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Green Synthesis of Silver Nanoparticles by Using Waste Vegetable Peel and its Antibacterial Activities

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Abstract

Aims: Green synthesis of nanoparticles is gaining more attention due to the eco-friendliness, least toxicity and ease in its synthesis. The present study was carried out to describe the bioreduction of silver nitrate ($AgNO_3$) by using vegetable peels waste and evaluation of antibacterial properties.

Methods: Vegetable peel wastes of five different plant sources (*Lagenaria siceraria*, *Luffa cylindrica*, *Solanum lycopersicum*, *Solanum melongena* and *Cucumis sativus*) were prepared. Silver nanoparticles (AgNPs) were synthesized by adding 50 mg lyophilized vegetable peel extract in 20 ml of $AgNO_3$ (2mM), which was then incubated at 80°C for 10 min. The formation of AgNPs was monitored by UV-visible spectroscopy and characterized using transmission electron microscope (TEM) and fourier transform infrared (FTIR). The antimicrobial activity of AgNPs against *Escherichia coli* and *Klebsiella pneumoniae* was investigated with variable amount of AgNPs solution by disc diffusion assay.

Results: UV-Vis spectra showed maximum absorbance at 430 nm which confirmed AgNPs synthesis. TEM analysis revealed spherical shape AgNPs having size up to 20 nm. FTIR spectra confirmed the presence of proteins bound to AgNPs act as reducing and stabilizing agent. AgNPs were found to be bactericidal against *E.coli* and *K.pneumoniae*.

Conclusion: To our best knowledge there is no report available on the use of vegetable waste in the synthesis of silver nanoparticles, depicting the eco-friendly nature of our study.

Keywords: AgNPs, Antibacterial, Vegetable peel waste,

INTRODUCTION

Nanotechnology is a field of modern research and scientific innovation that deals with the synthesis and manipulation of particle structure within the nanometer scale range. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process. AgNPs are of interest because of their unique properties (e.g., size and shape depending on optical, electrical, and magnetic properties), and have drawn attentions of many researchers due to their suitability of applications in different fields such as, electronics, material science and medicine [1].

A number of approaches are available for the synthesis of AgNPs including chemical and photochemical reactions in reverse micelles, thermal decomposition of silver compounds, radiation assisted, electrochemical, sonochemical, and microwave assisted process [2]. Physical and chemical syntheses are expensive, and due to presence of some toxic chemical absorbed on the surface that may have adverse effect in the medical applications. There is a growing attention to biosynthesize AgNPs using plant based substances because of its cost effectiveness, does not involve the use of toxic chemical and is considered eco-friendly [3]. Use of plant extract for the synthesis of nanoparticles could be advantageous over other environmentally benign biological processes as it does not require labour intensive maintainance of cell cultures. Plant substances are suitable to produce nanoparticles having diverse shapes and sizes, more stable and the rate of synthesis is much faster than microorganisms. Furthermore, plant extracts are more suitable for large scale production of stable nanoparticles compared to other organisms [4, 5].

In the past few years, there has been an increasing interest in AgNPs on account of the antimicrobial properties that they display [6]. They are even being projected as future generation antimicrobial agents [7]. AgNPs have potential antimicrobial effects against infectious organisms such as *E.coli, Bacillus subtilis, Vibrio cholerae, Pseudomonas aeruginosa, Syphilis typhus* and *Staphylococcus aureus* [8].

The present study was aimed the extracellular biosynthesis of AgNPs, using vegetable peel wastes. Furthermore, characterization and antimicrobial studies have also been carried out.

MATERIALS AND METHODS

Collection of waste vegetable peel and powder preparation

The waste vegetable peel of *L.siceraria*, *L.cylindrica*, *S.lycopersicum*, *S.melongena* and *C.sativus* were collected from the kitchen waste (Fig.1A) The vegetable peels were then washed and boiled in distilled water for 10 min at 95°C. Vegetable peels (100 g) were crushed in 200 ml of distilled water and the extract formed was filtered through a muslin cloth. Then the filtrate was treated with equal volumes of chilled ethanol (Fig.1B) and the resultant precipitate was lyophilized into powder (Fig.1C).

