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Research Article

Green Synthesis of Silver Nanoparticles Using Apple Extract and Its Antibacterial Properties

Zainal Abidin Ali,[1] Rosiyah Yahya,[2] Shamala Devi Sekaran,[3] and R. Puteh[1]

[1]*Corrosion and Coating Laboratory, Department of Physics, University of Malaya, 50603 Kuala Lumpur, Malaysia*
[2]*Department of Chemistry, University of Malaya, 50603 Kuala Lumpur, Malaysia*
[3]*Department of Medical Microbiology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia*

Correspondence should be addressed to R. Puteh; rustam@um.edu.my

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Silver nanoparticles (AgNPs) were synthesized using apple extract as a reducing agent and aqueous silver nitrate as the precursor. The AgNPs formation was observed as a color change of the mixture from colorless to dark-brownish. The X-ray diffraction pattern confirmed the presence of only Ag crystallites, and the dynamic light scattering estimates the average sizes of the AgNPs to be 30.25 ± 5.26 nm. Furthermore, Fourier Transform Infrared as well as UV-vis spectroscopy identifies ethylene groups as the reducing agent and capping agent for the formation of the AgNPs. This green synthesis provides an economic, eco-friendly, and clean synthesis route to AgNPs. AgNPs in suspension showed activity against Gram-negative and Gram-positive bacteria with minimum bactericidal concentrations (MBCs) to be in the range from 125 μg/mL to 1000 μg/mL.

1. Introduction

Silver nanoparticles (AgNPs) have been receiving broad interest for a large number of applications such as in optics [1], selective coatings for solar energy absorption [2], biolabeling [3], catalysts [4], and antibacterial agents [5] owing to their unique properties. In antibacterial application, for instance, apart from being effective, AgNPs still remain a popular choice due to their nontoxicity towards human [6] in comparison to other metals or materials. However, scarcity makes them expensive and limits their application. To overcome the problem, numerous synthesis methods have been developed [7–12]. Most of the conventional methods for producing AgNPs require numerous chemicals, which not only is expensive but also could produce hazardous residue. Therefore, a green synthesis of AgNPs is desirable to provide an economic, eco-friendly, and cleaner synthesis route.

A number of biomolecules in extracts have been shown to successfully act as reducing agents in the green synthesis of AgNPs. For example, black tea leaf extract has been used for the biosynthesis of AgNPs with sizes averaging 20 nm [13]. The extract of *Mangifera indica* leaf also produces AgNPs with sizes of about 20 nm [14]. Extracts from fruits such as the red fruits of the piquin pepper (*Capsicum annuum* var. *aviculare*) have also been shown to produce AgNPs in the range of 3–10 nm [15]. The aqueous extract of *Hovenia dulcis* fruit produces AgNPs with sizes of 45 nm [16].

Escherichia coli (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Klebsiella pneumoniae* (*K. pneumonia*) are the Gram-negative bacteria, whereas *Methicillin-Resistant Staphylococcus aureus* (*MRSA*) and *Staphylococcus aureus* (*S. aureus*) are the Gram-positive bacteria that are responsible for majority of hospital-acquired infections, namely, nosocomial infections. Urinary tract infections are the most common type of nosocomial infections [17]. Surgical site infections, bloodstream infections, and pneumonia are the other most common types [18]. Application of inorganic nanomaterial as possible antibacterial agents has been intensively studied [19, 20]. While numerous efforts are focusing on synthesizing materials other than AgNPs for this purpose, it has been proven that the AgNPs still outperform those nanomaterials [19] and thus remain relevant to the field of antibacterial studies.

In the present research work, we report the green synthesis of AgNPs using red-apple (*Malus pumila*) extract. The AgNPs were subsequently tested for their antibacterial properties against *E. coli*, *S. aureus*, *P. aeruginosa*, and *MRSA*.

