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Green Synthesis Of silver Nanoparticles Using *Elettaria cardamomum*: Characterization and Antimicrobial Potential

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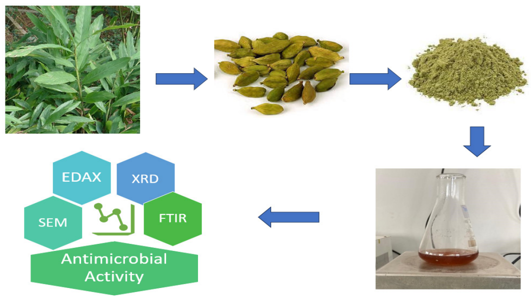
Graphical abstract



Abstract

Recently, the eco-friendly production of silver nanoparticles (AgNPs) has become a focal point for researchers. In India, the quest for cost-effective, non-hazardous compounds suitable for reducing and stabilizing AgNPs has been limited. This study explores the synthesis of AgNPs using *Elettaria cardamomom* seed extract. We evaluated the efficiency of silver ion (Ag+) reduction and AgNP formation from an aqueous AgNO3 solution combined with the cardamom seed extract. Characterization of the AgNPs was carried out through UV-vis spectroscopy, XRD, SEM, EDAX, and FTIR. This research presents a practical and efficient strategy for AgNP production. A formulation using readily available 1 mm aqueous AgNO3, known for its accessibility and therapeutic properties, was created with cardamom seed extract serving as both a capping and reducing agent. Within 30 minutes, the silver ions were reduced to AgNPs, resulting in a change from golden to dark brown-reddish tint. Various analytical techniques, including UV-vis spectroscopy and Fourier-transform infrared spectroscopy, were employed for characterization. This study introduces an affordable, robust, and renewable method using *E. cardamomum* seeds for AgNP synthesis, which is entirely eco-friendly and devoid of toxic chemicals. AgNPs hold promise for applications in cancer treatment and diagnosis, setting them apart from other noble metals.

Keywords: Green synthesis, Silver Nanoparticles, Elettaria cardamomum, Nanotechnology, Surface Plasmon Resonance

Introduction

The process of altering matter within a length range of 1 to 100 nm to create useful materials, tools, and systems is referred to as “nanotechnology.” At this scale, novel characteristics, and functions emerge(1). Nanotechnology is currently an expanding field of manufacturing processes. Notably, living cells represent some

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of the best examples of nanoscale machines capable of a wide range of activities, including energy generation and material extraction(2). Nanoparticles can be created using a chemical synthesis process in a short period of time; however, this approach requires capping agents to stabilize the size of the nanoparticles. The chemicals used in the creation and stabilization of nanoparticles are dangerous and generate environmentally harmful byproducts. Recently, research in nanoparticle synthesis using microbes and plant extracts has gained more importance due to its eco-friendliness, versatility, and, most importantly, the avoidance of hazardous chemicals (3).

When compared to microbes, plant-mediated synthesis is frequently used *by* researchers for its advantages, such as avoiding the need to maintain microbial cultures, saving time, and cost-effectiveness(4). The need for ecologically safe synthetic processes for producing nanoparticles has led to increased interest in biological techniques that do not produce dangerous chemicals as by-products. Consequently, “green nanotechnology” is becoming increasingly popular(5). Green chemistry has recently become essential for producing environmentally friendly products(6).

Cardamom (*E. cardamomum*), widely recognized as the ‘Queen of Spices’ and belonging to the Zingiberaceae family, is one of the most important and readily available spices. The seeds of cardamom are the source of its aroma and flavor. Plants synthesize a wide variety of secondary metabolites, with a significant portion consisting of phenolic compounds and flavonoid compounds(7). Nowadays, medicinal plants(8), fruits(9-11), weeds(12), and spices(13) have been used for the synthesis of nanoparticles. Spices are a new frontier in green synthesis, and Singh et al. have already reported the synthesis of silver nanoparticles using clove buds. The presence of aromatic flavor compounds in the clove buds is likely responsible for the reduction of silver ions to silver nanoparticles(13).

Materials and Methods

Collection and extraction of cardamom seeds

A variety of plants in the families Elettaria and Amomum in the family Zingiberaceae produce seeds that are used to make the spice known as cardamom. We bought fresh Elettaria cardamom seeds from a local market of Ahmedabad district, cleaned them with distilled water, and let them air dry. The seeds were pulverized to a fine powder. 20 g of newly made dry seed powder was suspended in 200 ml of distilled water and heated at 75 °C for 30 minutes. Whatman Filter Paper No. 1 was used to filter the final solution before using it as an extract in the following experiment.

