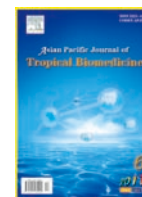




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## Biofabrication of Ag nanoparticles using *Moringa oleifera* leaf extract and their antimicrobial activity

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### ABSTRACT

**Objective:** To formulate a simple rapid procedure for bioreduction of silver nanoparticles using aqueous leaves extract of *Moringa oleifera* (*M. oleifera*). **Methods:** 10 mL of leaf extract was mixed to 90 mL of 1 mM aqueous of AgNO<sub>3</sub> and was heated at 60 – 80 °C for 20 min. A change from brown to reddish color was observed. Characterization using UV–Vis spectrophotometry, Transmission Electron Microscopy (TEM) was performed. **Results:** TEM showed the formation of silver nanoparticles with an average size of 57 nm. **Conclusions:** *M. oleifera* demonstrates strong potential for synthesis of silver nanoparticles by rapid reduction of silver ions (Ag<sup>+</sup> to Ag<sup>0</sup>). Biological methods are good competitors for the chemical procedures, which are eco-friendly and convenient.

## 1. Introduction

Nanomaterials have a long list of applicability in improving human life and its environment. The first relation between human life and nano scale was developed naturally in ayurveda, which is a 5000-year-old Indian system of medicine. It had some knowledge of nanoscience and technology before the term ‘nano’ was even formed. Modern science has just started exploring nanoscience in the 21st century[1]. Research and development in this field is growing rapidly throughout the world. A major output of this activity is the development of new materials in the nanometer scale, including nanoparticles. These are usually defined as particulate materials with at least one dimension less than 100 nanometers (nm), even the particles could be of zero dimension as in the case of quantum dots.

Metal nanoparticles which have a high specific surface area and a high fraction of surface atoms have been studied extensively because of their unique physicochemical characteristics including catalytic activity, optical properties, electronic properties, antibacterial properties and magnetic

properties[2]. Synthesis of noble nanoparticles for the applications such as electronics, environmental and biotechnology is an area of constant interest[3]. Generally metal nanoparticles are synthesized and stabilized by using chemical methods such as chemical reduction[4,5], electrochemical techniques[6], microwave assisted process[7] and now a days via green chemistry route[8]. Use of plants in synthesis of nanoparticles is quite novel leading to truly green chemistry which provide advancement over chemical and physical method as it is cost effective and environment friendly easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals. Bacteria and fungi could be used for the synthesis of nanoparticles[9,10] but use of leaf extract[11] reduce the cost and we do not require any special culture preparation and isolation techniques.

*Moringa oleifera* (*M. oleifera*) (Moringaceae, English: drumstick tree) has been used as ingredient of Indian diet since centuries. It is cultivated all most all over the country and its leaves and fruits are used as vegetables. Almost all parts of the plant have been utilized in traditional medicine properties. The leaves of plant have also been reported for its antitumor, hypotensive, cardioprotective, wound healing activities and use for eye diseases[12]. Here in, we report for the first time synthesis of Ag nanoparticles, reducing the silver ions present in the solution of silver nitrate by the

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aqueous extract of *M. oleifera* leaf. Further these biologically synthesized nanoparticles were found highly toxic against different pathogenic microorganisms tested.

## 2. Materials and methods

### 2.1. Materials

All chemicals used in this experiment were of highest purity and obtained from Sigma (Bangalore, India) and Merck (Mumbai, India). *M. oleifera* leaves were collected from Regional Agriculture Research Station, Acharya N.G. Ranga Agricultural University, Tirupati, Andhra Pradesh, India.

### 2.2. Plant extract and synthesis of silver nanoparticles

Plant leaf extract was prepared by mixing 10 g of dried leaf powder with 100 mL deionized water in 500 mL of Erlenmeyer flask and boiled for 20 min. For the reduction of Ag<sup>+</sup> ions, 10 mL of leaf extract was mixed to 90 mL of 1 mM aqueous of AgNO<sub>3</sub> and was heated at 60 – 80 °C for 20 min. A change from brown to reddish color was observed.

### 2.3. UV-VIS spectra analysis

The reduction of pure Ag<sup>+</sup> ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-VIS spectrophotometer UV-2450 (Shimadzu).

### 2.4. Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) (HITACHI, H-7500) is a microscopy technique whereby a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. Ag nanoparticle image was formed from the interaction of the electrons transmitted through the specimen; the image of Ag nanoparticles was magnified and focused onto an imaging device.

