UFSM

CIÊNCIA e NATURA

ISSN 2179-460X
OPEN ACCESS

Ci. e Nat., Santa Maria, v. 48, e88052, 2026 • https://doi.org/10.5902/2179460X88052
Submitted: 06/28/2024 • Approved: 04/24/2026 • Published: 06/18/2026

Chemistry

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)

Renato Barros Pereira[I], **Thamires de Jesus Teles Ribeiro**[I], **Américo Pinheiro Neto**[I], **Victor Elias Mouchrek Filho**[I], **Gustavo Oliveira Everton**[I]

[I] Universidade Federal do Maranhã, São Luís, MA, Brasil

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* (bay 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 CFT determination yielded 54.27 and 1.71 mg EAT/g 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 mg/L were quantified, respectively. It can be stated that the cross-EO and its bioproducts showed satisfactory results, which evidenced the effectiveness of the synergistic properties and its biotechnological potential.

Keywords: Essential oil; Antioxidant; Anti-inflammatory

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 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 mg/L. 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; Antioxidante; Anti-inflamatório

1 INTRODUCTION

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

Due to their physicochemical 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 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, 2004).

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) are widely used as condiments in domestic preparations, in folk medicine and by the food, cosmetics and pharmaceutical industries (Patrakar; Mansuriya; Patil, 2012; Tepe, 2015). They stand out for presenting biological activity, namely: antioxidant, antimicrobial, antifungal, anti-inflammatory and analgesic activities (Fernández et al., 2018; Ercin et al., 2022; Siriken et al., 2018; Mohammadi et al., 2015; Iannarelli, R. et al., 2018).

Essential oils (EOs), derived from various parts of aromatic plants, are composed of a wide range of volatile, low molecular weight secondary metabolites. These compounds have garnered significant scientific interest due to their multiple applications in the cosmetic, pharmaceutical, and food industries (Zengin et al., 2018; Dhara & Tripathi, 2013; Vasireddy et al., 2018; Khorshidian et al., 2018). Among the diverse biological properties attributed to these compounds, antioxidant activity stands out as one of the most relevant, representing a significant fraction of the chemical composition of EOs (Miguel, 2010).

The biological importance of natural antioxidants is intrinsically linked to their ability to counteract oxidative stress. This physiological imbalance occurs when there is a disruption in the pro-oxidant/antioxidant equilibrium, leading to an increased production of Reactive Oxygen Species (ROS) – such as alkoxyl (RO•), superoxide anion (O2•-), hydroxyl (HO•), and peroxyl (RO2•) radicals – and Reactive Nitrogen Species (RNS), such as nitric oxide (NO•) and peroxynitrite (ONOO−) (Poprac et al., 2017; Weidinger & Kozlov, 2015). Oxidative stress is implicated in the pathogenesis of various human diseases, including neurodegenerative disorders like Alzheimer's and cancer (Poprac et al., 2017).

In this context, bioactive compounds present in condiment and aromatic plants are considered promising sources of natural antioxidants capable of preventing or mitigating damage induced by oxidative stress (Qasim Barkat & Khalid Mahmood, 2018). The antioxidant activity of many of these compounds, such as thymol, carvacrol, menthol, eucalyptol, and eugenol, is often related to the presence of hydroxyl groups in their chemical structures, which act as electron donors to neutralize free radicals (Zhang, 2005). Ongoing investigation into these compounds not only validates their traditional uses but also paves the way for the development of novel pharmaceutical, cosmetic, and food applications, offering a natural alternative to synthetic antioxidants, which may pose adverse health effects (Vital et al., 2016; Olmedo et al., 2013).

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, 2016). Bioproducts from the species studied 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., 2016). 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).

2 METHODOLOGY

2.1 Obtaining plant material

The aerial parts of *Pimpinella anisum* and leaves of *Laurus nobilis* used in this study were obtained from the federally certified distributor. 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.

