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Chemistry

Formulation of a nanoemulsion of the cross-essential oil of *Schinus terebinthifolius*, *Illicium verum* and *Syzygium aromaticum* to obtain silver nanoparticles with antioxidant and anti-inflammatory potential

Formulação de nanoemulsão do óleo essencial cruzado de *Illicium verum*, *Schinus terebinthifolius* e *Sygyzium aromaticum* para obtenção de nanopartículas de prata com potencial antioxidante e anti-inflamatório

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ABSTRACT

This study evaluated the chemical profile, antioxidant, and anti-inflammatory activities of silver nanoparticles (AgNPs) synthesized from the nanoemulsion of cross-processed essential oil (CEO) derived from *S. terebinthifolius, I. verum*, and *S. aromaticum* (NOE). Chemical constituents were identified via GC-MS analysis. The nanoemulsions were prepared using the phase inversion method, and AgNP synthesis was achieved through the reduction of $AgNO_3$ utilizing NOE. Antioxidant activity was assessed by the ABTS radical scavenging assay, while anti-inflammatory activity was evaluated through albumin protein denaturation. The predominant compound identified in the OE was trans-anethole. The highest antioxidant activity, with an IC_{50} of 3.03 mg/L, was observed for AgNPs at pH 10. The most effective anti-inflammatory response had an IC_{50} of 0.12 mg/mL. This research provides novel insights into AgNPs synthesized from NOE, demonstrating their potential to enhance the tested biological activities and highlighting the influence of pH on these formulations.

Keywords: Anethole; Cross; Synthesis

RESUMO

Este estudo avaliou o perfil químico, a atividade antioxidante e anti-inflamatória de nanopartículas de prata (AgNPs) sintetizadas a partir da nanoemulsão do óleo essencial cruzado (OE) de *S. terebinthifolius, I. verum e S. aromaticum* (NOE). Os componentes químicos foram identificados por CG-EM. As nanoemulsões foram preparadas pelo método de inversão de fases e a síntese das AgNPs ocorreu por redução do $AgNO_3$

utilizando NOE. A atividade antioxidante foi avaliada pelo método de descoloração do radical ABTS, enquanto a atividade anti-inflamatória foi analisada através da desnaturação de proteína albumina. O principal constituinte identificado no OE foi o E-anetol. A melhor atividade antioxidante, foi obtida para AgNPs com IC_{50} de 3,03mg/L em pH 10. Já a melhor atividade anti-inflamatória apresentou um IC_{50} de 0,12mg/mL. Este estudo trouxe resultados inéditos para AgNPs sintetizadas a partir do NOE, demonstrando sua eficácia em potencializar as atividades testadas e evidenciando o impacto do pH nessas formulações.

Palavras-chave: Anetol; Cruzado; Síntese

1 INTRODUCTION

Silver nanoparticles (AgNPs) have a wide range of applications and are among the most versatile nanostructures. They can be synthesized through various methods; however, many conventional techniques involve extreme conditions and the use of toxic chemicals, which may limit their practical applications (Ebrahiminezhad et al., 2016). Alternative approaches leverage bioactive compounds derived from plants, capitalizing on the notable capacity of AgNPs to be produced via their antioxidant potential (Ebrahiminezhad et al., 2016). The development of such green synthesis routes is particularly relevant for minimizing environmental impacts and offers a cost-effective alternative, given their low production costs (Guimarães et al., 2021).

Medicinal plant species documented in the literature serve as promising sources for AgNP synthesis. Notably, *Schinus terebinthifolius*, *Illicium verum*, and *Syzygium aromaticum* have been identified, despite limited studies focusing on the production of AgNPs via their essential oils or bioproducts derived thereof.

Schinus terebinthifolius, traditionally used in folk medicine, has recently attracted scientific interest regarding its essential oil. Macedo et al. (2018) emphasized the plant's rich composition of bioactive compounds, notably phenolic compounds and terpenes, which underlie its antimicrobial, healing, anti-inflammatory, and antioxidant activities (Dannenberg et al., 2017).

