

Keywords

Silver nanoparticles. Green synthesis. Reducing agent. Eucalyptus leaves. Inhibition.

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Green Synthesis Of Silver Nanoparticles Using Eucalyptus Globulus Extracts And Evaluation Of Antimicrobial Catalytic Activities

ABSTRACT - The production of nanoparticles through green synthesis has become well established due to their wide applications in various fields, such as biomedical and food engineering. In this study, a plant extracts rich in bioactive compounds—phenols, tannins, flavonoids, alkaloids, and saponins—was used as a reducing and stabilizing agent during nanoparticle formation. The presence of AgNPs was confirmed by UV-Visible Spectrophotometry (4802 UV-Visible DOUBLE BEAM Spectrophotometer), showing a characteristic absorption band in the 500–700 nm range. The spherical morphology and average particle size were determined by dynamic light scattering (DLS, ISO 13321), yielding values between 30 and 40 nm, with some distributions extending up to 100 nm. Process optimization was carried out by varying the volume of Eucalyptus globulus extract, silver nitrate concentrations, pH, temperature, and reaction time. The bio-synthesized AgNPs exhibited antibacterial and catalytic activity against *Escherichia coli*, *Staphylococcus* sp., and *Botrytis cinerea*. The results showed that the nanoparticles displayed greater inhibitory capacity between 24 and 48 hours after application, demonstrating their potential for antimicrobial applications.

RESUMO – A produção de nanopartículas por meio da síntese verde tem se consolidado devido às suas amplas aplicações em diversas áreas, como a biomédica e a engenharia de alimentos. Neste estudo, extratos vegetais ricos em compostos bioativos — fenóis, taninos, flavonoides, alcaloides e saponinas — foram utilizados como agentes redutores e estabilizantes durante a formação das nanopartículas. A presença de AgNPs foi confirmada por espectrofotometria UV-Visível (espectrofotômetro UV-Visível de feixe duplo 4802), apresentando uma banda de absorção característica na faixa de 500–700 nm. A morfologia esférica e o tamanho médio das partículas foram determinados por espalhamento dinâmico de luz (DLS, ISO 13321), obtendo-se valores entre 30 e 40 nm, com algumas distribuições estendendo-se até 100 nm. A otimização do processo foi realizada variando-se o volume do extrato de Eucalyptus globulus, as concentrações de nitrato de prata, o pH, a temperatura e o tempo de reação. As AgNPs biossintetizadas apresentaram atividade antibacteriana e catalítica contra *Escherichia coli*, *Staphylococcus* sp. e *Botrytis cinerea*. Os resultados mostraram que as nanopartículas exibiram maior capacidade inibitória entre 24 e 48 horas após a aplicação, demonstrando seu potencial para aplicações antimicrobianas.

Palavras-chave: Nanopartículas de prata. Síntese verde. Agente redutor. Folhas de Eucalyptus. Inibição.

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INTRODUCTION

Nanobiotechnology is rapidly growing as an interdisciplinary field within ecological research and is used across a wide range of scientific areas such as biology, chemistry, physics, biomedicine, and materials and food engineering (IRAVANI; VARMA, 2020; BHARDWAJ et al., 2020; ZHANG et al., 2022). It involves various shapes and sizes of particles ranging from 1 to 100 nm, which over the past two decades have been used to produce metal nanoparticles with different morphologies, compositions, and structures. Today, many diseases are caused by pathogenic microorganisms which, intensified by the high levels of global pollution, contribute to the development and evolution of stronger and more resistant forms of viruses and bacteria. As a result, new generations are compelled to develop innovative technologies for microbial elimination and anti-inflammatory products—namely, non-toxic and novel methods within the field of nano research that involve chemical compounds and plant-based materials for nanomaterial synthesis (SINGH et al., 2018). Although nanoparticles can be synthesized using different physicochemical methods, their fabrication through biological approaches—such as the use of fruits or plant extracts—is a more attractive option, as these are non-toxic and therefore environmentally safe, especially for applications in food, pharmaceuticals, and medicine. Thus, plant extracts represent the most advantageous alternative because they are less expensive, simple, fast, require less energy, and can be carried out at room temperature (K. R. et al., 2017; SULEMAN et al., 2021; SHARMA, 2018).