Synthesis of silver nanoparticles

As a precursor, silver nitrate (AgNO3) was used to start the production of silver nanoparticles. 40 ml of various AgNO3 concentrations (0.16, 0.18, and 0.20 gm) and about 7 ml of *E. cardamomum* seed extract was mixed in a conical beaker. The solution’s colour shifted from light brown to dark brown during the 30 minutes that it was brought to a boil at 75°C. The reduction of Ag+ ions to AgO was noticed by observing the UV-Vis spectra of various concentrations of the reaction mixture (silver nitrate solution and seed extract).

Optimization of synthesized silver nanoparticles

With an increase in leaf extract concentration (10, 20, and 30 mL) in 10 mL of 1 mM silver nitrate, the concentration ratio of the leaf extract and silver nitrate was optimized (ratio1:1,2:1,3:1). The solution's absorbance was measured spectrophotometrically after two days of incubation. It was also done by changing quantities of the aqueous silver nitrate by 16,18 and 20ml. Also, the stability of the produced silver nanoparticles was checked after 10 days through UV spectrophotometry.

Antibacterial activity study

Antibacterial activity of synthesized AgNP was assessed by agar well diffusion method(14). In this method 0.2 ml of young test culture of *Bacillus subtilis* and *E. coli* was inoculated in sterile melted top agar previously cooled at 50^{o}C. After solidification, two wells of 8mm size were created by sterile cup-borer. One well is loaded with antibiotic neomycin which is designated as A and other well is loaded with AgNPs synthesized by aqueous cardamom extract which is designated as S.

Characterization studies

The ability of colored materials to selectively absorb energy within the visible portion of the electromagnetic spectrum is what gives color its appearance. Energy absorption causes an electron to move from its ground state to its excited state. As a result, the principal characterization instrument to investigate the creation of metal nanoparticles is the UV visible absorption spectroscopy. UV-visible spectroscopy, which has proven to be a very helpful method for the investigation of nanoparticles, was used to characterize the nanoparticles in the main. With a resolution of 1 nm, UV-visible spectroscopy (nanodrop) analysis was performed(15). Using a UV-visible Spectrophotometer, the reduction of silver metal ions to silver nanoparticles was first examined (Perkin-Elmer) at regular intervals with wavelengths ranging from 360 to 700 nm. Using a (Bruker VERTEX-70) FT-IR spectrophotometer with a resolution of 4 cm-, it was feasible to identify potential biomolecules that oversaw the reduction and stability of silver nanoparticles(16). FTIR Spectra of neem leaf broth and synthesized AgNPs were recorded on ALPHA-T –Bruker model in the range of 4000 – 400 cm-1.

Results and Discussion

The current investigation thoroughly examines biological synthesis, characterization, process optimization, size-based separation and purification, mechanism of synthesis of silver nanoparticles, antimicrobial activity of plant material synthesized silver nanoparticles, and their potential antimicrobial mechanism. A straightforward method for the creation of silver nanoparticles was used for the screening of various plant components. So, the identification of new and potential biological nanoparticle manufacturing systems was based on the results of this screening.

There is evidence in the literature that plant extracts have the ability to convert metal ions into metal nanoparticles. Due to the presence of bioactive phytochemicals in the aqueous date seed extract such as phenolics, flavonoids, polyphenols, aldehydes, carboxylic acids, anthraquinone, saponin, terpenoids tannin, and proteins, they were able to stabilize the produced silver nitrate salt into AgNPs. The change of color in the reaction mixture from orange red to dark brown provided early proof that silver nanoparticles had been produced because of the surface Plasmon resonance phenomenon. (Fig. 1).

An important method to investigate how metal NPs develop in aqueous solutions is UVVis spectroscopy. UV-vis spectroscopy was employed to examine the characteristics of nanoparticles. UV-Vis spectroscopy is a vital technique to confirm the nanoparticles' size, shape, and stability. through the development of a brown tint from a light orangish golden(17). Visualization of AgNPs generation was possible. From 350 to 800 nm, the measured spectrum values were obtained. A single, strong, and broad surface Plasmon Resonance (SPR) peak was seen at 429 nm throughout the reaction period, indicating that the NPs were disseminated 27 in the aqueous solution. This peak was measured by analyzing the absorption spectra at a wavelength range of 200 to 800 nm (Fig. 2). Due to the plasmon resonance exhibited by AgNPs, it is known that AgNPs exhibit a UV-Vis absorption spectrum maximum in the range of 400–500 nm. The AgNPs' Surface Plasmon Resonance (SPR) absorption band's nearly identical, symmetrical shape (Fig. 3) indicates

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that they formed into spherical nanoparticles without significant aggregation(18). Numerous reports have shown that Silver nanoparticles' resonance peak appears to be located between 300 and 480 nm(19,20).

