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Chemistry

Green synthesis and biotechnological profile of silver nanoparticles using *Piper nigrum* L. essential oil

Síntese verde e perfil biotecnológico de nanopartículas de prata utilizando o óleo essencial de *Piper nigrum* L.

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ABSTRACT

It evaluated the chemical profile, antioxidant and anti-inflammatory activity, in an unprecedented way, of silver nanoparticles (AgNPs) synthesized from the essential oil nanoemulsion (NEO) of *Piper nigrum*. For essential oil extraction (EO), the hydrodistillation technique was used, and the chemical constituents were identified by Gas Chromatography Coupled to Mass Spectrometry (GC-MS). The nanoemulsions were prepared using the phase inversion method, and the synthesis of the AgNPs were performed by the AgNO3 reduction method using NEO. The AgNPs was characterized in terms of chemical profile by UV-Vis Spectrophotometry and particle size by Dynamic Mirroring of Light. Antioxidant activity was evaluated using the ABTS radical discoloration method and anti-inflammatory activity by protein denaturation. The majority constituent of the EO was limonene (42.41%). The maximum SPR band was centered at 420 nm, indicating the characteristic peak of the AgNPs. The lowest IC50 16.26 mg/L for antioxidant activity was obtained for AgNP pH 11. The IC50 that demonstrated the best result for anti-inflammatory activity was pH 11 was 0.217 mg/mL. This study brought in unprecedented results for AgNPs of *P. nigrum*, demonstrating to be efficient in improving the activities tested in this study and also demonstrating the effect of pH in these formulations.

Keywords: Antioxidant; Anti-inflammatory; Characterization

RESUMO

Este avaliou o perfil químico, atividade antioxidante e anti-inflamatória, de forma inédita, de nanopartículas de prata (AgNPs) sintetizadas a partir da nanoemulsão do óleo essencial (NEO) de *Piper nigrum*. Para extração do óleo essencial (EO), utilizou-se a técnica de hidrodestilação e os constituintes químicos foram identificados por Cromatografia Gasosa Acoplada à Espectrometria de Massas (CG-EM). As nanoemulsões foram preparadas através do método de inversão de fases e a síntese das AgNPs foram

realizadas pelo método de redução de AgNO3 utilizando a NEO. As AgNPs foram caracterizadas quanto ao perfil químico por Espectrofotometria UV-Vis e quanto ao tamanho de partícula por Espelhamento Dinâmico de Luz. A atividade antioxidante foi avaliada através do método de descoloração de radicais ABTS e a atividade anti-inflamatória por desnaturação proteica. O constituinte majoritário do EO foi o limoneno (42,41%). A banda máxima de SPR foi centrada em 420 nm indicando o pico característico das AgNPs. A menor IC50 16,26 mg/L para atividade antioxidante foi obtida para a AgNP pH 11. A IC50 que demonstrou o melhor resultado para a atividade anti-inflamatória foi a do pH 11 foi de 0,217 mg mL-1. Este estudo trouxe de forma inédita resultados para AgNPs de *P. nigrum*, demonstrando ser eficiente na melhoria das atividades testadas neste estudo, demonstrando também o efeito do pH nessas formulações.

Palavras-chave: Antioxidante; Anti-inflamatória; Caracterização

1 INTRODUCTION

Silver is one of the most important particles known for its popular medicinal properties (Ebrahiminezhad et al., 2016). The multifaceted properties of silver nanoparticles (AgNPs) have gained incredible importance in all emerging fields, especially in medicine, improving the therapeutic applications of mankind (Shaheen et al., 2016; Burdusel et al., 2018; El-Dali et al., 2021).

In the synthesis protocol of AgNPs, the plant-mediated green process is deservedly used by the scientific community due to its harmless, safe and benign process for the ecosystem (Ahmed et al., 2016). The classical physical and chemical methods had certain disadvantages, including the process requiring high temperature, pressure and toxic ingredients (Chung et al., 2016).

The emergence of nanoparticles has created a great scientific revolution in all fields to provide a sustainable environment for humanity (Diallo et al., 2014). They are exploring tremendous applications to strengthen human power against various threatening dangerous diseases, energy storage requirements and drug/gene delivery systems (Fries et al., 2021), but the current focus is on finding alternative sources for their production.

