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DEPARTAMENTO DE QUÍMICA

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PERFIL QUÍMICO, ATIVIDADE QUÍMICA E BIOLÓGICA DO ÓLEO ESSENCIAL CRUZADO E NANOEMULSÃO ÓLEO-EM-ÁGUA (O/A) DE *Pimpinella anisum* (Anis) e *Laurus nobilis* (Louro)

SÃO LUÍS – MA
2022

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Trabalho de Conclusão de Curso apresentado ao Curso de Química da Universidade Federal do Maranhão como requisito para a obtenção do título de Bacharel em Química.

Orientador: Prof. Dr. Victor Elias Mouchrek Filho.

Coorientador: Prof. Dr. Gustavo Oliveira Everton.

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Aprovada em ___/___/___

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A Deus, pela minha vida, pois é ele que produz em nós tanto o querer quanto o efetuar, de acordo com sua boa vontade (Filipenses 2:13). Pois dele, por ele e para ele são todas as coisas. A ele seja a glória para sempre, amém (Romanos 11:36).

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“Ao investigar a criação majestosa e
impressionante de Deus, a ciência
pode realmente ser um meio de adoração”
- Francis Collins

RESUMO

Este trabalho teve por objetivo avaliar o perfil químico, atividade anti-inflamatória e antioxidante de bioprodutos produzidos a partir do óleo essencial cruzado de *Pimpinella anisum* (Anis) e *Laurus nobilis* (Louro). Para a extração do OE foi utilizada a técnica de hidrodestilação em um sistema extrator Clevenger modificado e as nanoemulsões foram formuladas por método de inversão de fases. A determinação dos compostos fenólicos totais foi realizada pelo método de Folin-Ciocalteu. A atividade antioxidante foi executada pelo método espectrofotométrico de eliminação de radicais hidroxilas do ácido salicílico. A atividade anti-inflamatória foi avaliada pelo método de desnaturação proteica de albumina por degradação térmica. A determinação de CFT quantificou 54,27 e 1,71 mg $EAT.g^{-1}$ para o OE cruzado e NOE, respectivamente. O OE cruzado e seus bioprodutos apresentaram atividade antioxidante, anti-inflamatória com valores satisfatórios para Concentração Eficiente (CE_{50}). Para a capacidade antioxidante do OE, NOE, MOE, MNOE, BOE e BNOE foram obtidos, respectivamente, os valores de CE_{50} 9,72, 7,34, 3,09, 7,90, 111,02 e 12,30. Na atividade anti-inflamatória do OE, NOE, MOE, MNOE, BOE e BNOE foram quantificados os valores de CE_{50} 12,02, 28,96, 4,39, 4,07, 4,27 e 2,99 ppm. Pode-se afirmar que o OE essencial cruzado e seus bioprodutos apresentaram resultados satisfatórios, evidenciando a eficácia das propriedades sinérgicas e de seu potencial biotecnológico.

Palavras-chave: óleos essenciais, atividade antioxidante, atividade anti-inflamatória.

ABSTRACT

This work aimed to evaluate the chemical profile and the anti-inflammatory and antioxidant activity of bioproducts produced from the cross-essential oil of *Pimpinella anisum* (Anise) and *Laurus nobilis* (Laurel). For the extraction of EO, the hydrodistillation technique was performed using a modified Clevenger extractor system, and the nanoemulsions were formulated by the phase inversion method. The determination of total phenolic compounds was performed by the Folin-Ciocalteu method. Antioxidant activity was performed by the spectrophotometric method of hydroxyl radical elimination from salicylic acid. The anti-inflammatory activity was evaluated using the heat-induced protein denaturation method of albumin. The determination of CFT quantified 54.27 and 1.71 mg EAT.g^{-1} for cross-EO and EON, respectively. The cross EO and its bioproducts showed antioxidant and anti-inflammatory activity with satisfactory values for Efficient Concentration (EC_{50}). For the antioxidant capacity of EO, EON, MEO, MEON, EOB, and EONB, EC_{50} values of 9.72, 7.34, 3.09, 7.90, 111.02, and 12.30 were obtained, respectively. For the anti-inflammatory activity of EO, EON, MEO, MEON, EOB, and EONB, EC_{50} values of 12.02, 28.96, 4.39, 4.07, 4.27, and 2.99 ppm were quantified, respectively. It can be stated that the cross EO and its bioproducts showed satisfactory results, evidencing the effectiveness of the synergistic properties and its biotechnological potential.

Keywords: essential oil, Antioxidant activity, anti-inflammatory activity.

