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# *Syzygium aromaticum* L. (Clove) Essential Oil as a Reducing Agent for the Green Synthesis of Silver Nanoparticles

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## Abstract

The present investigation details a green synthesis of silver nanoparticles (AgNP) using the essential oil of *Syzygium aromaticum* L. (clove) as reducing agent, which is a matrix with a high content of eugenol, an important compound for the reducing action of silver nitrate. The synthesis of AgNP was performed at different pH conditions (pH 7, 8, 9 and 10), and was monitored by UV-Vis Spectroscopy, Dynamic Light Scattering (DLS) and Transmission Electron Microscopy. The synthesized nanoparticles presented characteristic Surface Plasmon Resonance bands with maximum absorbance between 405 and 460 nm. The DLS analysis revealed particle sizes from 31 to 72 nm and zeta potential between −30.1 and −50.8 mV indicating good stability against the agglomeration of the particles in solution. The micrographs obtained by TEM showed different particle shapes and a predominance of spherical-shaped nanoparticles, and average size ranging from 27 to 94 nm. The clove-based silver nanoparticles were efficient in controlling the growth of *Escherichia coli* and *Staphylococcus aureus* bacteria, and the minimum inhibitory concentration ranged from 60 to 100 μL/mL. This study highlights the feasibility of clove essential oil as an alternative for the synthesis of silver nanoparticles by a simple, inexpensive and eco-friendly method.

## Keywords

Essential Oil, Clove, Eugenol, Silver Nanoparticles, Antibacterial Activity

# 1. Introduction

A vast number of physical, chemical and biological methods can be applied to obtain metallic nanoparticles. Biological methods are those employing microorganisms or plants for the nanoparticle biosynthesis [1] [2] [3]. The use of plants or plant derivatives for the synthesis of nanoparticles is widely used nowadays, and their compounds often have important biological functionalities that can act synergistically with the nanoparticles for various applications in different areas from the food to the pharmaceutical and medical industries. The concern involving the non-degradation of the environment is even more evident today, which highlights the importance of using sustainable industrial methods capable of proposing alternatives for the reduction of harmful chemical residues [4] [5] [6].

In this context, silver nanoparticles (AgNP) are prominent in applications as antimicrobial agents that can eliminate microorganisms by interacting with their cells, thus preventing the cellular respiration, and consequently its replication [7]. Whereas, they are non-toxic against eukaryotic cells, especially at the concentrations usually used. However, the AgNP synthesis using harmful chemicals may limit their application in some areas. Therefore, the search for alternative agents capable of reducing silver salts for the formation of nanoparticles, without causing health or environmental risks, is an important role in science [8].

The clove (*Syzygium aromaticum* L.) is a plant grown in several countries including Brazil. The clove essential oil is widely used for its antimicrobial, antifungal and antioxidant action, as well as anesthetic and analgesic effects. These characteristics are strongly attributed to its major compound, eugenol, present in the oil in concentrations that can reach up to 90% [5] [9]. The chemical composition suggests clove essential oil as a potential silver reducing agent for the synthesis of nanoparticles. The probable mechanism of reduction is given by the proton donation of the eugenol structure, reducing the $Ag^+$ to $Ag^0$ and thus forming the nanoparticles. In addition, the phytochemicals present in the essential oil can also interact on the surface of the nanoparticles, resulting in stabilization and preservation [10] [11].

The use of plant extracts in the synthesis of silver nanoparticles is quite common. However, there is still a shortage in the use of essential oils for this purpose. Therefore, the main objective of the present study was to evaluate a green synthesis of silver nanoparticles using the essential oil of *Syzygium aromaticum* L. (clove) as a reducing and stabilizing agent, as well as their characterization by transmission electron microscopy, dynamic light scattering, UV-Vis spectroscopy, besides the evaluation of the antimicrobial activity.

