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Research Article

BIOSYNTHESIS OF SILVER NANOPARTICLES USING POMEGRANATE JUICE EXTRACT AND ITS ANTIBACTERIAL ACTIVITY

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Abstract

Green synthesis of silver nanoparticles (AgNPs) from silver nitrate was carried out using aqueous Pomegranate juice extract (PJE) as a reducing agent. The formation of AgNPs was characterized by UV-visible (UV–vis) spectroscopy, transmission electron microscopy (TEM), Fourier transforms infrared spectroscopy and X-ray diffraction (XRD). Surface Plasmon resonance (SPR) of ~420-423 nm confirmed the earlier formation of AgNPs. TEM and XRD analysis showed that the AgNPs with an average diameter of 23 nm are crystalline in nature and have face-centered cubic geometry. The antibacterial efficiency of AgNPs against *Escherchia coli* and *Staphylococcus aureus* showed high level of inhibition. Further, the zone of inhibition increased with the increase in the concentration of silver nanoparticles. These studies are quite useful as it shows the utility of green nanotechnology for the synthesis of silver nanoparticles without any toxic residuals and byproducts. The efficient antimicrobial activity of biosynthesized AgNPs proves the application potential in the area of nano-medicine.

Keywords: Biological synthesis; Nano-particles; Antibacterial activity; X-ray diffraction

Introduction

Nanoscience is a rapidly growing field with potential application for generating new and unique types of nanoparticles (Juan and Tae-Hoo, 2016). Due to their unique properties, silver nanoparticles have been widely used in the fields of biomedical applications, forensic science, solar cells, home water purification systems and optoelectronics (López-Miranda *et al.*, 2016). Although a variety of physical and chemical methods are established for the synthesis of nanoparticles, the use of high cost, low stability and toxic chemicals significantly limits their biomedical applications (Vivek Dhand and Soumya, 2016). Hence, a new synthesis routes such as biological approaches remain a growth area. In this situation, microorganisms such as prokaryotic bacteria, fungi and medicinal plant extracts are used for the biological syntheses of AgNPs due to its cost-effective, eco-friendly and less time consuming process (Mohammadi *et al.*, 2016). However, the extracts from plants are potentially advantageous over conventional microorganisms, being free from toxic chemicals, providing natural capping agents for the stabilization of AgNPs, prevent the aggregation of nanoparticles. Because of these advantages, AgNPs have been synthesized by various types of plant extracts (Kumar *et a*l., 2014). AgNPs exhibit enhanced properties depending upon their size and morphology. Antimicrobial activity of AgNPs mostly depends on the relative surface area, since the smaller AgNPs can have greater toxic potentials (Sangjin and Minji Jang, 2016). Besides, the shape and size of AgNPs can be controlled by modifying the chemical concentrations within the growth solution. The ratio of plant extracts to silver nitrate concentration is the critical factor in the synthesis of AgNPs. However, the potential of plants as anti-microbial activity for the synthesis of AgNPs is not fully disclosed. Among the plants is the pomegranate (Fig. 1). Pomegranate's antioxidant activity is known to have good antimicrobial properties. In one study, pomegranate extract was found to inhibit the growth of human breast cancer cells by inducing cell death (James, 1971). The aim of the study was to synthesize silver nanoparticles using PJE and silver nitrate, as well as to examine the antimicrobial activity of silver nanoparticles against certain pathogenic bacteria found in food products.

Fig. 1: Pomegranate plants

Materials and methods

Synthesis of Silver Nanoparticles

In this work, the combination of microwave and biological sources were used for the green synthesis of AgNPs using silver nitrate $AgNO_3$ (purchased from Sigma-Aldrich) as metal precursor and extract of pomegranate juice. 0.25 g of silver nitrate $AgNO_3$ with 100 mL of double-distilled deionized water was stirred at room temperature for about 10 min, followed by filtration with what man filter paper No.4 to achieve clear solution. 60 ml of pomegranate juice in 100 mL distilled water was extracted using microwave oven (900 w power) heated to 65°C for 60 s. Aqueous extract was filtered and stored at -20°C for further testing. To mix the two portions, 15 mL of aqueous juice extracts were separately added to 20 mL of aqueous $AgNO_3$ solution in 100 mL Erlenmeyer flasks. The microwave heater was applied to prevent extreme boiling as well as aggregation. The processing temperature of extracts was carried out for five minutes in a cyclic mode (stopped periodically 2 sec after each switched on 2 sec heating). The temperature was increased slowly to 65 °C. A color change from light yellow to reddish brown was detected upon different incubation microwave exposure time. The reaction mixture was stopped by centrifugation at 12000 rpm for 10 min to collect particles, which disclosed the successful formation of AgNPs synthesis as reported earlier (Ahmed and Saifullah, 2016).

