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Research Article

Single-Step Acer pentapomicum-Mediated Green Synthesis of Silver Nanoparticles and Their Potential Antimicrobial and Antioxidant Activities

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The current investigation is aimed at synthesizing nonhazardous, ecofriendly silver nanoparticles (AgNPs) from aqueous leaf extract of *Acer pentapomicum* and at evaluating its antibacterial, antifungal, and antioxidant activities. In the present study, AgNPs were synthesized at room temperature by mixing 7 mL of 1 mM $AgNO_3$ with 1 mL of *A. pentapomicu*m leaf extract. The synthesized AgNPs were then characterized via various techniques, including UV/visible spectrophotometry showing maximum absorbance at 450 nm. Scanning electron microscopy (SEM) reveals a spherical shape of AgNPs with a size range of 19-25 nm, while the average crystalline nanosize of 9.5 nm and crystalline nature were confirmed by XRD. FTIR showing a broad signal of 3394.71 which confirmed the coating of phenolic and alcoholic compounds on AgNPs, indicating their possible role in the capping and stabilization of silver nanoparticles. EDX showed the elemental composition of the synthesized nanoparticles. Our AgNPs were also found stable at a temperature of 55°C and pH range of 6-7 and in the presence of a salt solution. Furthermore, the green synthesized AgNPs were found to exhibit potent antibacterial activity against various bacterial species, with a maximum of 66% inhibition against *Pseudomonas aeruginosa* and 50.5% against E. *coli* and *Xanthomonas campestris*. These nanoparticles also possess good antifungal activity against various fungal species. Regarding the antioxidant activity, the AgNPs were found to possess a maximum of 93% antioxidant activity against DPPH at a concentration of 250 μg/mL and a minimum of 74% scavenging activity at 5 μg/mL.

1. Introduction

Many pathogenic microbes are continuously gaining antibiotic resistance, against which traditional antibiotics are not effective. To combat this situation, nanoparticles are biogenically manufactured and effectively used as drug carriers. The nanoparticle synthesis is a rising world widely because of their broad range of utilization in various science fields such as biosensors, nanobiotechnology, energy conversion, and medicine [1, 2]

Nanobiotechnology is playing a significant role in the production of new drugs formulations. A variety of chemical, physical, and biological methods is utilized for the production of these nanoparticles. The chemical method produces a larger quantity of metal nanoparticles in a very less time, but this approach can also lead to nonecofriendly bioproducts due to the use of chemicals which are toxic in nature. Therefore, there is a need of a nontoxic and environment-friendly fabricated procedure for the synthesis of metal nanoparticles that do not involve any toxic chemicals or their by-products [3].

Silver nanoparticles are unique and important as compared to other metal nanoparticles because of their unique properties, chemical stability, good conductivity, and antifungal, antibacterial, anti-inflammatory, and antiviral, potency. They can be incorporated into food industry, superconducting materials, composite fibers, cosmetic products, etc. [3, 4]. Silver nanoparticles are also utilized in water filtration, water purification system, textile, and medical devices and in cancer diagnosis and treatment [5]. The utilization of plants for the synthesis of silver nanoparticles has drawn attention not only due to its nonpathogenic, economical, and ecofriendly protocol but also because of its facile, single-step procedure and for its potent applications in biomedical sciences.

Phytosynthesized silver nanoparticles exhibited remarkable significant antioxidant and anticancer activity as compared to other biosynthetic methods [6]. Bharathi and Bhuvaneshwari reported the potential antioxidant activity of silver nanoparticles using *Cassia angustifolia* flowers [7]. Bharathi et al. documented that the phytosynthesized AgNPs from *Cordia dichotoma* fruit extract exhibited more than 90% inhibitory activity against biofilm formed by *S. aureus* and *E. coli* [8]. AgNPs synthesized from *Annona muricata* and *Eriobotrya japonica* plant extracts could also be an alternative for preventing inflammation by enhancing autophagy and as a potent therapy for various cancer types [9, 10].The genus *Acer* belongs to *Aceraceae* and is mainly distributed in Asia and North America. Since ancient times, many species of *Acer* family are utilized for various medicinal properties. *Acer pentapomicum*, commonly known as Maple tree, is a small deciduous tree with a dark brownish smooth bark. It belongs to the family *Aceraceae*, locally known as Tarkana [10]. In our current study, we fabricated the silver nanoparticles from aqueous leaf extract of *Acer pentapomicum* and evaluated its antibacterial, anticandidal, antifungal, and antioxidant activity. This work to the best of our knowledge is the first report on the synthesis of silver nanoparticles using *Acer pentapomicum*.

2. Material and Methods

2.1. Chemical and Reagents. All the chemicals such as silver nitrate, DPPH, NaCl, Nutrient Agar media, Nutrient Broth media, and methanol were obtained from Sigma-Aldrich (St. Louis, USA).

2.2. Collection and Identification of Plant Extract. Acer pentapomicum plant was collected from the Swat district, located in the northern area of Pakistan. The identification of the plant was done by Prof. Mehbob ur Rahman of "Post Graduate Jehanzeb College Swat, Pakistan."

2.3. Preparation of Leaf Extract. Acer pentapomicum leaves were first shade dried and then ground to powder form. About 15-20 grams of leaf powder was then boiled in 150 mL of deionized water. This aqueous boiled extract was then filtered, stored, and further utilized for silver nanoparticle synthesis.