Green biochemical synthesis is advantageous not only because it is environmentally friendly but also because plant extracts act as reducing and stabilizing agents in nanoparticle synthesis. Eucalyptus is a species native to Australia and was introduced to Ecuador in 1865. In the central-northern region of Ecuador, several species grow in latitudes from (0°18'37" S, 78°30'58" W; 0°9'3" S, 78°25'36" W). Few species have been introduced in temperate and subtropical regions. The extract of Eucalyptus globulus Labill is known for its therapeutic properties—antirheumatic, astringent, expectorant, febrifuge, among others—and is widely used in alternative medicine due to its health benefits (KHAN et al., 2021). Studies on the application of eucalyptus extract in diabetes show that this disease can be controlled due to its antimicrobial, anti-inflammatory, antirheumatic, anti-asthmatic,

bactericidal, balsamic, and circulatory stimulant properties (SILVA et al., 2019).

The extract of Eucalyptus globulus Labill contains tannins, alkaloids, saponins, phenols, reducing compounds, among others. The aglycone, a free glycoside and part of the saponins known as sapogenin, acts as a reducing agent (MITTAL et al., 2018). Mittal reports that the carbonyl group present in the organic compounds of saponins forms a protective layer around the nanoparticles, preventing their aggregation and therefore stabilizing the synthesis (SINGH et al., 2018).

This research presents a room-temperature green synthesis method developed to produce AgNPs using an extract obtained from the leaves of Eucalyptus globulus Labill, which is non-toxic, environmentally friendly, and low-cost (HEERA; SHOBHA, 2019). Additionally, the antimicrobial effect of silver nanoparticles (AgNPs) was evaluated against strains of *E. coli*, *Staphylococcus*, and *Botrytis cinerea*.

MATERIALS AND METHODS

Silver nitrate was obtained from Laboratorio Luque C.A., Guayaquil, Ecuador. Sabouraud bacteriological agar (bipetri) and MacConkey agar (monopetri) were obtained from Laboratorio Saqmic, Guayaquil, Ecuador, for the identification of bacterial strains *E. coli*, *Staphylococcus*, and *Botrytis cinerea*, collected through environmental processes (water and fruits). The eucalyptus leaves used for the extract were obtained from the botanical garden of the Universidad de Chimborazo, located in the northern part of the city of Riobamba, on Avenida Antonio José de Sucre.

Preparation of the plant extract:

To obtain the extract of Eucalyptus globulus Labill, the following procedure was used: The leaves were crushed and heated at 65 °C with distilled water (1:1) for 20 minutes, followed by magnetic stirring (300–500 rpm) for 10 minutes to improve concentration. The mixture was then vacuum filtered.

Fresh crushed leaves (1:1) were added to the filtrate, heated at 60 °C for 10 minutes, and subjected to the same treatment described in step 1.

The mixture was macerated for 0–1 day, 0–3 days, and 0–5 days at a relative humidity of 70% and a temperature of 10 °C to preserve its components.

The phytochemical composition of the macerates was evaluated to determine the presence of reducing compounds, saponins, phenols, flavonoids, and alkaloids (NAGARAJU et al., 2019).

Table 1. Phytochemical tests of eucalyptus extract

Maceration time (days)	pH	temperature (°C)	Reducing compounds	Saponins	Phenolic compounds	Alkaloids
0-1	5	30	+	+	blue (tannins) +++	+
0-3	4	27	+	+	red (general phenols)	+
0-5		26	+	+	blue (tannins)	+

Positive results were observed at 0–1 day, whereas higher concentrations were detected at 0–3 and 0–5 days

Synthesis of AgNPs:

The synthesis of silver nanoparticles was carried out through the reduction of a precursor salt, silver nitrate (AgNO_3), using *Eucalyptus globulus* Labill extract as a reducing agent, as follows:

Two stock solutions of silver nitrate at different concentrations (0.1 N and 0.01 N) were initially prepared at room temperature (25 °C).

From each solution, aliquots of 5 mL, 10 mL, and 15 mL were taken and mixed with aqueous extract of

Eucalyptus globulus Labill (10 mL, 15 mL, 20 mL) from each macerate, and distilled water (25–35 mL) to obtain a final volume of 50 mL.

The temperature then increased to 65 °C in 10 minutes, during which color changes were observed.

The reaction mixture was subsequently separated and the AgNP formation was accelerated by magnetic stirring and centrifugation (ROY et al., 2019).