2. Methodology

2.1. Synthesis of AgNPs. Red apples were bought from a local grocery shop, and $AgNO_3$ was purchased from Sigma-Aldrich. The apple extract was prepared by cutting the apples into small pieces, which were then thoroughly washed with running tap water. Next, 100 g of the small cut apples was put in 200 mL of deionized water, which was heated for 1 hour at 80°C. The extract was filtered using filter paper, and the filtrate was later used as the reducing agent for AgNP preparation. The synthesis of AgNPs was carried out by using 20 mL of the apple extract in 180 mL of 0.1 M aqueous $AgNO_3$ solution. The mixture was stirred and heated at 80°C for different durations.

2.2. Characterisation of AgNPs. UV-vis spectroscopy was used to monitor the color changes of the mixture after 5, 10, 15, 20, 30, and 60 minutes. The UV-vis spectra in the wavelength region of 200–700 nm were recorded on a UV-2450 Shimadzu UV spectrophotometer. To observe the morphology of the synthesized AgNPs, images were obtained by a FESEM (HITACHI SU-6600 model) instrument. The green-synthesized AgNPs were centrifuged to obtain the residue and subsequently washed with deionized water. This process was repeated several times before the powder was dried in a hot air oven at 100°C for 24 h. The powdered AgNPs were analyzed by a Bruker model D8 advanced powder X-ray diffractometer to identify their crystalline structures. A PerkinElmer Fourier Transform Infrared (FTIR) Spectroscope was used to identify the possible functional groups involved in the synthesis of AgNPs. Zeta potential measurements were made by microelectrophoresis using a Malvern Zetasizer Nanoseries Nano ZS (Malvern Instruments, Herrenberg, Germany). Particle size and its distribution (dispersity) were assessed with a laser dynamic light scattering (DLS) instrument (Zetasizer Nanoseries, Malvern Instruments Ltd., Malvern, Worcestershire, UK).

2.3. Antibacterial Test. Minimum bactericidal concentrations (MBCs) by broth dilution method were used to test the antibacterial properties of the synthesized AgNPs against four bacterial strains, namely, *E. coli*, *S. aureus*, *P. aeruginosa*, and *MRSA*. AgNPs at 1000, 500, 250, and 125 μg/mL in suspensions were used to determine the lowest bactericidal concentration needed to prevent the growth of bacteria. Live cells of the bacteria at concentrations of 5×10^5 CFU/mL were inoculated with different concentrations of AgNPs for 24 hours. For each bacterial strain, three replications were done.

3. Results

3.1. Ag Nanoparticles (AgNPs) Analysis. AgNPs were successfully synthesized from the aqueous silver nitrate solution using apple extract in a continuously heated and stirred mixture. The colorless reaction mixture slowly changed to a dark-brownish suspension after several minutes of reaction (Figure 1). This color change is in accordance with other reports of green syntheses using different types of extracts [21–24].

Figure 1: Apple extract, aqueous solution of 0.1 M $AgNO_3$, and synthesized AgNPs (from left to right).

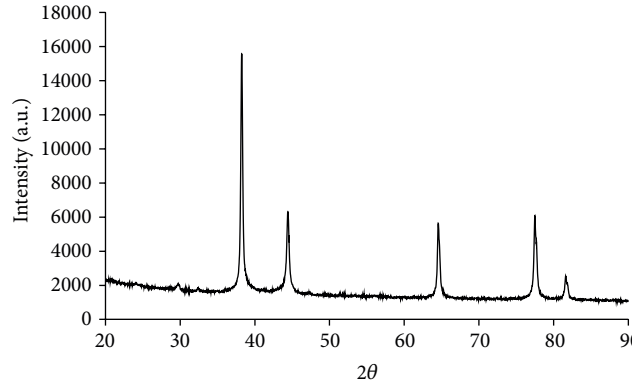


Figure 2: XRD spectrum of AgNPs green-synthesized using apple extract.