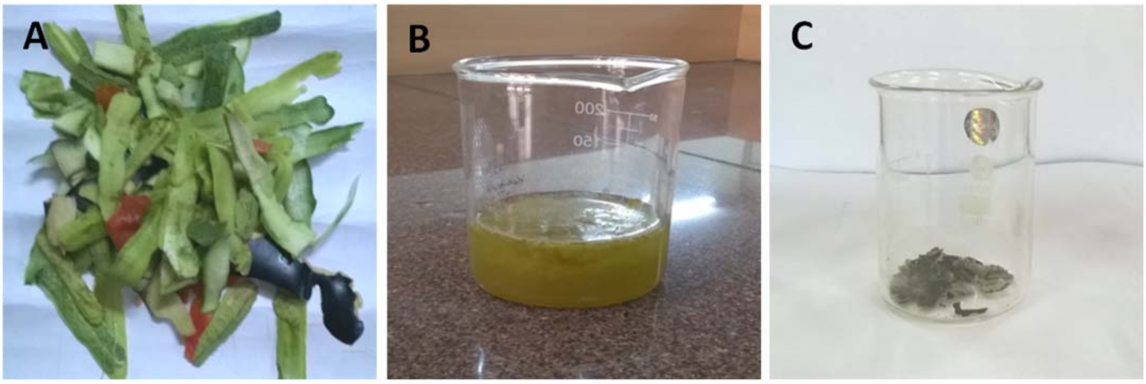

Fig.1 - Preparation of vegetable peel extract. (a) Waste vegetable peels, (b) Alcohol precipitated extract, (c) Lyophilized extract powder

Biosynthesis and optimization of AgNPs
The reaction was carried out by adding 50 mg of vegetable peel extract powder in 20 ml silver nitrate solution (2mM). This mixture was then incubated at 80 °C in a water bath for 10 min.

Characterization of AgNPs
Morphological Examination
The change in color of the incubated mixture was observed and recorded which indicated the synthesis of AgNPs.
UV-Vis Spectrometry Analysis
The AgNPs synthesized after were subjected to optical measurements, which were carried out by using a UV-Vis spectrophotometer (Thermo scientific/ Biomate 3S) and scanning the spectra between 300-800 nm at the resolution of 0.1 nm with the help of UV-Vis spectrophotometer.
TEM analysis
To determine shape and size of synthesized AgNPs, TEM (Jeol, USA) was done. TEM micrograph images of the synthesized AgNPs were taken for morphological characterization.
FTIR Analysis
FTIR analysis was carried out in order to determine the role of functional groups involved in the stabilization (capping) and synthesis of silver nanoparticles present in the vegetable peel extract. 10 mg of the powdered sample of the formed AgNPs was subjected to FTIR (Shimazdu, India) analysis at a resolution of 4cm^{-1}.

Assay for Antibacterial Activity
AgNPs synthesized from vegetable peel extract were tested for antimicrobial activity by standard disc diffusion method against human pathogenic microorganisms *E. coli* and *K. pneumoniae* (Clinically isolated). Fresh overnight of cultures (100 µl) were spread on to nutrient agar plates. Five sterile paper discs of 6 mm diameter containing sterile water, increasing amount of AgNPs (4 µl, 8 µl, 10 µl and 12 µl) were placed in each plate. Antibacterial assay of vegetable peel extract was also carried out using disc diffusion method, in order to check its activity against the target microbial species.

RESULTS

Synthesis of AgNPs
Visual observation of $AgNO_3$ treated with vegetable peel powder extract showed change in color into dark yellow color which indicates the formation of AgNPs. Fig. 2 shows the difference between the color of $AgNO_3$ solution and biosynthesized AgNPs.

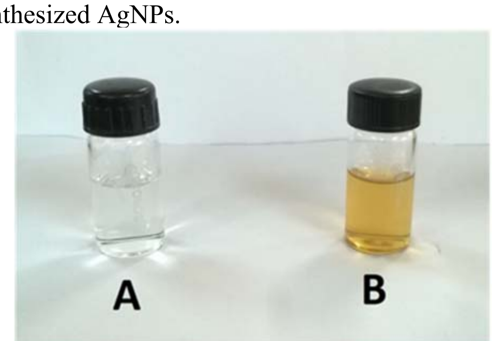

Fig. 2 - Synthesis of AgNPs. (a) $AgNO_3$ solution, (b) AgNPs solution.

Characterization of AgNPs
UV-Vis spectra
The UV-Vis spectrum of AgNPs after 4 hours of synthesis, as a function of time, is shown in fig. 3. The maximum peak was observed at 430 nm, which is the surface plasmon resonance of the AgNPs.

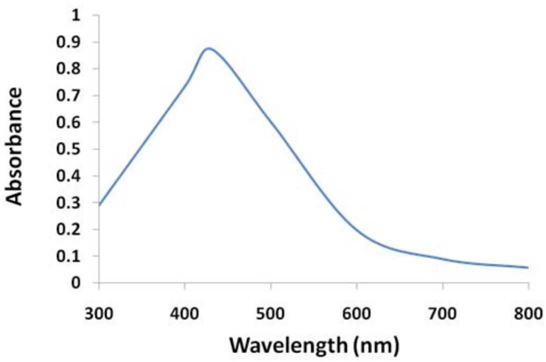

Fig. 3 - UV–Vis spectrum analysis. Plasmon resonance of silver nanoparticles reduced by vegetable peel extract at 430nm.