Table 2. Silver nanoparticle (AgNPs) synthesis at different maceration times

Maceration time (days)	test	AgNO_3 solution	eucalyptus extract	distilled water	final volume
0–1	#1	10 ml (0.1 N)	5 ml	35 ml	50 ml
0–1	#2	15 ml (0.01 N)	10 ml	25 ml	50 ml
0–1	#3	10 ml (0.1 N)	5 ml	35 ml	50 ml
0–1	#4	10 ml (0.01 N)	5 ml	35 ml	50 ml
0–3	#1	10 ml (0.1 N)	5 ml	35 ml	50 ml
0–3	#2	15 ml (0.01 N)	10 ml	25 ml	50 ml
0–5	#1	10 ml (0.1 N)	5 ml	35 ml	50 ml
0–5	#2	15 ml (0.01 N)	10 ml	25 ml	50 ml
0–5	#3	10 ml (0.1 N)	5 ml	35 ml	50 ml
0–5	#4	10 ml (0.01 N)	5 ml	35 ml	50 ml

Characterization of silver nanoparticles:

The characterization of silver nanoparticles (AgNPs) was performed using a 4802 UV–Visible Double Beam Spectrophotometer within a wavelength (λ) range of 340–1000 nm. Identification and size determination of AgNPs were conducted through DLS (ISO 13321), within a range of 30–40–100 nm, along with visual observation of color changes in the solution due to surface plasmon resonance excitation (VEERAPANDIAN; YUN, 2018). The spherical morphology of the AgNPs was confirmed by laser analysis (ISO 13321), determining an average particle size range. An X-ray diffractometer with Cu K α radiation ($\lambda = 0.1546$ nm) at a diffraction angle between 10° and 90° was used to determine the crystalline structure of the nanoparticles, characterize the surface chemistry of the AgNPs, and identify biomolecules present in *Eucalyptus globulus* Labill.

Antibacterial activity:

The membrane filtration method, using streaking and massive inoculation of fungi and bacteria, was used to evaluate the antibacterial activity of the biosynthesized AgNPs against two human pathogens, *E. coli* and

Staphylococcus (RAZA et al., 2019), as well as the fungus *Botrytis cinerea* present in strawberries (ZARE et al., 2019). Antibacterial activity was assessed against the three strains following the guidelines of the Clinical and Laboratory Standards Institute (ISO 15189:2012). For *E. coli*, massive inoculation was performed using freshly prepared agar to ensure visible growth. The plates were allowed to dry under sterile conditions for 24 h, after which different concentrations (50 μL , 100 μL) of AgNPs were applied. The plates were incubated at 35 °C for 24 h, and antibacterial activity was determined by measuring the inhibition zone around the well (6 mm in diameter).

For streak inoculation, *Staphylococcus* and *Botrytis cinerea* were used. AgNP solutions at concentrations of 50 μL and 100 μL were applied on the same day of inoculation, and plates were incubated at 35 °C for 48 h. Antibacterial activity was evaluated based on the absence of microbial growth around the well (4 mm in diameter) (ALI et al., 2020). To assess inhibitory effects under alkaline conditions, each AgNP sample was neutralized with minimal quantities of 0.1 N sodium hydroxide, achieving pH readings between 7 and 10, as described in Table 3.

Table 3. pH of the solutions before and after neutralization with 0.1 N NaOH

Maceration time (days)	test	Initial pH	Ph after neutralization
0-1	#1	4 (acidic)	7 (neutral)
0-1	#2	5 (acidic)	8 (basic)
0-1	#3	4 (acidic)	10 (basic)
0-1	#4	5 (acidic)	5 (acidic)
0-1	#5	2 (acidic)	2 (acidic)
0-3	#1	7 (neutral)	7 (neutral)
0-3	#2	4 (basic)	4 (basic)
0-3	#3	10 (acidic)	10 (acidic)
0-5	#1	4 (acidic)	7 (neutral)
0-5	#2	2 (acidic)	8 (basic)
0-5	#3	3 (acidic)	10 (basic)

0-5	#4	3 (acidic)	3 (acidic)
0-5	#5	4 (acidic)	4 (acidic)

RESULTS AND DISCUSSION

Observation of color change:

Color changes were observed in the solution, initially from light yellow to orange when silver nitrate (AgNO_3) was added to the aqueous extract of *Eucalyptus globulus* Labill at room temperature. When the temperature was increased to 65°C , a sudden shift to dark brown was noted due to an acceleration of the reaction. After centrifugation, the solution stabilized, showing color variations such as brown, yellow, dark pink, and orange. These color changes are attributed to chemical compounds present in the plant extract—such as alkaloids, flavonoids, saponins, tannins, and natural pigments—which act as reducing agents, converting silver ions (Ag^+) into metallic silver atoms (Ag^0). Similar color transitions have been reported by several researchers using different plant extracts (CHANDRASEKARAN et al., 2019; YADAV et al., 2020), associated with structural and molecular changes that alter the light absorption properties of the substances.