2.4. Biosynthesis of Silver Nanoparticles. One step facile method of Banerjee [11] was followed for the synthesis of silver nanoparticles. 1 mL of aqueous plant extract was mixed with 7 mL of 1 mM silver-nitrate solution. The 1 : 7 reaction mixture (1 mL aqueous extract + 7 mL $AgNO_3$) was placed on a shaker at 40°C for about an hour. An appearance of brownish color of the reaction mixture suggested the complete bioreduction of Ag^+ ions to Ag nanoparticles, which was then affirmed by UV-Vis spectroscopy after 24 hrs.

2.5. Stability Analysis of Biosynthesized AgNPs. The stability of green-manufactured nanoparticles was carried out against temperature, pH, and salt by following the method of Ateeq et al. [12]. The AgNPs were isolated at different temperatures (25-100°C) and pH ranges (3-8). 1 mL of 1 mM, 10 mM, 100 mM, and 1 M each of sodium chloride solution was added to the synthesized AgNPs to check its stability against salt stress. All the isolated samples at different stresses were then analyzed by UV-Vis spectroscopy.

2.6. Characterization of Biosynthesized Silver Nanoparticles. Parameters involved in the characterization of green synthesized silver nanoparticles provide a comprehensive view of nanoparticle morphology, particle size, crystalline nature, and potential functional groups responsible for the bioreduction of silver ions to silver nanoparticles.

2.6.1. UV-Visible Spectrophotometry. The synthesis of silver nanoparticles was observed by a UV-Vis spectrophotometer. The absorbance spectrum of reaction mixture was acquired by a U-2900 Spectrophotometer, HITACHI, Japan in the range of 300-800 nm.

2.6.2. Scanning Electron Microscope. The morphology and size of the greenly synthesized AgNPs were evaluated by SEM (JEOL Japan, JSM 5910) [11]. ImageJ software was then used for the analysis of obtained SEM images.

2.6.3. Energy-Dispersive X-Ray (EDX). Energy-dispersive X-ray (OXFORD, UK, Model No. INCA 200) was carried out to confirm the presence of elemental silver in silver nanoparticles.

2.6.4. Fourier Transform Infrared Spectroscopy. FTIR spectroscopy was performed for the identification of organic functional groups present in the aqueous extract responsible for the bioreduction of silver ions to AgNPs. The freeze-dried samples of aqueous leaf extract and green-synthesized AgNPs were blended with potassium bromide (KBR) and analyzed by FTIR (SHIMADZU, IR-Prestige-21, Japan) in a spectral range of 400-4000 cm^1 with a transmittance mode of 4 cm^1 resolution as explained by Banerjee [11].

2.6.5. X-Ray Diffraction Analysis. The average crystalline size and crystalline nature of our silver nanoparticles were investigated by X-ray diffraction pattern (JEOL, JDX-3532, Japan) using copper Kα radiation of 1.05404 A° operated at 30 m Ampere and current 40 kV voltage. The XRD pattern was recorded at Bragg's angle in a range of 10 theta to 70 theta and Debye equation; i.e., $D = 0.94\beta\cos\theta$was used to determine the average crystalline size as explained by [11].

