

Full Length Research Paper

Silver nanoparticles biogenic synthesized using an orange peel extract and their use as an anti-bacterial agent

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Accepted 29 January, 2014

Synthesis of nanoparticles by green methods with antibacterial properties is of great researchers' concern in the explored of new pharmaceutical and biomedical products. In this study, we synthesized a new product of nanosized particles of silver, non-toxic economy, clean, and conservator for energy. An environmentally friendly route is used for synthesizing silver nanoparticles (AgNPs) using an orange peel extract as both reducing and stabilizing agent at room temperature. The synthesized NPs were characterized using ultraviolet (UV)-Vis spectrophotometer, Zitasizer which measures the average size of the particles at about 91 nm, Fourier transform infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM) equipped with the energy dispersive spectroscopy (EDS) and characterization using Transmission electron microscopy (TEM). The results confirmed that the orange peel extract is a very good bioreductant for the synthesis of Ag NPs and we investigated the synthesized nanoparticles as an antibacterial which showed that the biogenic synthesized AgNPs exhibit inhibition, and had significant antibacterial against both gram-positive and gram-negative bacterial strains.

Key words: Silver nanoparticles, biogenic synthesis, orange peel, anti bacterial, gram-positive, gram-negative bacteria streamers.

INTRODUCTION

Nanotechnology mainly deals with the fabrication of nanoparticles having various shapes, sizes and managing their chemical and physical parameters for further use in human benefits with their growing

applications in various fields (Bhyan et al., 2007). Preparation of metal nano-sized, usually ranging in size from 1 to 100 nanometers (nm), is amongst the most emerging areas in the field of nanotechnology. Currently,

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the application of nano materials is becoming increasingly important in order to solve the problems associated with material sciences, including solar energy conversion, photonics (Calvo et al., 2006; Cao et al., 2010), catalysis (Chandan et al., 2011), microelectronics (Dastjerdi et al., 2010), antimicrobial functionalities (Du et al., 2009), and water treatment (Huang et al., 2007).

Nanoparticles usually have better or different properties than the bulk material of the same elements. The antibacterial effect of silver nanoparticles (AgNPs) is greatly enhanced because of tiny size. Nanoparticles have immense surface area relative to volume. Therefore, minuscule amounts of AgNPs can lend antimicrobial effects to hundreds of square meters of its host material. Nanomaterials are the leading requirement of the rapidly developing field of nanomedicine, and bionanotechnology. Nanoparticles are being utilized as therapeutic materials tools in infections against microbes thus, the properties of nanoparticles and their effect on microbes are essential to clinical applications. Among noble metal nanoparticles, AgNPs have received considerable attention owing to their attractive physicochemical properties (Ip et al., 2006).

The AgNPs have various and important applications. Historically, silver has been known having a disinfecting effect and has been found in applications ranging from traditional medicines to culinary items. It has been reported that AgNPs are non-toxic to human and most effective against bacteria, virus and other eukaryotic micro-organism at low concentrations and without any side effects (Jeong et al., 2005; Kamyar et al., 2012). Moreover, several salts of silver and their derivatives are commercially manufactured as antimicrobial agents (Khandelwal et al., 2010). A small concentration of silver is safe for human cells, but lethal for micro organisms (Krutyakov et al., 2008). Antimicrobial capability of AgNPs allows them to be suitably employed in numerous household applications such as textiles disinfection in water treatment, food storage containers, home appliances and in medical devices (Marambio-Jones and Hoek, 2010). The most important application of silver and AgNPs is in medical industry such as tropical ointments to prevent infection against burn and open wounds (Muhammad et al., 2012).

Biological synthesis of nanoparticles by plant extracts is at present under exploitation as some researchers worked on it (Palanivel et al., 2013; Savage and Diallo, 2005) and testing for antimicrobial activities (Savithamma et al., 2011; Saxena et al., 2010; Setua et al., 2007). For the last two decades, extensive work has been done to develop new drugs from natural products because of the resistance of micro-organisms to the existing drugs. Nature has been an important source of a products currently being used in medical practice (Sharma et al., 2009).