### 2.5. Antimicrobial activity study

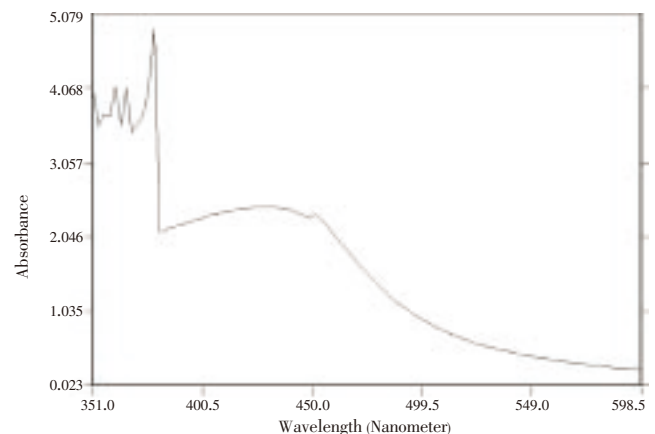
Antimicrobial activities of the synthesized Ag nanoparticles were determined, using the agar well diffusion assay method<sup>[13]</sup>. Approximately 20 mL of molten and cooled media (NA/SDA) was poured in sterilized petri dishes. The plates were left overnight at room temperature to check for any contamination to appear. The test organisms were grown in selected broth for 24 h. A 100 mL broth culture of each test organism ( $1 \times 10^5$  cfu/mL) was used to prepare lawns. Agar wells of 5 mm diameter were prepared with the help of a sterilized stainless steel cork borer. Two wells were prepared in the agar plates. The wells were labeled as A, B. 'A' well was loaded with 30  $\mu$ L of Ag nanoparticles suspended

'hydrosol' and 'B' well loaded with 30  $\mu$ L of positive control drugs (chloramphenicol/ketoconazole) (Table 1) were used as positive controls. The plates containing the test organism and Ag nanoparticles were incubated at 37 °C for 24 – 48 h. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the wells. The diameter of such zones of inhibition was measured using a meter ruler and the mean value for each organism was recorded and expressed in millimeter.

## 3. Results

### 3.1. UV-VIS spectra analysis

Reduction of Ag ion into silver particles during exposure to the plant extracts could be followed by color change. Ag nanoparticle exhibit dark yellowish – brown color in aqueous solution due to the surface plasmon resonance phenomenon. The result obtained in this investigation is very interesting in terms of identification of potential plants for synthesizing the Ag nanoparticles. UV-Vis spectrograph of the colloidal solution of silver nanoparticles has been recorded as a function of time. Absorption spectra of silver nanoparticles formed in the reaction media at 10 min has absorbance peak at 430 – 440 nm, broadening of peak indicated that the particles are polydispersed (Figure 1).



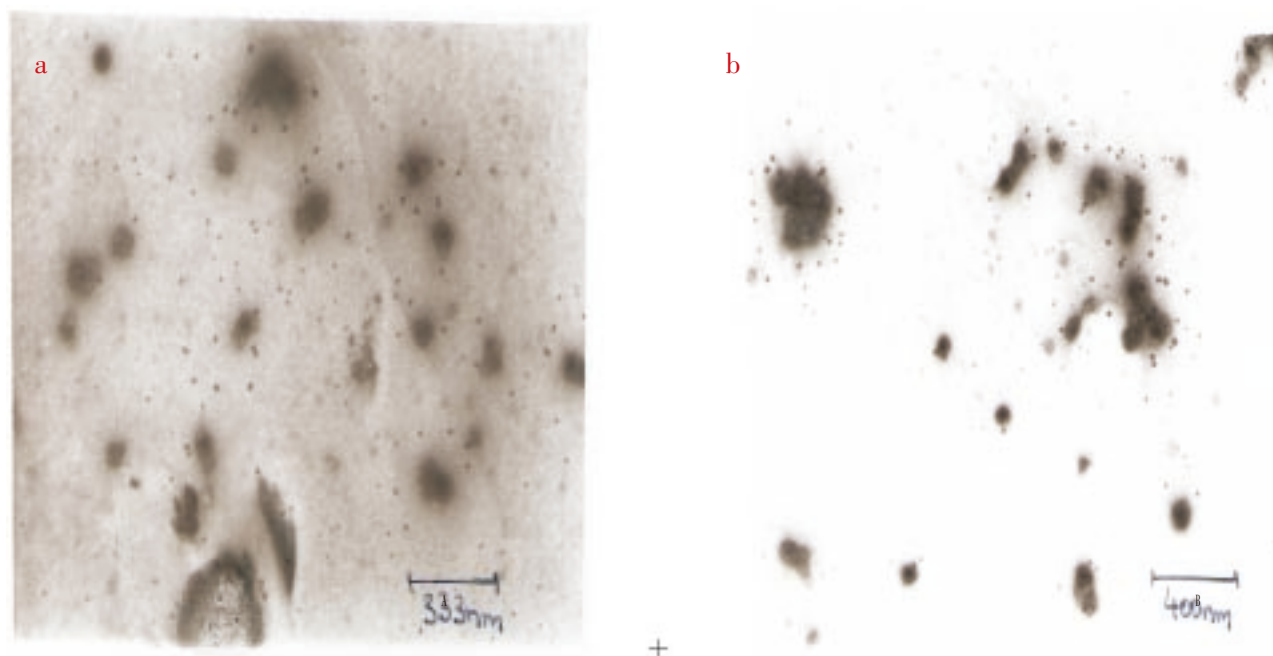
**Figure 1.** UV-VIS absorption spectra of Ag nanoparticle synthesized from *M. oleifera* leaves extract at 1 mM silver nitrate.

