

RESEARCH ARTICLE

FRUIT BIOWASTE MEDIATED GREEN ROUTE APPROACH SILVER NANOPARTICLES - AS ANTIBACTERIAL MATERIAL

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ABSTRACT

Environmental free approach or green chemistry synthesis of metallic nanoparticles has become new growing branch of nanobiotechnology. In this present work a simple and environmental free biosynthesis silver nanoparticles (AgNPs) were prepared using Musambi Peels (MPs) aqueous extract as the reducing agent guided by the principles of green chemistry. The fruit waste aqueous extract was challenged with silver nitrate solution for the production of AgNPs in room temperature. The crystalline phase and morphology of AgNPs were determined from UV-Vis spectroscopy, Fourier transform infrared (FTIR) spectra, X-ray diffraction (XRD), Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS). The UV-Vis spectrum indicated that the surface plasmon broad peak was observed nearby 450 nm throughout the reaction 30min-24h. XRD spectrum revealed that the average size of biowaste mediated AgNPs obtained approximately 46 nm by using the Debye-Scherrer equation. SEM image of AgNPs showed uniformly distributed on the surface of the cell with high agglomeration. EDS analysis revealed that the presence of silver was confirmed from the Ag peaks at 2.8-3.7 keV. In addition, the biowaste mediated AgNPs loaded disk were tested for antibacterial properties against *Escherichia coli* and *Staphylococcus aureus* and found that the obtained metallic AgNPs have been good antibacterial material for biological applications.

Keywords: Musambi Peels, silver nanoparticles, XRD, SEM, antibacterial.

1. INTRODUCTION

Nanoparticles frequently expression unique and considerably changed physical, chemical and biological properties compared to their macro scaled counterparts (1). Nanomaterials are naturally described as materials smaller than 100 nm in at least one dimension. The nanoparticles utilization is the most progressive at present, both in scientific knowledge and in commercial applications including water treatment process (2). Numerous noble metal nanoparticles for example copper, gold, silver and platinum were usually synthesized by using various methods including chemical, physical and biological route. Generally, the physical and chemical method nanoparticles synthesis have many disadvantages and this approach not consider as eco-friendly approach due to toxic effect that adversely effects the ecosystem. Later, researcher across the world has searched for novel and eco-friendly approach for the synthesis of biocompatible nanoparticles (3). Hence, biological route preparation of nanoparticles is less costly, less time consuming, and more environmentally free materials, therefore nowadays

researcher are looking forward to the possible biological approach to synthesis nanoparticles (4).

Recently many researcher reported synthesis of silver and gold nanoparticles via eco-friendly methods using a wide range of biological resources like fungi (5,6), bacterial (7,8) marine algae (9), marine yeast (10) Actinomycete (11) and plants (12,13). Conversely, production of metal nanoparticles using plants medicated green route approach, the reduction rate of metal salts is very fast and preparation routes itself requires no specific conditions unlike the physical and chemical method (14, 15). Hence, owing of the present results focuses on the green synthesis of silver nanoparticle (AgNPs) using aqueous extract of musambi peels (*Citrus limetta*) as reducing agent in aqueous solution of silver nitrate. The synthesized AgNPs were characterized by using UV-visible spectrophotometer (UV-vis), Fourier transform infrared (FTIR) spectra, X-ray diffraction (XRD), Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDS). Additional, the antibacterial properties of green synthesized AgNPs

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were test against human pathogens by disk diffusion method.

2 MATERIALS AND METHODS

2.1. Preparation of peel extract

Musambi Peels (MPs) were collected from local market nearby our college, Tamilnadu, India, on the basis of cost effectiveness and ease of availability. The fresh and healthy MPs were rinsed thoroughly first with tap water 5-10 min followed by distilled water 10-20 min to remove all the dust and unwanted visible particles, MPs were cut into small pieces and dried at room temperature. To prepare the aqueous extract of MPs about 20g of finely incised MPs were weighed and transferred in to 200 mL beakers containing 100 ml of distilled water and boiled for about 30 min at 80°C using heating mantel. After heating treatment, the colour of the MPs aqueous solution changed from watery to light yellow colour. The MPs aqueous extract was separated by filtered through normal filter paper followed by Whatman No.1 filter paper to remove particulate matter and to get clear aqueous extract solution. The MPs aqueous extract was stored at refrigerated (4°C) in 100 ml Erlenmeyer flaks to be used for biosynthesis of silver nanoparticles from silver nitrate. The effectiveness and accuracy in results without any contamination, each and every steps of the experiment were maintained under sterility conditions.