A number of synthetic methods have been employed for the synthesis of silver-based nanoparticles involving

physical, chemical (Singh et al., 2010) and biochemical techniques (Sinha et al., 2009). Chemical reduction method is widely used to synthesize AgNPs because of its readiness to generate AgNPs under gentle conditions and its ability to synthesize AgNPs on a large scale (Thirumurgan et al., 2010). However, these chemical synthesis methods employ toxic chemicals in the synthesis route which may have adverse effect in the medical applications and hazard to environment. Therefore, preparation of AgNPs by green synthesis approach has advantages over physical and chemical approaches as it is environmental friendly, cost effective and the most significant advantage is that the conditions of high temperature, pressure, energy and no toxic chemicals are required in this synthesis protocol (Thirumurgan et al., 2009; Yugang et al., 2003).

In this present work, we report the biogenic synthesis of AgNPs by using waste biomaterial orange peel extract, which was used as green reducing agent and stabilizer. The efficacy of the synthesized AgNPs as antibacterial agent was studied.

EXPERIMENTAL

Chemicals materials and bio extract

For green synthesis of AgNPs, the reagent in this work is of analytical grade and is used as received without further purification. Silver nitrate (AgNO_3) from Techno Pharmchem, India is used. Orange peel was washed and cut into small pieces, then boiled with deionized water for 3min then filtered.

Synthesis of silver nanoparticles

Green AgNPs were synthesized by bio reduction of Ag^+ by using fresh suspension of (5 ml) orange peel extract (greenish in color). The emulsion color was turned to dark brown after adding to 1 mM AgNO_3 and stirring at room temperature.

Microorganisms and antibacterial activity method

Pure culture of *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Salmonella* are types of bacteria. The antibacterial activities of biosynthesized AgNPs were carried out by disc diffusion method. Nutrient agar medium plates were prepared, sterilized and solidified. After solidification, bacterial cultures were swabbed on these plates. The sterile discs were dipped in AgNPs solution (5 mg/ml) and placed in the nutrient agar plate and kept for incubation at 37°C for 24 h. Zones of inhibition for control, were measured. The experiments were repeated 3 times and mean values of zone diameter were determined (Jeong et al., 2005).

Characterization of biogenic silver nanoparticles

Biogenic AgNPs were characterized spectrophotometrically using ultraviolet (UV)-Vis spectroscopy analyses as function of time at room temperature using Perkin Elmer UV-Vis spectrometer, Nicolet 6700, Fourier transform infrared (FT-IR) spectrophotometer was recorded, the size of synthesized AgNPs was analyzed through

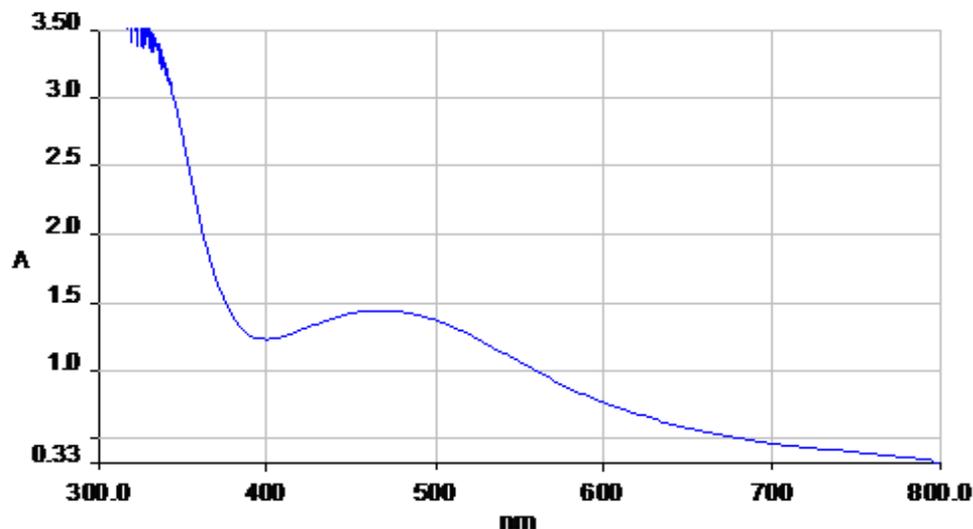


Figure 1. UV-vis spectra of reduced Ag ions to AgNPs with orange peel extract.

