxidant activity of five medicinal plants. Química nova, 30(2), 351-355. doi: https://doi.org/10.1590/S0100-40422007000200021.

Souza Filho, A. P. D. S. et al. (2009). Efeitos potencialmente alelopáticos dos óleos essenciais de Piper hispidinervium C. DC. e Pogostemon heyneanus Benth sobre plantas daninhas. Acta amazônica, 39(2), 389-395. doi: https://doi.org/10.1590/S0044-59672009000200018.

Sugumar, S. et al. (2014). Nanoemulsion of eucalyptus oil and its larvicidal activity against Culex quinquefasciatus. Bulletin of entomological research, 104(3), 393-402. doi: https://doi.org/10.1017/S0007485313000710.

Sundararajan, R., & Koduru, R. (2016). In vitro antioxidant activity on roots of Limnophila heterophylla. Free Radicals and Antioxidants, 6(2), 178-185. doi: https://doi.org/10.5530/fra.2016.2.8.

Syed, I., Banerjee, P., & Sarkar, P. (2020). Oil-in-water emulsions of geraniol and carvacrol improve the antibacterial activity of these compounds on raw goat meat surface during extended storage at 4 C. Food Control, 107, 106757.

Tadros, T. et al. (2004). Formation and stability of nano-emulsions. Advances in colloid and interface science, 108(20), 303-318. doi: https://doi.org/10.1016/j.cis.2003.10.023.

Takayama, C. et al. (2016). Chemical composition of Rosmarinus officinalis essential oil and antioxidant action against gastric damage induced by absolute ethanol in the rat. Asian Pacific journal of tropical biomedicine, 6(8), 677-681. doi: https://doi.org/10.1016/j.apjtb.2015.09.027.

Tiuzzi, M., & Furlan, M. R. (2016). Atividade antioxidante do alecrim. Revista Eletrônica

Thesis, 26(1), 99-114. https://silo.tips/download/atividade-antioxidante-do-alecrim#.

Wanderley, A. L. (2016). Atividade antioxidante e antimicrobiana do óleo essencial de Rosmarinus officinalis L., cultivado em sistema orgânico sob diferentes condições, frente a bactérias causadoras de mastite bovina. [Dissertação de Mestrado em Agronomia, Universidade de Brasília]. Repositório Institucional da UNB.

Wang YuZhu, W. Y. et al. (2018). Effects of a natural antioxidant, polyphenol-rich rosemary (Rosmarinus officinalis L.) extract, on lipid stability of plant-derived omega-3 fatty-acid rich oil. Food Science and Technology, 89(37), 210-216. doi: https://doi.org/10.1016/j.lwt.2017.10.055.

Waterhouse, A. (2006). Folin-ciocalteau micro method for total phenol in wine. American Journal of Enology and viticulture, 48(1), 357-363.

Zaouali, Y. et al. (2010). Essential oils composition in two Rosmarinus officinalis L. varieties and incidence for antimicrobial and antioxidant activities. Food and chemical toxicology, 48(11), 3144-3152. doi: https://doi.org/10.1016/j.fct.2010.08.010.

Zhuang, H., Tang, N., & Yuan, Y. (2013). Purification and identification of antioxidant peptides from corn gluten meal. Journal of Functional Foods, 5(4), 1810-1821. doi: https://doi.org/10.1016/j.jff.2013.08.013.

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