The XRD spectrum confirmed the crystalline structure of the precipitate as Ag (Figure 2). The peak values at 2θ = 38.15°, 44.35°, 64.59°, 77.47°, and 81.60° correspond to the (111), (200), (220), (311), and (222) lattice planes of the face-centered cubic crystal structure of AgNPs. The FESEM image in Figure 3(a) shows morphological structure of the AgNP. The DLS assesses the average sizes of the AgNPs to be 30.25 ± 5.26 nm. Figure 3(b) shows the size distribution. Overall, the synthesized AgNPs are spherical in shape and exhibit aggregation. Zeta potential spectrum of the AgNPs is shown in Figure 3(c). The zeta potential value was 5.68 ± 3.28 mV. This value range indicates that the AgNPs have strong agglomeration and precipitation [25].

UV-vis spectroscopy has been widely used to detect the presence of AgNPs during green syntheses [24, 26]. In particular, absorbance in the range of 420–450 nm has been used as an indicator to confirm the reduction of Ag^+ to metallic Ag [24, 27]. In this study, the formation of AgNPs was monitored by measuring UV-vis spectra at different time intervals (Figure 4). As the time increased, the intensity of this absorbance increased, indicating increases in the amount

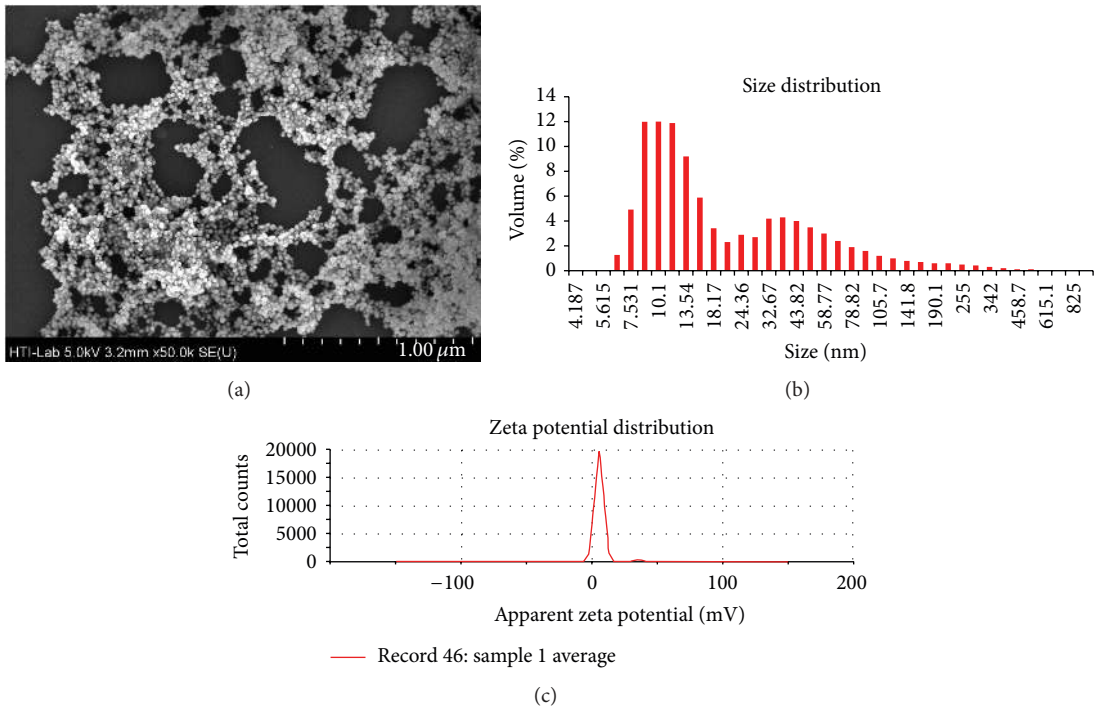


Figure 3: (a) FESEM images, (b) particle size distribution, and (c) zeta potential spectra of AgNPs.