Zetasizer, Nano series, HT Laser, ZEN3600 from Malvern Instrument, UK, Scanning electron microscopy (SEM) has been employed to characterize the shape and morphologies of formed biogenic synthesized of AgNPs, JEOL-FE-SEM, and Energy dispersive spectrometer (EDS) analysis for the confirmation of elemental silver was carried out for the detection of elemental silver. The samples were dried at room temperature and then analyzed for samples composition of the synthesized nanoparticles. Elemental analysis on single particles was carried out using Oxford Instrument, Incax-act, equipped with SEM. Transmission electron microscopy (TEM) has been employed to characterize the size, shape and morphologies of formed biogenic synthesized of AgNPs, which was prepared by drop of AgNPs solution on carbon coated copper grid and the film on grid was dried. The TEM was operated and the measurements were performed at accelerating voltage of 100 KV.

RESULTS AND DISCUSSION

The biogenic synthesis of AgNPs by an orange peel extract was carried out. Silver nitrate used has distinctive properties such as good conductivity, catalytic and chemical stability. The formation of AgNPs was found to be successful as suggested by initial changes in color. It is well known that AgNPs exhibit brown color in aqueous solution due to excitation of surface plasmon vibrations in AgNPs.

The synthesis of green AgNPs had been confirmed by measuring the UV-Vis spectrum of colloidal solution which has absorbance peak at 466 nm; and the expanding of peak indicated that the particles are mono-dispersed as shown in Figure 1.

The FT-IR measurements were provided to describe and confirm the possible formation of bio reduction and efficient stabilization of green synthesized AgNPs by using an orange peel extract. The reduction compounds of the extract were confirmed by FT-IR spectra. FT-IR

bands of orange peel were inferred at 3270.82 , and 1634.24cm^{-1} in blue color (Figure 2) and FT-IR spectrum of the AgNPs shows peaks at 3260.70 , 1634.62 , 1376.62 and 1243.76cm^{-1} in red color. Intense absorption is observed at 1634.24cm^{-1} and is characteristic of the C=C stretching aromatic ring and this result agree with the result of the Thin layer chromatography (TLC) test, which refers to the active ingredient in the orange peel that causes the reduction of Ag^+ ions, we found that the effective group is Flavonoids which led to the bio reduction of aqueous silver ions (Ag^+).

As shown in Figure 3, the average size of the formed biogenic AgNPs was measured by Zitasizer and it was 91.89nm with monodispersity.

Figure 4a and b illustrate TEM images recorded at high magnification. Morphology of the AgNPs synthesized by using an orange peel extract indicates that the nanoparticles are spherical in shape with a smooth surface morphology.

SEM is shown in Figure 5a was employed to analyze the structure and morphology of the nanoparticles to give further insight into the features of the AgNPs obtained from the proposed biogenic synthesis method, the image showed relatively spherical shape of the formed nanoparticles. The EDS microanalysis is shown in Figure 5b and confirms the presence of AgNPs which is known to provide information on the chemical analysis of the elements or the composition at specific locations. The spectrum analysis reveals signal in the silver region and then confirms the formation of AgNPs. Metallic silver nanocrystals generally show a typical optical absorption peak at approximately 3keV due to the surface plasmon resonance (Ip et al., 2006; Bar et al. 2009; Magudapathy et al., 2001). This result confirmed that the produced nano-structures are pure silver as shown in Table 1.

