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Characterization and Biosynthesis of Silver Nanoparticles by Clove Extract and its Application to Bacteria

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Annotation: This study investigates the synthesis of silver nanoparticles (AgNPs) using clove extract (Syzygium aromaticum), a plant known for its rich bioactive compounds. The research evaluates the physicochemical properties and antibacterial effects of the synthesized nanoparticles. Clove extract, abundant in phenolic compounds like eugenol, functions as both a reducing and stabilizing agent in converting silver ions (Ag^+) into silver nanoparticles. The synthesis process involved mixing the clove extract with silver nitrate and applying heat, resulting in a noticeable color change to brown, indicative of nanoparticle formation.

The presence of silver nanoparticles was confirmed using UV-Vis spectroscopy, which displayed a characteristic peak at 438 nm, corresponding to the surface plasmon resonance (SPR) of AgNPs. Fourier transform infrared (FTIR) spectroscopy was utilized to identify the functional groups involved in the synthesis and stabilization of the nanoparticles. Scanning electron microscopy (SEM) provided insights into the size distribution of the AgNPs, which

ranged from 9.8 nm to 27.6 nm, suggesting a high surface area and reactivity suitable for antimicrobial applications.

The antibacterial activity of the synthesized AgNPs was assessed against Enterococcus and Escherichia coli (E. coli) by measuring the inhibition zones. The results demonstrated significant antibacterial effects, with inhibition zones measuring 18 mm for Enterococcus and 20 mm for E. coli. These findings underscore the potential of silver nanoparticles derived from clove extract as effective antimicrobial agents.

Keywords: silver nanoparticles, clove, bacteria.

1. Introduction

Green synthesis is a new method for synthesizing nanoparticles, using biological sources. Interest in this method has increased recently because it is considered low-cost and environmentally friendly. Among the most vital, attractive, and widely used metallic nanoparticles are silver nanoparticles, which are used in biomedical applications and are involved in the treatment and prevention of many serious diseases, especially cancer. [1] The most important advantages of green nanoparticle synthesis are the short reaction time and stability of the prepared particles, improved control over crystal size and shape, and ease of scaling up the scope of use. The principle of nanoparticle synthesis in this way relies on the use of plants and microorganisms in the synthesis. In this research, we synthesized nanoparticles using clove extract. One of the most important active molecules in clove is eugenol, which reduces silver. Thus, silver nanoparticles coated with the plant extract are formed. [2]

One of the most important applications of cloves is its use as a local anesthetic, a dental analgesic, and a reducing and encapsulating agent in the synthesis of nanoparticles. The aim of this study is to use nanosilver as an antimicrobial and antibacterial agent and to determine its effectiveness. [3]

2. Materials and methods

2.1. Materials

All the used compounds are commercially available and they have been used with no further purification. Spectrophotometric measurements were made using a Shimadzu UV-Visible 1650PC double-beam spectrophotometer. The FTIR measurements were made using a Shimadzu 8400 Series Japan. The Scanning Electron Microscope measurements were made in Germany.

2.2. Methods

2.2.1. Preparation of Clove Extract

The cloves were thoroughly washed and dried before preparing the extract. A mixture was made using distilled water at a 1:10 weight-to-volume ratio, which was then stirred for 15 minutes at

90 °C using a magnetic stirrer. After stirring, the crude extract was allowed to cool and subsequently filtered.

2.2.2. Synthesis of Silver Nanoparticles

A 100 mL solution of 1 mM silver nitrate ($AgNO_3$) was combined with varying volumes of clove extract (0.5 mL, 1 mL, and 2 mL). This mixture was heated to a temperature range of 70-80 °C for 15 minutes while stirring continuously. The reaction was deemed complete when the solution exhibited a color change to brown, indicating the formation of nanoparticles. For consistency, a fixed volume of 0.5 mL of extract was used for further testing, based on the observed reaction time and color change. The synthesized nanoparticles were collected as a precipitate for subsequent analysis.

Figure (1) shows the synthesis of silver nanoparticles

2.2.3. Study of the Effect of Extracts and Silver Nanoparticles on Bacterial Growth

Preparation of Culture Medium (Mueller-Hinton Agar)

1. Dissolve 25 g of Mueller-Hinton agar in one liter of distilled water.
2. Sterilize the solution by placing it in an autoclave until fully dissolved.
3. Pour the agar medium into sterile Petri dishes and allow it to solidify.