Plant sources have been selected by researchers mainly for the rapid production of AgNPs. The different parts of the plants such as root, stem, bark, leaf, flower and

fruit have been used as potential sources for the production of AgNPs (Nayak et al., 2016; Hebeish et al., 2016; Yuan et al., 2017; Patil et al., 2018; Lakshmanan et al., 2018; Behravan et al., 2019; Jebril et al., 2020). Therefore, in the present study, we selectively selected the essential oil nanoemulsion of *Piper nigrum* L., which is of medicinal value, for the preparation of AgNPs (Kanniah et al., 2021).

P. nigrum is popularly known as black pepper and belongs to the Piperaceae family (Takooree et al., 2019). The phytochemicals present in this species include flavonoids, alkaloids, terpenoids, tannins, polyphenols, carbohydrates and phenolic acids. These can act as reducing agents and stabilisers in the production of silver nanoparticles (Shervani et al., 2011; Liu et al., 2018). From a green synthesis perspective, we believe that *P. nigrum* has the potential to act as a reducing agent in the synthesis of AgNPs (Kanniah et al., 2021).

Thus, this study aimed to evaluate the chemical profile, antioxidant and anti-inflammatory activity of AgNPs synthesized from the essential oil nanoemulsion (NEO) of *P. nigrum*.

2 METHODOLOGY

2.1 Obtaining plant material

Piper nigrum seeds used in this study were collected in August 2022 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 (EO), 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 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. Subsequently submitted to analyses.

2.3 Chemical Profile

Identification of chemical constituents was performed by gas chromatography coupled to mass spectrometry (GC-MS) using a QP 2010 Plus instrument (Shimadzu Corporation, Kyoto, Japan) equipped 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 the carrier gas at a flow rate of 1.0 mL/min. The injector and detector temperatures were 220°C and 240°C respectively. The sample injection volume was 0.5 µL, diluted in hexane (1%) and the injection volume split ratio (split) was 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 to 300 °C, with the final temperature maintained for 7 min. The column pressure was approximately 71.0 kPa.

The mass spectrometer was operated at an ionisation potential of 70 eV and an ion source temperature of 200 °C. Mass analysis was performed in full scan mode, in the range of 45 to 500 Da, with a sweep speed of 1000 Da/s and a scan interval of 0.5 fragments/s. Data were acquired 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 (nC9 - nC18) 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.,

2007). Comparisons of the mass spectra obtained with those in the FFNSC 1.2, NIST107 and NIST21 libraries were also made.

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

2.4 Preparation of the nanoemulsions

The preparation of the nanoemulsions was carried out according to the adapted methods described by Sugumar et al. (2014), Kubitschek et al. (2014) and Rodrigues et al. (2014).

The EO concentration (5% v/v) was fixed for the formulation. The required amounts of each component of the oil phase (oil + Tween20) were heated to 65 ± 5°C. The aqueous phase was heated separately to 65 ± 5°C, gently added and mixed with the oil phase to form a primary formulation by the phase inversion method. Final homogenisation was achieved using a magnetic stirrer, in which the formulation was kept under constant stirring at 6000 rpm until the temperature was reduced to 25°C ± 2°C.

To demonstrate stability, the formulated nanoemulsions were subjected to different stress tests according to the methodology described by Shafiq et al. (2007). They were tested for phase separation by centrifugation. The heating-cooling cycle was carried out by keeping the formulated nanoemulsions at 40 and 4°C, alternating each temperature for 48 h. The cycle was repeated three times. This was done to check the stability of the nanoemulsion at variable temperatures. The freeze-thaw stress was carried out by keeping the nanoemulsions alternately at -21 and 25°C for 48 h at each temperature. This cycle was repeated twice. The experiment was performed in triplicate.

2.5 Formulation and characterization of nanoparticles

The synthesis of silver nanoparticles was carried out according to the methodology adapted from Sena et al. (2019) and Vilas, Philip and Mathew (2014). To obtain them, a solution of silver nitrate ($AgNO_3$, 1 mmol/L) was prepared in distilled water. For synthesis, the pH of the $AgNO_3$ solution was adjusted to 8, 9, 10 and 11 using sodium hydroxide solution (NaOH 0.1 mol/L). For each condition tested, 10 mL of the AgNO3 solution with the respective corrected pH was heated to 50 °C on a heating plate with constant magnetic stirring. For the addition of the essential oil, a solution of 1000 mg/L was prepared, diluted in acetone 1% for the concentrations of 4-167 mg/L, and added in a volume of 5 mL to the reaction system, giving a total volume of 15 mL. After mixing, the solution was homogenised for 10 min and then incubated for 24 h at room temperature.