2.2. Synthesis of silver nanoparticles

In a typical reaction procedure, aqueous solution of silver nitrate (AgNO_3) at the concentration of 1 mM was prepared in 250 ml Erlenmeyer flasks and 10 ml MPs aqueous extract was added for reduction into Ag^+ ions at room temperature. The colour change of the reaction mixture from faint light to yellowish brown to reddish brown to colloidal brown was monitored occasionally for maximum 2 h using UV-visible spectrophotometer (JASCO V670, Japan) at the intervals of 30 min. The experimental reaction was carried out in darkness to avoid photo activation of AgNO_3 at room temperature. After 2 h incubation, the reduction of reaction mixture (MPs extract + AgNO_3) to Ag^+ ions was confirmed by the color change followed by the bioreduction, the reaction mixture was kept aside 24 h at room temperature for complete bioreduction. Then, the AgNPs solution obtained by MPs extract was centrifuged at 8,000 rpm for 30 min, the supernatant was discarded and final pellet was washed with distilled water 5-10

min, the pellet was dried at 60°C using hot air oven to get fine powder form of AgNPs.

2.3. Characterization of Silver nanoparticles

The sample (2mL) of the suspension was collected periodically to monitor the completion of bioreduction of Ag^+ in aqueous solution scan in UV-visible spectrophotometer (JASCO, V-670, Japan) between wavelengths of 300 to 800 nm, having a resolution of 1 nm. UV-vis spectra were recorded at intervals of 30min, 1h, 2h and 24h. Fourier transform infrared (FTIR) spectra for green synthesis silver nanoparticles was recorded on a Shimadzu FTIR spectrometer 8000 series, with a sample as KBr pellet method in the wavenumber region of 4,000 - 400 cm^{-1} . Crystalline nature of the nanoparticles was analyzed by XRD at 2θ ranges from 20 to 80°C (Philips PW 1830).The morphology and elemental composition of green synthesis silver nanoparticles was identified by Scanning Electron Microscopy (SEM, JEOL JSM-6390) along with Energy Dispersive X-ray Spectroscopy (EDS, Model No. 9582, Oxford Instruments) operating at an accelerating voltage of 20 kV.

2.4. Antibacterial activity

The green synthesized silver nanoparticles using musambi peels aqueous extract were tested for antibacterial activity by disc diffusion methods against human pathogenic organisms such as *Escherchia coli* and *Staphylococcus aureus*. Pure culture of bacteria Mueller Hinton, the bacterial test organisms were grown in nutrient broth at 37°C for 24 h. About 200 L of aliquot of each strain (1×10^6 cfu/mL) was spread uniformly onto the individual pre-sterilized petridishes plates using sterile cotton swabs and allowed to dry for 10 to 15 min. On other side, Whatman No. 1 filter paper discs (3 mm in diameter) were prepared and coated with 50 μ l of silver nanoparticles. The silver nanoparticles coated filter paper discs were placed on the surface of each cultured plate, streptomycin discs was used as positive control. Finally, the petridishes were incubated at 37°C for 24 h to find antibacterial properties of green synthesized silver nanoparticles coated filter paper inhibition zones were measured in millimeters.

3. RESULTS AND DISCUSSION

Due to the extensive applications of nanoparticles, a large number of methods have been developed for the controllable synthesis of nanoparticles. However, precise control on the size and distribution of nanoparticles remains a great task (6). In the present study, silver nanoparticles

(AgNPs) were rapidly synthesized using MPs aqueous extract as bio-reductants. The bio-reduction of AgNO_3 into AgNPs was completed within 24h of incubation turned the colour to dark brown (Fig. 1).