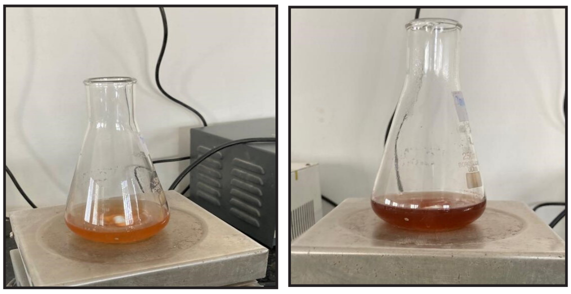

Fig. 1. Color change of leaf extracts containing silver before (left side) and after (right side) the synthesis of silver nanoparticles.

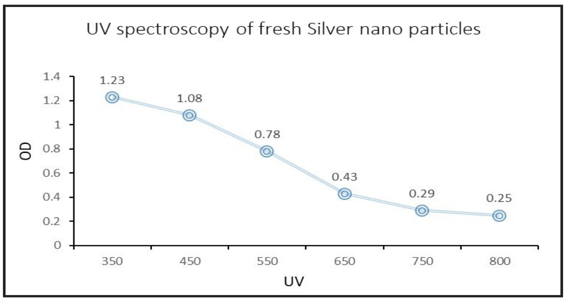

Fig. 2 UV-Visible absorption spectra of biosynthesized silver Nanoparticle from *E. cardamomum* depicting peak at 450nm.

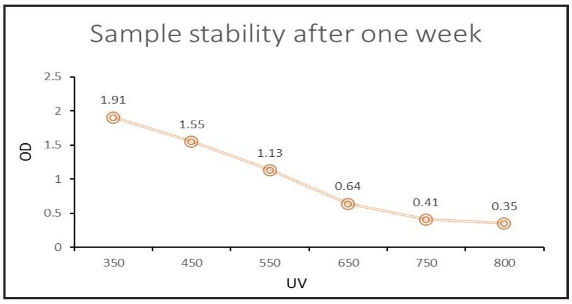

Fig. 3 UV-Visible absorption spectra of same biosynthesized silver nanoparticle after a week.

The FTIR spectra of the reduced AgNO3 solution with the plant extracts were found between the wave number ranges of 4000-600 cm1(Fig. 4). FTIR research was done at a resolution of 4 cm1 to determine the likely functional groups in plant extracts that are in charge of lowering the silver ion and capping the produced AgNPs. The occurrence of the FTIR peak at 3350 cm1 shows the presence of N-H bonds in amines, as opposed to the tiny peak at 2913 cm1, which reflects the H-C-H symmetric stretching of alkanes. Fig. 5 showing the FTIR spectra of silver nanoparticle synthesized using *E. cardamomom* seed broth. The alkaloid capsaicin with N-H stretch is responsible for both the coating of the nanoparticles and the bio-reduction of silver ions. The band at 1017 cm1 is caused by the ethers' C-O elongation in plant extract. Previous research has found that the harmonics of alcoholic O-H, alkanes C-H, phenolic O-H, and C-O stretches, respectively, are 3325-3198 cm1, 2917-2833 cm1, 1380-1360 cm1, and 1050-1025 cm(21,22).

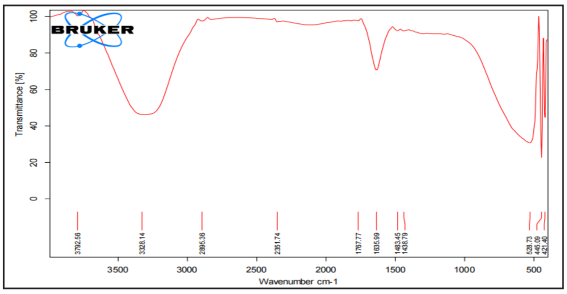

Fig. 4. FTIR spectra of *E. cardamomom* seed broth extract

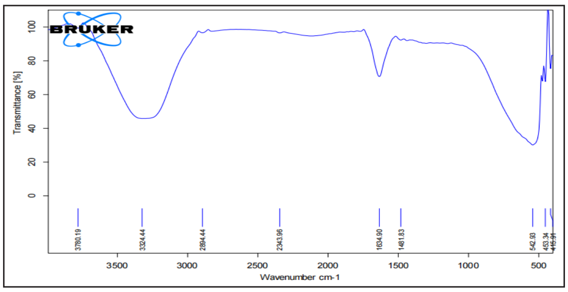

Fig. 5. FTIR spectra of silver nanoparticle synthesized using *E. cardamomom* seed broth