TEM analysis

In order to confirm the UV-Vis data, the TEM study was carried out. TEM micrograph revealed that the synthesized nanoparticles were more or less spherical in shape with an average size of 20 nm (Fig.4).

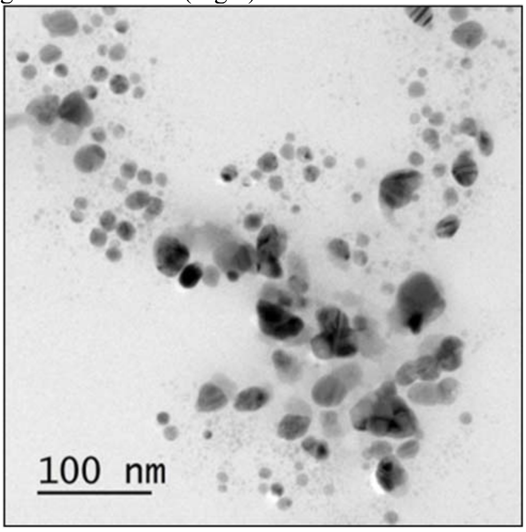

Fig. 4 - TEM micrograph of biosynthesized AgNPs.

FTIR spectrum

FTIR analysis was used for the characterization of the extract and the synthesized nanoparticles for which the absorption spectra of vegetable peel extract before and after reduction of Ag^+ ions are shown in fig. 5. FTIR analysis confirmed that the bioreduction of Ag^+ ions to AgNPs are due to the reduction by capping material of plant extract. Absorbance bands in Fig. 5A (before bioreduction) are observed in the region of 400–4000 cm^{-1} are 3852.68, 3727.65, 3697.55, 3403.99, 2924.73, 1735.17, 1685.44, 1654.23, 1560.50, 1546.37, 1438.29, 1407.67, 1236.92, 1051.64, and 425.64 cm^{-1} respectively. The broad peak at 1236.92 cm^{-1} and 1051.64 cm^{-1} is attributed to the stretching vibration of –C-N (aliphatic amines) and their total disappearance of these bands after bioreduction can be attributed to the certain proteins responsible for the capping of the nanoparticles. A very strong and sharp peak at 1384.28 cm^{-1} after $AgNO_3$ addition (Fig. 5B) can be attributed to the presence of nitrate ions after its reduction. The broad peaks at 492.08 cm^{-1} and 413.95 cm^{-1} (Fig. 5.B) are related to AgNP banding with oxygen from hydroxyl groups of vegetable peel extract. Based on the FTIR analysis, it can be assumed that phytochemicals and certain proteins present in the vegetable peel extract may be involved in capping and stabilizing the nanoparticles.

Antibacterial Assay

The antimicrobial activity of AgNPs was investigated against human pathogens, viz., *E. coli* and *K. pneumoniae* using disc diffusion method (Table 1). The zones of inhibition were increased with increase in amount of AgNPs poured on sterile discs against both of the microbial strains.

