Green Synthesis and Antibacterial Activities of Silver Nanoparticles Mediated by Extract of *Cucurbita maxima* (Pumpkin) Fruit Pith

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Abstract

Synthesis of silver nanoparticles (AgNPs) has become a necessary field of applied science. Biological method for synthesis of AgNPs by *cucurbita maxima* fruit pith extracts was used. The AgNPs were identified by UV–visible spectrometry; X-ray diffraction (XRD), scanning electron microscopy (SEM) and Fourier transform infrared spectrometry (FT-IR). The presence of surface plasmon band around 420 nm indicates AgNPs formation. Parameter optimization showed the smallest size of AgNPs (2.86 ± 0.3 nm) was obtained with 0.1 M AgNO₃ at 40°C. The present study provides the proof that the molecules within aqueous *cucurbita maxima* fruit pith extracts facilitate synthesis of AgNPs and highlight on value-added from *cucurbita maxima* fruit pith and fruit peel extracts for cost effectiveness. Also, eco-friendly medical and nanotechnology-based industries could also be provided. Size of prepared AgNPs could be controlled by temperature and AgNO₃ concentration. Further studies are required to study effect of more parameters on size and morphology of AgNPs as this will help in the control of large scale production of biogenic AgNPs. The synthesized silver nanoparticles are screened antibacterial activities.

*Keywords:* Green synthesis, Silver nanoparticles, UV, FTIR, XRD, SEM, TEM and Antibacterial Activities

1. Introduction

Nanotechnology is a fast growing branch of science that deals with synthesis and development of varied nanomaterials. Now, various kinds of metal nanomaterials are being prepared by copper, zinc, titanium, magnesium, gold, alginate and silver [1]. AgNPs became the main focus of intensive research because of their wide selection of applications in areas like catalyst, optics, antimicrobials, and biomaterial production [2-6]. Silver nanoparticles showed new or improved properties due to their unique size, morphology, and distribution. At the
moment, there’s a growing demand to develop eco-friendly nanoparticles using safe chemicals in the synthesis protocol. Researcher turned to biological system for synthesis of nanoparticles as alternatives to chemical and physical methods. These as a result of several unicellular and multicellular organisms are well-known producing inorganic materials either intra- or extracellular[7-8]. Biosynthesis of nanoparticles such as nanosilver and control in their size composition and mono-disparity are important areas of research in nanoscience. Silver nanoparticles are widely used among all nanomaterials. So biological and biomimetic approaches for biological synthesis of AgNPs are under research. Biomass or extracellular materials from microorganisms like Fusarium oxysporum, Escherichia coli, Aspergillus flavus, licheniformis are used for biotransformation of silver ions to AgNPs [9-11]. The aim of this study is to biosynthesize AgNPs using fungi Rhizopus stolonifer which is a cheap, safe, nonpolluting and acceptable method. Filamentous fungi are more preferred than bacteria and unicellular organisms as they are easy to handle and able to synthesize AgNPs extracellular [12]. Synthesized nanoparticles by fungi are more stable with better mono-disparity[13]. In this study we prepared stabilized AgNPs by aqueous extract of cucurbita maxima fruit pith, characterized by UV–Visible absorption spectra, XRD, FTIR and confirmed by SEM.

2. Material methods

2.1 Sample collection

The waste pith of Cucurbita maxima fruit were collected from kitchen waste.

![Figure 1a Pumpkin Fruit](image1a)

![Figure 1b Waste Pith of Pumpkin Fruit](image1b)

2.2 Reagents

All analytical grade chemical and solvents used in the sample preparation were purchased from local suppliers of Singma-Aldrich chemical company-Pondicherry, Precession scientific company-Trichy.

2.3 Extraction

Extraction from dried pith of Cucurbita maxima, these pith are done using water as the extraction solvents. via direct hot extraction (DHE) method [14]. 100 ml of solvent with 100 gm
of the sample (waste peel and pith) in separate around bottomed flasks was kept on a heating mantle covering at 60°C for two days followed by ensuing filtration by means of whatman filter paper # 1. Then, the obtained filtrates were collected and dried with vacuum evaporator pending a rudimentary glutinous extract is obtained. Then, the collective supernatants are filtered and the filtrates are cooled and dried using hot air oven until a crude glutinous extract is obtained. After vanishing of organic solvents, these are stored at – 20°C till scrutiny

2.4 Synthesis of silver nanoparticles

2.4.1. Green synthesis of AgNPs mediated by pith extract of pumpkin fruit

Aqueous solution of silver nitrate was prepared by adding 1 mM of AgNO₃ to 250 ml of distilled water at room temperature and stored in an amber colored bottle to evade auto oxidation of silver ions. Silver nitrate (10 mL; 1 mM) was added drop wise into Extract (100 mL) while stirring and heated (45 °C) in a water bath at pH 9. The resulting solution became brown after 30 min of heating, indicating the formation of silver nanoparticles [15], as showing in figure 1. The colloidal suspension thus obtained was centrifuged at 4000 rpm for 30 min and the pellet obtained after discarding the supernatant was re dispersed in deionized water. The centrifugation process was repeated 2 to 3 times for the removal of any adsorbed substances on the surface of silver nanoparticles. The synthesized nanoparticles were lyophilized and recovered in powdered form.