AgNps demonstrated antimicrobial efficacy against *E. coli* and *B. subtilis*, as demonstrated by the findings and visible zones of inhibition on (Figure 6(a, b)). AgNps exhibited behavior that was dose dependent. At a concentration of 30 g/disc, generic Neomycin and nanoparticles were compared side by side. Biosynthesized AgNps displayed larger zones of inhibition at 30 g dosages. The ability of the AgNps to inhibit both Gram-positive and Gram-negative bacteria at the cellular level was amply demonstrated by this action in other words, compared to traditional antibiotics, AgNps were more efficient at preventing the growth of pathogenic germs. Numerous studies have discussed how effective silver's antibacterial action is against various infections(23,24). However, a few studies have produced some data and hypotheses: I (i) To interact with bacteria, they should have a spherical shape; (ii) Nanoparticles can enter bacteria and cause damage by likely working in conjunction with substances containing sulphur and phosphorus(25). All silver nanoparticles with a diameter of 1 to 10 nm attach to the cell membrane surface of bacteria and interfere with their ability to function(26). The antibacterial effect is likely generated from the electrostatic interaction between the positive charged Nanoparticles and the negative charged cell membrane of the bacterium(27,28). On the other hand(29), observed that the antibacterial activity of silver nanoparticles on Gram-negative bacteria was dependent on the concentration of Ag nanoparticles and was directly related to the creation of pits in the bacterial cell wall.

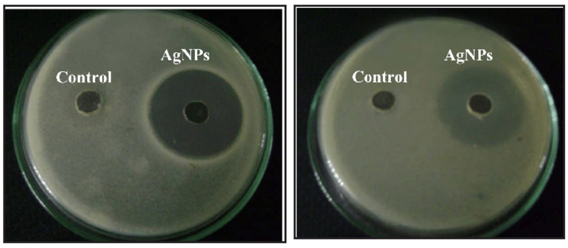

Fig. 6. The different zones formed by taking neomycin as control and (a) *Bacillus subtilis* (b) *E. coli.*

This research developed a simple, quicker, and more environmentally friendly method for creating AgNPs from cardamom seed aqueous extracts. monitoring of shifting FTIR peak locations of Spectra suggest that various functional categories of plant secondary metabolites may serve as capping and stabilizing agents. The Ag ion reduction will be complete in 4 hours due to how fast metal ions are reduced. Successfully produced silver nanoparticles underwent UV-vis and FTIR analysis. Two samples were tested in UV-VIS and compared to one another to show that as samples age, the particles tend to become denser. The functional group contained in the cardamom seeds is found using FTIR. The graph demonstrated the presence of the two major functional groups, 1,8 cineole and a-terpinyl acetate.

Conclusion

The objective of our study was to explore the antimicrobial, antioxidant, and synthesis capabilities of AgNPs while also investigating the effects of sulfidation on these nanoparticles.

Our experimental results demonstrate the successful achievement of green synthesis of AgNPs utilizing *E. cardamomum* extract as both a capping and reducing agent. In terms of accessibility, environmental sustainability, affordability, and the simplicity of the reduction process, green synthesis outperformed traditional chemical reduction methods. Notably, the green synthesis approach has been optimized to yield AgNPs rapidly and in substantial quantities. Characterization of the synthesized AgNPs revealed nearly spherical shapes with sizes averaging around 50 nm, as confirmed through a series of comprehensive characterization techniques. AgNPs synthesized via green methods hold significant promise for various applications, including drug delivery, DNA analysis, gene therapy, cancer treatment, antimicrobial agents, biosensors, catalysis, surface-enhanced Raman spectroscopy (SERS), and magnetic resonance imaging (MRI). This study introduces a straightforward, simplified, and environmentally friendly method to produce AgNPs using aque-

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ous extracts of cardamom seeds. The successful derivation of silver nanoparticles was confirmed through UV-vis spectroscopy, and further insights into the functional groups present in cardamom seeds were obtained through FTIR analysis. Notably, our FTIR analysis revealed the presence of two key functional groups, namely, 1,8 cineole and a-terpinyl acetate, as evident from the generated spectra.

Acknowledgments

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