The presence of silver nanoparticles in the solution was confirmed using a 4802 UV-Visible Double Beam Spectrophotometer, with characteristic peaks between 500 and 700 nm, corresponding to AgNP concentrations ranging from 200 to 900 mg/L. Due to molecular and structural modifications, color shifts in the samples correlate with changes in their ability to absorb light within the visible region of the electromagnetic spectrum.

Effect of Leaf Extract Concentration on AgNP Size and Concentration:

Silver ions above a certain threshold can be toxic and may cause precipitation. To prevent this, ionic forms are transformed into nanoparticles by plant extract. The effect on the size and concentration of AgNPs in the reaction mixture was evaluated by varying the extract concentration. It was observed that the size and shape of the AgNPs can be controlled by adjusting the concentration. When the concentration of *Eucalyptus globulus* Labill extract was increased, the sample absorbance also increased. This indicates that the particle size of AgNO_3 decreases as the extract concentration increases until reaching the optimal extract level, producing nanoparticles with higher concentration in mg. Similar results have been reported in previous studies (CHANDRASEKARAN et al., 2013).

Effect of AgNO_3 Concentration on AgNP Size and Concentration:

A higher ratio of reducing agent to substrate accelerates the reduction of Ag^+ to Ag^0 , followed by limiting aggregation due to the reducing agent. Therefore, changes in the absorption peak of the reaction mixture were observed at different concentrations of the stock silver salt solutions, ranging from 0.1 N to 0.01 N. It was observed that both the absorption peak and intensity decreased from 0.1 N to 0.01 N, as shown in Figures 1 and 2.

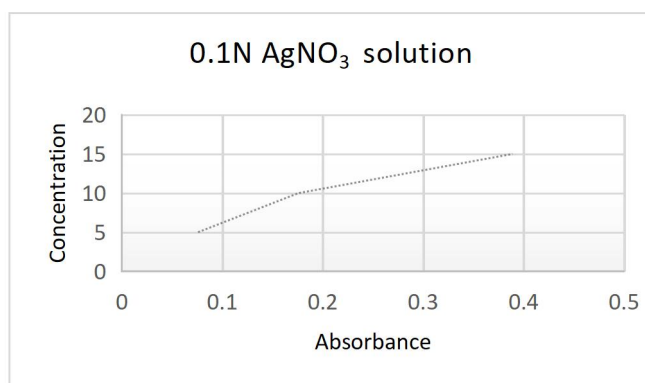


Figure 1. Effect of Concentration in 0.1 N AgNO_3 Solution and Absorbance

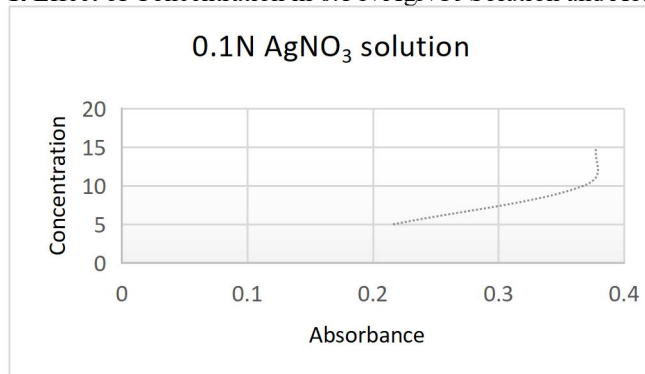


Figure 2. Effect of Concentration in 0.01 N AgNO_3 Solution and Absorbance

Effect of pH on AgNP Size and Concentration:

The pH of the solution is an important factor to consider in nanoparticle formation. As the pH increases from acidic to basic, the intensity of the absorption peak increases. The concentration of AgNPs in the solution also increases due to the higher reduction rate of Ag^+ , resulting in highly uniform nanoparticles with an average size of 40 ± 30 nm, as shown in Figures 3, 4 and 5. Similar results have been reported in other studies (HUSSAIN et al., 2020).