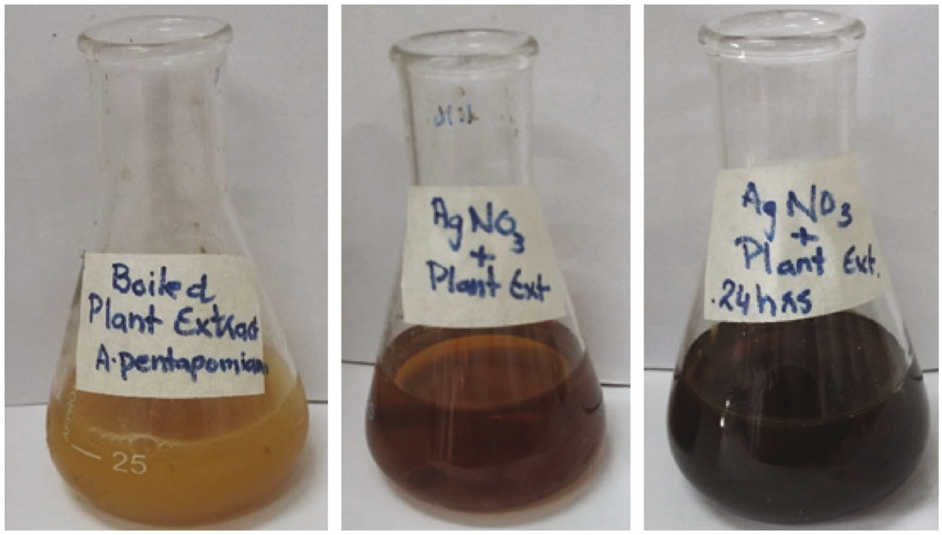
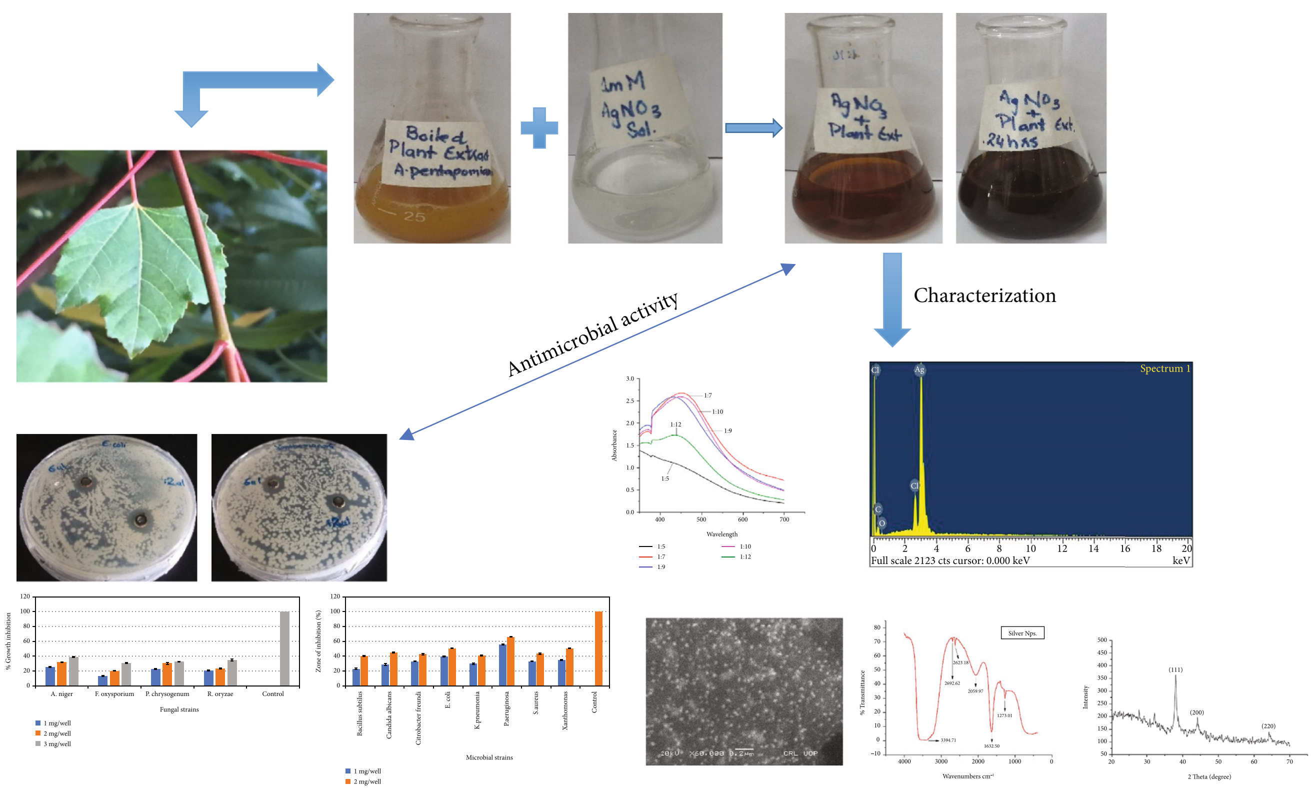


Figure 1: Color change of *Acer pentapomicum*-mediated AgNPs. The color of the solution changes to reddish brown after complete nanoparticle synthesis.



Scheme 1: Graphical abstract of green synthesis of silver nanoparticles.

2.7. Biological Activity. Antibacterial and anticandidal activity against *Bacillus subtilis*, *E. coli*, *P. aeruginosa*, and *X. campestris* was tested by the well diffusion method of Ali et al. [13]. Bacterial and candida experiments were carried out in nutrient-agar and nutrient-broth media. Briefly, 100 μL of each microbial culture (1×10^6 CFU/mL) was spread evenly on media plates and wells of 6 mm in size were bored in the agar plates. AgNP solution in a concentration of 6 and 12 μL was poured in the wells. The petriplates were then incubated for 24 hrs at 37°C, and the percent inhibitory zone of growth was recorded.

Antifungal activity was investigated by following the method of Ramdas et al. [14].

2.8. DPPH Radical Scavenging Assay. In vitro antioxidant assay of the silver nanoparticles was investigated according to the protocol of Mensor et al. [15]. In brief, 0.1 mM

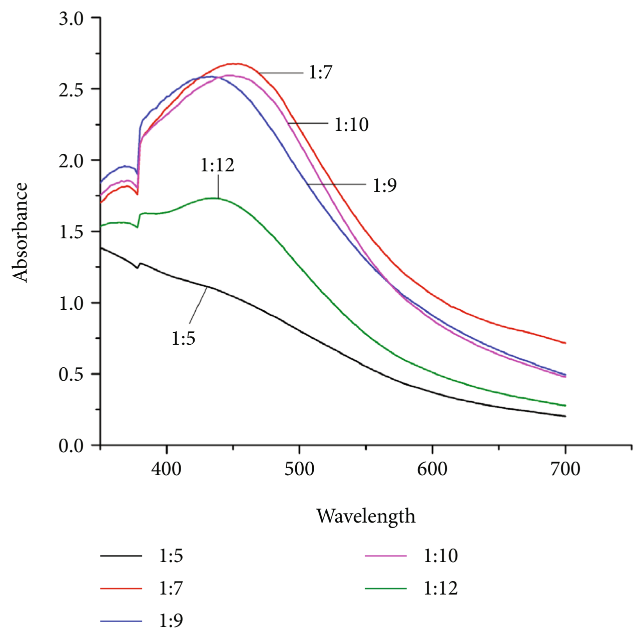


Figure 2: UV-Vis spectrum of *A. pentapomicum*-mediated silver nanoparticles, depicting the highest peak at 1 : 7.

