Original Article

Green Synthesis of Silver Nanoparticles Utilizing *Eriobotrya japonica* L. Seed Extract and Evaluation of their Antibacterial Activity

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Abstract

Background: Silver nitrate AgNPs nanoparticles have garnered significant attention for their unique antibacterial properties, making them a valuable addition to the medical field. Extracellular biosynthesis of AgNPs was performed by employing an aqueous extract of *Eriobotrya japonica* seeds to reduce silver nitrate ions in a short period. **Objectives:** We aimed to prepare AgNPs from the aqueous extract of fruit seeds and detect its antibacterial effect. **Materials and Methods:** AgNP formation was attested by altering the color of the plant extracts' single-nucleotide polymorphisms (SNPs) to coffee and several spectroscopic and microscopic analyses. The AgNP formation was also confirmed by spectroscopy. These silver nanoparticles of plant origin were tested for antibacterial activities using the disk-diffusion method, with three concentrations of 50, 100, 150 mg/mL, using *Klebsiella*, *Escherichia coli*, and *Streptococcus mutans*. **Results:** The inhibitory antimicrobial property of silver nanoparticles was assessed by measuring the area of inhibition. Dishes treated with silver nanoparticles made from extracts of *E. japonica* seeds encountered toxicity. It yielded the highest inhibition diameters at a concentration of 150 mg/mL toward *E. coli* and *Klebsiella*, which were 20 mm, whereas the growth of *S. mutans* was 15 mm. The antibacterial activity of silver was magnified by SNPs synthesized from the seed extract compared with that of anti-penicillin; the results suggested that AgNPs may have a good antibacterial advantage over conventional antibiotics. **Conclusion:** Antibacterial research on the *E. japonica* seed extract shows its potential as a natural antimicrobial. The study indicated that higher seed extract concentrations are more effective in limiting bacterial growth. The extract showed broad-spectrum antibacterial activity against *Klebsiella*, *E. coli*, and *S. mutans*, suggesting that *E. japonica* may be a good alternative or supplement to antibiotics. These findings suggest that *E. japonica* seed extract can be used to create novel antibacterial medicines, especially given the issue of antibiotic resistance. The study also shows that addition of natural ingredients can promote synthesis of physiologically active nanoparticles.

Keywords: Antimicrobial activity, *E. coli*, *Eriobotrya japonica*, *S. mutans*, silver nanoparticles

Introduction

Natural, plant-based, and bioprinted sources such as flavonoids, proteins, terpenoids, polysaccharides, polyphenes, alkaloids, and ketones act as reducing agents in stabilizing the nanoparticles and diffusing and converting metals into metallic nanoparticles, causing the generation of desired nanoparticles with properties that have been optimized as favorable in antibacterial applications.[1] *Eriobotrya japonica* is a non-deciduous tree belonging to the family Maloideae of Rosaceae; it is also an ornamental tree and a key source of nectar for rearing bees. It is 10 m tall and also may be smaller, ranging from 3 to 4 m.[2] This plant has medicinal and nutritional importance, that is, it protects from diseases and contains acids and organic fatty acids.[3] Amino

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acids, sulforaphane, etc. are nutrient trace elements. Sulforaphane compound, a phytochemical, has anti-inflammatory and antibacterial inhibitory properties.[4] Nanoparticles are synthesized in different ways, and plants contain natural chemical ingredients such as aldehydes and alkaloids contributing to the reduction and conversion of metallic nanoparticles.[5,6] Photo-silver nanoparticles (AgNPs), in addition, have greater selectivity and heat resistance, in various microorganisms, such as *Staphylococcus aureus* and *Escherichia coli*, and *Candida albicans*.[7]