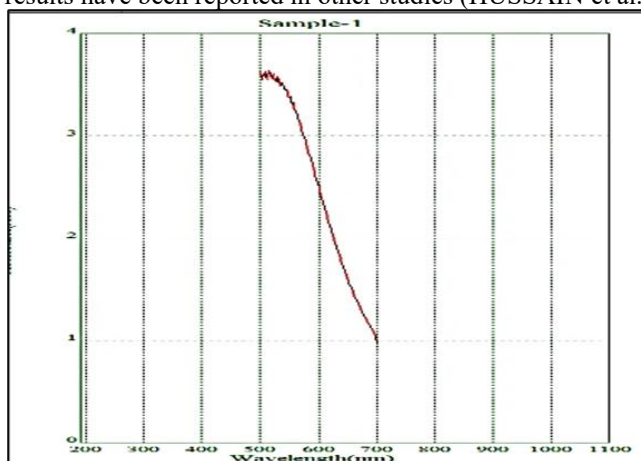


Figure 3. UV–Vis absorbance spectrum of silver nanoparticles synthesized at pH 7 (test #1, 0–1 day maceration), showing the highest absorbance peak at 700 nm and a concentration of 683 mg/L with an average particle size of 40 nm.

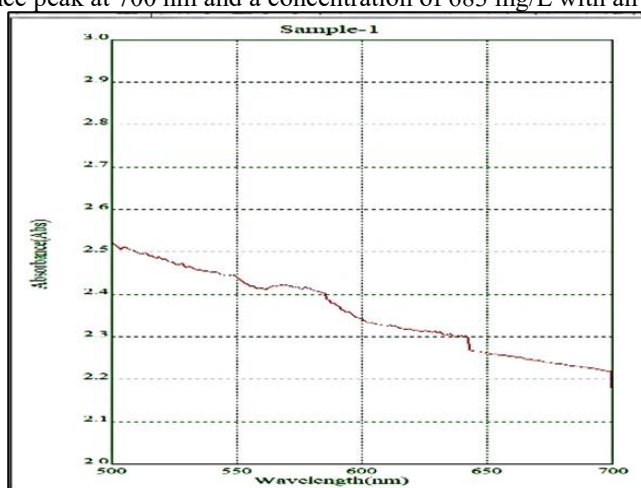


Figure 4. UV–Vis absorbance spectrum showing the lowest absorbance peak and concentration at pH 4 for test #2 (0–3-day maceration). Yellow spherical particles with an average size of 100 nm, concentration of 139.87 mg/L, and maximum absorbance at 579 nm.

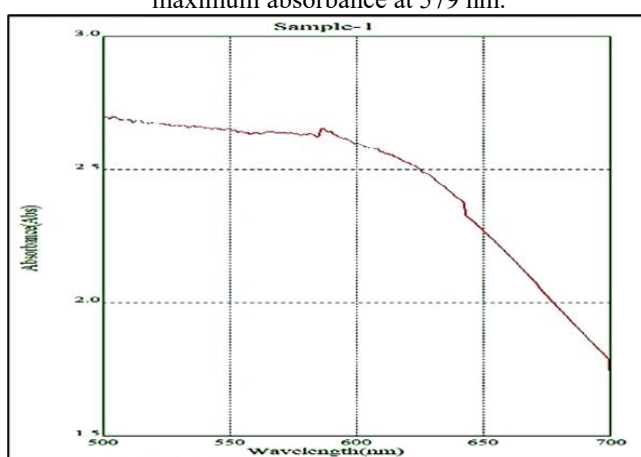


Figure 5. UV–Vis absorbance spectrum showing the highest absorbance peak and concentration at pH 10 for test #3 (0–5-day maceration). Black spherical particles with an average size of 30 nm, concentration of 988 mg/L, and maximum absorbance at 598 nm.

Effect of Temperature on AgNP Size and Concentration

Temperature played a crucial role in determining the size and shape of AgNPs. The effect of temperature was evaluated by varying it from 25 °C to 65 °C, showing an increase in the reduction rate of Ag^+ ions and a very broad absorbance peak that indicated an increase in particle size, as shown in Figures 6 and 7.