# 2. Materials and Methods

## 2.1. Materials

Silver nitrate ($AgNO_3$) and acetone were obtained from Sigma Aldrich (Brazil). Clove (*Syzygium aromaticum* L.) essential oils were purchased from the local market of Florianópolis (Santa Catarina, Brazil). All chemicals used were of analytical grade, and the solutions were prepared with deionized water.

## 2.2. Green Synthesis of AgNPs Using Clove Essential Oil

Initially, the clove essential oil was diluted in acetone at a ratio of 1:170. A stock solution of 0.31 mmol.$L^{-1}$ silver nitrate ($AgNO_3$) was prepared. Four aliquots of this solution were collected, and their pH was adjusted to 7, 8, 9 and 10 with a 0.1 mol.$L^{-1}$ NaOH solution in order to evaluate the synthesis under the different conditions. A 30-mL aliquot of each solution was heated under constant magnetic stirring, and 2 mL of the diluted clove essential oil was added dropwise into the boiling solution. Afterward, the solution remained under heating and vigorous stirring for 30 min. A brown-yellow color was observed indicating the formation of silver nanoparticles (AgNP).

## 2.3. UV-Vis Spectroscopy

The UV-Vis spectroscopy absorption study was performed on a Hitachi spectrophotometer, model U-1800 (Tokyo, Japan) at the wavelength range of 300 to 800 nm. The readings were performed at ~25˚C, and the results were used to build the curves of the Surface Plasmon Resonance (SPR) bands of the samples.

## 2.4. Dynamic Light Scattering (DLS) Analysis

For the dynamic light scattering analysis, the AgNPs samples were evaluated in a Zetasizer Nano Series (Malvern Instruments Worcestershire, UK) at a fixed angle of 173˚ at room temperature (25˚C). The results of particle size (Z-ave), polydispersity index (PI) and the zeta potential (ζ) were obtained.

## 2.5. Transmission Electron Microscopy (TEM)

Transmission electron microscopy was performed to elucidate the morphology of AgNPs in the different synthesis conditions. A JEOL model JEM-1011 (Tokyo, Japan) microscope was used for the TEM evaluation under 80 kV. The obtained images and the size distribution of the particles were analyzed with the aid of the Quantikov Image Analyzer software.

## 2.6. Antimicrobial Activity of AgNPs

The evaluation of the antimicrobial activity of AgNPs was performed according to a methodology adapted from the Clinical and Laboratory Standards Institute (CLSI) [12]. The AgNP samples were diluted with ultrapure water to obtain solutions at final concentrations in the wells of 40, 60, 80 and 100 μL·$mL^{-1}$. The following bacterial cultures were used: *Escherichia coli* (ATCC 8739) (gram negative), *Staphylococcus aureus* (ATCC 25923) (gram positive) and *Bacillus cereus* (ATCC 10987) (gram positive). Bacterial cells were suspended in 0.9% (w/v) saline solution (NaCl) and the inoculum density was standardized using the McFarland turbidity comparison standard to achieve a final plate concentration of ~$10^5$ CFU/mL.

Sterile 96-well polypropylene microplates were used for analysis. In each well were added 5 µl of inoculum, 90 µL of Mueller-Hinton broth and 10 µL of nanoparticles at different concentrations. Plates were incubated at 35˚C for 16 - 20 h. Afterward, the turbidity was observed (to the naked eye) and compared to the negative control (without inoculum) and to the positive control (without nanoparticles), being considered efficient in controlling the microorganism when the turbidity was not observed.

### 2.7. Statistical Analysis

All assays were performed at least in triplicate, and the data were expressed as mean and standard deviation.

## 3. Results and Discussion

To verify the efficiency of AgNP synthesis at different pHs using clove essential oil as reducing agent, the samples were analyzed by UV-Vis spectroscopy from 300 to 800 nm and the particle's formation was observed by the Surface Plasmon Resonance bands.