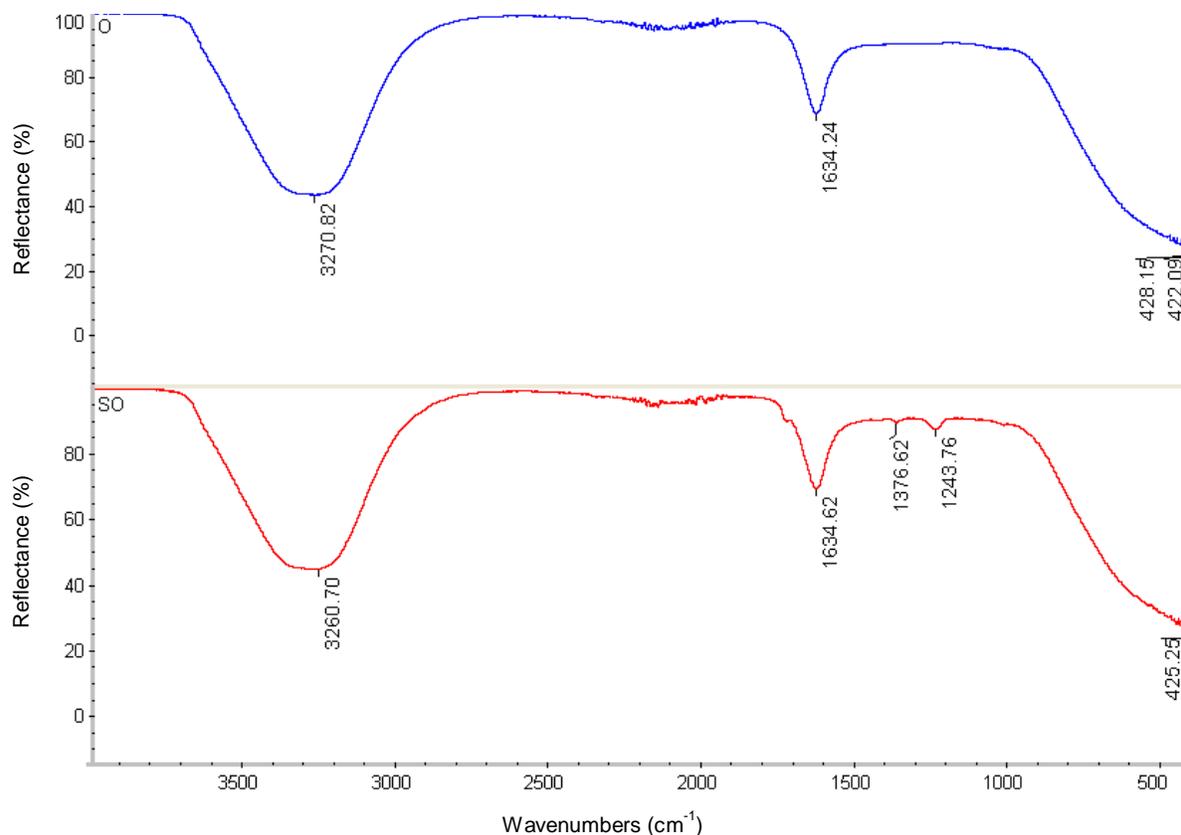
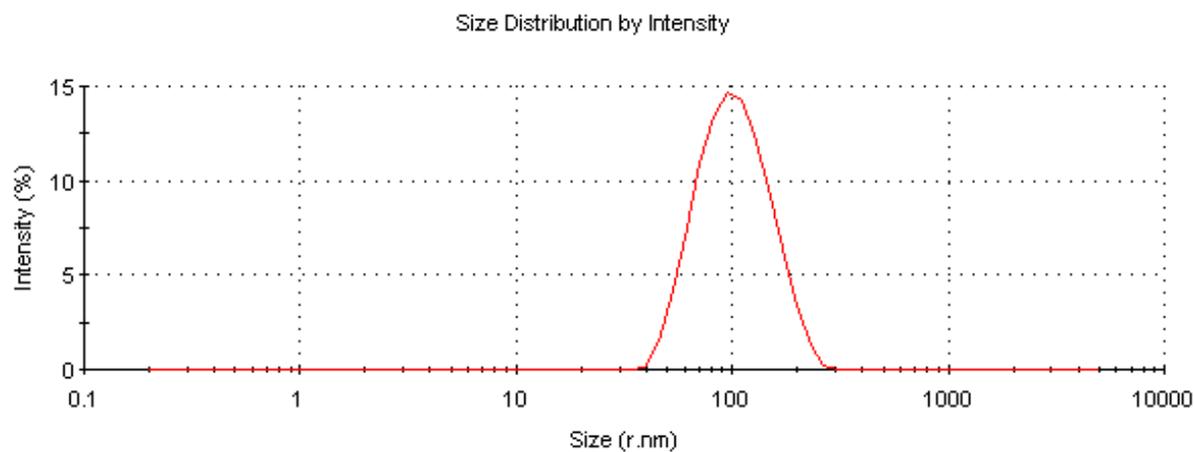


Figure 2. FTIR adsorption spectra of AgNPs prepared by orange peel.