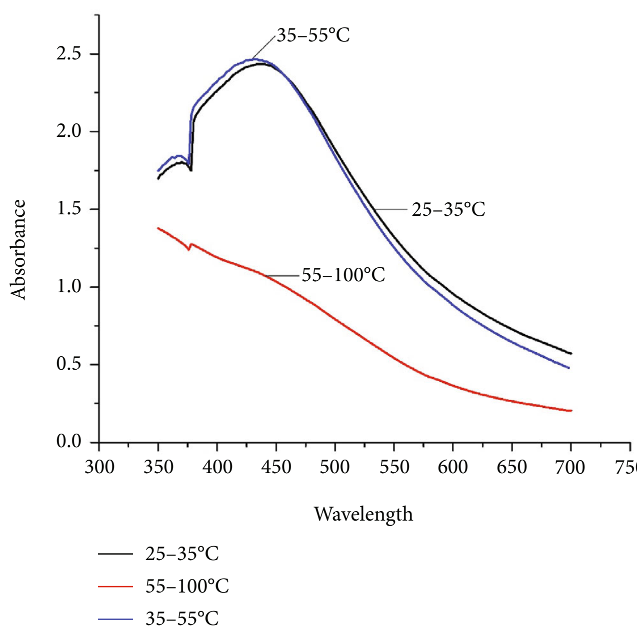


Figure 3: UV-visible spectrum of AgNPs isolated at different temperature Ranges.

solution of DPPH was added to different concentrations of AgNPs and to a reference standard gallic acid. The reaction mixture was then incubated in the dark for 30 minutes. Absorbance was then noted at 517 nm. Percent antioxidant activity was calculated by the following formula [16]:

$$AA = 100\frac{(Ao - As)}{Ao}, \quad (1)$$

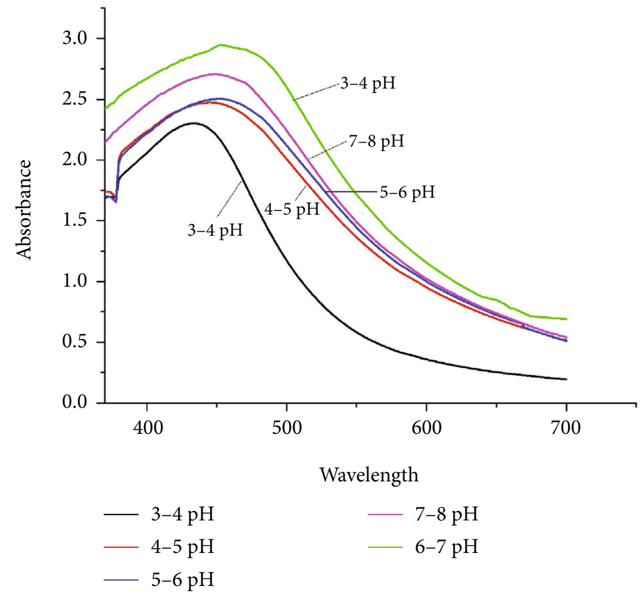


Figure 4: UV-visible spectrum of AgNPs at different pH levels ranging from 3 to 8.

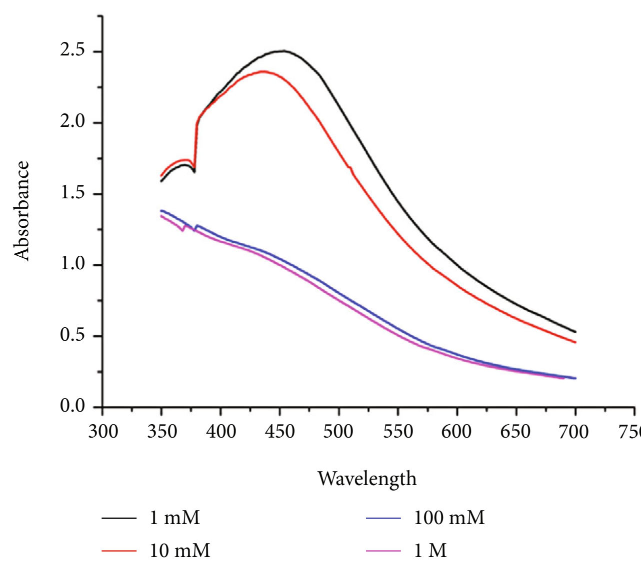


Figure 5: UV-visible Spectrum of AgNPs treated with different Salt (Nacl) solution.

where AA is the percent antioxidant activity, Ao is the absorbance of control, and As is the absorbance of the sample.