The formation of AgNPs occurred due to the reduction of $Ag^+$ ions into Ag atoms by the clove essential oil added to the $AgNO_3$ solution, which was confirmed after the colorless solution was turned into a yellowish brown. The maximum formation of AgNPs occurred at pH 8 (max absorbance at 435 nm), followed by pH 9, 10 and 7 (**Figure 1**). The clove essential oil has 70% - 90% of the eugenol compound in its chemical composition, in addition to other compounds in smaller amounts. These compounds present a variety of functional groups with high affinity for noble metals aiding in the stabilization of the nanoparticles [10]. Additionally, the presence of a phenolic ring in the eugenol structure allows it to act as a reducing agent by donating protons ($H^+$). Thus, it is

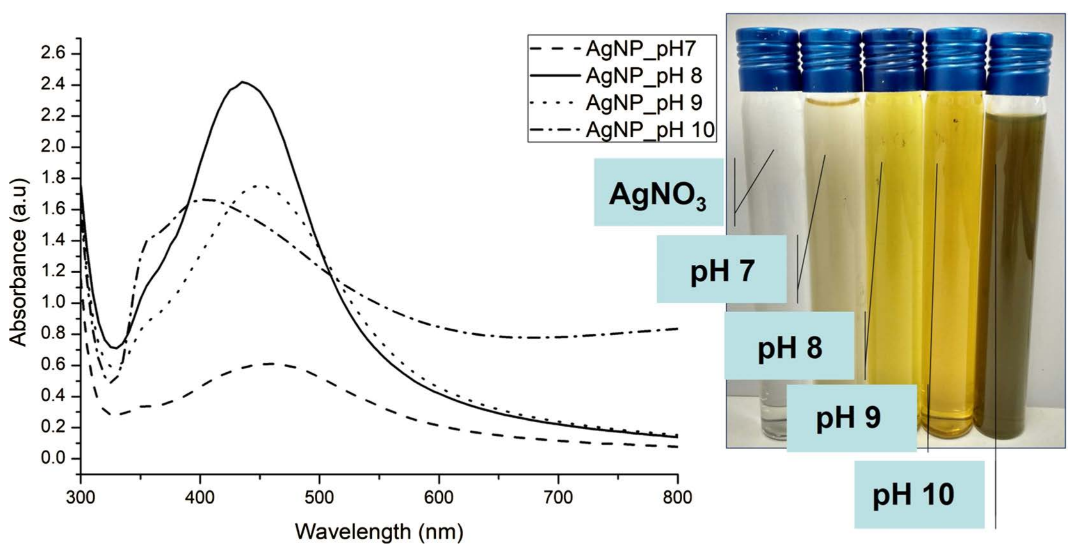


**Figure 1.** The SPR band for AgNPs using different pH condition and matching color.

suggested that the reduction of silver nitrate by clove essential oil occurs mainly by the action of the eugenol, forming the nanoparticles [13].

Bands of SPR with maximum absorbance at 430 nm are reported for the synthesis of nanoparticles using an aqueous clove extract [10], while a maximum absorbance of 435 nm is [14] reported for *Cinnamon zeylanicum* silver nanoparticles, which are similar to those found in the present work.

From **Figure 1** is possible to observe the difference in the color of the samples compared to the $AgNO_3$ solution which indicates the formation of the nanoparticles. This was corroborated by the SPR absorption bands that confirmed the formation of AgNPs in all pH conditions. In addition to the higher peak absorbance intensity for the sample synthesized at pH 8, its SPR band is more well-defined and indicates a better formation of nanoparticles, consistent with other results found in the literature [4] [6]. The full width at half-height (FWHH) indicates the particle size distribution, *i.e.*, wider bands are representative of high dispersion index and diverse formats [3], which can be observed in the band of AgNP synthesized at pH 7 and 10.

## 3.1. Size Distribution and Characterization

The samples were characterized for the elucidation of their size, polydispersity index and zeta potential by DLS, while the morphology of silver nanoparticles was studied by transmission electron microscopy (TEM) to verify their shape. The results of the DLS measurements are shown in **Table 1**.