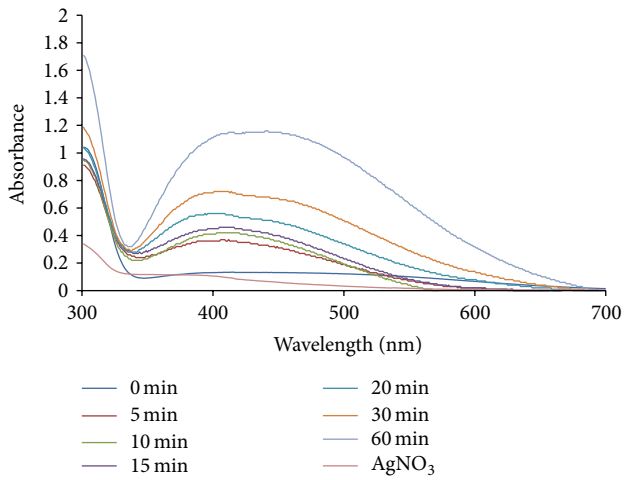


Figure 4: UV-vis spectra of AgNPs measured at different time intervals.

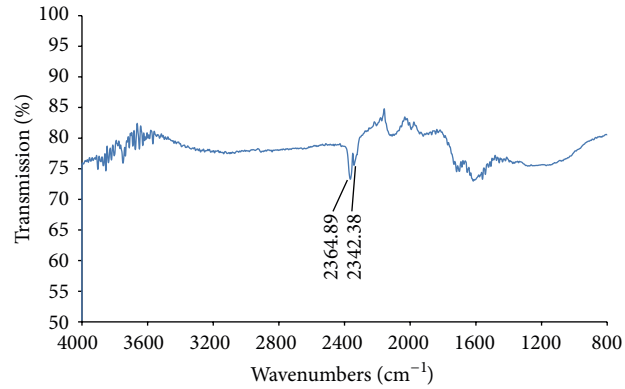
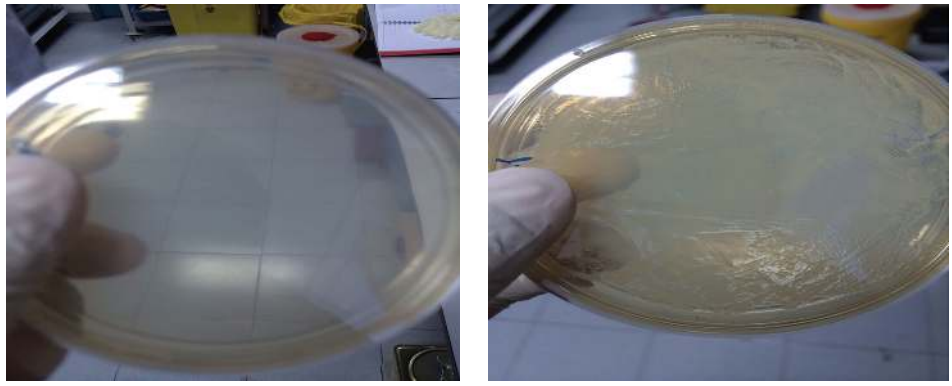


Figure 5: FTIR spectrum of the synthesized AgNPs.

of AgNPs produced from the mixture. The broad spectrum could probably be attributable to several reasons: (i) encapsulation of AgNPs by organic elements which originated from apple extract and (ii) extra fine nature [28] or high homogeneity [29, 30] of the AgNPs. The presence of organic elements encapsulating the AgNPs was identified by FTIR and their possible role is discussed in the next section. Moreover, it is noteworthy that the UV-vis spectra could be affected by many other different factors such as size and shape [31].

The FTIR spectra of the AgNPs were also recorded in order to identify the functional groups of the extract involved in the reduction of the synthesized AgNPs. The medium-intensity bands at 2364.89 cm^{-1} and 2342.38 cm^{-1} in the IR spectrum of the AgNPs indicate the presence of ethylene groups in the material bound to the AgNPs (Figure 5). Studies by [14, 26, 32] have also identified numerous organic extracts in the samples and proposed that these groups could serve as organic reducing or capping agents.