3. Results and Discussion

3.1. Visible Confirmation. Various concentrations of 1 mM silver nitrate solution were added separately to 1 mL aqueous leaf extract, which immediately initiated the silver nanoparticle synthesis. A visible change from yellow to brown color of the reaction mixture confirmed the synthesis of AgNPs [17]. After 24 hrs, the color of the reaction mixture further changed

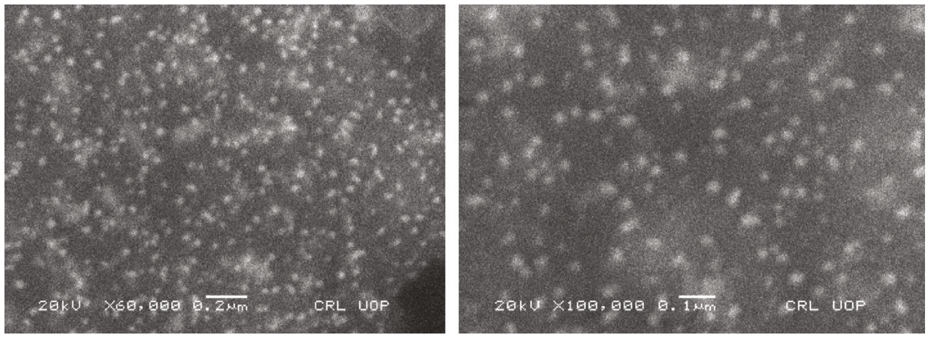


Figure 6: SEM of green synthesized AgNPs at different magnification an average particle size of 19-25 nm.

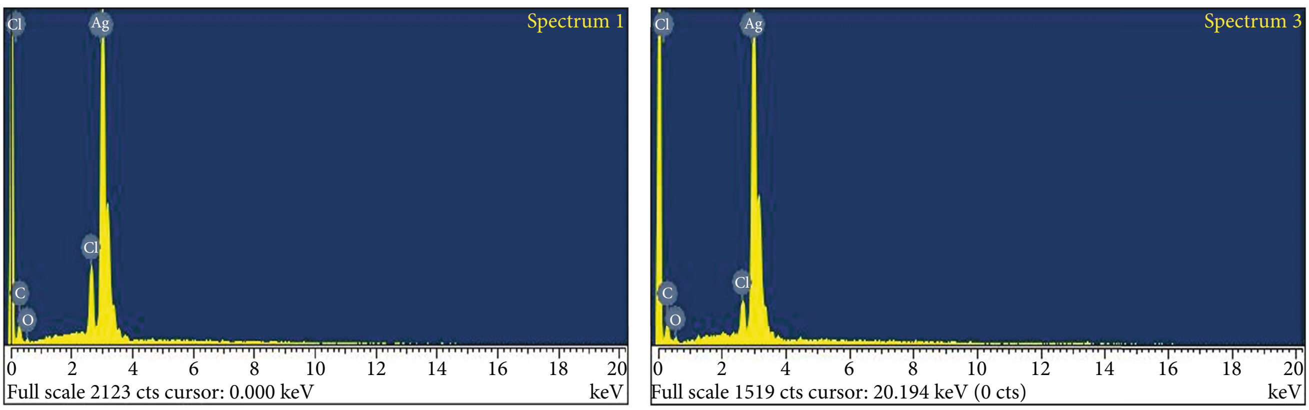


Figure 7: EDX spectrums of silver nanoparticles synthesized with *A. pentapomicum* aqueous leaf extracts.

to dark brownish which represents the complete bioreduction of Ag^+ ions to AgNPs (Figure 1 and Scheme 1) [18, 19].

3.2. UV-Vis Spectrophotometry. The nanoparticles were also affirmed by UV-Vis spectrophotometry, which is the 1st characterization tool utilized for the confirmation of our green synthesized silver nanoparticles. Samples from various combinations (1 : 1 to 1 : 16) of aqueous extract and 1 mM $AgNO_3$ solution were observed for the synthesis of silver nanoparticles. The nanoparticle solution was scanned from 300 to 700 nm. Figure 2 depicts the various combinations of nanoparticle mixture showing absorption bands of different intensities in a definite region which is because of the surface plasmon-resonance of AgNPs. The 1 : 7 combination of reaction mixture observed the highest surface plasmon absorption band at 450 nm, which is the absorbance range of AgNPs. Similar surface plasmon resonance peak for AgNPs was also reported [20, 21]. The 1 : 7 combination of AgNPs was further stabilized and characterized for size, surface morphology, functional group, and crystalline nature by various techniques.

3.3. Stability Tests of Silver Nanoparticles. Greenly synthesized AgNPs were also tested for their stability against various stresses such as temperature, pH, and salt stress. The AgNP synthesis was found highly dependent on the temperature and pH of the reaction mixture. Figure 3 depicts that as the temperature of the AgNP solution is increased from 25 to 55°C, the absorption band is also increased, suggesting the enhanced synthesis of silver nanoparticles. Thus, it is concluded from our findings that for large-scale production of AgNPs, a temperature range of 35-55°C is required. This finding is in full conformity with the findings of [22, 23] on *Tinospora cordifolia*, Neem, and banana peel-based AgNPs.

Previous studies reported that pH is another important parameter which greatly affects the nanoparticle synthesis and that the most favorable pH for plant-mediated silver nanoparticles is neutral pH [24, 25]. Our results are in complete accordance with these studies. The green synthesized silver nanoparticles from *A. pentapomicum* leaf extract were subjected to both acidic and basic pH stresses. As shown in Figure 4, our findings reveal that with an increase in the pH of AgNP solution, the absorption intensity also increases. At pH 6-7, the maximum intense absorption band was recorded which suggests that this neutral pH is the optimum pH for AgNP synthesis as it increases the bioavailability of the functional group present in plant aqueous extract to completely and efficiently reduce the Ag ions to AgNPs. Our investigations are in correlation with the findings of [26, 27]. Regarding the salt stress, our AgNPs were found to be more stable at a 1 mM salt stress (Figure 5).