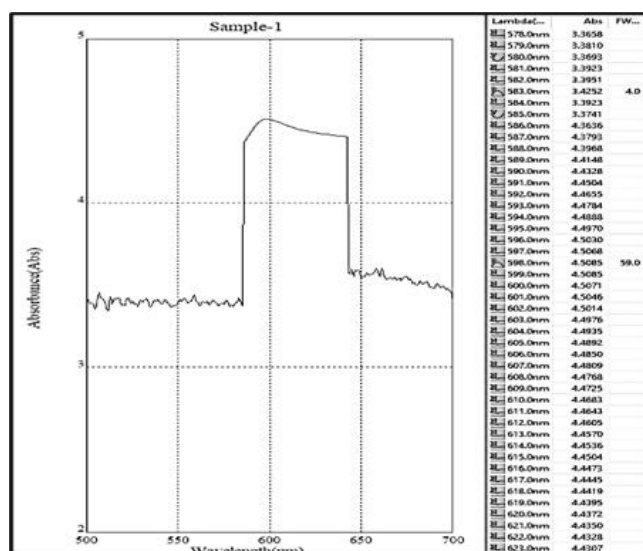


Figure 6. UV–Vis spectrum corresponding to silver nanoparticles synthesized at 30 °C (test #2, 0–1 day maceration), showing the lowest absorbance intensity.

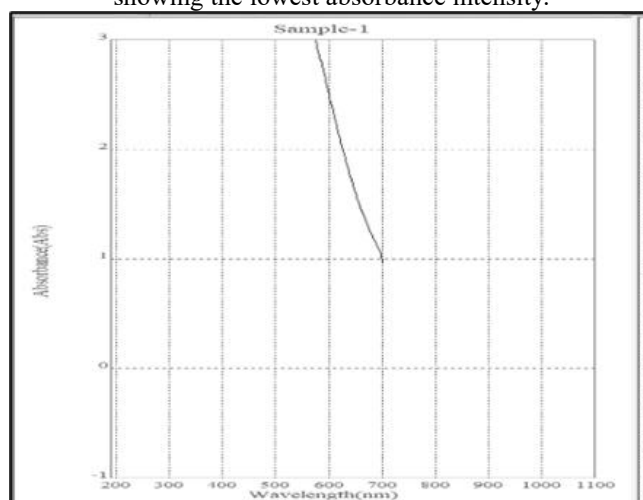


Figure 7. UV–Vis absorbance spectrum of silver nanoparticles synthesized at 65 °C (test #1, 0–1 day maceration), showing the highest absorbance intensity.

Effect of extract maceration time

UV–Vis spectra were used to monitor the effect of eucalyptus extract maceration time on the AgNPs concentration in the reaction mixture at different time intervals. The absorption peak intensity gradually increased, as shown in Figures 8, 9, and 10.

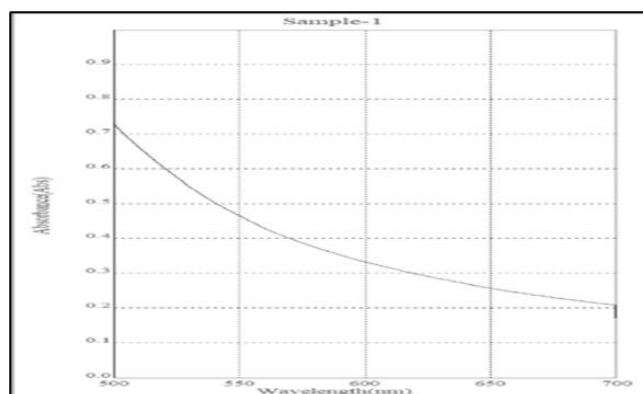


Figure 8. UV–Vis absorbance spectrum showing the lowest absorbance intensity for test #4 (0–1 day maceration). Yellow spherical particles with an average size of 100 nm, a concentration of 259.4 mg/L, and a maximum absorbance at 500 nm.