Bacterial Inoculation:

1. A bacterial suspension was prepared for each isolate at a concentration of 0.1 ml.
2. The Mueller-Hinton Agar medium was inoculated with the bacterial suspension using a sterile inoculating tool.
3. Bacteria were distributed evenly in the dishes using the diffusion plate method.

Addition of Extract and Silver Nanoparticles:

1- Three wells were created in each plate using a sterile 6 mm diameter cork punch.

2- 1.0 mL of different concentrations of extract (5%, 10%, 20%) and silver nanoparticles were added to the wells.

Incubation and Observation

The plates were incubated at 37°C for 24 hours.

After incubation, the inhibition zones around each well were measured in millimeters.

3. Results and Discussion

3.1. UV-Vis spectroscopy

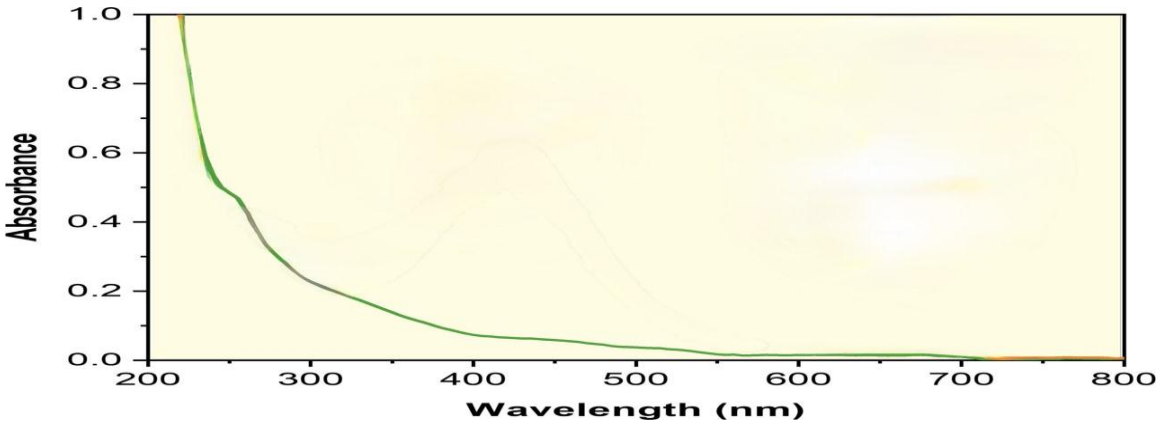

Figure (4-1): UV-Vis spectroscopy of the clove extract

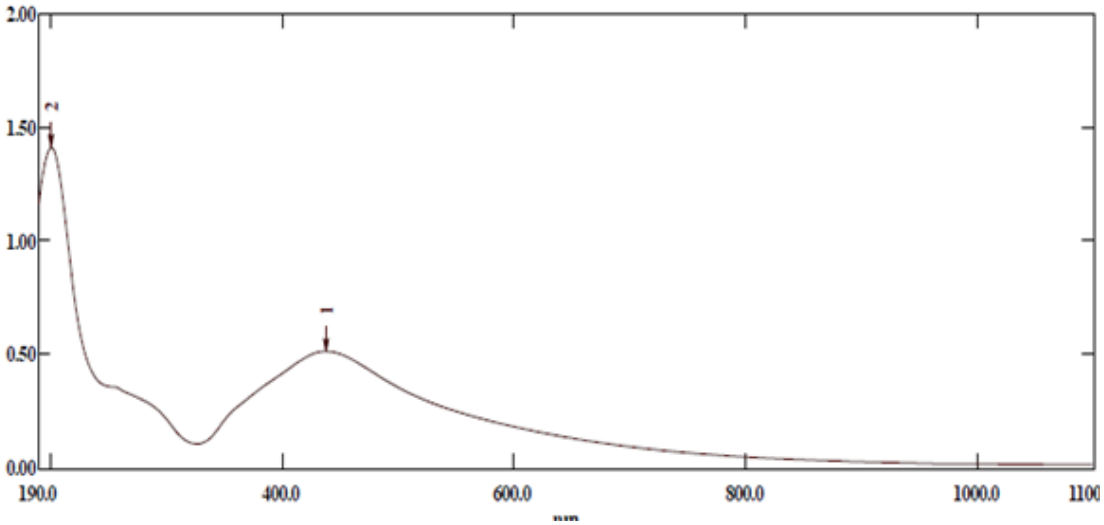

Figure (4-2): UV-Vis spectroscopy of AgNPs