Illicium verum, commonly known as star anise, possesses essential oil primarily composed of trans-anethole. This compound is responsible for its diverse biological properties, including antibacterial, anti-inflammatory, anticancer, antioxidant, and antifungal activities (Lima et al., 2008; Pereira, 2016).

Syzygium aromaticum, popularly known as clove, is traditionally used for its healing properties, including alleviation of toothache and halitosis. Its essential oil predominantly contains eugenol, a compound with applications in perfumery, analgesics, biocides, and antioxidants (Radünz et al., 2017).

The present study aimed to evaluate the chemical profile, antioxidant capacity, and anti-inflammatory activity of silver nanoparticles synthesized via the nanoemulsion of the cross-essential oil of *S. terebinthifolius*, *I. verum*, and *S. aromaticum*.

2 METHODOLOGY

2.1 Obtaining plant material

The fruits of *S. terebinthifolius*, aerial parts of *I. verum*, and floral shoots of *S. aromaticum* used in this study were collected in August 2022 from a federally certified supplier. Post-collection, the plant materials were transported to the Laboratory for Research and Application of Essential Oils (LOEPAV/UFMA). The leaves were weighed, crushed, and stored prior to essential oil extraction.

2.2 Essential Oil Extraction

Essential oils (EOs) were extracted via hydrodistillation using a Clevenger-type apparatus connected to a round-bottom flask heated with a heating blanket. For each sample, 100g of dried plant material—dried in a FANEM 520 convection oven at 45°C—was combined with distilled water at a 1:10 ratio. Hydrodistillation was conducted at 100°C for 3 hours, after which the EO was collected. The extracted EO was dried by percolation with anhydrous sodium sulfate (Na_4SO_4) and centrifuged. All procedures were performed in triplicate. The oils were stored in amber glass ampoules at 4°C until further analysis.

2.3 Chemical Profiling

Chemical constituents were identified by gas chromatography-mass spectrometry (GC-MS) using a Shimadzu QP 2010 Plus system equipped with a fused silica capillary column (30 m × 0.25 mm, DB-5 bonded phase, 0.25 µm film thickness). Helium served as carrier gas at a flow rate of 1.0 mL/min. The injector and detector temperatures were set at 220 °C and 240 °C, respectively. Sample injections (0.5 µL) were prepared in hexane (1%) and split at a ratio of 1:100. The temperature program commenced at 60°C, increasing at 3 °C/min to 240 °C, then at 10°C/min until reaching 300 °C, which was maintained for 7 minutes. The total pressure was approximately 71.0 kPa.

Mass spectra were acquired in full scan mode from 45 to 500 Da, with a scan rate of 1000 Da/sec and a scan interval of 0.5 seconds. Data processing was performed using LabSolutions LC/GC Workstation 2.72 (Shimadzu). Retention indices were calculated relative to a homologous series of n-alkanes (C9–C18) using the Van den Dool and Kratz method (Van den Dool & Kratz, 1963). Compound identification was confirmed by comparing retention indices and mass spectra against literature references (Adams et al., 2007) and spectral libraries (FFNSC 1.2, NIST 107, and NIST 21).

Quantitative analysis was performed via GC-FID on a Shimadzu GC-2010, under conditions identical to the GC-MS analysis, except for the detector temperature set at 300°C. Relative percentages of constituents were calculated by area normalization.

2.4 Preparation of Nanoemulsions

Nanoemulsions were formulated following adapted protocols from Sugumar et al. (2014), Kubitschek et al. (2014), and Rodrigues et al. (2014). The EO concentration was fixed at 5% v/v. The oil phase components (oil + Tween 20) were heated to 65 ± 5°C, and the aqueous phase was heated separately to the same temperature.

The aqueous phase was gradually added to the oil phase with gentle mixing to produce a primary emulsion via phase inversion. Final homogenization was achieved using a magnetic stirrer at 6000 rpm while cooling to 25 ± 2°C.

Stability assessments involved centrifugation for phase separation, thermal stress testing through cycles of heating at 40°C and cooling at 4 °C over 48 hours (repeated thrice), and freeze-thaw cycles at -21°C and 25°C for 48 hours each (repeated twice). All tests were performed in triplicate.