LISTA DE TABELAS

SUMÁRIO

1. INTRODUÇÃO

Os óleos essenciais são biosintetizados no metabolismo secundário das plantas medicinais aromáticas e armazenados em estruturas específicas (FEIJÓ *et al.*; CUNHA *et al.*, 2012). Muitos destes possuem diferentes propriedades farmacológicas decorrentes do conteúdo de vários compostos bioativos , alguns dos quais possuem potente efeito antioxidante e anti-inflamatório (AVOLA *et al.*, 2020; FERREIRA, 2014).

Devido as suas características físico-químicas como volatilidade e lipofilicidade, a aplicação dos óleos essenciais se torna limitada, dessa forma sistemas de encapsulamento que confiram proteção a esses compostos podem atuar melhorando a estabilidade, dispersão e até mesmo a atividade dos óleos essenciais (ASBAHANI *et al.*, 2015).

Dentre as emulsões, as nanoemulsões destacam-se por serem sistemas com tamanho de partícula entre 20 e 100 nm, apresentando maior estabilidade a separação e coalescência que emulsões convencionais (MCCLEMENTS, 2015).

O interesse em investigar plantas com atividade anti-inflamatória e antioxidante reside no fato de que as elas têm uma vasta aplicação em vários processos patológicos. Estas pesquisas têm aberto múltiplas oportunidades para intervenção terapêutica (FERREIRA, 2014).

Dentre as plantas aromáticas, a *Pimpinella anisum (*Anis*)* e *Laurus nobilis (*Louro*)* são amplamente utilizados como condimentos em preparos domésticos, na medicina popular e pelas indústrias de alimentos, cosméticos e farmacêutica (PATRAKAR, 2012; TEPE, 2015). Destacam-se por apresentar atividade biológica, a saber: atividades antioxidante, antimicrobiana, antifúngica, anti-inflamatória e analgésica (ERCIN *et al.*, 2022 ; SIRIKEN *et al.*, 2018; MOHAMMADI, S. *et al.*, 2015; IANNARELLI, R. *et al.*, 2018).

Existem diversos estudos na literatura que avaliam as possíveis interações sinérgicas dos óleos essenciais na eficácia de suas atividades biológicas (BASSOLÉ, 2012; AUMEERUDDY-ELALFI *et al*, 2018; CAPUTO *et al.*, 2017). Os bioprodutos provenientes das espécies estudadas tem comprovado suas ações antioxidante e anti-inflamatória, tornando-as relevante neste estudo (RU *et al.*, 2022; FADEL, *et al.*, 2020; AUMEERUDDY-ELALFI, *et al*., 2018). Neste contexto, o presente trabalho objetivou avaliar o perfil químico, atividade anti-inflamatória e antioxidante de bioprodutos produzidos a partir do óleo essencial cruzado de *Pimpinella anisum* (Anis) e *Laurus nobilis* (Louro).

2. OBJETIVOS

2.1. Objetivo Geral

- Avaliar o perfil químico, atividade anti-inflamatória e antioxidante de bioprodutos produzidos a partir do óleo essencial cruzado de *Pimpinella anisum* (Anis) e *Laurus nobilis* (Louro).

2.2. Objetivos Específicos

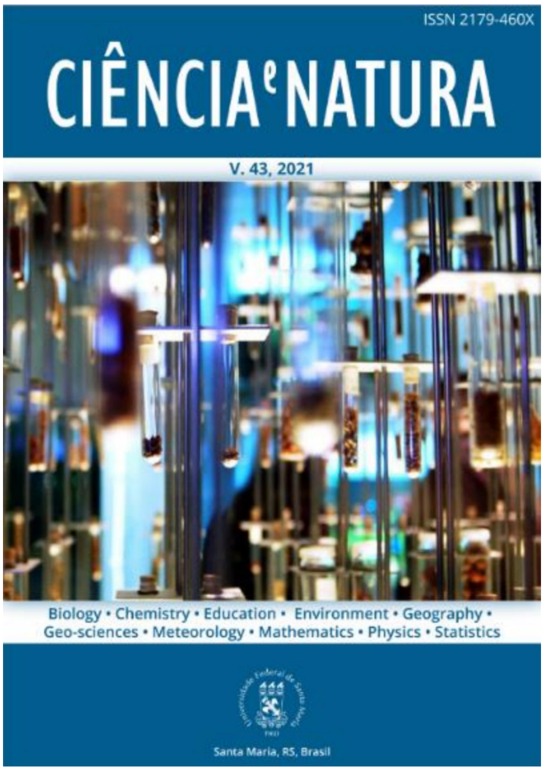
- Obter uma nanoemulsão (O/A) estável a partir do óleo essencial cruzado extraído de *Pimpinella anisum* e *Laurus nobilis*;
- Microencapsular, por casting, o óleo essencial cruzado de *Pimpinella anisum* e *Laurus nobilis* obtido e nanoemulsão (O/A) estável;
- Produzir, por casting, um biofilme a partir do óleo essencial cruzado de *Pimpinella anisum* e *Laurus nobilis* obtido e nanoemulsão (O/A) estável;
- Quantificar o teor de fenólicos totais do óleo essencial cruzado e nanoemulsão de *Pimpinella anisum* e *Laurus nobilis*;
- Determinar a Concentração Eficiente 50% para atividade antioxidante do óleo essencial cruzado, microencapsulado, biofilme, e nanoemulsão de *Pimpinella anisum* e *Laurus nobilis*;
- Determinar a Concentração Eficiente 50% para atividade anti-inflamatória do óleo essencial cruzado, microencapsulado, biofilme e nanoemulsão de *Pimpinella anisum* e *Laurus nobilis*;