Spectroscopic analyses in the UV-Vis range were performed on a spectrophotometer in the 100-320 nm range. 3 mL of each sample was pipetted into a 10 mm optical path quartz bucket at room temperature.

Measurements of the size and distribution of nanoparticles in the colloids were performed by the technique of dynamic light scattering - DLS, which evaluates the hydrodynamic beam using a Zetasizer System Nano ZS90 (Malvern Instruments, UK), according to the methodology described by Sena et al. (2019). Measurements were performed under the following conditions: laser wavelength (He-Ne) of 633 nm, fixed scattering angle of 173º and temperature of 25ºC, and normal resolution mode. Measurements were made using a polystyrene beaker (DTS0012) with a volume of 1.5 mL and a dilution factor of 3x.

2.6 Total Phenolics

The determination of 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 sample was read in a UV-VIS spectrophotometer at 760 nm. The standard curve was expressed in milligrams equivalent to grams (mg EAT/g) of tannic acid.

2.7 Antioxidant activity by elimination of ABTS radicals

A determination of antioxidant activity by the ABTS [2,2-azinobis-(3-ethylbenzothiazolin-6-sulphonic)] method was adapted according to the methodology proposed by Re et al. (1999). From the concentrations of essential oils and silver nanoparticles (5 to 100 mg/L) in ethanol, the reaction mixture with the ABTS radical cation was prepared. In the dark, an aliquot of 100 µL of each concentration of samples containing 3.0 mL of abts radical cation was transferred and after 6 minutes the absorbance of the reaction mixture was read in a spectrophotometer at a wavelength of 730 nm. Analyses were performed in triplicate. The elimination of ABTS radicals was expressed as a percentage and the 50% (EC_{50}/IC_{50}) effective concentrations capable of inhibiting 50% of the elimination, respectively, were expressed in mg/L.

2.8 Anti-inflammatory activity by albumin protein denaturation

The anti-inflammatory activity was evaluated using 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 and silver nanoparticles (100-500 mg/L) diluted in PBS and 3000 µL of a solution to 10% albumin diluted in PBS and incubated at (37±1) °C for 15 min. Denaturation was induced by keeping the reaction mixture in a water bath at 70°C for 10 minutes. After cooling, the absorbance was measured at 660 nm in a UV/VIS spectrophotometer. The inhibition of protein denaturation was expressed as a percentage and the 50% effective concentration (EC_{50}/IC_{50}) capable of inhibiting 50% of the denaturation was expressed in mg/L.

3 RESULTS AND DISCUSSION

3.1 Chemical profile

Table 1 presents the chemical composition of The EO of *P. nigrum* extracted in this study.

Table 1 – Chemical composition of the essential oil of *Piper nigrum*

Compound	IR[b]	IR[c]	(%)[a]
a-pinene	925	926	5,95
β-pinene	972	973	14,28
β-myrcene	992	993	2,72
a-phellandrene	1003	1002	3,49
3-carene	1010	1009	15,81
p-cymene	1024	1023	1,39
limonene	1028	1027	42,47
α-terpinolene	1088	1088	0,96
β-linalool	1100	1100	1,62
α-terpineol	1192	1192	0,21
α-copaene	1379	1380	1,01
β-caryophyllene	1426	1427	8,53
α-humulene	1461	1462	0,47
β-selinene	1493	1494	0,23
δ-cadinene	1529	1530	0,35
caryophyllene oxide	1591	1592	0,51

a- Percentages obtained by peak area normalization FID; b- Linear Kovats retention indexes (column DB-5) experimental; c- Theoretical linear Kovats retention indexes; Source: Authorship (2024)

Sixteen compounds were quantified with limonene being the major compound (42.47%). In their studies, Costa et al. (2010) quantified 17 compounds for *P. nigrum* EO using the CG/MS technique and reported E-caryofilena as the major compound (24.2%), in addition to quantified caryophilic oxide (20.1%), sabinee (19.9%) and limonene (13.0%).

Melo et al. (2021), also when performing GC/MS to quantify and identify chemical compounds, totalled 38 compounds, with four monoterpenes as main components, including: sabinee (30.62%), limonen (21.43%), β-pineno (9.62%), α-pineno (5.31%) and faire (2.37%), for *P. nigrum* EO obtained by hydrodistillation.