AgNPs have nanoscale dimensions, better antimicrobial efficacy on account of their increased surface-to-volume ratio, and peculiar chemical and physical attributes.[8] This product contains organic matter particles, in addition to botanical elements, which are of natural quality.[9] Large small particle mass and high surface area lead to increased antimicrobial activity against pathogenic microorganisms.[10] Thus, AgNPs can be investigated for their antimicrobial effects.[11] The antibacterial activity is effective and potent, as high as 40% for all tested strains as AgNPs demonstrated significant toxicity for the examined strains, and they ascribed that nanoparticle activity could be linked with their intrinsic toxicity.[12] Bacterial cell permeability results in bacteria losing their viability, and the activity of AgNPs against *Pseudomonas* and *Aspergillus niger* has been investigated.[13] The inhibition zone shows that zinc nanoparticles from *Mangifera indica* seeds substantially increase the rate of infectious inhibition and reduce inflammation. AgNPs were synthesized from *Carum carvi* extracts.[14]

Pathogenic *Staphylococcus epidermidis*, *S. aureus*, *E. coli,* and *Proteus vulgaris* were employed to examine the antibacterial properties. The synthesized AgNPs were potent against Staph and harmless to the ecosystem upregulation of AgNP production using the aqueous extract of *Erythrophyllum comparitum* leaves. Ultraviolet–visible (UV-Vis), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy, X-ray diffraction (XRD), and dynamic light scattering methods were employed to characterize the AgNP antibacterial ability. Thus, we aim to produce AgNPs from the aqueous extract of fruit seeds and test it on some pathogenic bacterial isolates.[15,16]

Materials and Methods

Preparation of the plant extract and preparation of silver nanoparticles

The extract related to the plant was meticulously taken from the seeds of *E. japonica* from the local markets and classified by the Natural History Museum. The seeds were dried after extracting them from the fruits and crushed using an electronic pulverizer. About 100 g of seed powder was weighed, and an adequate quantity of water was added to it and brought to a boil at a boiling point at 80 m for 60 min with constant stirring, and after cooling down, it was filtered with Whatman No. 1 paper.

To purify the extract for later use by preparing AgNPs,[17] 5 mL of 10 mM $Ag(NO_3)_2$ $6H_2O$ was drizzled into the prepared homogenous leaf extracts, and the mixture was incubated at 65°C for 20 min, and the obtained thick yellow paste was completely dehydrated at 400°C for 2 h prior to collecting and packaging individually for further characterization. The sample contaminations were removed by calcination, and a filtered form of NP is obtained.[18]

Analytical methods

UV–Vis spectrophotometry, XRD, and scanning electron microscopy (SEM) were performed and measured to firmly maintain as AgNPs:

To analyze the synthesized silver oxide nanoparticles, we used a UV–Vis spectrophotometer. First, 1.7 g of the nanoparticles was dissolved in 17 mL of dimethyl sulfoxide. The solution was then subjected to UV–Vis spectrophotometric analysis. Both visual observations and measurements of absorbance were recorded during the process. The spectroscopic readings showed changes in the sample, confirming the successful synthesis of silver oxide nanoparticles. This method is a key step in verifying the synthesis of the nanoparticles.[19]

1. The structure of the synthesized Ag NPs was ascertained by XRD. The crystal size of the Ag NPs synthesized was calculated using Scherrer's formula ($D = 0.9\ \lambda/\beta\ \cos\theta$, where θ stands for the diffraction angle, $\lambda = 1.5406$ Å, and β stands for the full width at half maximum. $D - 0.942,/\beta \cos\theta$, where D is the average crystal size perpendicular to the reflecting planes, à is the X-ray wave-length. A Zn–NP laminated film was created upon a glass plate, and the XRD pattern was examined in the extent of 10 to 80.
2. SEM: This tool relies on a process where the sample surface gets meticulously scanned with a concentrated electron beam precisely for getting microscopic photoimages of the organized AgNPs.

The antibacterial activity

The disk-diffusion method was employed to test AgNPs' antibacterial properties. Plates of nutrient agar were prepared, sanitized, and solidified. Bacterial isolates were swabbed on these plates after they had solidified. The sterilized disks were dipped in AgNP solution (50, 100, and 150 mg/mL), a well was created in the culture media, and the prepared solution was added to it on the nutrient agar plate and incubated for 24 h at 37°C. Control, single-nucleotide polymorphisms, and silver nitrate inhibition zones were all measured. The investigations

were carried out three times, with the mean values of the zone diameter being provided each time.[17,20]

Ethical approval

It was attained from the Research Ethics Committee of the Department of Biotechnology by Order 23 on Feb 5, 2023.