UV-Vis absorption spectroscopy was utilized for the preliminary characterization of the synthesized nanoparticles. This technique assesses the synthesis and stability of the silver nanoparticles (AgNPs).The distinct optical properties of AgNPs make them highly reactive to certain wavelengths of light. Due to the surface plasmon resonance phenomenon, AgNPs exhibit good absorption in the visible spectrum in the range of 438 nm in the figure (4-2) which belongs to the prepared nano silver, as this peak was not present in the UV-via spectrum of the clove extract in the figure (4-2). [4]

3.2 Fourier transform infrared spectroscopy (FTIR)

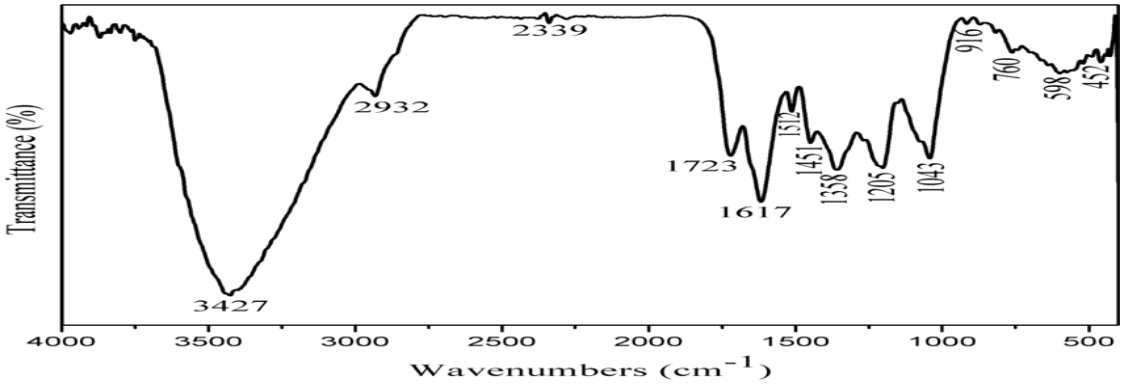

Figure (4-3): FTIR spectrum of the clove extract

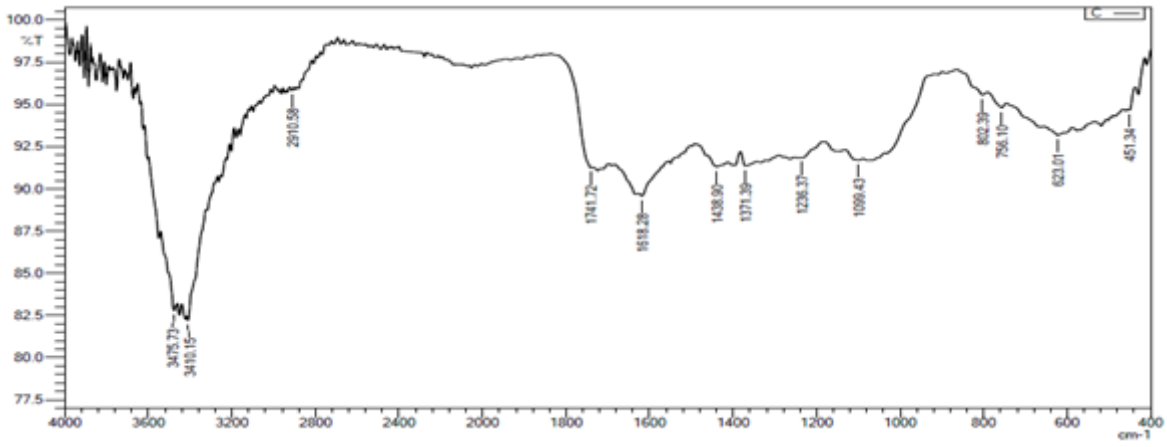

Figure (4-4): FTIR spectrum of AgNPs

The FTIR spectrum confirms the successful biosynthesis of silver nanoparticles (AgNPs) using clove extract, displaying peaks that indicate the presence of biomolecules involved in nanoparticle formation and stabilization. The observed peaks reveal biomolecules responsible for reducing and stabilizing silver ions:

1. O–H Stretch (~3400 cm^{-1}): Corresponds to hydroxyl groups from phenolic compounds like eugenol, which reduce Ag^{+} to Ag^{0}.
2. C=O Stretch (1700–1600 cm^{-1}): Indicates carbonyl groups from aldehydes, ketones, or carboxylic acids, contributing to reduction and stabilization.[5]
3. C–O and C–N Stretch (1200–1000 cm^{-1}): Suggests ester or ether linkages from polyphenols, flavonoids, and proteins, aiding in nanoparticle stabilization.
4. C–H Stretch (~2900 cm^{-1}): Reflects aliphatic hydrocarbons, possibly from organic molecules adsorbed on the nanoparticle surface.
5. Fingerprint Region (<1000 cm^{-1}): Represents complex vibrations of organic compounds, further confirming plant-derived molecules on the AgNP surface. [6]

Figures (3-3) and (3-4) we can see the presence of all these peaks with a slight shift, which indicates the role of the clove plant in reducing and encapsulating the silver nanoparticles.

3.3 Scanning Electron Microscope (SEM

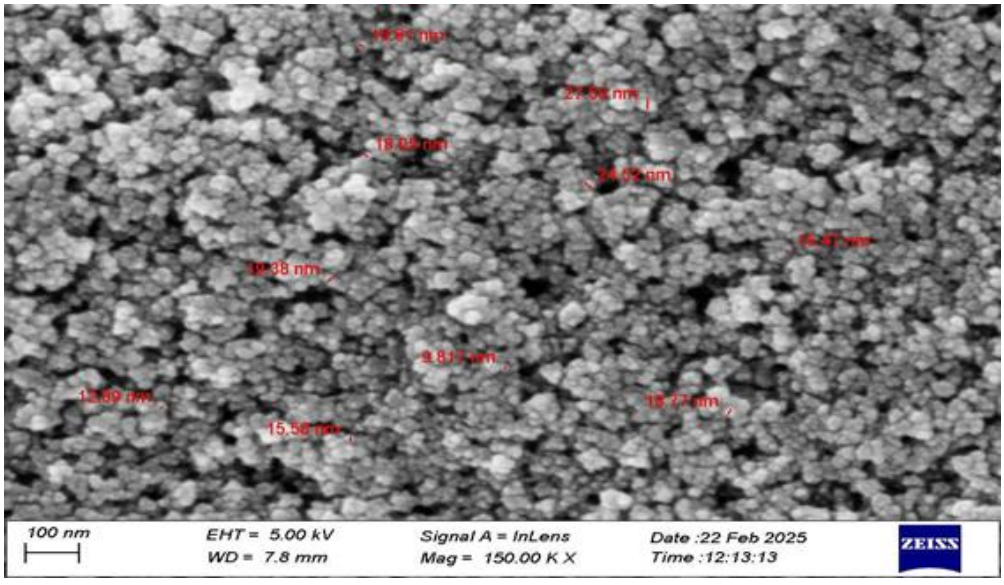

Figure 4-5 The SEM analysis of silver nanoparticles (AgNPs) synthesized with clove extract

The SEM analysis of silver nanoparticles (AgNPs) synthesized with clove extract revealed a particle size range from 9.8 nm to 27.6 nm. The Working Distance (WD) was 7.8 mm, and the Electron High Tension (EHT) was 5.00 kV. These results offer valuable insights into the nanoparticle characteristics:

1. Particle Size (9.8 nm to 27.6 nm): The observed size range is typical for silver nanoparticles and suggests high surface area and reactivity. Smaller nanoparticles (around 9.8 nm) may exhibit enhanced antimicrobial and catalytic properties, while the larger particles (up to 27.6 nm) could have different optical or chemical behaviors. This size distribution indicates relatively uniform synthesis, though slight variations might be present.[7]