	Diam. (nm)	% Intensity	Width (nm)
Z-Average (r.nm): 91.89	Peak 1: 107.4	100.0	39.86
PdI: 0.147	Peak 2: 0.000	0.0	0.000
Intercept: 0.869	Peak 3: 0.000	0.0	0.000
Result quality : Good			

Figure 3. Zitasizer of the formed AgNPs.

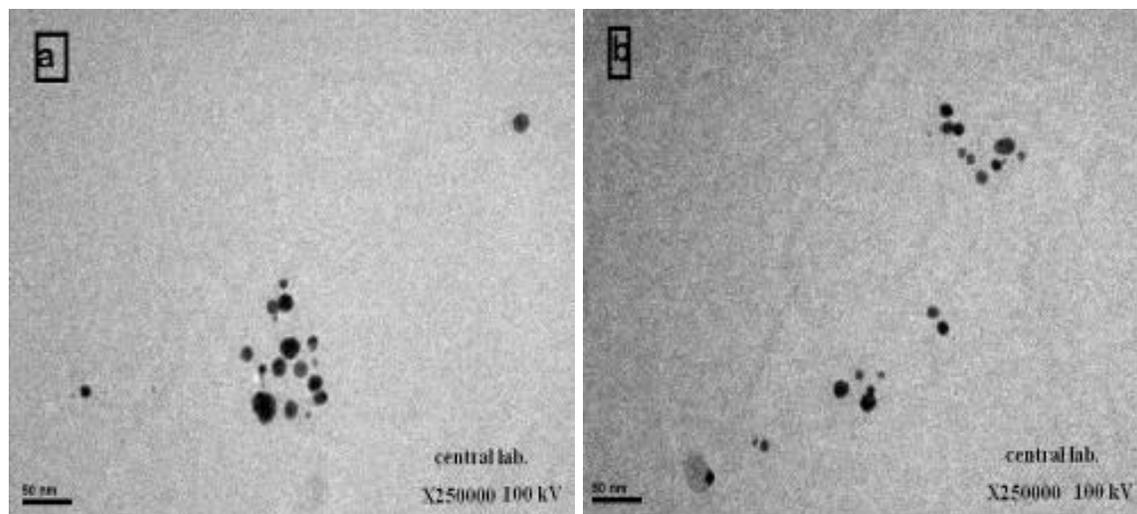


Figure 4a and b. TEM images of the formed biogenic AgNPs.