3. CAPÍTULO 1- ARTIGO

ARTIGO A SER SUBMETIDO NA REVISTA CIÊNCIA E NATURA

Chemical profile, chemical and biological activity of cross essential oil and oil-in-water (O/W) nanoemulsion of *Pimpinella anisum (Anise)* and *Laurus nobilis (Laurel)*

Área: Química



Chemical profile, chemical and biological activity of cross essential oil and oil-in-water (O/W) nanoemulsion of *Pimpinella anisum (Anise)* and *Laurus nobilis (Laurel)*

Perfil químico, atividade química e biológica do óleo essencial cruzado e nanoemulsão óleo-em-água (O/A) DE *Pimpinella anisum (Anis)* e *Laurus nobilis (Louro)*

Resumo

Este trabalho teve por objetivo avaliar o perfil químico, atividade anti-inflamatória e antioxidante de bioprodutos produzidos a partir do óleo essencial cruzado de *Pimpinella anisum* (Anis) e *Laurus nobilis* (Louro). Para a extração do OE foi utilizada a técnica de hidrodestilação em um sistema extrator Clevenger modificado e as nanoemulsões foram formuladas por método de inversão de fases. A determinação dos compostos fenólicos totais foi realizada pelo método de Folin-Ciocalteu. A atividade antioxidante foi executada pelo método espectrofotométrico de eliminação de radicais hidroxilas do ácido salicílico. A atividade anti-inflamatória foi avaliada pelo método de desnaturação proteica de albumina por degradação térmica. A determinação de CFT quantificou 54,27 e 1,71 mg $EAT.g^{-1}$ para o OE cruzado e NOE, respectivamente. O OE cruzado e seus bioprodutos apresentaram atividade antioxidante, anti-inflamatória com valores satisfatórios para Concentração Eficiente (CE_{50}). Para a capacidade antioxidante do OE, NOE, MOE, MNOE, BOE e BNOE foram obtidos, respectivamente, os valores de CE_{50} 9,72, 7,34, 3,09, 7,90, 111,02 e 12,30. Na atividade anti-inflamatória do OE, NOE, MOE, MNOE, BOE e BNOE foram quantificados os valores de CE_{50} 12,02, 28,96, 4,39, 4,07, 4,27 e 2,99 ppm. Pode-se afirmar que o OE essencial cruzado e seus bioprodutos apresentaram resultados satisfatórios, evidenciando a eficácia das propriedades sinérgicas e de seu potencial biotecnológico.

Palavras-chave: óleos essenciais, atividade antioxidante, atividade anti-inflamatória

Abstract

This work aimed to evaluate the chemical profile and the anti-inflammatory and antioxidant activity of bioproducts produced from the cross-essential oil of *Pimpinella anisum* (Anise) and *Laurus nobilis* (Laurel). For the extraction of EO, the hydrodistillation technique was performed using a modified Clevenger extractor system, and the nanoemulsions were formulated by the phase inversion method. The determination of total phenolic compounds was performed by the Folin-Ciocalteu method. Antioxidant activity was performed by the spectrophotometric method of hydroxyl radical elimination from salicylic acid. The anti-inflammatory activity was evaluated using the heat-induced protein denaturation method of albumin. The determination of CFT quantified 54.27 and 1.71 mg $EAT.g^{-1}$ for cross-EO and EON, respectively. The cross EO and its bioproducts showed antioxidant and anti-inflammatory activity with satisfactory values for Efficient Concentration (EC_{50}). For the antioxidant capacity of EO, EON, EOM, EONM, EOB, and EONB, EC_{50} values of 9.72, 7.34, 3.09, 7.90, 111.02, and 12.30 were obtained, respectively. For the anti-inflammatory activity of EO, EON, EOM, EONM, EOB, and EONB, EC_{50} values of

12.02, 28.96, 4.39, 4.07, 4.27, and 2.99 ppm were quantified, respectively. It can be stated that the cross EO and its bioproducts showed satisfactory results, evidencing the effectiveness of the synergistic properties and its biotechnological potential.