Fig. 1. Biosynthesis of AgNPs using MPs aqueous extract a) Musambi peels, b) Aqueous extract of MPs, c) 1mM AgNO_3 , d) Reaction mixture 0th min and e) Reaction mixture after 24h.

3.1. UV-Vis Spectrum Studies

The green route synthesized AgNPs using MPs aqueous extract was confirmed by the UV-Vis spectrum analysis at different nm scale. The colour changed into light yellowish brown to dark brown was due to excitation of Surface Plasmon Vibration which indicated the formation of AgNPs at room temperature.

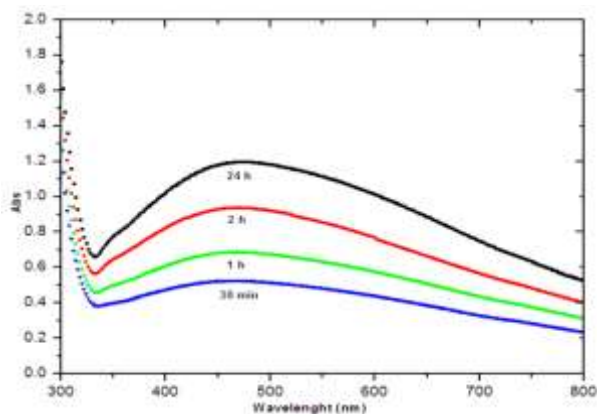


Fig. 2. UV Spectrum of biosynthesized AgNPs

3.2. FTIR study

FTIR spectrum analysis has helped to understand the nature of biomolecules present in the MPs extract that involved in the formation of AgNPs. The FT-IR spectrum of MPs extract mediated green synthesized AgNPs showed sharp peaks located at 3371.54 , 1635.64 , 1373.32 and 1256.87 cm^{-1} and light peaks located at 2121.70 , 2314.58 , 2546.04 , 2638.62 , 2723.49 and 2846.93 cm^{-1} . The sharp peak at 3371.54 , 1635.64 , 1373.32 , 1256.87 cm^{-1} may be assigned to the O-H, C-N and C-O stretch bonding function group of secondary alcohols respectively. The light peaks at 2121.70 and 2314.58 cm^{-1} indicated C-O bonding function group of phenols and

alcohols and the peaks at 2546.04 , 2638.62 , 2723.49 and 2846.93 indicated the presence of N-H primary amine and O-H stretch the function group of carboxylic acid (Fig.3).

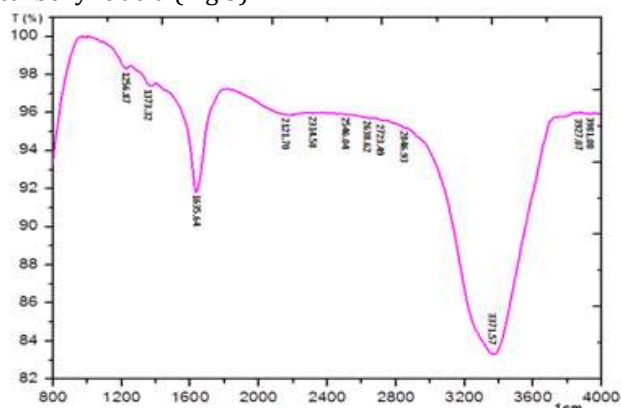


Figure 3: FTIR Spectrum of MPs extract mediated AgNPs

The FTIR spectrum revealed that the different functional groups present at different position and functional biomolecules like phenols, alcohols and carboxylic acid are involved in the reduction of silver ions (16).