2.2 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 mantle as a source of heat. 100 g of each plant material were used, previously dried in a FANEM 520 convective air oven at 45°C, adding distilled water

(1:10). 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 before being submitted to analyses.

2.3 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. 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 to 240 °C, followed by an increase of 10 °C/min 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 and a scan interval of 0.5 fragments/s. 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 Dool and Kratz equation (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., 2017). 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.

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 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 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.

2.5 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.

2.6 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.

2.7 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, 2002). 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) of tannic acid.

2.8 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).

Essential oil solutions (10-100 mg/L) in 0.2% DMSO, biofilms (10-100 mg/L) 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 mg/L.

2.9 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 mg/L) diluted in PBS, biofilms (10-100 mg/L) 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 mg/L.

3 RESULTS AND DISCUSSION

3.1 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 cross-EO 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-cineol	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

Source: Autorship (2024)

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-cineol 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)

3.2 Total Phenolics

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

Table 2 – Total phenolic content - CFT (mg EAT/g) for essential oil and nanoemulsion, from *Pimpinella anisum* and *Laurus nobilis*

ID	CFT (mg EAT/g)	Equation	R^2
EO	54.27	y=0.0586x+0.06	0.9999
NEO	1.71		

Note: EO- essential oil; NEO- nanoemulsion. Source: Autorship (2024)

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 literature search was carried out 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, 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 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, 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 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 e Angelis-Pereira, 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.

3.3 Antioxidant activity

Table 3 presents results obtained for the antioxidant capacity of the cross essential oil and nanoemulsion, as well as their by- products, relative to the 50% efficient concentration (EC_{50}) in mg/L, 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} (mg/L)	Equation	R^2
OE	9.72	y=50.104x+0.5107	0.9897
NEO	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; NEO- essential oil nanoemulsion; MEO- microencapsulation of essential oil; MEON- microencapsulation of essential oil nanoemulsion; EOB – essential oil biofilm; EONB – essential oil nanoemulsion biofilm. Source: Autorship (2024)

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 mg/L 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 mg/L, 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 mg/L for *L. nobilis*, a result also lower than that obtained in this work.

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

3.4 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 mg/L. The results are shown in Table 4.

Table 4 – Anti-inflammatory capacity for cross essential oil, nanoemulsion, biofilm and microencapsulation of *Pimpinella anisum* and *Laurus nobilis*

ID	CE_{50} (mg/L)	Equation	R^2
EO	12.02	y=44.201x+2.2622	0.9756
NEO	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; NEO- essential oil nanoemulsion; MOE- microencapsulation of essential oil; MEON- microencapsulation of essential oil nanoemulsion; OEB – essential oil biofilm; EONB – essential oil nanoemulsion biofilm. Source: Autorship (2024)

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 mg/L 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, 2012; 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 individual essential oils, such as Almijalli et al. (2022), who obtained an EC_{50} value of 48.31 mg/L for *L. nobilis essential oil* using a different method. While Alumar et al. (2022) studying the anti-inflammatory action of essential oils from three Apiaceae fruits against *Helicobacter pylori* obtained an EC_{50} of 10.7 mg/L 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.

4 CONCLUSIONS

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 remain 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.

REFERENCES

Aala, J., Ahmadi, M., Golestan, L., Shahidi, S. A., & Shariatifar, N. (2023). Effect of multifactorial free and liposome-coated of bay laurel (Laurus nobilis) and rosemary (Salvia rosmarinus) extracts on the behavior of Listeria monocytogenes and Vibrio parahaemolyticus in silver carp (Hypophthalmichthys molitrix) stored at 4° C. *Environmental Research*, 216, 114478. doi: https://doi.org/10.1016/j.envres.2022.114478

Adams, R. P. (2017). Identification of essential oil components by gas chromatography/mass spectrometry. 5 online ed. *Gruver, TX USA: Texensis Publishing*. http://www.juniperus.org/uploads/2/2/6/3/22639912/bk4frontisbnpreface-contents5thedonline2017.pdf