Keywords: essential oil, Antioxidant activity, anti-inflammatory activity.

Introduction

Essential oils are biosynthesized in the secondary metabolism of aromatic medicinal plants and stored in specific structures (FEIJÓ et al.; CUNHA *et al*., 2012). Many of these have different pharmacological properties due to the content of various bioactive compounds, some of which have potent antioxidant and anti-inflammatory effects (AVOLA *et al*., 2020; FERREIRA, 2014).

Due to their physical-chemical characteristics such as volatility and lipophilicity, the application of essential oils becomes limited, thus encapsulation systems that provide protection to these compounds can act by improving the stability, dispersion and even the activity of essential oils (ASBAHANI *et al*., 2015). Among emulsions, nanoemulsions stand out for being systems with particle sizes between 20 and 100 nm, with greater stability to separation and coalescence than conventional emulsions (MCCLEMENTS, 2015).

The interest in investigating plants with anti-inflammatory and antioxidant activity lies in the fact that they have a wide application in various pathological processes. This research has opened multiple opportunities for therapeutic intervention (FERREIRA, 2014).

Among the aromatic plants, *Pimpinella anisum* (anise) and *Laurus nobilis* (laurel) they are widely used as condiments in domestic preparations, in folk medicine and by the food, cosmetics and pharmaceutical industries (PATRAKAR, 2012; TEPE, 2015). They stand out for presenting biological activity, namely: antioxidant, antimicrobial, antifungal, anti-inflammatory and analgesic activities (ERCIN *et al*., 2022; SIRIKEN *et al*., 2018; MOHAMMADI, S. *et al*., 2015; IANNARELLI, R. *et al*., 2018).

There are several studies in the literature that evaluate the possible synergistic interactions of essential oils in the effectiveness of their biological activities (BASSOLÉ, 2012; AUMEERUDDY-ELALFI *et al*, 2018). Bioproducts from the studied species have proven their antioxidant and anti-inflammatory actions, making them relevant in this study (RU *et al*., 2022; FADEL, *et al*., 2020; AUMEERUDDY-ELALFI, *et al*., 2018). In this context, the present work aimed to evaluate the chemical profile, anti-inflammatory and antioxidant activity of bioproducts produced from the crossed essential oil of *Pimpinella anisum* (anise) and *Laurus nobilis* (laurel).

Methodology

Obtaining plant material

The aerial parts of *Pimpernel anisum* and leaves of *Laurus nobilis* used in this study were obtained in August 2022, from the federally certified distributor - Produtos Naturais Muniz LTDA (CNAE 4729-6/99). After collection, the plant species were transported to the Laboratory for Research and Application of Essential Oils (LOEPAV/UFMA), where the leaves were weighed, crushed and stored for the extraction of essential oil from the plant.

Extraction of essential oils

For the extraction of the 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. 100g of each plant material were used, previously dried in a FANEM 520 convective air oven at 45°C, adding distilled water (1:10 m/v). Hydrodistillation was performed at 100°C for 3 h and the EO extract was collected. The EO was dried by percolation with anhydrous sodium sulfate (Na_2SO_4) and centrifuged. These operations were carried out in triplicate and the samples were stored in amber glass ampoules under refrigeration at 4°C. Subsequently submitted to analyses.

Chemical Profile

The identification of chemical constituents was performed by gas chromatography coupled to mass spectrometry (GC-MS), using a QP 2010 Plus model equipment (Shimadzu Corporation, Kyoto, Japan) operating with a fused silica capillary column (30 m × 0. 25 mm). with a DB-5 bonded phase (film thickness, 0.25 µm). Helium was used as a carrier gas with a flow rate of 1.0 mL min^{-1}. The injector and detector temperatures were 220 and 240 °C, respectively. The injection volume of the sample was 0.5 µL, diluted in hexane (1%) and injection volume partition ratio (split) of 1:100. The temperature ramp started at 60°C, with an increase at a rate of 3 °C min^{-1} to 24°C, followed by an increase of 10°C min^{-1} until reaching 300 °C, with the final temperature maintained for 7 min. The column pressure was around 71.0 kPa. The mass spectrometer was operated with an ionization potential of 70 eV and an ion source temperature of 200 °C. Mass analysis was performed in full scan mode, ranging from 45 to 500 Da, with a sweep speed of 1000 Da s^{-1} and a scan interval of 0.5 fragments s^{-1}. Data were obtained and processed using Lab software Solutions LC/GC Workstation 2.72 (Shimadzu, Kyoto, Japan). The retention index of the compounds was calculated in relation to a homologous series of n-alkanes (nC_9 – nC_{18}), using the Van den equation Dool and Kratz (VAN DEN DOOL & KRATZ, 1963). The identification of the compounds was carried out by comparing the calculated retention indices with those described in the literature (ADAMS et al., 2007)). Comparisons of the mass spectra obtained with those existing in the FFNSC 1.2, NIST107 and NIST21 libraries were also performed.