3.4. Characterization of Silver Nanoparticles

3.4.1. Scanning Electron Microscopy (SEM). Greenly synthesized AgNPs could be of various shapes such as pentagonal, hexagonal, or spherical and of different sizes [28]. SEM

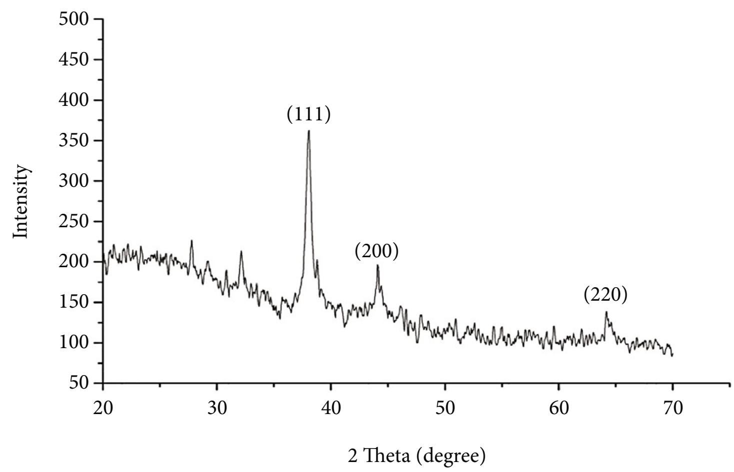


Figure 8: X-ray diffraction spectrum of green-synthesized silver nanoparticles.

Table 1: Crystal sizes of silver nanoparticles (AgNPs) synthesized from *A. pentapomicum* leaves.

Sample	Peak position (miller indices)			Average crystalline size (nm)
	38.1 (111) (nm)	44.1 (200) (nm)	64.15 (220) (nm)	
AgNPs	12	10	6.6	9.5

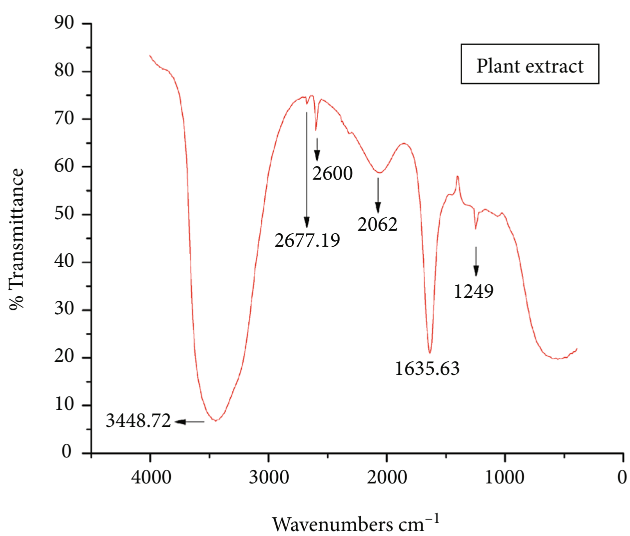


Figure 9: FTIR spectrum of plant extract.

analysis was performed to investigate the size and morphology of our green manufactured AgNPs. The scanning electron micrographs (Figure 6) revealed the spherical morphology of the AgNPs with the average size range of 19-25 nm. These results ascertain that the *A. pentapomicum*-mediated AgNPs are in nanorange. The same results were also reported by [29, 30].

3.4.2. Energy-Dispersive X-Ray (EDX). The EDX graph (Figure 7) is showing absorption spectrum of AgNPs that were prepared from naturally occurring bioactive components present in *A. pentapomicum* aqueous extract. The spectrum showed a strong absorption peak of 3.4 keV, which is the typical absorption peak for AgNPs [17, 31]. Elemental signals for oxygen and carbon were also observed in the EDX spectrum which possibly represents the enzymes and proteins present in our aqueous extract and involved in capping of silver nanoparticles [11, 32].

3.4.3. X-Ray Crystallography Diffraction (XRD). X-ray diffraction was carried out to determine the crystalline nature and average crystalline size of *A. pentapomicum*-mediated AgNPs. The recorded XRD pattern displayed three major peaks at 38.1°, 44.1°, and 64.15° which correspond to Bragg's reflection and is the characteristic diffraction pattern for silver. (Figure 8). Bragg's reflection indicated the presence of (111), (200), (220) sets of lattice planes known as miller indices. These miller indices represent the face-center cubic structure of silver. Our reported results perfectly correspond with the "International Centre of Diffraction Data," ICCD-card No. 04-0783 for the standard silver.

Debye–Scherrer equation was used to calculate the average crystalline size of all the peaks at 2θ by determining the full width half maximum of the (111) (200), (220) which came out to be 9.5 nm (Table 1). It is clearly demonstrated from our findings that the silver salt had been reduced by *A. pentapomicum* plant extract to AgNPs under different reaction conditions. The presence of the structural peaks and the average crystalline nanosize of 9.5 nm from XRD spectrum clearly indicate the purity and nanocrystalline nature of our greenly manufactured AgNPs. These findings are in accordance with the previous reports [33, 34].