Al-Mijalli, S. H., Mrabti, H. N., Ouassou, H., Flouchi, R., Abdallah, E. M., Sheikh, R. A., ... & Faouzi, M. E. A. (2022). Chemical composition, antioxidant, anti-diabetic, anti-acetylcholinesterase, anti-inflammatory, and antimicrobial properties of Arbutus unedo L. and Laurus nobilis L. essential oils. *Life*, 12(11), 1876. doi: https://doi.org/10.3390/life12111876

Aumeeruddy-Elalfi, Z., Gurib-Fakim, A., & Mahomoodally, M. F. (2016). Kinetic studies of tyrosinase inhibitory activity of 19 essential oils extracted from endemic and exotic medicinal plants. *South African Journal of Botany*, 103, 89-94. doi: https://doi.org/10.1016/j.sajb.2015.09.010

Avola, R., Granata, G., Geraci, C., Napoli, E., Graziano, A. C. E., & Cardile, V. (2020). Oregano (Origanum vulgare L.) essential oil provides anti-inflammatory activity and facilitates wound healing in a human keratinocytes cell model. *Food and Chemical Toxicology*, 144, 111586. doi: https://doi.org/10.1016/j.fct.2020.111586

Bassolé, I. H. N., & Juliani, H. R. (2012). Essential oils in combination and their antimicrobial properties. *Molecules*, 17(4), 3989-4006. doi: https://doi.org/10.3390/molecules17043989

Belasli, A., Ben Miri, Y., Aboudaou, M., Aït Ouahioune, L., Montañes, L., Ariño, A., & Djenane, D. (2020). Antifungal, antitoxigenic, and antioxidant activities of the essential oil from laurel (Laurus nobilis L.): Potential use as wheat preservative. *Food science & nutrition*, 8(9), 4717-4729. doi: https://doi.org/10.1002/fsn3.1650

Campos, M. G., Webby, R. F., Markham, K. R., Mitchell, K. A., & Da Cunha, A. P. (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

da Cunha, A. P., Nogueira, M. T., & Roque, O. R. (2012). *Plantas aromáticas e óleos essenciais: composição e aplicações*. Lisboa (Portugal): Fundação Calouste Gulbenkian.

de Lima, T. C. P., de Almeida, A. F., de Oliveira, E. C. P., Carrera Silva Júnior, J. O., Ribeiro Costa, R. M., Pena Matos, A., & Fonseca Gomes, M. R. (2020). Desenvolvimento de nanogel de copaifera reticulata sobre a lesão muscular em ratos usando fonoforese. *Saúde e Pesquisa*, 13(1). doi: 10.17765/2176-9206.2020v13n1p181-192

Dhara, L., & Tripathi, A. (2013). Antimicrobial activity of eugenol and cinnamaldehyde against extended spectrum beta-lactamase producing enterobacteriaceae by in vitro and molecular docking analysis. *European Journal of Integrative Medicine, 5*(6), 527–536. doi: https://doi.org/10.1016/j.eujim.2013.08.005

Dhifi, W., Bellili, S., Jazi, S., Nasr, S. B., El Beyrouthy, M., & Mnif, W. (2018). Phytochemical composition and antioxidant activity of Tunisian Laurus nobilis. *Pak. J. Pharm. Sci*, 31(6), 2397-2402. https://www.researchgate.net/profile/Wissem-Mnif/publication/328654107_Phytochemical_composition_and_antioxidant_activity_of_Tunisian_Laurus_nobilis/links/5bdab17aa6fdcc3a8db5112e/Phytochemical-composition-and-antioxidant-activity-of-Tunisian-Laurus-nobilis.pdf

El Asbahani, A., Miladi, K., Badri, W., Sala, M., Addi, E. A., Casabianca, H**., ...** & Elaissari, A. (2015). Essential oils: From extraction to encapsulation. *International journal of pharmaceutics*, 483(1-2), 220-243. doi: https://doi.org/10.1016/j.ijpharm.2014.12.069