Quantitative analysis was performed by gas chromatography with flame ionization detector (GC-FID), using equipment model GC-2010 (Shimadzu Corporation, Kyoto, Japan), with identical experimental conditions to those used in the qualitative analysis, except for the temperature of the detector, which was 300°C. The relative percentages of each constituent were obtained by the area normalization method.

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 concentration (5% v/v) was fixed for the formulation. The required amounts of each oil phase constituent (oil+Tween20) were heated to 65 ± 5 °C. The aqueous phase was separately heated to 65 ± 5°C, gently added and mixed with the oil phase, providing a primary formulation, by the phase inversion method. Final homogenization was achieved using a magnetic stirrer, in which the formulation remained under constant agitation at 6000 rpm, until reaching a temperature reduction of 25 °C ± 2 °C. To prove the stability, the formulated nanoemulsion was submitted to different stress tests according to the methodology described by Shafiq et al., (2007). They were reassessed for phase separation by centrifugation. The heating cycle was performed by keeping the formulated nanoemulsion at 40 and 4 °C, alternating each temperature for 48 h. The cycle was repeated three times. This was done to verify the stability of the nanoemulsion at different temperatures. Frozen stress-thawing was performed by keeping the nanoemulsion alternately at -21 and 25 °C for 48 h at each temperature. The cycle was repeated twice. The experiment was carried out in triplicate.

Microencapsulation
The encapsulation test followed the methodology described by Dubey et al. (2009). Reagents of sodium alginate, anhydrous calcium chloride, the EO and NOE under study, distilled water and tween 80 surfactant were used for the synthesis of alginate microparticles. For the synthesis of encapsulated EO and NOE particles, 60 g of sodium alginate solution (3.5% w/v) were mixed with 15 g of tween 80 and 25 µL of essential oil. The mixture was homogenized at 10,000 rpm and then dropped onto a $CaCl_2$ solution to harden the particles via crosslinking.

Biofilm
The preparation of biofilms followed the method developed by Zactiti&Kieckbusch (2006), according to the casting technique, which consists of preparing a filmogenic solution and applying it to a support. The formulation consists of 30g of sodium alginate in 1000 mL of distilled water under constant mechanical agitation at 1000 rpm. Then the glycerol is added, keeping the mixture under mechanical agitation for 30 minutes at room temperature. After this period, the essential oil or nanoemulsion at 2% of the total composition is slowly added, keeping the filmogenic solution at 45 °C for 30 minutes under mechanical agitation at 1000 rpm. At the end of this procedure, it was poured into a 15.0 cm diameter petri dish and placed in an oven for solvent evaporation (casting) at a temperature of 45 °C for 24 h. After drying the films, they were left in a desiccator for 24 hours.

Total Phenolics
The determination of the total phenolic compounds of the crossed essential oil and nanoemulsion was performed by the Folin-Ciocalteu spectrophotometric method (WATERHOUSE, 2012). 5 mg of samples diluted in 1 mL of ethanol were used. 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 milligrams equivalent to grams (mg EAT g^{-1}) of tannic acid.

Evaluation 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&Cumbes (1989) and Sundararajan et al. (2018).
Essential oil solutions (10 – 100 ppm) in 0.2% DMSO, biofilms (10 – 100 ppm) and nanoemulsions (no dilution) were prepared. In these solutions, 2 mL of distilled water, 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 in a UV/VIS spectrophotometer.

The scavenging of hydroxyl radicals was expressed in percentage and the 50% Efficient Concentration (EC_{50}/IC_{50}) capable of inhibiting 50%, respectively, of the scavenging was expressed in ppm.

Anti-inflammatory activity by albumin protein denaturation

The anti-inflammatory activity was evaluated by the albumin protein denaturation method by thermal degradation (PADMANABHAN&JANGLE, 2012).