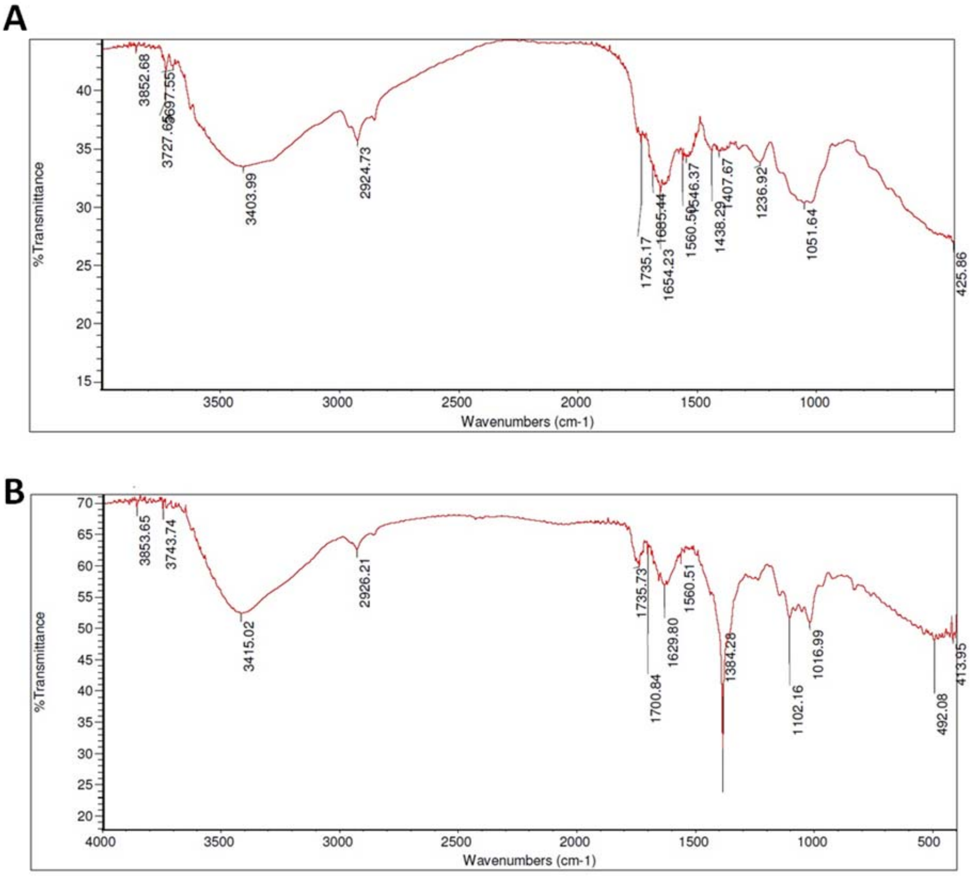

Fig. 5 - FTIR spectra from 400 cm-1 to 4000 cm-1 of (a) Vegetable Peel Extract, (b) Biosynthesized AgNPs

Table 1 - Zone of inhibition of AgNPs produced by the *Pseudomonas* sp. against MDR *E. coli* and *K. pneumoniae.*

S. No	MDR bacterial strains	Zone of inhibition (mm) ± SEM			
		4 µl	8 µl	10 µl	12 µl
1.	*E. coli*	7 ± 0.4	10 ± 0.8	13 ± 1.1	18 ± 1.1
2.	*K. pneumoniae*	6 ± 0.5	11 ± 0.6	15 ± 0.9	19 ± 0.9

DISCUSSION

Present study demonstrates green synthesis of AgNPs using vegetable peel wastes. It also mention antibacterial activity of synthesized AgNPs against MDR *E.coli* and *K .pneumoniae*.

It is well known that AgNPs exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles [9]. The probable mechanism for the nanoparticles synthesis have been reported by Mata et al., (2009) [10] and Kemp et al., (2009) [11] suggesting that polysaccharide plays an important role in the bioreduction of metallic nanoparticles. Furthermore, Sahu et al., (2013) [12] and Arunachalam et al., (2013) [13] reported the biosynthesis of AgNPs due to the presence of phytochemicals in the plant extracts. However, the biochemical pathways responsible for the production of AgNPs using plants are yet to be elucidated and are probably the future promising area of research.

Presence of protein as capping material helps in stabilization of AgNPs [14]. In FTIR spectra, the broad and strong bands at 3403.99 cm^{-1} was due amine groups (-NH). The sharp band at 2924 cm^{-1} is assigned to –C-H stretch and variable peaks at 1654.23 cm^{-1} and 1685.44 cm^{-1} represents the stretching vibrations of –C=C, arises most probably due to phytochemicals such as lycopene and carotene present in vegetable peel extract [15]. The medium bands at 1407.67, 1438.29, and 1546.37 cm^{-1} can be attributed to the stretching vibration of –C=C and –C-C (aromatic ring) and their disappearance after AgNP synthesis is due to the fact that certain phytochemicals such as flavones, flavanoids, tannis and quercetin are present in the vegetable peel extract, which may be involved in its synthesis.

Silver is known to be potent antibacterial in nature [16]. AgNPs have been used as biocide in different formulations as well as in consumer and medical products [17]. Several bacterial species including both Gram positive and Gram negative have been susceptible to AgNPs [18]. In our study we found AgNPs as strong anti bacterial aginst *E.coli* and *K.pneumoniae* in increasing concentrations. Similar findings were observed by Lara et al., (2010) [19] and Percival et al. (2007) [20].

CONCLUSION

The present study described AgNPs with mean diameters of 20 nm were synthesized using waste vegetable peel extract of five different plant sources (*L.siceraria*, *L.cylindrica*, *S.lycopersicum*, *S.melongena* and *C.sativus*), as a reducing agent. The detailed characterization of the nanoparticles was carried out using UV-Vis spectroscopy, TEM and FTIR spectrometry. The UV-Vis spectrum shows the characteristics plasmon absorption peak for silver nanoparticles at 430 nm. TEM micrograph images shows that the nanoparticles synthesized were nearly spherical and with average size of 20 nm. FTIR study was carried out to detect the possible functional groups responsible for imparting stability to nanoparticles and its synthesis. FTIR results suggest that the phytochemicals and certain proteins present in the vegetable peel extract may be involved in synthesis, capping and stabilizing the nanoparticles respectively. Furthermore, antibacterial activity, as a function of nanoparticles amount was carried out against the two most pathogenic Gram negative bacteria, *E. coli* and *K. pneumoniae*. From the results we can conclude that AgNPs showed a strong antibacterial potential.

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