Characterization

The AgNPs formation was examined by using the UV–vis spectral analysis (JASCO, V-630 spectrophotometer) in the range of 300-800 nm with a resolution of 1 nm. FT-IR analysis was carried out using a Perkin Elmer FTIR spectrophotometer with a resolution of $4cm^{-1}$. The synthesized AgNPs were subjected to powder XRD analysis using Cu-Kα radiation (λ = 0.15418 nm) within 2θ range of 20°–90°. Techno Philips HR-TEM with the selected area electron diffraction (SEAD) facility was carried out to characterize the size and morphological features of AgNPs. AgNPs were analyzed by Scanning Electron Microscopy (SEM) (FE-SEM, model: S-4800, HITACHI) in backscattered electron (BSE) mode with Energy Dispersive X-ray Spectrometry (EDS).

Antibacterial Activity of Biosynthesized AgNPs

The tested bacterial strains was: gram-negative (Esherichia coli ATCC 25922), gram-positive (enterotoxigenic S. aureus- ATCC 25923). Both of these was collected from Department of Medical Microbiology, Zagazig University, Egypt. Bacterial cultures were maintained on Nutrient Agar plates and Slants. They were sub-cultured and subsequently stored at 4°C. The strains were inoculated in the nutrient broth (Peptone, 10 grams; beef extract, 3 grams; NaCl, 3 grams; DW, 1000ml; pH 7.0) and incubated at 37°C for 24 hrs. Müller– Hinton agar plates (negative control) were inoculated with bacterial strains. Then 80 µl of the tested samples were placed into the wells under sterile conditions. Plates were incubated aerobically at 37°C in 24 h for bacterial growth. Inhibition zones were measured (mm) and experiments were carried out in triplicates.

Results and discussion

Visual Observation and UV-Vis Spectroscopy

The synthesis of AgNPs from $AgNO_3$ solution using PJE was rapid in case of reaction mixture. Since the mixture was exposed to microwave heater for a few minutes, the formation of AgNPs was monitored with color change from light yellow to reddish brown during incubation at 65°C (Fig. 2 inset). The UV–vis spectra and its corresponding absorption peaks as well as the wave length of AgNPs at different extraction time have been shown in Fig. 2. The PJE was used as a reference for baseline corrections. As seen, surface plasmon resonance (SPR) of Ag was appeared at the absorbance value of 423 nm. The microwave exposure time higher than 4 min shifted the SPR peak to ~417 nm. The increase in the intensity of SPR with the rise of microwave irradiated time is due to an increasing number of AgNps formed, size and morphology of the synthesized AgNPs. Besides, there is no significant peak showing a sign of pure PJE. However, it is generally recognize that the best results of UV–vis analysis were obtained for the sample corresponding to the kinetic formation of AgNps recorded at 4 min interval.

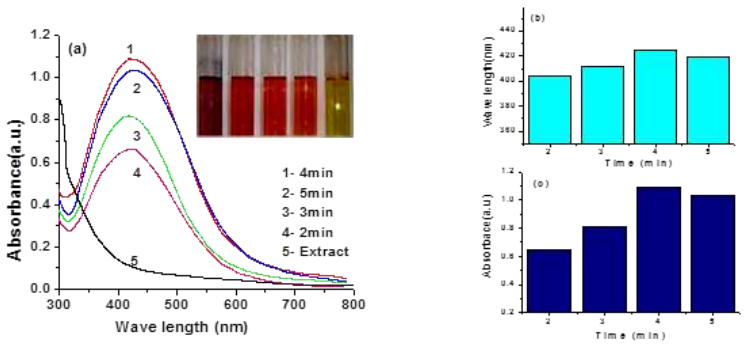

Fig. 2: (a) UV–Vis absorption spectra of Ag NPs recorded as a function of extraction time, (b and c) the wavelength and absorption peaks as a function of microwave exposure time.