Ercin, E., Kecel-Gunduz, S., Gok, B., Aydin, T., Budama-Kilinc, Y., & Kartal, M. (2022). Laurus nobilis L. essential oil-loaded PLGA as a nanoformulation candidate for cancer treatment. *Molecules*, 27(6), 1899. doi: https://doi.org/10.3390/molecules27061899

Fadel, H. H. M., El-Ghorab, A. H., Hussein, A. M., El-Massry, K. F., Lotfy, S. N., Ahmed, M. Y. S., & Soliman, T. N. (2020). Correlation between chemical composition and radical scavenging activity of 10 commercial essential oils: Impact of microencapsulation on functional properties of essential oils. *Arabian Journal of Chemistry*, 13(8), 6815-6827. doi: https://doi.org/10.1016/j.arabjc.2020.06.034

Feijó, A. M., Bueno, M. E. N., Ceolin, T., Linck, C. L., Schwartz, E., Lange, C**., ...** & Heiden, G. (2012). Plantas medicinais utilizadas por idosos com diagnóstico de Diabetes mellitus no tratamento dos sintomas da doença. *Revista Brasileira de Plantas Medicinais*, 14, 50-56. doi: https://doi.org/10.1590/S1516-05722012000100008

Ferreira, A. R. A. (2014). *Uso de óleos essenciais como agentes terapêuticos* (Doctoral dissertation, [sn]). http://hdl.handle.net/10284/4513

Fidan, H. et al. (2019). Composição química e atividade antimicrobiana dos óleos essenciais de Laurus nobilis L. da Bulgária. *Moléculas*, v. 24, n. 4, pág. 804. https://www1.ibb.unesp.br/Home/Departamentos/Botanica/RBPM-RevistaBrasileiradePlantasMedicinais/artigo10_v6_n2.pdf

Figueredo, G., Özcan, M. M., Chalard, P., Özcan, M. M., Uslu, N., & Al Juhaimi, F. (2020). Chemical composition of essential oil of anise (Pimpinella anisum), cumin (Cuminum cyminum), fennel (Foeniculum vulgare) and parsley (Petroselinum crispum Mill.) seeds. *J. Agroaliment. Pro-cesses Technol*, 26(1), 01-05. https://journal-of-agroalimentary.ro/admin/articole/79799L1_Guilles_Figueredo_2020_26(1)_1-5.pdf

Goudjil, M. B., Ladjel, S., Bencheikh, S. E., Zighmi, S., & Hamada, D. (2015). Study of the chemical composition, antibacterial and antioxidant activities of the essential oil extracted from the leaves of Algerian Laurus nobilis Lauraceae. *Journal of Chemical and Pharmaceutical Research*, 7(1), 379-385. https://www.researchgate.net/profile/Mohamed-Goudjil-2/publication/271643791_Study_of_the_chemical_composition_antibacterial_and_antioxidant_activities_of_the_essential_oil_extracted_from_the_leaves_of_Algerian_Laurus_nobilis_Lauraceae/links/54ce4dd00cf29ca810fb8c8a/Study-of-the-chemical-composition-antibacterial-and-antioxidant-activities-of-the-essential-oil-extracted-from-the-leaves-of-Algerian-Laurus-nobilis-Lauraceae.pdf

Iannarelli, R., Marinelli, O., Morelli, M. B., Santoni, G., Amantini, C., Nabissi, M., & Maggi, F. (2018). Aniseed (Pimpinella anisum L.) essential oil reduces pro-inflammatory cytokines and stimulates mucus secretion in primary airway bronchial and tracheal epithelial cell lines. *Industrial crops and products*, 114, 81-86. doi: https://doi.org/10.1016/j.indcrop.2018.01.076