Furthermore, Costa et al. (2020), when performing GC/MS on *P. nigrum* EO obtained through hydrodistillation, quantified 29 volatile compounds and obtained a percentage of characterisation, especially monoterpenes with 51.66%, whose main representatives are sabinene (14.96%), wild (14.17%) and α-pipeno (5.28%), in addition to the main component e-caryoene (34.87%).

3.2 Characterization of silver nanoparticles

The colour change of the silver nitrate solution and the EO nanoemulsion is the preliminary identification to confirm the formation of AgNPs. Here, after adding the *P. nigrum* nanoemulsion to the silver nitrate solution, the colour of the solution changed to pale yellowish brown within 5-10 minutes. In addition, the solution changed to dark brown after 30 minutes of incubation, indicating the production of AgNPs by the reduction of silver metal ions.

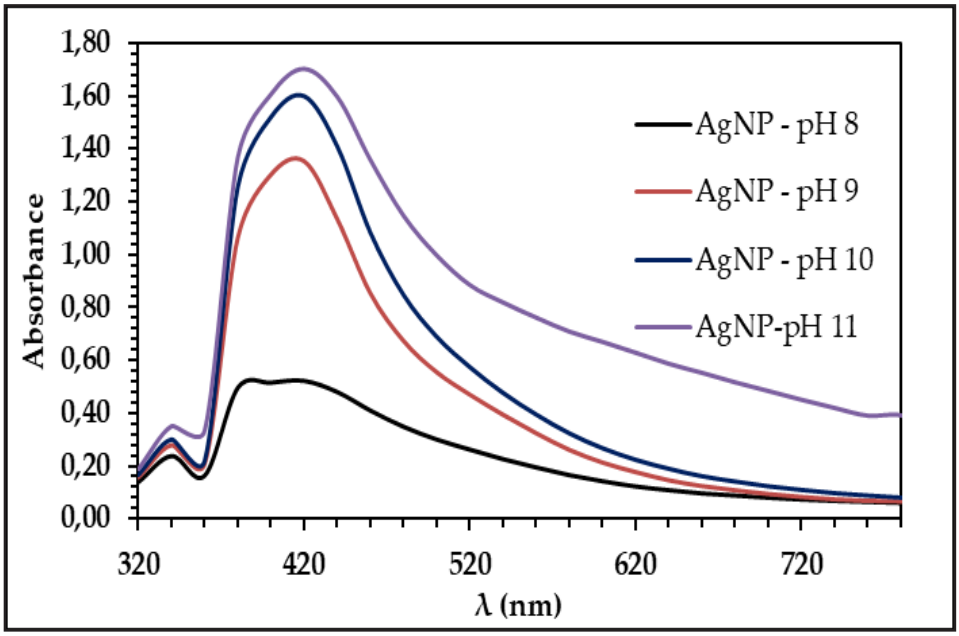
To verify the efficiency of AgNP synthesis at different pH values using *P. nigrum* EO nanoemulsion as reducing agent, the samples were analysed by UV-Vis spectroscopy from 320 to 800 nm and particle formation was observed by surface plasmon resonance (SPR) bands as shown in Figure 1.

Figure 1 shows that as we increase the pH the intensity of PRS increases directly proportional to it.

According to Figure 1, the maximum SPR band was increased by 380 nm and centered at 420 nm indicates the spr peak characteristic of the AgNPs. In all silver nitrate concentration (1-5 mM) the SPR peak was observed at 430 nm. It is emphasized that considering that the peak prS observed for the concentration of 1 mM was achieved more absorbance intensity compared to other concentrations of silver nitrate from

other autres, they reveal that the AgNPs increasingly synthesized at 1 mM of silver nitrate have higher intensity.

Figure 1 – UV-Vis spectroscopic analysis of silver nanoparticles synthesized with *P. nigrum* EO nanoemulsion



Source: Authorship (2024)

According to the stability of the synthesised AgNPs, the absorbance was measured and the peak of the SPR was observed at 420-435 nm. The stabilisation is based on the presence of existing stabilising agents in the EO of *P. nigrum*. Carboxylic acids and ketanacids have been reported to act as reducing and stabilising agents (Ortega-Arroyo et al., 2013; Gomes et al., 2015; Sambalova et al., 2018; Masum et al., 2019). Thus, due to the presence of a large number of stabilising agents, the AgNPs obtained excellent stability up to 30 days.