FT-IR Analysis

PJE is known to have total phenolic compounds, which provides high antioxidant potential. The functional groups present in PJE and PJE /AgNps were analyzed using FT-IR spectroscopy. According to the FTIR analyses shown in Fig. 3, the results of both aqueous PJE and AgNPs exhibit minor changes in the positions and magnitude of the vibrational bands due to the interaction of phenolic compounds with AgNPs. The band pattern of PJE was detected at the peaks 3456, 2973, 2782, 1790, 1331 and 956 cm^{-1}, which was similar to those of polyphenols specified by Reddy et al., (Jayachandra Reddy and Nagoor, 2014). Absorption peaks located at 3456 and 2973 cm^{-1} indicate the presence of O-H stretching vibrations of phenol group and C-H stretching of aromatic compound, respectively. These absorption peaks changed to 3427 and 2931 cm^{-1} for the PJE /AgNps. While the C=O band at 1331 cm^{-1} showed minor change to 1343 cm^{-1}, the carbonyl band at 1790 cm^{-1} changed its position to 1797 cm^{-1} for the same sequence. Moreover, the band at 956cm^{-1} shifted to 890 cm^{-1} for PJE /AgNps.

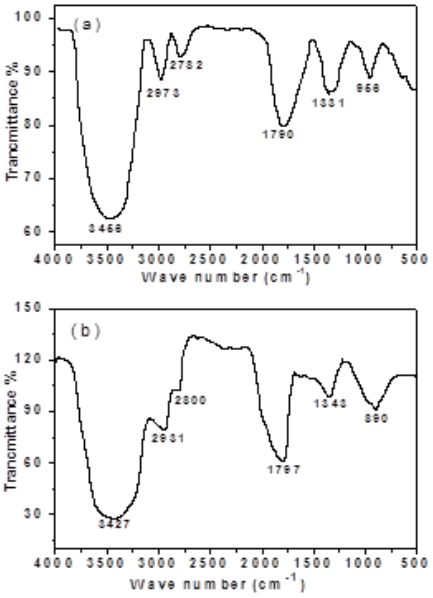

Fig. 3: FT-IR spectra for the pomegranate juice extract (a) PJE and (b) PJE /AgNps

SEM and EDS Analysis

SEM image of the samples (Fig. 4) captured the morphology of AgNPs with different sizes. The green synthesized Ag NPs were spherical in shape. The presence of some large particles was due to the high surface activity and aggregation of smaller particles. The average size of these aggregates is 1.3µm. The elemental profile of synthesized nanoparticle using EDS analysis confirms the formation of silver nanoparticles. EDS analysis of AgNPs revealed highest percentage of Ag followed by O, C and Al. The optical absorption peak appeared at 3 keV was due to the surface plasmon resonance of AgNPs.

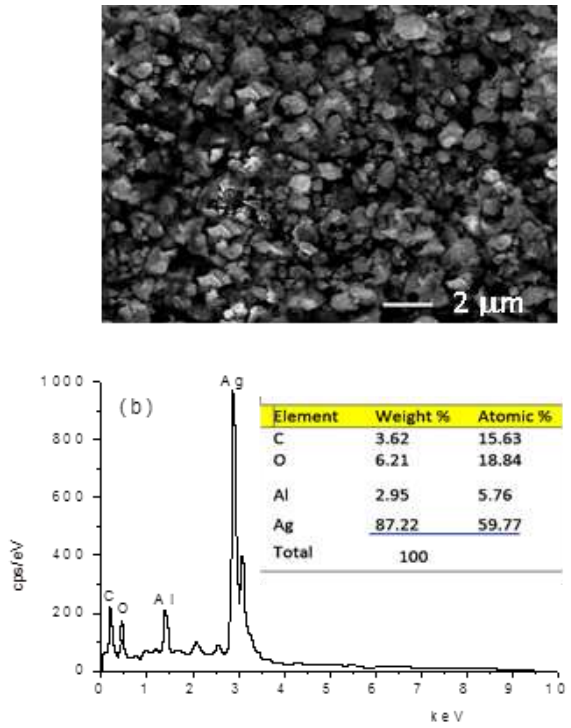

Fig. 4: (a) SEM and (b) EDS analysis of Ag NPs using pomegranate juice extract.