2.5 Formulation and Characterization of Silver Nanoparticles

Silver nanoparticles (AgNPs) were synthesized following the adapted method of Sena et al. (2019) and Vilas, Philip, and Mathew (2014). A 1 mM $AgNO_3$ solution was prepared in distilled water, with the pH adjusted to 9 or 10 using NaOH (0.1 mol/L). For each pH condition, 10 mL of $AgNO_3$ solution was heated to 50°C under magnetic stirring.

Then, 500 µL of the nanoemulsion containing EO was added directly. The mixture was homogenized for 10 minutes and incubated at room temperature for 24 hours.

UV-Vis spectra (100–320 nm) were recorded using a spectrophotometer with quartz cuvettes (10 mm path length). Particle size and distribution were measured via dynamic light scattering (DLS) using a Zetasizer Nano ZS90 (Malvern Instruments, UK) at 633 nm wavelength, with a fixed scattering angle of 173°, at 25°C in triplicate, using 1.5 mL of diluted sample (dilution factor 3×).

2.6 Total Phenolics

Total phenolic content was quantified using the Folin-Ciocalteu method (Waterhouse, 2012). Samples (5 mg) were diluted in 1 mL ethanol. To each sample, 7 mL distilled water, 800µL Folin-Ciocalteu reagent, and 2 mL of 20% sodium carbonate were added. After 2 hours of incubation, absorbance was measured at 760 nm using a UV-Vis spectrophotometer. Results were expressed as mg of tannic acid equivalents per gram of sample (mg GAE/g).

2.7 Antioxidant Activity via ABTS Radical Scavenging

Antioxidant activity was assessed using the ABTS assay (Re et al., 1999). Sample solutions (EOs and AgNPs) at concentrations ranging from 5 to 100mg/L in ethanol were reacted with ABTS radical cation solution. In darkness, 100μL of each sample was mixed with 3mL of ABTS•+ solution, and absorbance was measured at 730nm after 6 minutes. All measurements were performed in triplicate. The percentage of radical scavenging was calculated, and the IC_{50} (concentration inhibiting 50% of radicals) was determined in mg/L.

2.8 Anti-Inflammatory Activity via Protein Denaturation

The anti-inflammatory potential was evaluated by the protein denaturation method (Padmanabhan & Jangle, 2012). Reactions (4 mL) contained 1 mL of sample solutions (EOs and AgNPs at 100–500 mg/L in PBS) and 3 mL of 10% bovine serum albumin (BSA) solution in PBS. The mixtures were incubated at 37°C for 15 minutes, then denatured by heating at 70°C for 10 minutes. After cooling, absorbance was measured at 660 nm. The percentage inhibition of protein denaturation was calculated, and the IC_{50} (mg/L) was determined as the concentration inhibiting 50% of denaturation.

3 RESULTS AND DISCUSSION

3.1 Chemical profile

Table 1 shows the chemical constituents identified in the essential oil of *S. terebinthifolius x I. verum x S. aromaticum*. Twenty-four compounds were quantified, with the majority E-anethole (29,83%), followed by Eugenol (23,75%) and δ-3-Carene (21,32%).

Carvalho *et al*. (2017) identified 13 components in the essential oil of the fruits of *Schinus terebinthifolius*, the majority component being Delta-3-Careno (40.52%). In turn, Silva *et al*. (2020) quantified 28 components in the essential oil of the fruits of *S. terebinthifolius*, with alpha-pinene being the majority component, present in 24.3%. Lima *et al*. (2014) quantified 5 components in the essential oil from the dried fruits of *Illicium verum*, the main component being (E)-anethole (90.41%). Corrêa (2017) identified 18 components in the essential oil of *I. verum.,* the majority being anethole (88.32%). Gomes *et al*. (2018) identified 5 components in the essential oil *S. aromaticum*, obtained from flower buds, with eugenol being the one that was present in the largest quantity (52.53%). Pereira *et al*. (2008) quantified 4 compounds in the essential oil of *S. aromaticum* and eugenol (86.3%) was the major component.