![Figure:2(a) Extract, 2(b) 0.1M AgNO₃ solution and 2(c) AgNPs Suspension](image)

2.4. UV–visible spectrometry measurement

Biotransformation of silver ions was monitored by UV–visible spectroscopy measurement of the reaction medium. Three milliliters of supernatant were taken after 6, 12, 24, 36 and 48 h and absorbance was scanned by Labomed, UV–vis double beam (Labomed, Inc, USA) within the wave length ranging from 200 to 600 nm. The absorption of the visible depends directly on color of the chemicals in solution. 2.5. X-ray diffraction (XRD) measurement XRD technique was used for checking quality of prepared nanoparticles. XRD pattern of drop-coated films of synthesized nanoparticles on glass material was recorded in a wide selection of Bragg angles 2h at a scanning rate of 20 min⁻¹, using Philips PW 1830 instrument (Philips, Inc, USA) adjusted at 40 kV and 30 mA with metal Cu ka radiation (k= 1.5405 Å ° ).
2.5. Fourier rework Infrared (FT-IR) spectrometry analysis

The sample was scanned by FT-IR spectrometry using PerkinElmer spectrophotometer (Los Angeles, CA). Briefly 2 mg of sample was mixed 200 mg Potassium bromide (KBr) (FT-IR grade) and pressed into a pellet and placed into the sample holder and FT-IR spectra were scanned in rang 4000–400 cm\(^{-1}\) in FTIR spectrometry at a resolution of 1 cm\(^{-1}\).

2.5. X-ray diffraction (XRD) measurement

XRD technique was used for checking quality of prepared nanoparticles. XRD pattern of drop-coated films of synthesized nanoparticles on glass material was recorded in a wide selection of Bragg angles 2h at a scanning rate of 20 min\(^{-1}\), using Philips PW 1830 instrument (Philips, Inc, USA) adjusted at 40 kV and 30 mA with metal Cu ka radiation (k = 1.5405 Å\(^{\circ}\)).

2.6 SEM Analysis

SEM micrograph shows the synthesized nanoparticles were (0.5 and 1 mM) spherical and aggregated shape. The EDX attachment with the FESEM was known to provide information on the chemical analysis of the fields that are being investigated or the composition at specific locations. In representative profile of the spot EDX analysis was obtained by focusing on Ag NPs.

3. RESULT AND DISCUSSION

3.1 UV spectroscopy

![Figure 3](http://ymerdigital.com)

**Figure 3**: UV–visible absorption spectra of produced AgNPs using extract of waste Pith in Pumpkin Fruit at different incubation times, synthesis of AgNPs is the function of time.

UV–Visible spectrometry showed optical absorption spectra of AgNPs ranging from 250 to 650 nm. The absorption spectra show one outstanding symmetric peak around 449 nm. That is
as a result of surface plasmon resonance of AgNPs (Fig. 3). This spectroscopic pattern results from interactions of free electrons limited to tiny metallic spherical objects with episode electromagnetic wave. To study effect of time on AgNPs production, we measured UV–Vis spectra at different time, we found that absorbance at 449 nm increased with the incubation time of the silver nitrate with the pith extract. The statistical analysis showed a significant difference (P value =0.001) in the production of AgNPs (Fig. 3) the highest production of AgNPs was after 60 mints of incubations. UV-spectroscopy showed increased absorbance with time and AgNPs were synthesized by 24 h and there was almost no increase in absorbance after 1 hr for the four tested pith extract [16]. An increase in intensity of the absorbance peak with time indicates the continued reduction of the silver ions and an increase in concentration of AgNPs [17]. Electronic style of AgNPs is notably sensitive to their form and size, resulting in clear effects in its visible spectrum pattern. One of interesting criteria is increasing bandwidth of resonance with the decrease in the dimensions of the particles as a result of electron scattering induction at the surface. Resonance shifting and the variation of its bandwidth are important information for nanoparticles characterization. The presence of Plasmon band at 449 nm due to dipole plasmon resonance [18] shows that the AgNPs have a spherical shape.