TEM and XRD Analysis

TEM analysis was employed to evaluate the shape and size of AgNPs (Fig. 5). The individual AgNPs are mostly spherical shape with diameters of <30 nm. The average size of nanoparticles was found to be 22 nm. The XRD pattern of AgNPs shown in Fig. 5 indicates the four distinct diffraction peaks at 38.2◦, 44.5◦, 64.6◦ and 77.8° respectively. The diffraction peaks show good match with JCPDS, file no. 04-0783, which are indexed as (111), (200), (220) and (311) planes of a cubic system with an face-centered cubic (fcc) of AgNPs. The Ag nanocrystals are highly anisotropic, since the ratio between the intensity of the (200) and (111) diffraction peaks (~ 0.33) is lower than that of Ag bulk intensity ratio (~ 0.5). According to Debye–Scherrer's equation $D = K\lambda/B_s \cos\theta$, where K is constant (0.94), λ is X-ray wavelength (1.5406 A°), B_s is X-ray line width (FWHM) and θ is the Bragg angle, the average crystal size D of AgNPs was calculated and found to be 23 nm, which is reasonably correlates with the TEM measurement

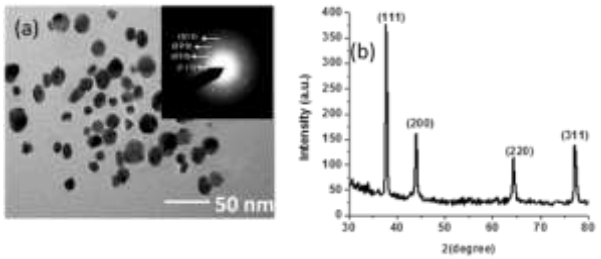

Fig. 5: (a) TEM image of AgNPs synthesized using pomegranate juice extract and (b) X-ray diffraction analysis of synthesized silver nanoparticles.

Antibacterial Activity of Silver Nanoparticles

In this study, the biogenic extract and those refereed AgNps were immediately tested for imperative antibacterial activities towards both the gram positive (*S. aureus*) and gram negative (*E. coli*). The inhibition zone of juice extract and Ag NPs against fungal strains are shown in Fig. 6 and. While Ag NPs exhibited good antibacterial activity against both S. aureus and E. coli, the zone of inhibition show that Ag NPs have higher antibacterial activity against *E. coli* over *S. aureus*. Table 1 shows that the zone of inhibition with different concentrations of nanoparticles produced in extract against *S. aureus* and *E. coli* was around 12 mm and 14 mm for 50 µg/ml, 15 mm and 16 mm for 100 µg/ml, respectively. From the zone area we can also observe that inhibition zone of juice extract did not show any antibacterial activity. These results provide a considerable insight to deliver a new way to synthesize nanoparticles and simultaneously enhance its antimicrobial property, as the antimicrobial results of nanoparticles show more effective results than that of the juice extracts. Although the exact mechanism of antibacterial activity of Ag NPs is still unknown phenomenon, many researchers have suggested four possible mechanisms:(1) interference during cell wall synthesis; (2) suppression during protein bio-synthesis (translation); (3) interference or disruption of transcription process; and (4) disruption of primary metabolic pathways (Dhand *et al*., 2016; Bindhu and Umadevi, 2015). However, there are many concerning features, which are still on the verge of experimental verification, are yet to be explored.

Table 1: Antimicrobial activity of silver nanoparticles against gram negative bacteria (Escherichia coli) and gram positive bacteria (Staphylococcus aureus).

Zone of inhibition (mm) obtained by disc diffusion method

Components	Zone of inhibition (mm)		
	Extract	Concentration 50µg/ml	Concentration 100µg/ml
E. coli	NA	14±.33	16±.54
S. aureus	NA	12±.05	15±.35

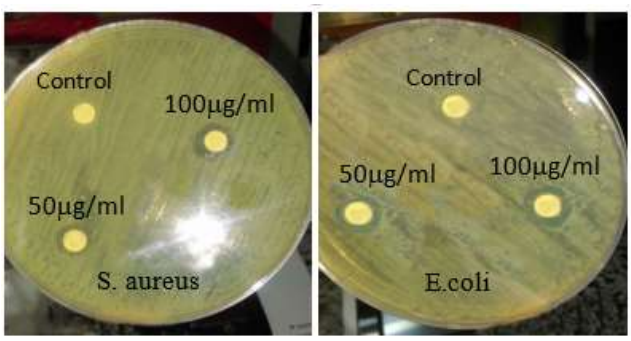

Fig. 6: Zone of inhibition of AgNPs against S. aureus and E.coli pathogenic microorganisms.

Conclusions

The present study is regarding the green synthesis of Ag NPs and their antimicrobial activity against gram negative and gram positive bacteria *i.e. Echerichia coli* and *Staphylococcus aureus.* This study has reported a biosynthesis of AgNPs using Pomegranate juice extract as a reducing agent. The prepared nanoparticles were monodispersed and spherical in shape with an average size of 23 nm. The Characterization study supports the formation of AgNPs. The synthesized AgNPs have antibacterial activity and thus has a potential to use in biomedical applications.

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