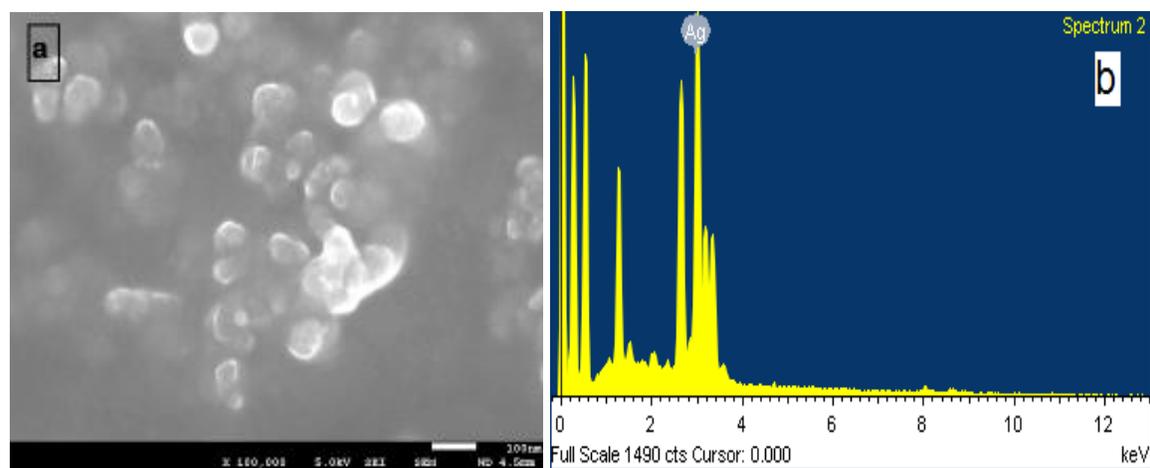


Figure 5. Green synthesis method (a) SEM image, (b) EDS pattern of spherical AgNPs prepared.

Silver nanoparticles as antibacterial agent

Silver, a naturally occurring element, is non-toxic, hypoallergenic, and does not accumulate in the body to cause harm and is considered safe for the environment. Many manufactured goods like washing machines, air conditioners and refrigerators are using linings of AgNPs for their antimicrobial qualities. Sportswear, toys and baby articles, food storage containers, HEPA filters, laundry detergent etc. are made with AgNPs. The products also with AgNPs were used, such as heart valves and other implants, medical face masks, wound dressings and bandages.

Nanomaterials are the leaders in the field of nanomedicine, bio-nanotechnology and have a great importance in nano toxicology research. Silver exhibits

the strong toxicity in various chemical forms to a wide range of microorganism that is very well known and AgNPs have recently been shown to be a promising antimicrobial material (Ip et al., 2006).

In this work, the antibacterial activity of the biogenic synthesized AgNPs. The analysis results showed that nanoparticles exhibited low toxicity against *Klebsiella* which the zone of inhibition around AgNP saturated disc for bacterial culture, and the numerical value of diameter of inhibition zone was presented in Table 2, also the results showed maximum sensitivity against *E. coli*, *Pseudomonas*, and *Salmonella*.

The results in Table 2 confirmed that the successfully biogenic synthesized AgNPs showed antibacterial activity on both gram-positive and gram-negative bacteria and the analysis of bacterial growth showed that the toxicity of

Table 1. EDS elemental micro-analysis of the AgNPs.

Element	Weight (%)	Atomic (%)
Ag L	100.00	100.00
Total	100.00	

Table 2. Zone of inhibition (mm) of nanoparticles against different bacterial strains

Reagent	<i>E. coli</i> interpretation zone diameters (mm)			<i>Klebsiella</i> interpretation zone diameters (mm)			<i>Pseudomonas</i> interpretation zone diameters (mm)			<i>Salmonella</i> interpretation zone diameters (mm)		
AgNPs	6	6	6	3	2	3	6	6	6	6	6	6

AgNPs spherical shape are higher than that of gold nanoparticles spherical shape.

Hence, AgNPs synthesized by this method should be prospect further for antimicrobial applications for examples in wastewater treatment, food and water storage and manufacturing of medical supplies such as wound dressings or beds, bandages. The biological method used here in preparation is recognized by saving huge amount of energy, eco-friendly, economic, clean, and has no any toxic chemicals for the synthesis.

Conclusion

Present work demonstrated the rapid extracellular biogenic synthesis of green AgNPs using an orange peel extract and their use as an antibacterial agent. The used biogenic method here is non-toxic, environmentally friendly, simple, low cost and has no toxic chemicals. The results confirmed that orange peel plays an important role in the reduction and stabilization of silver. The formation of AgNPs was determined by UV-Vis spectroscopy where surface plasmon absorption maxima can be observed at 466 nm from the UV-Vis spectrum. Zitasizer shows the average size of the produced nanoparticles to be 91 nm. The bio produced AgNPs were characterized using FT-IR spectroscopic, TEM, SEM and EDS techniques.

For technical view, the successfully biogenic synthesized AgNPs showed antibacterial activity on both gram-positive and gram-negative bacteria and this may be useful in a wide variety of applications in pharmaceutical, biomedical fields, industrial appliances like bandage, food and water storage and wastewater treatment in a low price.

ACKNOWLEDGEMENTS

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through research group no RGP- VPP-278.

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