3.2 FTIR spectroscopy

Fourier transform infrared spectrum indicted that extract of waste pith in Pumpkin Fruit contain active biomolecules which are responsible for biotransformation of silver ion to metallic AgNPs, that revealed distinct peak within the range of 4000–500 cm\(^{-1}\) (Fig. 4). The broad peak at

![Figure:4 FTIR spectrum of AgNPs](image)

3440 cm\(^{-1}\) is resulting from strong stretching vibration of phenolic hydroxyl OH [20]. The band at 2920-2640 is due to an NH group from peptide linkage in the mycelia aqueous extract of waste Pith of Pumpkin Fruit [21]. The peak at 1440 cm\(^{-1}\) is characteristic of amid group NHCO.
Infrared (IR) analysis study has confirmed that carbonyl group resulted from amino acid residue and peptide protein can strongly bind to metal, so protein may act as capping protein of AgNPs which prevents agglomeration and stabilizes particles within the medium. This proof suggests that the biological molecules are responsible for biotransformation of silver ions to AgNPs and its stabilization in aqueous medium. It is a well-known character of protein that it can bind to AgNPs through a free amine group and stabilization of AgNPs may be due to the surface bound protein [22]. The peaks at 1040 cm\(^{-1}\) is corresponding to carbonyl stretch vibrations in the amide linkages of proteins [23]. The carbonyl group from amino acid residues and peptides remains has strong capability to bind to silver [24]. Also it is reported that proteins will bind to nanoparticles either through free amino or cysteine group in proteins [25]. The peaks at 879 and 728 cm\(^{-1}\) are corresponding AgNPs binding between oxygen from hydroxyl group and amid carbonyl groups of extract of waste Pith of Pumpkin Fruit [26]. 445 cm\(^{-1}\) is represent Ag-O stretching vibration. From FTIR results we conclude that the presence of protein in reaction medium provide reducing agent and coat covering for AgNPs known as capping proteins. Capping protein prevents agglomeration of AgNPs in the medium and responsible for forming high stable AgNPs. Similar results are obtained with bacteria [27] and Algae [28]. However polymers and surfactants were widely used as capping agent in preparation of AgNPs, protein capping provides advantage over polymer and surfactant as it is cost effective, safe, ecofriendly and does not need special conditions. Several harmful chemical byproducts, metallic aerosol, irradiation, etc. are commonly produced during use of chemical and physical in AgNPs synthesis processes. These, along with the facts that these processes are expensive, time consuming, and typically done on small laboratory scale, render these methods less suitable for large-scale production [28]. Another advantage of protein capping when compared to polymer and surfactant is it acts as the anchoring layer for drug or genetic materials to be transported into human cells [29]. The presence of a nontoxic protein cap also increases uptake and retention inside human cells [30]. The presence of natural capping proteins eliminates the postproduction steps of capping which is necessary form most of applications of nanoparticles in the field of medicine [31].

3.3 XRD Analysis

![XRD Pattern of AgNPs using extract of waste Pith of Pumpkin Fruit](image-url)
The XRD pattern showing crystallinity of the nanoparticles. The full-width at half-maximum (FWHM), the intensity and position we calculate from the XRD data. The (100), (002), (101), (102), (110), (103), (112) give the broad diffraction peak in 31.67°, 34.35°, 36.18°, 47.44°, 56.48°, 62.77°, 67.83° at 2θ range in 20–80°. The hexagonal structure was confirmed by (JCPDS card no. 04-0783). The XRD data gave the size of the AgNPs using extract of waste Pith of Pumpkin Fruit gives 36.03 nm by using scherer equation.

\[ D = \frac{K\lambda}{\beta \cos \theta} \]

Where \( \lambda \) is the wavelength of Cu Kα radiation (1.5406 Å), \( \beta \) is full width half maximum (FWHM) of (101) plane and \( \theta \) is Bragg’s diffraction angle.

### 3.4 SEM analysis

The scanning electron microscope uses a beam of high-energy electrons to produce a variety of signals at the surface of specimens used. The signals show information about the sample including chemical composition, and crystalline structure, external morphology (texture) and orientation of materials which make up the sample. SEM analysis is normally considered to be non-destructive because the x-rays generated do not lead to loss of volume of the sample, so it becomes possible to repeatedly analyse the same materials. A scanning electron microscope is a kind of electron microscope which images a sample by scanning it using a high-energy electron beam. The electrons then interact with the atoms making up the sample, thus producing signals which reveal information about the sample's composition, surface topography and other properties such as electrical conductivity. The SEM analysis was used to determine the structure of the reaction products that were formed. Thin films of the sample were prepared on a gold coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a
mercury lamp for 5 min. SEM image has showed individual Silver particles as well as a number of aggregates. SEM analysis is done to visualize shape and size of nanoparticle. The SEM image of synthesized Silver nanoparticles is shown in Fig.6. From the SEM images were seen in different magnification ranges like 2μm–80μm which clearly demonstrated the presence of spherical and mountain rock shaped nanoparticles.