The reaction mixture (4000 µL) consisted of 1000 µL of different concentrations of essential oils (100 – 500 ppm) diluted in PBS, biofilms (10 – 100 ppm) and nanoemulsions (without prior dilution in solvent) and 3000 µL of a solution to 10% albumin diluted in PBS and incubated at (37±1) °C for 15 minutes. Denaturation was induced by keeping the reaction mixture at 70°C in a water bath for 10 minutes. After cooling, absorbance was measured at 660 nm in a UV/VIS spectrophotometer. Inhibition of protein denaturation was expressed in percentage and the 50% Efficient Concentration (EC_{50} /IC_{50}) capable of inhibiting 50% of denaturation was expressed in ppm.

Results and discussion

Chemical profile

The analysis of the chemical constituents of the cross-EO extraction of *Laurus nobilis* and *Pimpinella anisum plants* performed in GC/SM equipment are shown in Table 1.

Table 1. Chemical composition of EO cross from *Laurus nobilis* and *Pimpinella anisum.*

IR_{exp}	IR_{tab}	Constituents	percentage (%)
930	931	α-pinene	0.12
944	943	Sabinene	0.44
950	951	β-Pinene	0.63
1020	1020	β-Myrcene	2.95
1201	1200	Damage	0.85
1248	1251	1,8-cineole	20.14
1280	1285	(E)- anethole	74.21
1206	1207	Methyl chavicol	0.17
1454	1454	Humulene	0.15
1534	1534	Isoeugenol acetate	0.34

In this study, 10 compounds were identified in the cross-EO sample, the major constituent being (E)-anethole with 74.21%, followed by 1,8 cineole with 20.14%. Studies report 1,8-cineole as the major constituent of *L. nobilis*, as Mssillou et al. (2020) who identified 25 compounds in flowers of *L. nobilis* grown in Morocco, quantifying 45.01% of 1.8-cineole. Like Fidan et al. (2019) who identified 38 constituents in the fruits, branches and leaves of *L. nobilis* cultivated in Bulgaria, quantified 33.3%, 48.5% and 41.0% of 1,8-cineole, respectively. On the other hand, some studies in the literature indicate (E)-anethole as the main component of *Pimpinella anisum*, as Figueredo et al. (2020), who, analyzing the chemical profile of some plants, identified 18 constituents in the *Pimpinella anisum,* quantifying 93% of (E)-anethole.

These differences may be due to the harvest time and local, climatic and seasonal factors, as well as the storage time of the medicinal plant used (MSSILLOU et al., 2020)

Total Phenolics

The results obtained in the determination of total phenolics (FT) by the Folin-Ciocalteu method, expressed as tannic acid equivalent (mg EAT g^{-1}) per gram are shown in Table 2.

Table 2. Total phenolic content - CFT (mg EAT g^{-1}) for essential oil and nanoemulsion, from *Pimpinella anisum* and *Laurus nobilis.*

ID	CFT (mg EAT g^{-1})	Equity straight	R^2
EO	54.27	y=0.0586x+0.06	0.9999
EON	1.71		

Note: EO – essential oil; NEO – nanoemulsion.

Since there is no similar work in the literature with the essential oil crossed from *Pimpinella anisum* and *Laurus nobilis* for the purpose of direct comparison, a search was carried out in the literature to investigate the potential of the species individually to analyze whether the total phenolic content was favorable for the crossing in the extraction.

In the study by Dhifi et al. (2018), the authors evaluated the CFT of the essential oil of the leaves of *L. nobilis* at 174.1 mg EAG g^{-1}, which is a higher quantity than that observed in this study. Still following this perspective, Aala et al. (2023) reported a value of 257.66 mg EAG g^{-1} for the CFT of the extract of *L. nobilis leaves,* also higher than that presented in this work.

P. anisum seed extract shows a lower result, as Rebey et al. (2020), who evaluated the effect of initial drying techniques on the composition of phenolic compounds and obtained values ranging from 31.15 to 42.70 mg EAG g^{-1}, concluding that the drying method in the shade increases phenolic compounds. Still, Martins et al (2021) evaluating the bactericidal potential of several essential oils, among them the EO of *P. anisum*, obtained a value of 112.45 mg EAT g^{-1} for CTF, quantitatively higher than that obtained in this work.

The phenolic compounds evaluated and quantified are part of the secondary metabolites of the plants studied, they are one of the classes responsible for the anti-inflammatory activity and for several antioxidant properties observed (SÉFORA-SOUSA, 2013). The results obtained in this study demonstrate that the crossed EO has potential natural antioxidant action. This work describes in an unprecedented way an essential oil obtained from a cross extraction of *Pimpinella anisum* and *Laurus nobilis*, which makes it very relevant.

Antioxidant activity

Table 3 presents results obtained for the antioxidant capacity of the cross-essential oil and nanoemulsion, as well as their bioproducts, relative to the 50% efficient concentration (EC_{50}) in ppm, from the method of scavenging hydroxyl radicals.