Table 1 - Chemical composition of the essential oil of *S. terebinthifolius x I. verum x S. aromaticum*

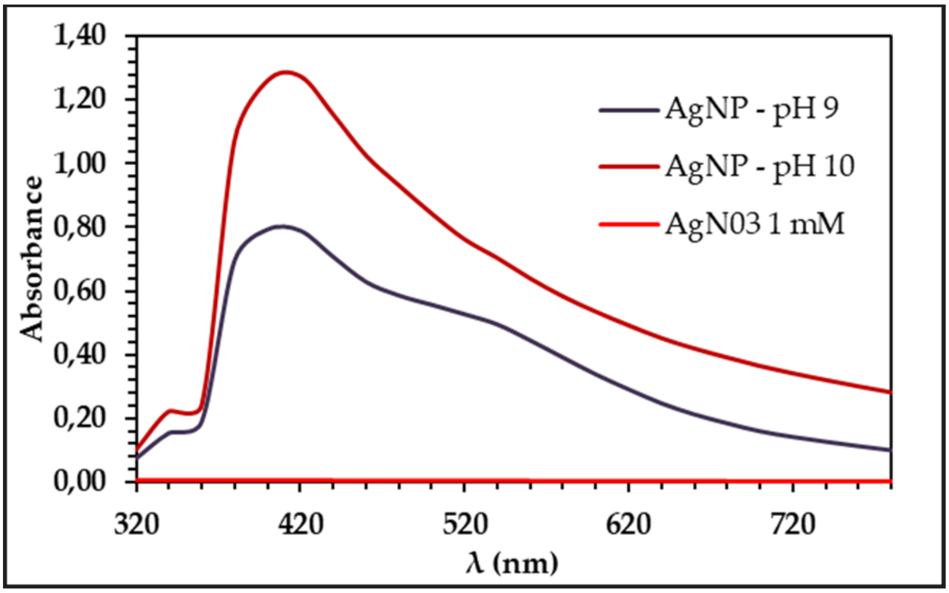
Compound	**IR^b**	**IR^c**	**(%)^a**
α-pinene	939	938	5,58
Myrcene	990	989	0,62
δ-3-carene	1011	1010	21,32
Silvestrene	1030	1029	3,24
Limonene	1034	1031	0,89
Terpinolene	1088	1087	0,58
Linalool	1115	1098	0,36
4-terpineol	1183	1177	0,10
Metyl chavicol	1209	1195	0,43
(E)-anethole	1293	1283	29,83
δ-elemene	1338	1337	0,17
Eugenol	1359	1358	0,63
Copaene	1367	1368	0,84
β-patchoulene	1381	1380	0,77
Eugenol	1388	1389	23,75
Sativene	1391	1390	0,19
β-cedrene	1420	1419	0,34
γ-elemene	1436	1435	0,16
Germacrene D	1481	1480	0,96
Humulene	1498	1498	1,32
Eugenil	1515	1515	0,92
Caryophyllene	1537	1538	6,45
Hedicariol	1548	1547	0,26
cis-α-Santalol	1675	1674	0,30

Note: a- Percentages obtained by peak area normalization FID; b- Linear Kovats retention indexes (column DB-5) experimental; c- Theoretical linear Kovats retention indexes
Source: Authors (2025)

3.2 Characterization of silver nanoparticles

Figure 1 shows the results regarding the UV-Vis characterization of the silver nanoparticles synthesized by *S. terebinthifolius x I. verum x S. aromaticum*.

Figure 1 – UV-Vis spectroscopic analysis of silver nanoparticles synthesized with *S. terebinthifolius x I. verum x S. aromaticum* EO nanoemulsion



Source: Authors (2025)

A gradual variation in color from light yellow to brown was observed during the reaction, due to the excitation of the plasmonic band (Mulfinger et al., 2007), as the pH elevation occurs.

Table 2 presents the data related to the size of the particles characterized by DLS assay.