3.2. Antibacterial Test. Minimum bactericidal concentration (MBC) is the lowest concentration of antibacterial agents which prevents visible microorganism growth after overnight incubation. The observed MBC values for the AgNPs were summarized by Table 1. The MBCs for each particular set of the bacteria show the same concentration, therefore giving zero standard deviation value (±0.0). Figure 6 shows



(a) (b)

Figure 6: Petri dish of *E. coli* plated (a) containing 125 μg/mL AgNPs and (b) control.

Table 1: Minimum bactericidal concentration (MBC) against 5 bacteria.

Bacteria	Minimum bactericidal concentration (MBC) (μg/mL)
E. coli	125 ± 0
S. aureus	1000 ± 0
P. aeruginosa	500 ± 0
MRSA	1000 ± 0

the comparison of plating between the one containing Ag (125 μg/mL) and the one without Ag (control) for *E. coli*. Figure 6(a) shows clear and transparent petri dish, indicating that the presence of AgNPs has prevented survival of the bacteria, whereas in Figure 6(b) an obscured and blurry dish was obtained, indicating the survivability.

4. Discussion

The present study reports the green synthesis of AgNPs using widely available fruit, apple, as its reducing agent. While there have been numerous methods on synthesizing nanoparticles, most of the methods use expensive chemicals and therefore are not cost effective. Moreover, the residues produced are hazardous and toxic. This will result in pollution which could lead to disastrous effects on our environment. Recently the usage of flowery and plant extracts has become an interest due to its clean and simpler approaches.

The XRD peaks revealed that the structure corresponds to face-centered cubic crystal of Ag. In this study, pure Ag crystallite was obtained, while some studies reported the presence of peaks corresponding to unassigned peaks [24], weak peaks [22], oxide of Ag [15], or incomplete peaks [33]. Philip et al. proposed that unassigned peaks were probably observed due to the fact that the crystallization of bioorganic phase occurs on the surface of the nanoparticles [34], which in our case does not occur. The absorption peak in UV-vis test in the range of 420–450 nm confirmed the formation of Ag. The sizes of the AgNPs are in the range of other reports [16, 32]. Ethylene groups detected by FTIR have been reported by [35] to be capable of acting as reducing or capping agent.

AgNPs are most notably known for their antibacterial properties. The widespread cases of multidrug resistant bacteria against the standard antibiotics have led researches to potentially incorporate AgNPs and other nanomaterials as ingredient to boost the antibiotic effects. In our cases, all the bacteria have been totally eliminated and the MBCs were comparable to the one reported by Sathishkumar et al. on *E. coli* [36]. Some report showed a better antibacterial performance which could probably be attributable to the smaller sizes of AgNPs. For example, Saxena et al. [33] reported the usage of average mean size of 16 nm and antibactericidal property at 45 μg/mL on *E. coli*. There have been several proposed mechanisms on how AgNPs work as antibacterial although the exact mechanism is still unknown. Several reports [37–40] suggested that the AgNPs could produce Ag ions which will damage the cell membrane, interrupt the metabolic activity, and subsequently lead to denaturation of protein and finally cell death. AgNPs could also produce reactive oxygen species (ROS) such as singlet oxygen $^1O_2$, hydroxyl radical $^\bullet OH$, and peroxide radical $O_2^{\bullet-}$ which are toxic to the bacteria [41].

5. Conclusion

AgNPs were synthesized using apple extract and $AgNO_3$ aqueous solution. The crystalline nature of the AgNPs is evident from sharp peaks in the XRD spectrum, and the average size was 30.25 ± 5.26 nm. The zeta potential value of 5.68 ± 3.28 mV indicates strong agglomeration and precipitation. FTIR analysis suggests that ethylene groups from the apple extract could act as the reducing agent responsible for the reduction of Ag^+ into Ag. This method is environmentally friendly, of low cost, and simple and therefore can promote the application of green technology for the production of AgNPs. Our studies have shown that the range from 125 to 1000 μg/mL was required to eliminate the Gram-positive and Gram-negative bacteria. *E. coli* required the lowest MBC which was 125 μg/mL of AgNPs.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

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