Results

The results exhibited a finding showing "adding 50 mL of aqueous extract of the lower link seeds to 5 mL of silver sulfate produced 0.4 g of AgNPs", and this proved the color change of the extract from milky white to coffee, after a day of incubation in the dark place at room temperature, showing the generation of AgNPs, as in Figure 1.

UV/VIS spectrophotometry

It was maintained by taking UV perceptible spectrum analysis in the range of 100–900 nm using Shimadzu UV/VIS 2401PC. The maximum absorption exhibited a high absorption from 440 to 500 nm and a distinct peak at 420 nm [Figure 2].

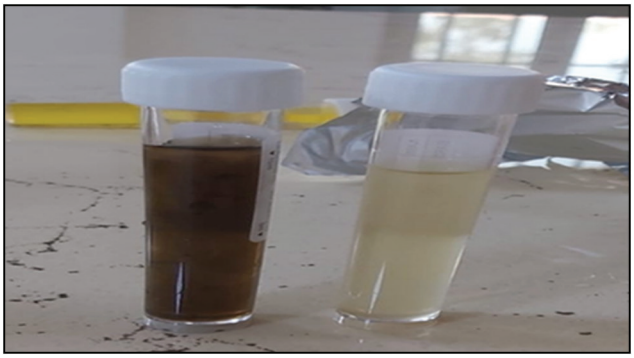

Figure 1: Color change of silver nitrate to silver nanoparticles (brown, left) by adding *Eriobotrya japonica* seed extracts (milky color, right)

Characterizing nanoparticles with the XRD technique

The XRD results revealed that the AgNPs prepared by reducing Ag ions with aqueous extract were naturally crystalline and that the X-ray diffraction represented the presence of the characteristic peaks of the XRD pattern that the synthesized material consisted of nanoscale particles. The diffraction pattern of JCPDS No. 89-3722. Few unmapped peaks were present 28.04°, 31.52°, 45.67°, 55.91°, and 66.95°. These Bragg peaks could possibly be attributable to the organic compounds present in the extract and responsible for the reduction of silver ions and the stabilization of the resulting nanoparticle size average for the synthesized AgNPs. By biological analysis, as being within the hexagonal phase, and the diameter of AgNP nanoparticles was calculated using the Debye–Scherrer formula, with the crystalline size of the created AgNPs evaluated and concluded to be 52.24 nm. Peaks corresponding to ICDD card number 01-079-0207. The AgNPs were hexagonal, with lattice parameters confirming their crystalline structure a (= b) = 3.3267 A 0 and c = 5.2114 A 0, and the average size was about 15 nm for AgNPs from the aqueous seed extract [Figure 3].

Characterizing nanoparticles with the field emission scanning electron microscopy device

SEM was meticulously employed to detect and specify the shapes of nanoparticles' surface with a diameter calculation, the agglomerations of individual AgNPs with the presence of many nanoscale aggregates [Figure 4]. AgNPs revealed the accumulation of certain single crystals; TEM assay uncovered that the size of Zn–NPs ranged from 30 to 53 nm [Figure 4].

The antibacterial activity of the *E. japonica* extract

The present study evaluated the antibacterial efficacy of the *E. japonica* (Loquat) seed extract against *Klebsiella*, *E. coli*, and *Streptococcus mutans*, using penicillin as a control as shown in Figure 5.