Table 2 – Average size of the particle diameter of the AgNPs

S. terebinthifolius x I. verum x S. aromaticum	**Average diameter (nm)**	**PDI**	**Zeta potential (mV)**
NPAg-NEO pH 9,0	88,22	0,214	-2,01
NPAg-NEO pH 10,0	76,99	0,299	-2,11
NPAg-NEO pH 11,0	55,55	0,333	-7,09

Note: PDI-Polydispersion index; NPAg-NEO- silver nanoparticles
Source: Authors (2025)

Results discordants are presented by Ferreira et al. (2022) identified the size of the silver nanoparticle at 204 nm, synthesized from the aqueous extract of *Schinus terebinthifolius*, by the light scattering analysis (DLS) method. Luna et al. (2015) found the value of silver particle size 19 nm synthesized from the aqueous extract of *Illicium verum*, by the method of Transmission Electron Microscopy. Maciel et al. (2019) identified the silver particle size synthesized from *Sygyzium aromaticum* essential oil at 4 pH conditions (7, 8, 9 and 10), ranging from 31.4 nm to 71.9 nm, by the light scattering analysis method (DLS).

3.3 Total Phenolic Content

Table 3 presents the result regarding the quantification of the Total Phenolic Content (TPC).

Table 3 – Determination of Total Phenolic Content (TPC) of *S. terebinthifolius x I. verum x S. aromaticum* essential oil

EO	TPC mg EAT/g	Equation	R^2
S. terebinthifolius x I. verum x S. aromaticum	343,45	y=0,0586x+0,06	0,9998

Note: EO-Essential oil
Source: Authors (2025)

Similar results were observed by Jeribi et al. (2014) when analyzing the essential oil of *S. terebinthifolius* and quantifying a TPC content of 16 mg/L, collected in Tunisia, and extracted by the hydrodistillation method. Carneiro et al. (2022) also quantified the TPC content in the essential oil of *S. terebinthifolius*, obtained in Carmopólis (SE), Brazil, finding a value of 4572–4582 mg GAE/kg.

Similarly, Asif et al. (2016) found the value of 63.51 µg GAE/mg when quantifying TPC in essential oil *Illicium verum* from Malaysia. Costa (2015), in turn, found the value of 0.0528 µg EAG/µg when quantifying TPC in essential oil of *I. verum* from Vargem Grande Paulista, SP, Brazil. Radünz et al. (2019) quantitatively identified a TPC content of 9.07 GAE mg/g in *S. aromaticum* essential oil, collected in Pelotas (RS), Brazil, obtained by the hydrodistillation method. Costa (2015) obtained the value of 0.9690 µg EAG/µg when analyzing essential oil of *S. aromaticum,* from Vargem Grande Paulista (SP), Brazil.

3.4 Evaluation of antioxidant activity

Table 4 presents the results regarding the antioxidant activity of the nanoemulsion and silver nanoparticles synthesized through the essential oil of S. *terebinthifolius x I. verum x S. aromaticum.*

Table 4 – Antioxidant capacity of essential oil and silver nanoparticles

S. terebinthifolius x I. verum x S. aromaticum	**IC_{50} mg/L**	**Equation**	**R^2**
NEO	1,58	a=65,325; b=36,943	0,9981
NPAg-NEO pH 9,0	3,74	a=54,6069; b=18,7204	0,9984
NPAg-NEO pH 10,0	3,03	a=98,0448; b=2,7636	0,9996

Note: EO- Essential oil; NEO - Nanoemulsion; NPAg- NEO pH 9,0- Silver nanoparticles of *S. terebinthifolius x I. verum x S. aromaticum* pH 9,0; NPAg- NEO pH 10,0- Silver nanoparticles of *S. terebinthifolius x I. verum x S. aromaticum* pH 10,0
Source: Authors (2025)

According to Table 4, where the values for the antioxidant activity of the nanoemulsion of the essential oil of *S. terebinthifolius x I. verum x S. aromaticum* and of the formulated bioproduct were quantified, the best result for the nanoemulsion is noted, since it presents the lowest $IC_{50.}$ According to Campos et al. (2003), to be considered active, the IC_{50} must be quantified in values lower than 500 mg/L. Thus, it was observed that the nanoemulsion of the cross-essential oil of *S. terebinthifolius x I. verum x S. aromaticum* and the silver nanoparticles were active.