3.3. X-ray diffraction study

Green synthesized MPs extract mediated AgNPs were further analysis through X-ray diffraction was carried out to confirm the crystalline nature of the AgNPs. The XRD pattern indicates numbers of Bragg reflections that may be indexed on the basis of the face centered cubic structure of silver. A comparison of obtained XRD spectrum with the standard confirmed that the silver particles formed in present experiment were in the form of nanocrystals, as evidenced by the peaks at 2θ values of 38.14° , 44.33° , 64.48° and 77.44° corresponding to (111), (200), (220) and (311) Bragg reflections,

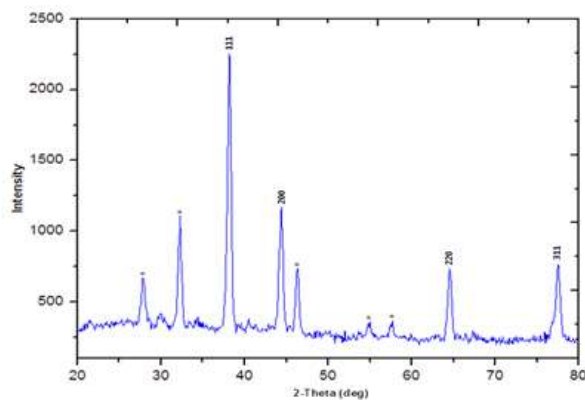


Fig. 4. XRD analysis of AgNPs

respectively, which may be indexed based on the face-centered cubic structure of silver (JCPDS file nos. 04-0783). X-ray diffraction results clearly show that the silver nanoparticles formed by the reduction of Ag⁺ ions by the carob leaf extract are crystalline in nature. The unassigned peaks at 2θ = 27°, 32°, 46° 54° and 57° denoted by (*) were also observed signifying that the crystallization of bioorganic phase occurs on the surface of the nanoparticles (Fig. 4).

The average particle size of AgNPs synthesized by the green route method can be calculated using the Debye-Scherrer equation $D = \frac{K\lambda}{\beta \cos\theta}$; where D is the crystallite size of AgNPs, λ is the wavelength of the X-ray source (0.1541 nm), β is the full width at half maximum of the diffraction peak, K is the Scherrer constant with a value from 0.9 to 1, and θ is the Bragg angle. From the XRD analysis the MPs aqueous extract mediated green synthesized AgNPs was found that the average size found to be approximately 46 nm using the Debye-Scherrer equation.

3.4. SEM-EDS analysis

Scanning electron microscopy images shows the shape of the green synthesized AgNPs using MPs extracts (Fig. 5a). The shape of surface morphology of the AgNPs was observed at different magnification and revealed that the SEM images of MPs extracts mediated AgNPs shown uniformly distributed on the surface of the cell with high agglomeration was noted. The large particles may be due to the aggregation of the smaller ones (16). The SEM was equipped with energy dispersive spectroscopy (EDS) analysis indicated that the presence of silver was confirmed from the Ag peaks (Fig. 5b).

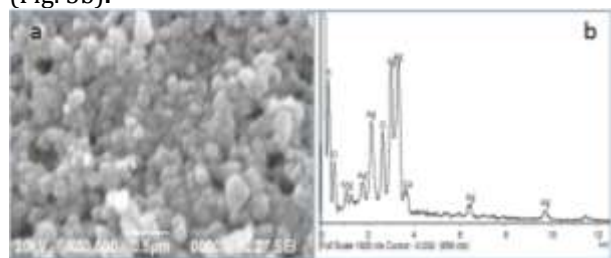


Fig. 5. SEM-EDS image of biosynthesis AgNPs

Silver nanoparticles revealed a strong signal of metallic silver at 2.8-3.7 keV and presence of oxygen, calcium and Mg in the EDS spectrum indicates that organic moieties exist in the aqueous extract of MPs (17). Therefore, these organic constituents are partially involved in the reduction of silver metallic nanoparticles at room temperature.