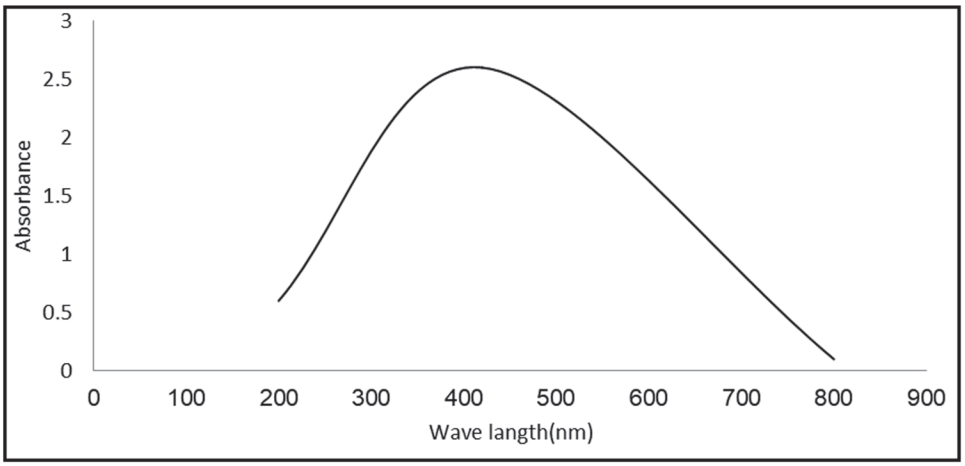

Figure 2: The ultraviolet–visible absorption spectrum of silver nanoparticles synthesized by *Eriobotrya japonic*a seeds

The diameters of the zones of inhibition were measured at three different concentrations of the seed extract: 150 mg/mL, 100 mg/mL, and 50 mg/mL, and these values provide insights into the dose-dependent activity of the extract. The diameters of inhibition reveal significant antibacterial activity of *E. japonica* seed extract across all bacterial strains tested. At the highest concentration of 150 mg/mL, the seed extract ultimately uncovered the greatest inhibition zones, with *Klebsiella* demonstrating the maximum susceptibility. More particularly, the inhibition diameter at 150 mg/mL was approximately 20 mm, which is generally the highest observed in the experiment. This undoubtedly shows that the seed extract at this concentration is highly effective against *Klebsiella*. For *E. coli*, the inhibition diameter at 150 mg/mL was slightly lower, about 17 mm, whereas for *S. mutans*, the inhibition diameter was nearly about 15 mm. These values, although slightly lower compared to those of *Klebsiella*, still reflect a strong antibacterial effect, indicating the broad-spectrum potential of the seed extract.

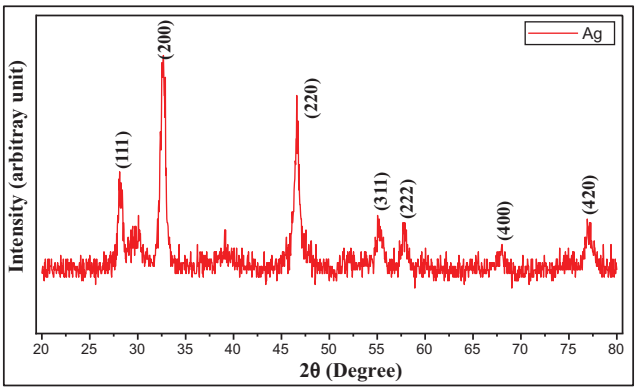

Figure 3: X-ray diffraction spectrum of synthesized silver nanoparticles

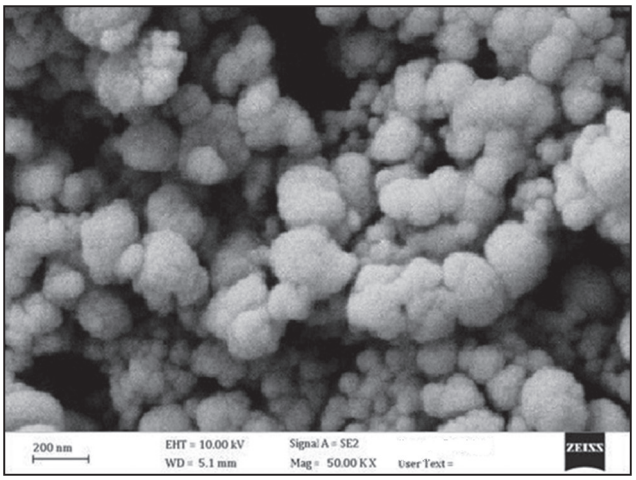

Figure 4: Field emission scanning electron microscopy image of silver nanoparticles