3.4.4. Fourier-Transform Infrared Spectroscopy (FTIR). The functional groups of organic compounds present in *A. pentapomicum* that facilitates the bioreduction of silver ions to

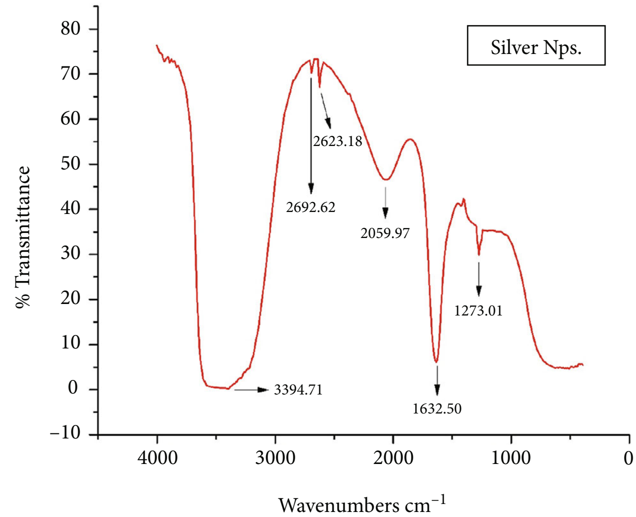


Figure 10: FTIR spectrum of green synthesized silver nanoparticle showing absorption bands with % transmittance at different wavenumbers cm^{1}.

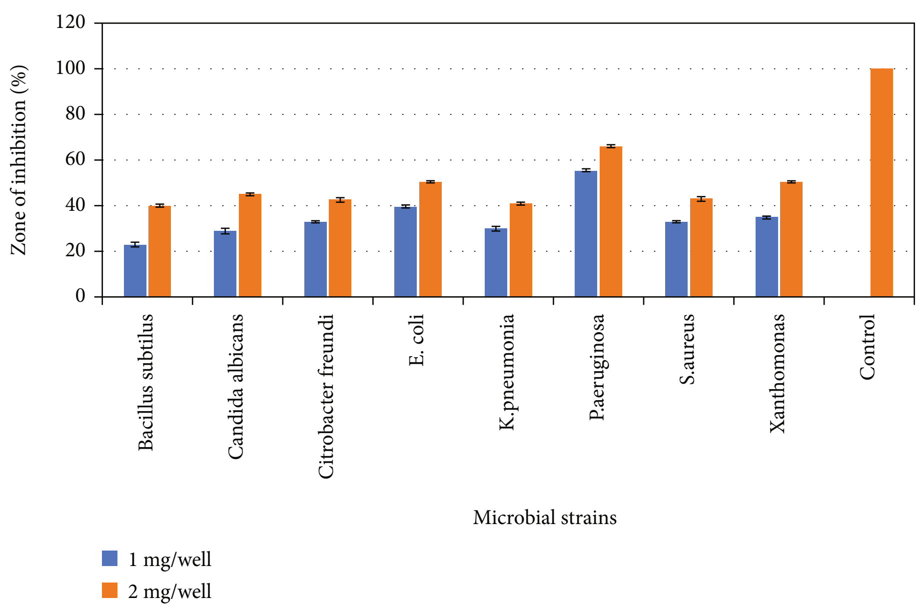


Figure 11: Antibacterial/anticandidal activity of green synthesized AgNPs against various microbes (mean ± SD of 3 replicates).

silver nanoparticles were identified by FTIR analysis. Various shifts in the wave numbers of multiple absorption bands were noted upon closer comparison of the FTIR spectrum of our silver nanoparticles and plant extract (Figures 9 and 10). The larger shift of 3394.71 cm^{-1} is associated with phenolic compounds and OH- group of alcohols. Other shifts of 1249.87 cm^{-1} to 1273.01 cm^{-1}, 1635.63 cm^{-1} to 1632.50 cm^{-1}, 2062 cm^{-1} to 2059.97 cm^{-1}, 2600 cm^{-1} to 2623.18 cm^{-1}, and 2677.19 cm^{-1} to 2692.62 cm^{-1} that indicate the specific group of biocomponents such as terpenoids and flavonoids were also noted. These shifts showed that the functional groups associated with these bands were mainly responsible for the bioreduction and stabilization of the Ag^{+} ions to AgNPs [35, 36].

3.4.5. Antibacterial and Anticandidal Bioassay. The antibacterial activity of silver nanoparticles has widely been studied and is suggested as a good alternative of synthetic antibiotics. The antibacterial potency of the silver nanoparticles is probably mediated by producing holes in the cell wall of bacteria. Due to these holes, the cell content of the bacteria is lost, and ultimately bacterial cell death occurred [37]. Our greenly synthesized silver nanoparticles from *A. pentapomicum* plant extract were found to possess potent antibacterial activity against different gram positive, gram-negative bacterial pathogens (Figure 11). The highest growth inhibition of 66% at 2 mg/well concentration was recorded against *P. aeruginosa*, *E. coli*, and *Xanthomonas campestris* exhibited 50.5% of growth inhibition at the