Jonville, M. C., Kodja, H., Strasberg, D., Pichette, A., Ollivier, E., Frédérich, M., ... & Legault, J. (2011). Antiplasmodial, anti-inflammatory and cytotoxic activities of various plant extracts from the Mascarene Archipelago. *Journal of ethnopharmacology*, 136(3), 525-531. doi: https://doi.org/10.1016/j.jep.2010.06.013

Khorshidian, N., Yousefi, M., Khanniri, E., & Mortazavian, A. M. (2018). Potential application of essential oils as antimicrobial preservatives in cheese. *Innovative Food Science & Emerging Technologies, 45*, 62–72. doi: https://doi.org/10.1016/j.ifset.2017.09.020

Kubitschek-KM, A. R. J., & Zero, J. M. (2014). Development of jojoba oil (Simmondsia chinensis (Link) CK Schneid.) based nanoemulsions. *Lat. Am. J. Pharm*, 33(3), 459-63. https://www2.unifap.br/quimica/files/2020/02/Development-of-Jojoba-Oil-Simmondsia-chinensis-Link-C.K.-Schneid.-Based.pdf

Martins, T. G. T., Rosa, P. V. S., de Araújo Neto, A. P., Carvalho, A. M. A. S., da Silva Silveira, L., Neves, I. R., ... & Mouchrek Filho, V. E. (2021). Chemical profile, bactericidal in vitro potential and toxicity against Artemia salina Leach of essential oils obtained from natural condiments. *Research, Society and Development*, 10(2), e58310212898-e58310212898. doi: https://doi.org/10.33448/rsd-v10i2.12898

McClements, D. J. (2004). *Food emulsions: principles, practices, and techniques*. Florida (USA): CRC press.

Miguel, M. G. (2010). Antioxidant and anti-inflammatory activities of essential oils: a short review. *Molecules*, 15(12), 9252-9287. doi: https://doi.org/10.3390/molecules15129252

Mohammadi, S. (2015). The Evaluation of the Analgesic Effects and Acute Toxicity of Methanol Extract of Pimpinella anisum. L in Male Wistar Rats. *Journal of Babol University of Medical Sciences*, 17(5), 59-65. https://www.researchgate.net/publication/281708623_The_Evaluation_of_the_Analgesic_Effects_and_Acute_Toxicity_of_Methanol_Extract_of_Pimpinella_anisumL_in_Male_Wistar_Rats#full-text

Mssillou, I., Agour, A., El Ghouizi, A., Hamamouch, N., Lyoussi, B., & Derwich, E. (2020). Chemical composition, antioxidant activity, and antifungal effects of essential oil from Laurus nobilis L. flowers growing in Morocco. *Journal of Food Quality*, 2020(1), 8819311. doi: https://doi.org/10.1155/2020/8819311

Olmedo, R. H., Nepote, V., & Grosso, N. R. (2013). Preservation of sensory and chemical properties in flavoured cheese prepared with cream cheese base using oregano and rosemary essential oils. *LWT - Food Science and Technology, 53*(2), 409–417. doi: https://doi.org/10.1016/j.lwt.2013.04.007

Ouis, N., & Hariri, A. (2021). Chemical analysis, antioxidant and antibacterial activities of Aniseeds essential oil. *Agriculturae Conspectus Scientificus*, 86(4), 337-348. https://hrcak.srce.hr/267393

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. http://cibtech.org/J-MEDICAL-SCIENCES/PUBLICATIONS/2012/JMS-02-01-PDF/22%20JMS%2043%20Preeti%20Padmanabhan.pdf

Patrakar, R., Mansuriya, M., & Patil, P. (2012). Phytochemical and pharmacological review on Laurus nobilis. *International journal of pharmaceutical and chemical sciences*, 1(2), 595-602. https://api.semanticscholar.org/CorpusID:25417289