At the lower concentration of 50 mg/mL, the inhibition diameters were reduced across all strains. The smallest inhibition zone was observed for *S. mutans*, with a diameter of about 12 mm, while *E. coli* showed an inhibition zone of about 13 mm, and *Klebsiella* maintained a slightly higher diameter of about 15 mm. These results affirmatively state the concentration-dependent nature of antibacterial activity, with high levels of concentrations resulting in more significant inhibition. The zones of inhibition produced by penicillin served as a control for comparison. The zones of inhibition for penicillin were relatively consistent across bacterial strains, with values around 14–15 mm, which were generally lower than those observed for the highest concentration of the seed extract. Specifically, the zone of inhibition for *Klebsiella* by penicillin was around 14 mm, which is significantly smaller than the 20 mm inhibition seen with the 150 mg/mL seed extract. This comparison highlights that the *E. japonica* seed extract, particularly at 150 mg/mL, exhibits superior antibacterial activity against *Klebsiella*, *E. coli*, and *S. mutans* in comparison

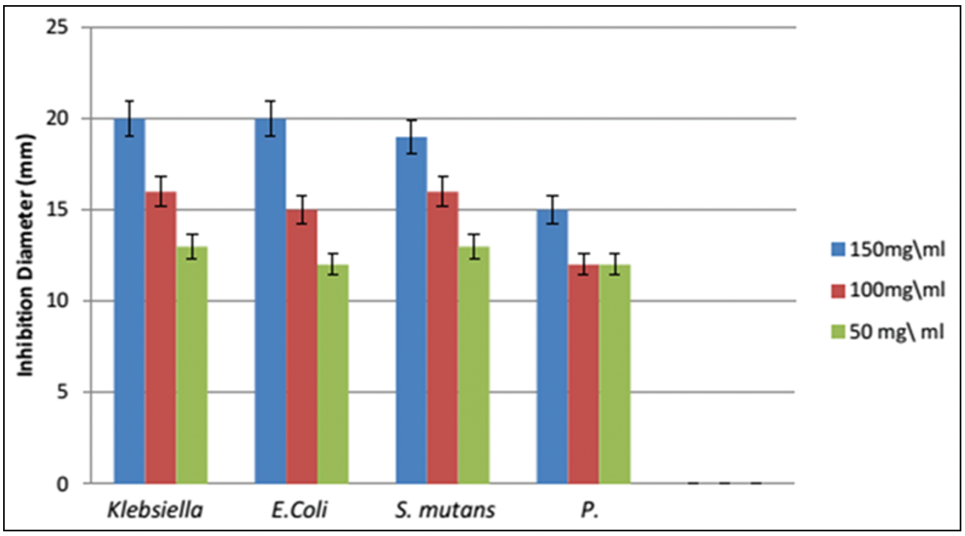

Figure 5: Antibacterial activity of *Eriobotrya japonica* seed extract, (P. = penicillin antibiotic-control; least significant difference 0.05 = 0.0210)

to penicillin. The ability of the seed extract to achieve larger zones of inhibition suggests that it may serve as an effective natural antimicrobial, particularly in contexts where antibiotic resistance is a concern.

Discussion

The results uncover that adding 50 mL of the aqueous extract of the lower limb seeds to 5 mL of silver sulfate produced 0.4 g of (AgNPs), and this proved the color change of the extract from milky white to coffee, after a day of incubation in the dim place at room temperature, indicating the generation of AgNPs [Figure 1]. This change in color was previously detected by numerous authors.[21,22] Authors proposed that the change in color was attributed to the surface resolution of the plasmon.