The mean size obtained by DLS indicates the hydrodynamic radius of the particle. A higher deviation is verified for the sample AgNP_pH 7, confirming what was observed in the SPR band. The AgNP_pH 10 sample showed the smallest average particle size; however, the highest polydispersity index (PI) was also observed for this sample. In this case, PI values lower than 0.4 indicate populations with a narrower distribution [15]. This result reinforces the results observed in the UV-Vis analysis. All the AgNP samples showed zeta potential values between −30.1 and −50.8 mV indicating good stability against agglomeration.

**Figure 2** shows the micrographs of AgNP obtained by TEM. Dense particles of various shapes and a thin layer on the surface of silver nanoparticles were observed. This may be evidence of a binding between the oil's phytochemicals, responsible for promoting steric hindrance stability. The different particle shapes observed herein can be attributed to the pH of the solutions that influences the

**Table 1.** Results of the dynamic light scattering analysis (DLS) for the AgNPs samples at different pHs represented by mean values and standard deviation.

| Sample | Average Size (nm) | Polydispersity Index | Zeta (mV) |
|---|---|---|---|
| AgNP_pH 7 | 71.9 ± 14.5 | 0.269 ± 0.043 | −31.9 ± 8.5 |
| AgNP_pH 8 | 55.4 ± 4.1 | 0.383 ± 0.019 | −34.8 ± 3.6 |
| AgNP_pH 9 | 74.8 ± 2.5 | 0.232 ± 0.011 | −30.1 ± 0.7 |
| AgNP_pH 10 | 31.4 ± 5.8 | 0.543 ± 0.004 | −50.8 ± 0.8 |