Table 3 Antioxidant capacity for cross essential oil, nanoemulsion, biofilm and microencapsulation of *Pimpinella anisum* and *Laurus nobilis*.

ID	CE_{50} ppm	Equity straight	R^2
OE	9.72	y=50.104x+0.5107	0.9897
EON	7.34	y=56.977x+0.6909	0.9867
MEO	3.09	y=14.087x+43.080	0.9891
MEON	7.90	y=55.379x+0.2824	0.9974
EOB	111.02	y=75.252x-103.92	0.9530
EONB	12.30	y=45.382x+0.3462	0.9942

Note: EO – essential oil; EON – essential oil nanoemulsion; MEO – microencapsu lation of essential oil; MEON – microencapsulation of essential oil nanoemulsion; EOB – essential oil biofilm; EONB – essential oil nanoemulsion biofilm.

According to Table 3, cross essential oil, microencapsulated, biofilm and nanoemulsions present significant values, proving the effective action and being classified as active, according to the criteria established by Campos et al. (2003), which establishes that natural products with EC_{50} less than 500 ppm are considered active.

The antioxidant activity of a plant compound is inversely proportional to EC_{50} values, in which the lowest concentration indicates a greater ability of the oil to reduce the hydroxyl radical by 50% (SOUSA et al., 2007). The nanoemulsion obtained showed an EC_{50} slightly higher than that of the EO, thus demonstrating that the nanoemulsion increases the efficiency of the antioxidant activity of the EO, proving the existence of an increase in the potentiality of the EO when working with nanoemulsions. Among the evaluated products, the MEO presented the best result, compared to the other products, regarding the antioxidant activity. It was also evidenced that all analyzed bioproducts have biotechnological potential, despite EOB presenting moderate activity.

However, as there are no studies with cross-EO, we compared results of studies in the literature that report the action of essential oils from *Pimpinella anisum* (anis) and *Laurus nobilis* (laurel) plants individually as an antioxidant agent.

Belasli et al. (2020), using the DPPH method, reported that *L. nobilis* EO, extracted from dried leaves harvested in Algeria, had an antioxidant capacity of 602 ppm, a result inferior to that obtained in this study. While Al-mijalli et al. (2022) identified, using the hydroxyl radical test, an EC_{50} value of 354 ppm for *L. nobilis*, a result also lower than that obtained in this work.

Research with the EO of *P. anisum* present inferior results, as Rebey et al. (2020), who quantified in their DPPH radical scavenging tests an EC_{50} of 114.87 ppm and 287.56 ppm, respectively, for the shade and oven drying method. Furthermore, the work by Ouis & Hariri (2021) quantified an EC_{50} of 118 ppm determined at a concentration of 1000 ppm by the DPPH method. Such values ensure the reliability of the values obtained in this study.

These differences in EC_{50} can be attributed to analytical methodologies and to the various factors that influence the chemical composition of the cross-EO, such as variety, plant growth conditions, EO storage conditions and the extraction methods used (BELASLI et al., 2020).

As a result of research in the literature, the antioxidant activity of the essential oil is related to its phenolic content; A comparative study evaluating the ability to capture free radicals between different classes of chemical compounds indicated that phenolic compounds present, in vitro, much higher antioxidant activity than non-phenolic compounds (GOUDJIL et al., 2015).

In this study, remarkable antioxidant activity was obtained. This significant antioxidant activity of EO can be attributed to the presence of compounds such as monoterpenes and oxygenated sesquiterpenes, both of which have been reported to have an inhibitory oxidation power (PEIXOTO, et al., 2017).

In view of the results, it was evident that *L. nobilis and P. anisum* combined have important antioxidant characteristics and, therefore, can be considered a promising source of natural antioxidants. The antioxidant activity of an essential oil is the ability to scavenge free radicals. If essential oils can scavenge some free radicals, they can also act as anti-inflammatory agents, as one of the inflammatory responses is the oxidative explosion that occurs in various cells-monocytes, neutrophils, eosinophils and macrophages (MIGUEL, 2010). These facts encourage the continuation of studies to evaluate the anti-inflammatory activity of the cross-EO.

Anti-inflammatory activity

The albumin denaturation method was used to evaluate the anti-inflammatory capacity of the cross-essential oil and nanoemulsion, as well as their bioproducts, relative to the 50% efficient concentration (EC_{50}) in ppm. The results are shown in Table 4.