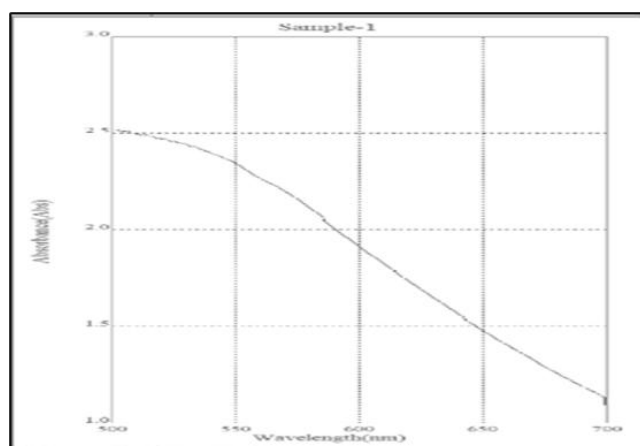


Figure 9. UV-Vis absorbance spectrum showing a medium absorbance intensity for test #1 (0–3-day maceration). Yellow and blue spherical particles with average sizes of 100 and 40 nm, a concentration of 613.4 mg/L, and a maximum absorbance at 500 nm.



Figure 10. UV-Vis absorbance spectrum showing the highest absorbance intensity for test #4 (0–5-day maceration). Black spherical particles with an average size of 30 nm, a concentration of 838.33 mg/L, and a maximum absorbance at 586 nm.

Antimicrobial activity of AgNPs

In the present study, the antibacterial activity of biosynthesized AgNPs was evaluated against two different human pathogens and a strawberry fungus (Figures 11, 12, and 13).



Figure 11. Inhibition of *Escherichia coli* after 48 h



Figure 12. Inhibition of *Staphylococcus* after 24 h of incubation



Figure 13. Inhibition of *Botrytis cinerea* after 24 h of incubation

It is evident that the AgNPs exhibited inhibition zones against the two tested organisms and the strawberry fungus.

Table 4. Inhibition time of AgNPs against bacteria and fungi

Bacterium or fungus	Concentrations (neutralized at pH 10)	Inhibition time
<i>Escherichia coli</i>	0.1 N and 0.01 N – 2 mg/L	48 h
<i>Staphylococcus</i>	0.1 N and 0.01 N – 5 mg/L	24 h
<i>Botrytis cinerea</i>	0.1 N and 0.01 N – 3 mg/L	24 h

The AgNPs concentrations used as positive controls were 2 mg/mL and 5 mg/mL, respectively, and pH was also an important factor.

After penetration into the cells, AgNPs released silver ions; these ions interacted with DNA, proteins, and sulfur-containing cellular constituents, thereby inhibiting microorganisms (BARABADI et al., 2020).

CONCLUSION

Currently, several reports describe methods that use plant extracts for nanoparticle synthesis as an alternative to conventional approaches, since plant extracts are economical and non-toxic compared to traditional chemical synthesis processes. In this study, a green technique for AgNPs fabrication was carried out using *Eucalyptus globulus* extract. This new technology for AgNPs synthesis appears to be low-cost, non-toxic, and feasible at room temperature. Some natural bio compounds present in the leaf extract, as confirmed by absorption peaks, are responsible for reducing and stabilizing the reaction.

The nanoparticles obtained through the green approach were smaller in size than those produced by chemical and physical methods and showed a uniform, monodispersed nature, being synthesized very rapidly. This method does not require tedious post-processing and can be scaled up to develop a viable technology for silver nanoparticle synthesis. The biosynthesized AgNPs exhibited a well-dispersed spherical morphology in solution, with an average size of 30 nm, as demonstrated by DLS analysis.

Furthermore, the biosynthesized AgNPs proved to be excellent antimicrobial agents against *Escherichia coli*, *Staphylococcus*, and *Botrytis cinerea*. It was found that AgNPs showed strong inhibitory activity against the two pathogenic bacteria and the strawberry fungus, requiring a longer exposure time only in the case of *E. coli* (48 h). The present work demonstrated a high inhibition potential against these bacteria and fungi, suggesting possible applications in medicine using plant-based media such as eucalyptus. This study provides a basis for future research in biomedicine,

particularly in therapies for rheumatism and blood sugar control in diabetic patients.