Peixoto, L. R., Rosalen, P. L., Ferreira, G. L. S., Freires, I. A., de Carvalho, F. G., Castellano, L. R., & de Castro, R. D. (2017). Antifungal activity, mode of action and anti-biofilm effects of Laurus nobilis Linnaeus essential oil against Candida spp. *Archives of oral biology*, 73, 179-185. doi: https://doi.org/10.1016/j.archoralbio.2016.10.013

Poprac, P., Jomova, K., Simunkova, M., Kollar, V., Rhodes, C. J., & Valko, M. (2017). Targeting free radicals in oxidative stress-related human diseases. *Trends in Pharmacological Sciences, 38*(7), 592–607.https://www.cell.com/trends/pharmacological-sciences/abstract/S0165-6147(17)30097-4

Qasim Barkat, M., & Khalid Mahmood, H. (2018). Phytochemical and antioxidant screening of Zingiber officinale, Piper nigrum, Rutag raveolanes, and Carum carvi and their effect on gastrointestinal tract activity. *Matrix Science Medica, 2*(1), 09–13. doi: http://doi.org/10.26480/MSM.01.2018.09.13

Rebey, I., Bourgou, S., Ben Kaab, S., Aidi Wannes, W., Ksouri, R., Saidani Tounsi, M., & Fauconnier, M. L. (2020). On the effect of initial drying techniques on essential oil composition, phenolic compound and antioxidant properties of anise (Pimpinella anisum L.) seeds. *Journal of Food Measurement and Characterization*, 14, 220-228. doi: https://doi.org/10.1007/s11694-019-00284-4

Rego, E. A. (2012). *Avaliação da actividade anti-inflamatória de plantas dos açores* (Master's thesis, Universidade dos Acores (Portugal)). https://www.proquest.com/openview/36d21f1367aac329137c644cdd42649b/1?pq-origsite=gscholar&cbl=2026366&diss=y

Rodrigues, E. D. C., Ferreira, A. M., Vilhena, J. C., Almeida, F. B., Cruz, R. A., Florentino, A. **C., ...** & Fernandes, C. P. (2014). Development of a larvicidal nanoemulsion with Copaiba (Copaifera duckei) oleoresin. *Revista Brasileira de Farmacognosia*, 24, 699-705. doi: https://doi.org/10.1016/j.bjp.2014.10.013

Ru, Q., Hu, Q., Dai, C., Zhang, X., & Wang, Y. (2022). Formulation of Laurus nobilis essential oil nanoemulsion system and its application in fresh-cut muskmelons. *Coatings*, 12(2), 159. doi: https://doi.org/10.3390/coatings12020159

Séfora-Sousa, M., & Angelis-Pereira, D. (2013). Mecanismos moleculares de ação anti-inflamatória e antioxidante de polifenóis de uvas e vinho tinto na aterosclerose. *Revista Brasileira de Plantas Medicinais*, 15, 617-626. doi: https://doi.org/10.1590/S1516-05722013000400020

Shafiq, S., Shakeel, F., Talegaonkar, S., Ahmad, F. J., Khar, R. K., & Ali, M. (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

Sırıken, B., Yavuz, C., & Güler, A. (2018). Antibacterial Activity of Laurus nobilis: A review of literature. *Medical Science and Discovery*, 5(11), 374-379. doi: https://doi.org/10.17546/msd.482929

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

Sugumar, S., Clarke, S. K., Nirmala, M. J., Tyagi, B. K., Mukherjee, A., & Chandrasekaran, N. (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

Tepe, A. S., & Tepe, B. (2015). Traditional use, biological activity potential and toxicity of Pimpinella species. *Industrial Crops and Products*, 69, 153-166. doi: https://doi.org/10.1016/j.indcrop.2015.01.069

Van Den Dool, H. A. N. D., & Kratz, P. D. (1963). A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *Journal of chromatography*. https://worldveg.tind.io/record/1766/

Vasireddy, L., Bingle, L. E. H., & Davies, M. S. (2018). Antimicrobial activity of essential oils against multidrug-resistant clinical isolates of the *Burkholderia cepacia* complex. *PLOS ONE, 13*(8), e0201835. doi: https://doi.org/10.1371/journal.pone.0201835