Table 4. Antioxidant capacity for cross essential oil, nanoemulsion, biofilm and microencapsulation of *Pimpinella anisum* and *Laurus nobilis.*

ID	CE_{50} ppm	Equity straight	R^2
EO	12.02	y=44.201x+2.2622	0.9756
EON	28.96	y=64.71x-44.593	0.9880
MEO	4.39	y=32,296x+29,239	0.9918
MEON	4.07	y=31.101x+31.052	0.9931
EOB	4.27	y=27.27x+32.808	0.9926
EONB	2.99	y=33.706x+33.945	0.9882

Note: EO – essential oil; EON – essential oil nanoemulsion; MOE – microencapsu lation of essential oil; MEON – microencapsulation of essential oil nanoemulsion; OEB – essential oil biofilm; EONB – essential oil nanoemulsion biofilm.

In agreement with Table 4, the essential oil crossed, microencapsulated, biofilm and nanoemulsion present significant values, proving the effective anti-inflammatory action and being classified as very active, according to the criteria established by

Jonville et al. (2011), which establishes that natural products with EC_{50} less than 50 ppm are considered very active.

The assay is based on the denaturation of albumin caused by temperature. The consequences of structural loss of denatured proteins result in decreased water solubility. These characteristics are evaluated in the spectrophotometer. The higher the absorbance, the greater the degree of denaturation (REGO, 2013; PADMANABHAN&JANGLE, 2012).

To date, no study assessing the anti-inflammatory activity of cross-EO has been published. However, there are studies in the literature with the essential elements separated, such as Al-mijalli et al. (2022), who obtained an EC_{50} value of 48.31 ppm for *L. nobilis essential oil* using a different method. While Alomar et al. (2022) studying the anti-inflammatory action of essential oils from three Apiaceae fruits against *Helicobacter pyroli* obtained an EC_{50} of 10.7 ppm for *P. anisum*, also using a different method than that used in this study. Therefore, the results obtained in this study can be evaluated as promising.

Among the analyzed bioproducts, EONB was the one with the highest anti-inflammatory capacity. The bioproducts, MEO, MEON and BOE had very close EC_{50} values, and possibly with similar anti-inflammatory capacity. The anti-inflammatory capacity of EON was the one with the lowest performance, but both the oil and its byproducts have anti-inflammatory potential.

Final considerations

In this study we report the chemical composition as well as the antioxidant and anti-inflammatory activity of the cross essential oil and its bioproducts. The chromatographic analysis revealed a diversity of volatile compounds in the cross EO, such compounds are responsible for the biological activity of the plants.

Based on the obtained results, the nanoemulsion presented stability and biotechnological potential. It was possible to conclude that the crossed EO and its bioproducts have antioxidant and anti-inflammatory potential.

The mechanisms involved are complex and poorly understood, as the EO contains a mixture of several dozen chemical constituents of various structures and modes of action that can interact to reduce or increase its effectiveness. This study, unprecedented for this combination, brings new scientific evidence, however, to fully evaluate the potential applications of cross-essential EO and its bioproducts, it is necessary to carry out studies on its toxicological effects to verify its safety.

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4. CONSIDERAÇÕES FINAIS

Neste estudo relatamos a composição química, bem como a atividade antioxidante e anti-inflamatória do óleo essencial cruzado e seus bioprodutos. A análise cromatográfica revelou uma diversidade de compostos voláteis no OE cruzado, tais compostos são responsáveis pela atividade biológica das plantas. Com base nos resultados obtidos, a nanoemulsão apresenta estabilidade e potencial biotecnológico. Foi possível concluir que o OE cruzado e seus bioprodutos possuem potencial antioxidante e anti-inflamatório. Os mecanismos envolvidos são complexos e pouco compreendidos, pois o OE contém uma mistura de várias dezenas de constituintes químicos de modos de ação que podem interagir de forma a reduzir ou aumentar sua eficácia. Este estudo, inédito para essa combinação, traz novas evidências cientificas, no entanto, para avaliar completamente as aplicações potenciais do OE essencial cruzado e seus bioprodutos são necessários realizar estudos sobre seus efeitos toxicológicos para verificar sua segurança.

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ANEXO A - NORMAS DA REVISTA

Submission Preparation Checklist

As part of the submission process, authors are required to check off their submission's compliance with all of the following items, and submissions may be returned to authors that do not adhere to these guidelines.

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If not, justify on “Editor’s Comments”.

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Add "HIGHLIGHTS" of the article (submitted as supplementary material), which are the main contributions of the article. Example: https://www.elsevier.com/authors/journal-authors/highlights;

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6. Prior to publication, the Authors will receive the Final Proof of the articles. At this moment, no modification will be allowed. Only typographical errors due to diagramation will be accepted. If the Final Proof cannot be sent for any reason, the Editorial Team will do this review.

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