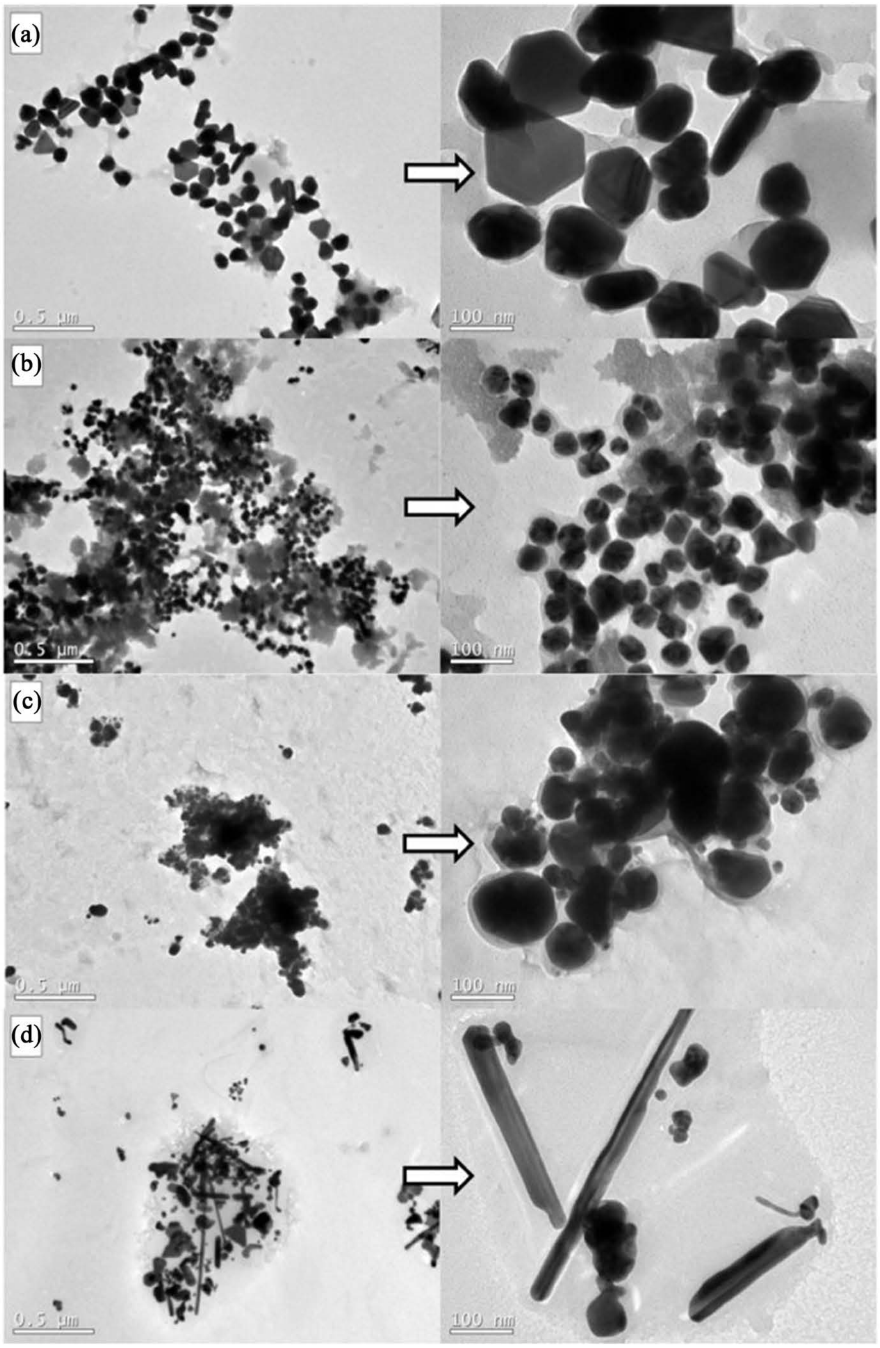


**Figure 2.** Transmission electron microscopy (TEM) for AgNPs synthesized with clove essential oil at different pH conditions. (a) pH 7; (b) pH 8; (c) pH 9; (d) pH 10.

reduction and stabilization of the nanoparticles system [16] [17].

The sample AgNP_pH 7 (**Figure 2(a)**) showed mainly triangles, squares, hexagons, and spheres, while pentagons, hexagons, and spherical shapes were observed for AgNP_pH 8 (**Figure 2(b)**), and little number of triangular shapes. In AgNP_pH 9 (**Figure 2(c)**) the spherical shapes were predominant, but a larger agglomeration of the particles was observed, which in this case, can contribute to the coalescence of the particles. The largest variety of shapes was observed in

AgNP_pH 10 (**Figure 2(d)**) which presented hexagon, square, spherical and needle formats.

The micrographs were analyzed to obtain the size distribution with the aid of the Quantikov Image Analyzer software, and the histograms of size distribution are represented in **Figure 3**. Overall, the samples presented a mean particle diameter between 27 to 94 nm. The AgNP_pH 7 sample had the highest average diameter and a substantial heterogeneity of sizes (78.0 ± 16.3 nm), this result reinforces what was observed by UV-Vis (**Figure 1**) and resembles the results obtained by DLS (**Table 1**). The lowest mean diameter was observed for the sample AgNP_pH 8 (35.5 ± 8.5 nm), which showed 76% of the particles with a diameter in the 40 nm range, showing a high homogeneity in its particle sizes. The AgNP_pH 9 sample had a mean diameter of 44.0 ± 18.9 nm indicating a high degree of heterogeneity like AgNP_pH 7, which can be evidenced in TEM micrographs (**Figure 2**). Furthermore, the average diameter of the AgNP_pH 10 sample (37.9 ± 18.7 nm) was quite similar to that obtained by DLS (**Table 1**), and its high standard deviation indicates the significant dispersion of the particles, with a great variety of shapes, which reinforces the results of SPR and TEM.