Vital, A. C. P., Guerrero, A., Monteschio, J. D. O., Valero, M. V., Carvalho, C. B., De Abreu Filho, B. A., Madrona, G. S., & Do Prado, I. N. (2016). Effect of edible and active coating (with rosemary and oregano essential oils) on beef characteristics and consumer acceptability. *PLOS ONE, 11*(8), e0160535. doi: https://doi.org/10.1371/journal.pone.0160535

Waterhouse, A. L. (2002). Determination of total phenolics. *Current protocols in food analytical chemistry*, 6(1), I1-1. https://www.researchgate.net/profile/Roberto-Molteni/post/Does_Folin-Ciocalteu_total_phenolic_compound_analysis_include_tannins/attachment/5eaadefff155db0001f88a68/AS%3A886026180521987%401588256511570/download/totalphenolsmethods.pdf

Weidinger, A., & Kozlov, A. V. (2015). Biological activities of reactive oxygen and nitrogen species: Oxidative stress versus signal transduction. *Biomolecules, 5*(2), 472–484. doi: https://doi.org/10.3390/biom5020472

Zactiti, E. M., & Kieckbusch, T. G. (2006). Potassium sorbate permeability in biodegradable alginate films: Effect of the antimicrobial agent concentration and crosslinking degree. *Journal of Food Engineering*, 77(3), 462-467. doi: https://doi.org/10.1016/j.jfoodeng.2005.07.015

Zengin, G., Senkardes, I., Mollica, A., Picot-Allain, C. M. N., Bulut, G., Dogan, A., & Mahomoodally, M. F. (2018). New insights into the in vitro biological effects, in silico docking, and chemical profile of clary sage (*Salvia sclarea* L.). *Computational Biology and Chemistry, 75*, 111–119. doi: https://doi.org/10.1016/j.compbiolchem.2018.05.005

Zhang, H.-Y. (2005). Structure-activity relationships and rational design strategies for radical-scavenging antioxidants. *Current Computer-Aided Drug Design, 1*(3), 257–273. https://www.benthamdirect.com/content/journals/cad/10.2174/1573409054367691

Authorship contributions

1 – Renato Barros Pereira

Graduated in Industrial Chemistry from the Federal University of Maranhão
https://orcid.org/0000-0001-6570-3450 • renato.pereira@discente.ufma.br
Contribution: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Visualization, Writing– original draft, Writing– review editing

2 – Thamires de Jesus Teles Ribeiro

Undergraduate student in Industrial Chemistry at the Federal University of Maranhão
https://orcid.org/0000-0003-1495-9282 • thamiresbelle@gmail.com
Contribution: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Visualization, Writing– original draft, Writing– review editing

3 – Américo Pinheiro Neto

Graduating in Industrial Chemistry from the Federal University of Maranhão
https://orcid.org/0009-0005-6095-0336 • americo.pn@discente.ufma.br
Contribution: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Visualization, Writing– original draft, Writing– review editing

4 – Victor Elias Mouchrek Filho

PhD in Chemistry from the University of São Paulo
https://orcid.org/0000-0003-2855-7292 • victor.mouchrek@ufma.br
Contribution: Funding acquisition, Project administration

5 – Gustavo Oliveira Everton

PhD in Chemistry from the Federal University of Maranhão
https://orcid.org/0000-0002-0457-914X • gustavooliveiraeverton@gmail.com
Contribution: Conceptualization, Data, Curation, Formal Analysis, Funding, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review editing

How to quote this article

Pereira, R. B., Ribeiro, T. J. T., Pinheiro Neto, A., Mouchrek Filho, V. E., & Everton, G. O. (2026). 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). *Ciência e Natura*, 48, e88052. DOI: 10.5902/2179460X88052. Available in: https://doi.org/10.5902/2179460X88052