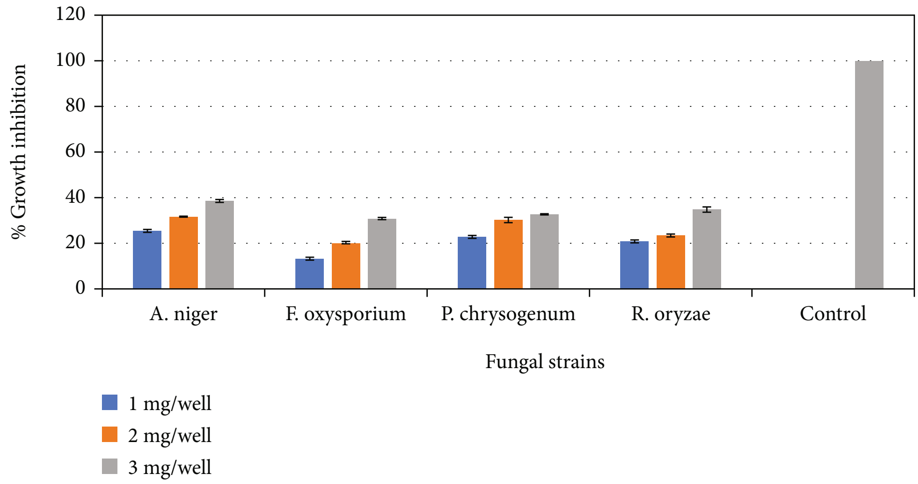


Figure 12: Antifungal activity of green-synthesized AgNPs against various fungal species (mean ± SD of 3 replicates).

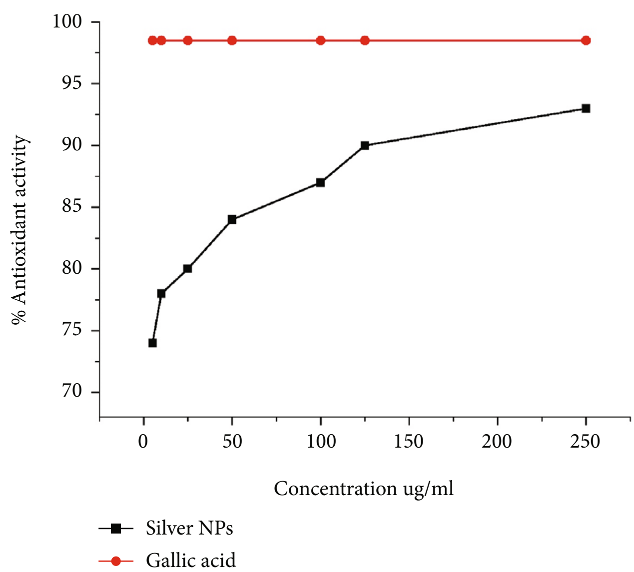


Figure 13: DPPH-radical scavenging assay of green synthesized silver nanoparticles from *A. pentapomicum*.

same concentration. Our results reported that silver nanoparticles were effective inhibiting the growth of all tested bacterial species. However, it was found out that gram negative microbes were more susceptible to AgNPs than the tested gram-positive *B. subtilis* and *S. aureus* [38, 39]. The strong antibacterial activity of our silver nanoparticles against gram negative bacteria may be due to the fact that gram negative bacteria have thin cell wall which is easily to disrupt as compared to gram positive bacteria which has rigid cell wall [40–42]. Sathiyaraj et al. also reported the significant antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, and *K. pneumoniae* [43, 44]. Similar results of AgNPs against *B. subtilis* and *E.coli* were also reported by Nandana et al. [39]. *Candida albicans* also showed good sensitivity to silver nanoparticles.

3.4.6. Antifungal Bioassay. The antifungal activity of different concentrations of greenly synthesized silver nanoparticles were also tested against various fungal species such as "*A. niger*, *F. oxysporum*, *Penicillium chrysogenum*, and *Rhizopus oryzae*" (Figure 12). The nanocrystalline silver nanoparticles were found to be highly effective against *A. niger* specie showing 25.6%, 32%, and 38.8% of growth reduction. The second most susceptible fungal species to all concentration of silver nanoparticles was found to be *Rhizopus oryzae* exhibiting 21, 23.7, and 35% growth inhibition. *F. oxysporum* and *Penicillium chrysogenum* also showed sensitivity to green synthesized AgNPs. Previous studies of *T. cordifolia*-based AgNPs also reported the antifungal activity against *Fusarium oxysporum* [45, 46]. Silver nanoparticles from *Aloe barbadensis* leaf extract were found to possess fungicidal activity against *Aspergillus* and *Rhizopus spice* [47–49], while *Thevetia peruviana*-based AgNPs exhibited toxicity against *A. niger* [50, 51].

3.5. Antioxidant Activity. The antioxidant activity of the *A. pentapomicum*-mediated AgNPs was evaluated by utilizing the DPPH-radical scavenging bioassay (Figure 13). According to our findings, these nanocrystals possess good DPPH-radical scavenging activity at all different tested concentrations. The highest DPPH-radical scavenging activity of 93% is noted at a higher concentration of 250 μg/mL while a minimum of 74% was noted at 5 μg/mL. Our results confirmed that *A. pentapomicum*-mediated AgNPs have the ability to quench free DPPH radicals. Similar results of antioxidant activity of green synthesized silver nanoparticles were also reported by other authors [42, 48, 50].

4. Conclusion

To the best of our knowledge, this work is the first report on single-step green synthesis of AgNPs using aqueous *Acer pentapomicum* leaf extract. Silver nanoparticles were successfully synthesized, characterized, and evaluated for various biological activities. The results indicated an encouraging antimicrobial and antioxidant efficacy of AgNPs. Thus, the outcome of this work revealed that the green synthesized AgNPs could be useful for various biomedical applications, specifically in the development of effective antimicrobials against various antibiotics resistance microbes.

Data Availability

Available data are presented in the manuscript.

Consent

All authors read and agreed on the final version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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