UV/VIS spectrophotometry was confirmed via taking UV–visible spectrum analysis in the range of 100–900 nm using Shimadzu UV/VIS2401PC. The maximum absorption spectra ranged from 440 to 500 nm and a distinct peak at 420 nm [Figure 2]. which reduces the secondary metabolites in plants like phenols and flavonoids and silver ions in silver oxide solution.[23] The extract of the plant serves as reducing agents and stabilizing agents, which can be considered the intrinsic absorption peak of AgNPs because of the transitions of electron from the valence to conduction bands.[24,25]

The XRD results uncovered that the AgNPs synthesized by reducing Ag ions with the aqueous extract were highly crystalline and that the X-ray diffraction pattern represented the formation of the specific lines of the XRD peaks that the previously formed material consisted of nanoscale particles. Standard diffraction pattern of JCPDS No. 89-3722. Few unmapped peaks were 28.04°, 31.52°, 45.67°, 55.91°, and 66.95°; these Bragg peaks could probably be attributed to the organic compounds present in the extract and are hold accountable for the decrease in silver ions and their stabilization of the resulting nanoparticle size average for the synthesized AgNPs. By biological means, as being within the hexagonal phase of the particles, the diameter of AgNP nanoparticles was calculated using the Debye–Scherrer formula, and the average crystalline size of the formed nanoparticles was assessed to be 52.24 nm. Peaks corresponded with ICDD card number 01-079-0207. The nanoparticles were hexagonal, with specific lattice parameters confirming their crystalline structure a $(= b) = 3.3267$ A 0 and $c = 5.2114$ A 0, corresponds to the previously stated values,[26] and the average size was about 15 nm for AgNPs from the aqueous extract of the seed extract.

SEM was employed to specify the nanoparticle's surface shapes with the diameter calculated. The agglomerations of individual AgNPs correlated with the presence of many nanoscale aggregates [Figure 4]. AgNPs uncovered accumulation with some single crystals. TEM analysis discovered that the mass of ZN-NPs fluctuated from 30 to 53 nm [Figure 4], and the presence of irregular aggregations of particles is attributed to the secondary metabolites in the extract,[17] which is consistent with what was found.[14]

AgNPs exhibit strong antimicrobial activity, primarily attributable to their maximum surface area-to-volume ratio and unique chemical and physical properties. According to studies, AgNPs can enter cells through processes such as cellular proliferation and endocytosis. Once inside, they interact with the cytoplasm and disrupt the mitochondrial function, leading to the reactive oxygen species (ROS) and silver ion (Ag^{2+}) release. These ions can penetrate cell membranes, reach the deoxyribonucleic acid, and cause irreversible chromosomal damage, ultimately resulting in cell death.[27] Pasquet *et al*. suggested that the release of ROS into the aqueous medium plays a significant role in causing cell death. Additionally, exposure to visible light enhances the antimicrobial effect of AgNPs increasing the rate of cell death.[28]

There is also a notable difference in how AgNPs affecting Gram-positive and Gram-negative bacteria as Gram-positive bacteria have more susceptibility to AgNPs because of their peptidoglycan layer, which facilitates the nanoparticles' attack on the cell. In contrast, Gram-negative bacteria, with their lipopolysaccharide-rich outer membrane, are more resistant and require higher concentrations of AgNPs for inhibition.[29-31] Furthermore, Gram-positive bacteria are highly sensitive to hydrogen peroxide than Gram-negative bacteria.[32,33] Certain microorganisms develop resistance to external factors due to their protective outer membrane and structural complexity.[28,34] For example, AgNPs have been shown to inhibit growth of *E. coli* by damaging the bacterial cell membrane and increasing its permeability.[15,35]

Conclusion

The research into the antibacterial activity of *E. japonica* seed extract demonstrates its significant potential as a natural antimicrobial agent. The study found that the efficacy of the seed extract is concentration-dependent, with higher concentrations yielding a larger inhibition of bacterial growth. The extract exhibited broad-spectrum antibacterial activity, particularly against strains like *Klebsiella*, *E. coli*, and *S. mutans*, suggesting that *E. japonica* could be an effective alternative or complement to conventional antibiotics. Those findings interestingly aid further exploration regarding the use of the *E. japonica* seed extract in developing novel antibacterial therapies, particularly in the context of increasing antibiotic resistance. Nonetheless, this paper sheds light on the potential of natural products in the synthesis of biologically active nanoparticles.

Data availability

No data are available for sharing.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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