4. ANTIMICROBIAL STUDIES

The nanoparticle of AgNPs mediated by Aqueous extract of cucurbita maxima(pumpkin) pith extract were taken. The test solutions were prepared by 100 mg/ml of by dissolving the nanoparticle in pure dimethylsulfoxide (DMSO) (Sigma, USA-Pondy). Test organisms were collected from the Bacteriology Unit of (CAS) Central advanced studies of Marine biology in (Parangipettai) Annamalai University, Tamil nadu. The organisms used in this research were Three Gram positive (S. aureus S. pyogenes and B.subtilis). Upon receipt, all isolates were subculture onto selected culturing media to ensure purity and confirm their identification. The strains were maintained and tested on Mueller Hinton Agar (MHA) (Merck, Germany), which was stored at 4°C. The test organisms were cultured overnight at 37°C before being used in the antibacterial assay described below. The AgNPs were tested different concentrations (100%, 50% and 25%) for antibacterial activity using the disc diffusion method [32]. Sterile commercial blank discs (Oxoid), 6.0 mm diameter, were impregnated with test solutions of AgNPs. Discs were stored at −5°C prior to use. During the night broth cultures were adjusted using a turbidometer to yield approximately 1.0 X 10 cfu per ml. Nanoparticles impregnated discs (20 μl) were placed on agar plates and incubated at 37°C for 24 hours. Pure DMSO (20 μl) was used as a negative control, while Ciprofloxacin discs (30 μl) were used as a positive control. Antibacterial activities were then determined by measuring the clear zone of inhibition to the nearest millimetre (mm). An in vitro test for antibacterial activity revealed that AgNPs mediated by the cucurbita maxima (Pumpkin) fruit pith extrac. They show good antibacterial effect against GPB (Gram positive bacterial) strains S.aureus, B.subtills and S. pyogenes in 100% concentration solutions, decreases the test solution concentration decreases the antibacterialal activity, remaining concentration solution have shown in moderate activity comparing standard Ciprofloxacin, Table:1 have shown zone of inhibition values of AgNPs against gram positive
Figure: 7 Plate of Antibacterial activities of WNPs, SnNPs and PbNPs against Gram positive Bacterial strains
Bacterial strains, **Figure:7** have shown gram positive bacterial strain against AgNPs, **Figure:8** have shown in the Cluster column chart of AgNPs against bacterial strains.

![Cluster column chart of AgNPs against bacterial strains](image)

**Figure:8** The Cluster column chart of AgNPs against bacterial strains.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Zone of Inhibition (mm)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>100%</td>
<td>15</td>
</tr>
<tr>
<td>50%</td>
<td>13</td>
</tr>
<tr>
<td>25%</td>
<td>09</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>22</td>
</tr>
<tr>
<td>Control (DMSO)</td>
<td>-</td>
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</tbody>
</table>

**Table: 1 Zone of inhibition on Antibacterial activities of AgNPs against Bacterial strains**

**5. CONCLUSION**

Nanotechnology will have large impact on rural development. Synthetic biology can revolutionize food production threatening traditional methods of agriculture. It is necessary to create international standards for nanotechnology and in addition special international organizations in the area of nanotechnologies to reduce national differences in assessing of nanotechnologies and risk governance practices. For these purposes it is necessary to create the research infrastructure for toxicology and risk assessment. In aspects of nanotechnology study
courses it is necessary to define what kind of skills and knowledge are needed in a small, agricultural country to take advantage of nanotechnology and to manage risks that are likely to emerge with increasing commercialization of nanotechnology. Ultimately, nanotechnology innovations may enable the agricultural industry to precisely control and improve production by reducing the disease incidence and increasing the nutrient availability. The green synthesis of Silver nanoparticles from *Cucurbita maxima* (Pumpkin) fruit pith extract was studied. The reduction of the metal ions led to the formation of Silver nanoparticles of fairly well-defined dimensions using the extract. This green chemistry approach towards the synthesis of Silver nanoparticles has many advantages such as environmental friendly, cost effective and easily scaled up to large scale synthesis. Antibacterial studies of silver nanoparticles on human being pathogen open a door for a fresh variety of antibacterial agents.

**REFERENCE**