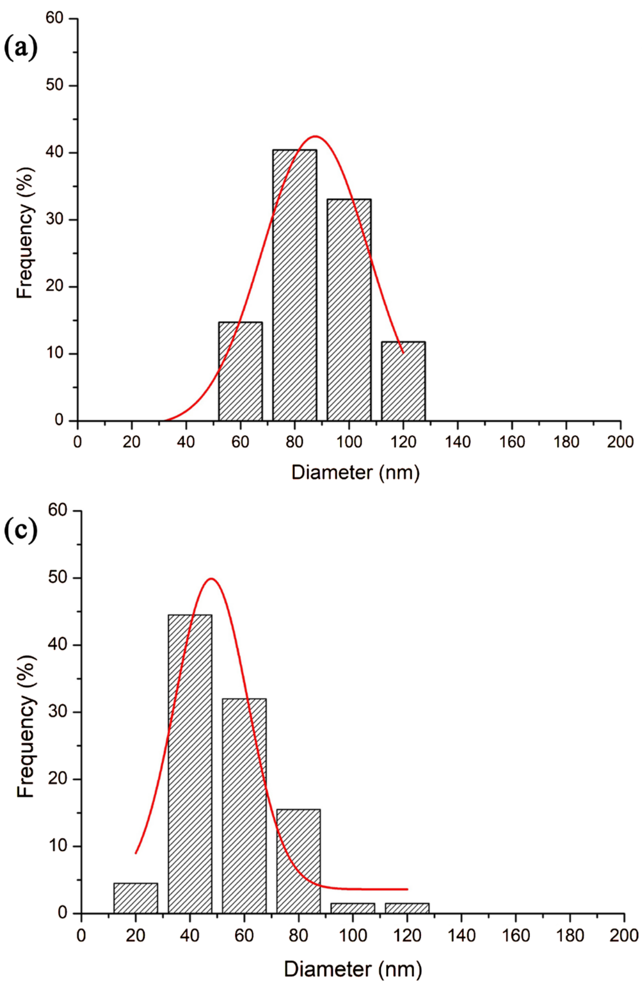


**Figure 3.** Size distribution histograms of AgNPs synthesized at different pH using clove essential oil.

### 3.2. Evaluation of Antimicrobial Activity

The broth microdilution test was used to determine the minimum inhibitory concentration (MIC) of the AgNPs. Turbidity was the parameter used to verify the growth of the microorganism in the wells. The sample AgNP pH 7 presented MIC of 60 µL/mL against *E. coli*, which is the same found for AgNP pH 8. For *S. aureus* the MIC of AgNP pH 8 was 80 µL/mL. The AgNP pH 9 sample presented a MIC of 100 µL/mL for both microorganisms. At pH 10 no inhibition of bacterial growth was observed. This result can be attributed to the higher variation on the particles' shapes and lower volume of formation in this condition. Additionally, inhibition did not occur in any of the samples tested against *B. cereus*. Reference [18] reports that triangular shaped particles exhibit almost total inhibition of bacterial growth, which decreases for spherical-shaped particles and is even smaller for the stem-shaped particles. It is also reported antimicrobial activity of silver nanoparticles against *S. aureus* and *E. coli* by the agar diffusion method [4]. Similar results are demonstrated by [13] against the same bacteria using polymer films impregnated with silver nanoparticles. The MIC of 8 µg/mL is reported for nanoparticles measuring ~20 nm against *E. coli* [7]. The results observed in this study are consistent with those found in the literature, regarding the particularities of particle size and shape.

## 4. Conclusion

The present work demonstrates a green synthesis of silver nanoparticles using the essential oil of clove which is a matrix rich in eugenol compound, essential for the action of silver nitrate reduction and synthesis of the nanoparticles. Particles of different shapes and predominance of spherical shapes were obtained by a simple, low cost and eco-friendly method. The antimicrobial activity of AgNPs was tested and proven to be effective against bacteria such as *E. coli* and *S. aureus* at pH 7, 8 and 9, being more effective at pH 8, reinforcing the effect of the shape associated with volume and size. These results were achieved using low concentrations of the sample, showing the advantage of using clove essential oil with a high concentration of the active compound.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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