2. Working Distance (WD = 7.8 mm): The moderate WD allows for a balance between high resolution and sufficient depth of field, ideal for imaging nanoparticles without distortion or charging effects.[8]

3. Electron High Tension (EHT = 5.00 kV): The low EHT minimizes damage to the particles, focusing on surface features and providing clear images while reducing beam-induced changes to the nanoparticles.[9]

4. Applications: The AgNPs in this size range are ideal for applications like antimicrobial agents, drug delivery, and sensors due to their high surface area and reactivity. Optimizing the synthesis process to narrow the size distribution could enhance their consistency for specific applications.[10]

The SEM results show promising nanoparticle characteristics with a size range of 9.8 nm to 27.6 nm, a moderate working distance of 7.8 mm, and a low EHT of 5.00 kV. These findings highlight the potential of the AgNPs for various applications, with future work focusing on refining synthesis conditions for more uniform particles.

3.4 Biological activity of silver nanoparticles:

The silver nanoparticles prepared from the Myrtle extract showed high effectiveness in inhibiting bacterial growth, with clear zones of inhibition observed around the wells where they were added. For the Pseudomonas bacteria used, the inhibition zone diameters were (16, 14, 14) mm for (500, 1000, 2000) µl, respectively.

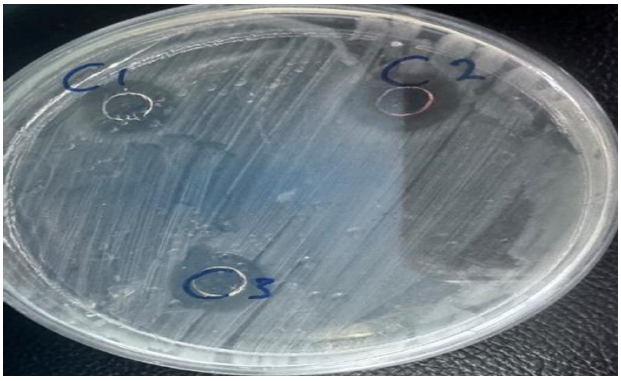

Figure 4.6 shows that the inhibition zone diameters increased with increasing extract concentration

The antibacterial efficacy of silver nanoparticles synthesized using clove extract was evaluated against *Enterococcus* and *Escherichia coli* by measuring the inhibition zones.

- **Enterococcus**: The inhibition zone measured 18 mm, indicating a significant antibacterial effect of the silver nanoparticles.
- **E. coli**: A slightly larger inhibition zone of 20 mm was observed, suggesting a higher susceptibility to silver nanoparticles compared to *Enterococcus*.

The variation in susceptibility can be attributed to differences in cell wall structure. *Enterococcus*,

a Gram-positive bacterium, possesses a thick peptidoglycan layer that acts as a barrier, limiting nanoparticle penetration and reducing antibacterial efficacy. In contrast, *Escherichia coli*, a Gram-negative bacterium, has a thinner peptidoglycan layer and an outer membrane that facilitates interactions with nanoparticles, leading to enhanced antibacterial activity and a larger inhibition zone .[11]

These results demonstrate the effectiveness of the prepared nanoparticles in combating bacterial infections and highlight their role as effective antimicrobial agents.

Figure (4-7) show the inhibition zone on enterococcus and E.coli

4.5. Conclusion

The successful biosynthesis of silver nanoparticles (AgNPs) using clove extract has been validated through several characterization techniques. UV-Vis spectroscopy identified the presence of AgNPs, displaying a distinct peak at 438 nm. absent in the clove extract alone. FTIR analysis revealed key functional groups responsible for nanoparticle formation, including hydroxyl, carbonyl, and phenolic compounds, highlighting their role in stabilization and reduction. SEM analysis determined that the synthesized nanoparticles had a size range of 9.8 nm to 27.6 nm, indicating a relatively uniform distribution suitable for various applications. The biological activity assessment showed significant antibacterial properties, with notable inhibition zones against Enterococcus (18 mm) and E. coli (20 mm), demonstrating the effectiveness of AgNPs in combating microbial infections. These results demonstrate the effectiveness of plant-derived nanoparticles as an environmentally friendly and effective antimicrobial agent.

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