Similar results were observed by Jeribi et al. (2014) when quantifying the IC_{50} of 82 µg/mL, also obtained by the ABTS method and considered the active essential oil of *S. terebinthifolius* collected in Tunisia. Data obtained by Carneiro et al. (2022) were also found, who quantified the IC_{50} 65.7 mM TE/kg in samples from 2019 and 67 mM TE/kg in a 2020 sample, in the case of samples of *S. terebinthifolius* obtained by cold pressing in Carmopólis - SE. Asif et al. (2016) identified the IC_{50} of

75.90 µg/mL using the ABTS method for *I. verum* essential oil obtained in Malaysia. It was observed in the studies of Teles et al. (2021), the value of IC_{50} of 78.98 µg/mL of the essential oil of *S. aromaticum* from São Luís - MA, Brazil.Costa (2015) observed the IC_{50} of 2.13 µM/µg Trolox for the essential oil of *S. aromaticum* from Vargem Grande Paulista (SP), Brazil.

The function of the secondary metabolites of medicinal and aromatic plants, which may be in the structure of terpenes, phenols, aldehydes, esters, alcohols and ketones, are strongly correlated with their antioxidant characteristics Bakkali *et al*. (2008). The above-mentioned biological activities of EOs are generally associated not only with the main components, but also with the secondary components of the EOs (Calo et al., 2015).

3.5 Anti-inflammatory capability

Table 5 presents the results regarding the anti-inflammatory activity of nanoemulsion and silver nanoparticles synthesized through the cross-essential oil of *S. terebinthifolius x I. verum x S. aromaticum.*

Table 5 – Anti-inflammatory capacity of essential oil and silver nanoparticles

S. terebinthifolius x I. verum x S. aromaticum	**IC_{50} mg/L**	**Equation**	**R^2**
NEO	0,3660	a=0,1521; b=-5,6671	0,9909
NPAg-NEO pH 9,0	0,1725	a=0,1514; b=23,879	0,9964
NPAg-NEO pH 10,0	0,1244	a=0,0604; b=42,487	0,9926

Note: EO- Essential oil; NEO - Nanoemulsion; NPAg- NEO pH 9,0- Silver nanoparticles of *S. terebinthifolius x I. verum x S. aromaticum* pH 9,0; NPAg- NEO pH 10,0- Silver nanoparticles *S. terebinthifolius x I. verum x S. aromaticum* pH 10,0
Source: Authors (2025)

According to Jonville et al. (2011), to be considered active, the IC_{50} must be quantified in values lower than 0.13 mg/mL and greater than this value is considered moderate and of interesting activity. Thus, it was observed that the nanoemulsion of the essential oil of *S. terebinthifolius x I. verum x S. aromaticum* and silver nanoparticles were active.

Results proving the anti-inflammatory action are described by Luís et al.(2019) when identifying the IC_{50} of 12.03 mg/mL for *Illicium verum essential oil*. El-Nashar et al. (2021) found the IC_{50} value of 63.09 μg/mL for the hexane extract of *Schinus terebinthifolius.*Manaswini et al. (2019) found the IC_{50} value of 45.39 μL/mL for *Syzygium aromaticum* essential oil. The activity observed for the essential oil has long been known to have anti-inflammatory, immunomodulatory, bronchodilator and antiviral properties, as they contain several active phytochemicals (Asif et al., 2020).

4 CONCLUSIONS

In this study, silver nanoparticles were synthesized using an unprecedented approach, alongside the characterization of the chemical profiles of the essential oils from *Schinus terebinthifolius*, *Illicium verum*, and *Syzygium aromaticum*. Analysis identified 24 constituents, with trans-anethole emerging as the predominant component.

Evaluation of the biological activities of the formulations revealed that both the nanoemulsion and the silver nanoparticles (AgNPs) exhibited antioxidant activity. The most notable result was observed with the nanoemulsion at pH 9.

Additionally, the AgNPs demonstrated the highest anti-inflammatory activity at pH 10, which was classified as active according to literature criteria, while the other formulations showed moderate activity.

These unprecedented findings highlight the potential of the green synthesis of silver nanoparticles from essential oils as a promising and sustainable alternative. The results demonstrate significant potential for applications requiring anti-inflammatory and antioxidant properties. Furthermore, the study aligns with Green Chemistry principles by offering a method that reduces or eliminates the use of toxic chemicals in nanoparticle production.

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