3.5. Antibacterial properties

The antibacterial properties of the silver nanoparticles and silver nanoparticles coated product have been exploited for a long time in the biomedical field (18,15). In this present study, antibacterial properties of MPs aqueous extract mediated AgNPs material was evaluated by using standard Zone of Inhibition (ZOI) microbiology assay against *E. coli* and *S. aureus* and found that MPs extracted mediated AgNPs achieved significant antibacterial activity against tested pathogens (Fig. 6). MPs extract mediated AgNPs loaded disk showed maximum ZOI was found to be 17mm for *S. aureus* and 14mm for *E. coli*, whereas, standard antibiotic disk streptomycin 19, 20mm against *S. aureus* and *E. coli* respectively. The AgNPs shown inhibition zone against both studied bacteria and the present study achieved the maximum activity against *S. aureus*. Previous study by Subbaiya *et al.* (19) reported that the *Nerium oleander* mediated biological synthesized silver nanoparticles the zone of inhibition was found more at *B. subtilis* and *E. coli*. Another study by Ranjithkumar *et al.* (2013b) reported that the areca nut mediated biogenic synthesized silver nanoparticles coated cotton fabric showed significant antibacterial properties against both Gram positive and Gram negative pathogens.

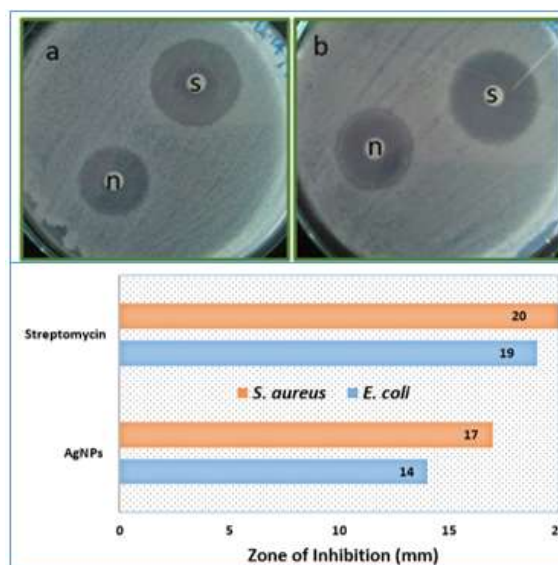


Fig. 6. Antibacterial activity of AgNPs by disk diffusion method. a) *E. coli* and b) *S. aureus*

Likewise, our present results suggested that the plant mediated green approach obtained metallic silver nanoparticles have been good antibacterial material for biological applications.

4. CONCLUSION

Green chemistry nanoparticles is gaining important due to the free form toxic chemicals and provides effective synthesis of expected products in an economic manner. In this present work, we developed an environmental free and convenient green chemistry method of the synthesis of silver nanoparticles from fruit waste as reducing agent and found MPs aqueous extract is found suitable for the production of AgNPs at room temperature by green approach. Production of AgNPs after inhibition the colour change occur due to surface Plasmon resonance during the reaction with the organic compounds present in the MPs extract resulting in the formation of AgNPs which was confirmed by UV-vis spectrum and Surface Plasmon broad peak was observed nearby 450nm. The FTIR spectrum indicated the different functional biomolecules present at different position such as phenols, alcohols and carboxylic acid are involved in the reduction of silver ions. XRD and SEM-EDS indicated the MPs extract mediated AgNPs shown uniformly distributed on the surface of the cell with high agglomeration. In addition, antibacterial activity of this green route synthesized AgNPs showed potential antibacterial activity against test pathogens. The overall graphical abstract of present work shown in figure 7.

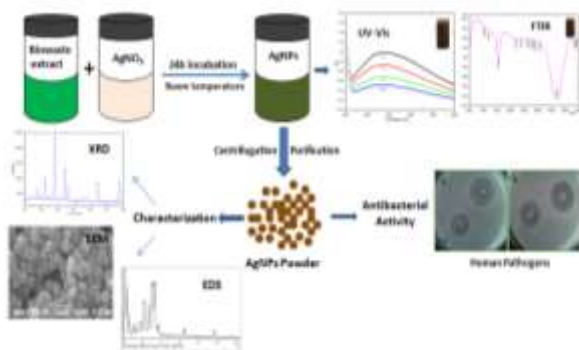


Fig. 7. Graphical abstract of preparation and characterization and applications nanoparticles

The growing need to develop environment friendly processes through green synthesis and other biological approaches to preparation of zero toxicity nanoparticles for biomedical applications. Hence, "Green synthesis" of nanomaterials makes use of environment friendly and non-toxic reagents.

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Conflict of Interest

We, the authors declare that they have no conflict of interests.

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