Table 2 shows the characterisation of the AgNPs synthesised from the nanoemulsion of the EO of *P. nigrum* by dynamic light microscopy.

It was observed that the higher the pH, the larger the size of the formulated nanoparticles and, consequently, the lower the zeta potential.

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

Piper nigrum	Average diameter (nm)	PDI	Zeta potential (mV)
NPAg-NEO pH 8,0	49,12	0,555	-13,87
NPAg-NEO pH 9,0	35,14	0,377	-10,44
NPAg-NEO pH 10,0	29,44	0,321	-8,35
NPAg-NEO pH 11,0	12,25	0,222	-4,26

Note: PDI-Polydispersion index; NPAg-NEO- silver nanoparticles; Source: Authorship (2024)

3.3 Total Phenolic Content

Table 3 presents the spectrophotometric quantification of total phenolic content (TPC) for *P. nigrum* EO.

Table 3 – Determination of Total Phenolic Content (TPC) of *Piper nigrum* essential oil

EO	TPC mg EAT/g	Equation	R^2
P. nigrum	220,99	y=0,0586x+0,06	0,9999

EO-Essential oil; Source: Authorship (2024)

Comparing the results presented in Table 3, lower results were observed by Andrade (2015), when extracting *P. nigrum* EO from a local market in Porto Alegre, Brazil, by supercritical extraction using CO2¬, demonstrating a TPC of 14-22.5 mg GAE/g. In the study by Abdul-Hafeez et al. (2014)_Referência não encontrada nas bibliografias, using EO collected in Egypt and extracted by hydrodistillation, lower results were also observed when quantifying the TPC for *P. nigrum* EO at 14.12 mg GAE/g.

Furthermore, Oboh et al. (2013) conducted studies using *P. nigrum* EO obtained from western Nigeria and extracted by the hydrodistillation technique, and obtained the TPC of 4.41 mg GAE/g, a result lower than this study. Sruthi et al. (2013), using the EO from eleven localities of Kerala, India, quantified the TPC in the range of 3.0-6.3 mg GAE g/, lower than that used in this study, also extracted by hydrodistillation.

The phenolic compounds quantified in this study are known to exert beneficial effects on human health due to their antioxidant, anti-inflammatory, cardioprotective, anticancer and antimicrobial properties (Acquaviva et al., 2016). They are considered to be excellent antioxidants that can eliminate excessive damage to the body caused by free radicals and chronic diseases. The centre of the antioxidant capacity of phenolics is in the hydroxyl, so the number and position of phenolic hydroxyls are directly related to their antioxidant activity (Farhoosh et al., 2016; Rodriguez et al., 2017).

3.4 Evaluation of antioxidant activity

Table 4 presents the antioxidant capacity of *P. nigrum* EO and silver nanoparticles.

Table 4 – Antioxidant capacity of essential oil and silver nanoparticles

P. nigrum	**IC_{50} mg/L**	**Equation**	**R^2**
NEO	66,89	a = 71,526; b=-80,561	0,9932
NPAg-NEO pH 8,0	42,91	a = 72,553; b=-68,448	0,9946
NPAg-NEO pH 9,0	40,17	a = 55,296; b=-38,692	0,9980
NPAg-NEO pH 10,0	16,29	a = 45,872; b=-5,5941	0,9937
NPAg-NEO pH 11,0	16,26	a = 40,477; b=-0,9806	0,9944

EO- Essential oil; NEO - Nanoemulsion; NPAg- NEO pH 8,0- Silver nanoparticles of *P. nigrum* pH 8,0; NPAg- NEO pH 9,0- Silver nanoparticles of *P. nigrum* pH 9,0; NPAg- NEO pH 10,0- Silver nanoparticles of *P. nigrum* pH 10,0; NPAg- NEO pH 11,0- Silver nanoparticles of *P. nigrum* pH 11,0; Source: Authors (2023)

As shown in Table 4, where the antioxidant activity values of NEO and the formulated bioproducts were quantified, the best result was observed for the silver nanoparticle at pH 11.0, since it has the lowest IC_{50}.

According to Campos et al. (2003), to be considered active, the IC_{50} must be quantified at values lower than 500 mg/L. Thus, NEO and all NPAg-NEO were found to be active. It is emphasised that this study presents in an unprecedented way the antioxidant activity of NEO *P. nigrum* and silver nanoparticles obtained from it by ABTS assay.