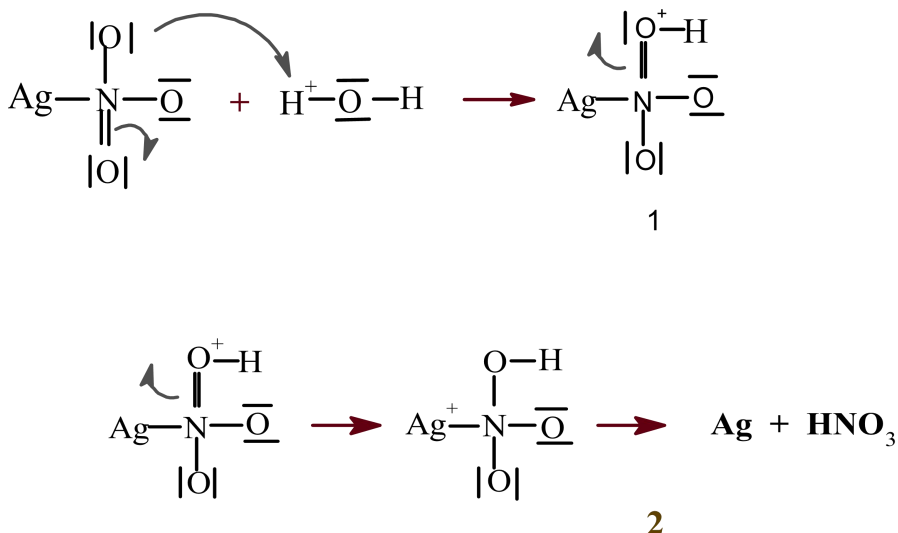
In addition, this work allowed the practical application of knowledge in organic chemistry, leading to the

development of our own chemical formulation based on the properties of eucalyptus leaves and the release of tannins, saponins, and phenolic compounds, as demonstrated in the following chemical reaction:

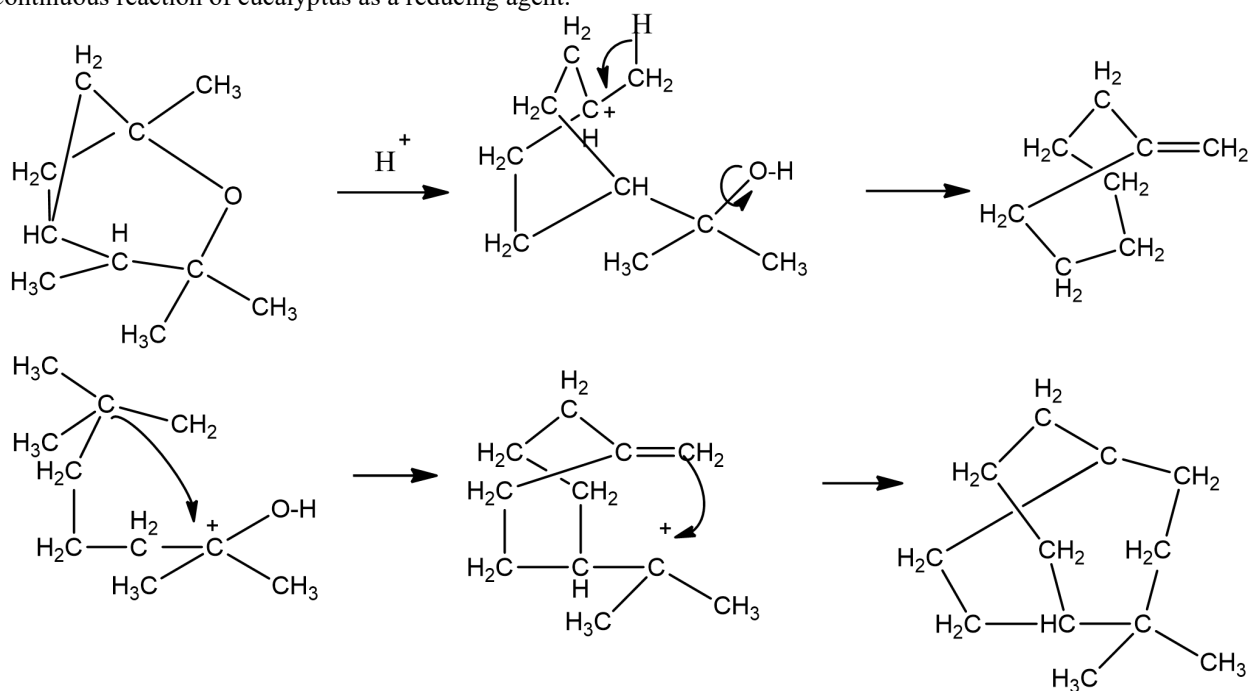


Reaction Mechanism:

Reduction of silver nitrate to zero-valent silver:



Continuous reaction of eucalyptus as a reducing agent:



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