According to the values found in Table 4 of the studies of Yusuf et al. (2019), using EO of *P. nigrum* collected in Japan, they quantified the IC_{50} in 1740 mg/L, a value higher than that found in this study. Li et al. (2020) also found superior results when performing the antioxidant activity of *P. nigrum* EO obtained in five different provinces of China, quantifying the IC_{50} approximate of 9065.47 mg/L.

Furthermore, the study conducted by Loizzo et al. (2014), using the BTS method and *P. nigrum* EO obtained in the local trade in the United Kingdom, observed lower results when quantifying the IC_{50} at 5.12 mg/L. However, when analysing the antioxidant activity of EO obtained in India, the work of Johari et al. (2022) found results with an IC_{50} higher than that of this study, with a IC_{50} quantified at approximately 377.184 mg/L.

Antioxidants benefit health by neutralising the effects of free radicals that cause cancer, skin ageing and cardiovascular disease. Eliminating free radicals that prevent lipid peroxidation and other free radical processes protects the body and prevents oxidation of processed foods (Anwar et al, 2009). And bioproducts obtained from the EO of *P. nigrum*, as well as those obtained in this study, show improved antioxidant potential, highlighting the unprecedented synthesis of silver nanoparticles from the nanoemulsion of this EO with high application potential.

3.5 Anti-inflammatory capability

Table 5 presents the anti-inflammatory capacity of *P. nigrum* EO and silver nanoparticles.

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

P. nigrum	**IC_{50} mg/L**	**Equation**	**R^2**
NEO	28,09	a = 7,0133; b=-18,8000	0,9999
NPAg-NEO pH 8,0	0,964	a = 0,0432; b=-8,3513	0,9999
NPAg-NEO pH 9,0	0,888	a = 0,0507; b=-4,9367	0,9915
NPAg-NEO pH 10,0	0,475	a = 0,0849; b=-9,6253	0,9589
NPAg-NEO pH 11,0	0,217	a = 0,0535; b=-0,38397	0,9513

EO- Essential oil; NEO - Nanoemulsion; NPAg- NEO pH 8,0- Silver nanoparticles of *P. nigrum* pH 8,0; NPAg- NEO pH 9,0- Silver nanoparticles of *P. nigrum* pH 9,0; NPAg- NEO pH 10,0- Silver nanoparticles of *P. nigrum* pH 10,0; NPAg- NEO pH 11,0- Silver nanoparticles of *P. nigrum* pH 11,0; Source: Authors (2023)

As shown in Table 5, where the values for the anti-inflammatory activity of NEO and the formulated bioproducts were quantified, and the best result for the silver nanoparticle with pH 11.0 was observed, because it has the lowest $IC_{50.}$

According to the classification of Jonville et al. (2011), to be considered moderately active, it should present IC_{50} < 130 mg/L, therefore the quantified values for all bioproducts of this study are considered interesting and moderately active following this criterion.

Comcanting the values found in Table 5 to the studies of Nagavekar&Singhal (2018), using solvent extract from *P. nigrum* grains, it quantified the IC_{50} in approximately 55 mg/L, a value higher than that found in this study. In their studies, Lomarat et al. (2015) quantified the IC_{50} of *P. nigrum* EO at 61.63 mg/L, a value higher than those found in all bioproducts formulated investigated in this study.

It is emphasized that this study presents in an unprecedented way the anti-inflammatory activity by the protein denaturation method of NEO of *P. nigrum* and silver nanoparticles from it.

4 CONCLUSIONS

Finally, silver nanoparticles (AgNPs) were synthesised in an unprecedented way from the nanoemulsion of *P. nigrum* essential oil. There was a directly proportional increase in the surface plasmon resonance of silver nanoparticles with increasing pH. Sixteen chemical constituents were quantified by CG/MS, with limonene being the major constituent of *P. nigrum* essential oil. In addition, the IC_{50} for the antioxidant activity of silver nanoparticles, classified as active, the best result for this assay was observed in the nanoparticle with pH 11. In this study, the quantification of the IC_{50} for the anti-inflammatory activity of silver nanoparticles was also carried out in an unprecedented way, where all the samples tested were shown to be efficient for antioxidant activity, highlighting the best result for the formulation with pH 11. Therefore, it can be concluded that this study has brought in an unprecedented way

the results for silver nanoparticles synthesised from the nanoemulsion of the essential oil of *P. nigrum*, where it proved to be efficient in improving the activities tested in this study, also demonstrating the effect of pH in these formulations.

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