### 3.2. TEM analysis of Ag nanoparticles

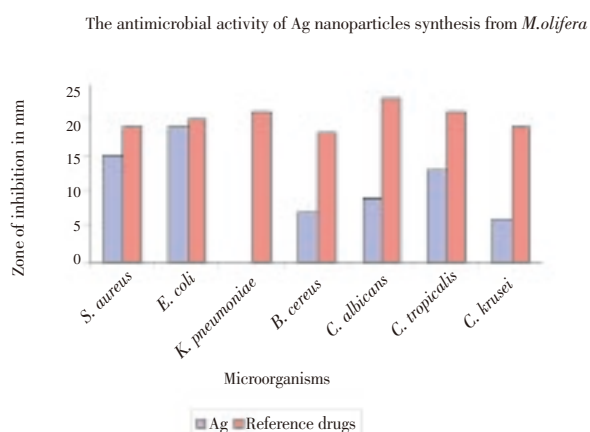
The silver nanoparticles synthesized with the help of *Moringa* leaf extract were scanned using TEM (HITACHI, H-7500) from which we can conclude that the average mean size of Ag nanoparticles was 57 nm and seems to be spherical in morphology as shown in Figure 2(a, b).

### 3.3. Antimicrobial activity study

Biosynthesis of Ag nanoparticles were studied for antimicrobial activity against pathogenic microorganisms (Clinical isolate) by using standard zone of inhibition (ZOI) microbiology assay, with a well size of 5 mm diameter and 30  $\mu$ L



**Figure 2.** TEM image of the silver nanoparticles synthesised from *M. oleifera* leaf extract (a, b). A:333nm; B:400nm



**Figure 3.** Antimicrobial activity of silver nanoparticles synthesized from *M. oleifera* leaf extract against pathogens.

**Table 1**

The antimicrobial activity of silver nanoparticle synthesised from *M. oleifera* leaf extract (mm).

| Name of the test organisms | Zone of inhibition |                 |
|----------------------------|--------------------|-----------------|
|                            | Ag nanoparticle    | Reference drugs |
| <i>S. aureus</i>           | 15                 | 19              |
| <i>E. coli</i>             | 9                  | 20              |
| <i>K. pneumoniae</i>       | 6                  | 21              |
| <i>B. cereus</i>           | 7                  | 18              |
| <i>C. albicans</i>         | 9                  | 23              |
| <i>C. tropicalis</i>       | 13                 | 21              |
| <i>C. krusei</i>           | 6                  | 19              |

Reference drug: chloramphenicol /ketoconazole.

of samples. Chloramphenicol / Ketoconazole of 10 mg/mL concentration were used as a control antimicrobial agent. The Ag nanoparticles synthesized showed inhibition zone

against all the test organisms. Maximum zone of inhibition was found to be *Staphylococcus aureus* (*S. aureus*) (15 mm); *Candida tropicalis* (*C. tropicalis*) (13 mm) and minimum of zone of inhibition was found to be *Candida krusei* (*C. krusei*) (6 mm); *Klebsiella pneumoniae* (*K. pneumoniae*) (11 mm) in all the tested organisms (Table 1, Figure 3).

#### 4. Discussion

Antibiotic resistance by the pathogenic bacteria has been observed since last decade; hence, the researchers are focusing on the development of new antibacterial agents. In the present scenario, Ag nanoparticles as antimicrobial agents have come up as a promising candidate in the medical field<sup>[14]</sup>. The extremely small size of nanoparticles exhibits enhanced or different properties when compared with the bulk material. There are different physical and chemical methods for the synthesis of nanoparticles, but there is always a need for the development of eco-friendly route for the synthesis process<sup>[15,16]</sup>. Hence, our current study proves to be an important step in this direction.

Formation and stability of Ag nanoparticles in aqueous colloidal solution are confirmed using UV-Vis spectral analysis. It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations<sup>[17]</sup>. As the *M. oleifera* leaf extract was mixed with aqueous solution of the silver nitrate, it started to change the color from watery to reddish brown due to reduction of silver ion, which indicated the formation of silver nanoparticles. It is generally recognized that UV-Vis spectroscopy could be used to examine size and shape-controlled nanoparticles in aqueous suspensions<sup>[18]</sup>. Figure 1 shows the UV-Vis spectra recorded from the reaction

medium after heating the solution at 80 °C for 20 min. Absorption spectra of silver nanoparticles formed in the reaction media has absorbance peak at 430–440 nm, which correspond to silver and broadening of peak indicated that the particles are polydispersed.

Silver nitrate which is readily soluble in water has been exploited as an antiseptic agent for many decades. Dilute solution of silver nitrate has been used since the 19th century to treat infections and burns. The exact mechanism of the antibacterial effect of silver ions is partially understood. Literature survey reveals that the positive charge on the Ag ion is crucial for its antimicrobial activity. The antibacterial activity is probably derived, through the electrostatic attraction between negative charged cell membrane of microorganism and positive charged nanoparticles<sup>[19]</sup>.

Lee *et al* investigated the antibacterial effect of nanosized silver colloidal solution against *S. aureus* and *K. pneumoniae* after padding the solution on textile fabrics<sup>[20]</sup>. Shrivastava *et al* studied antibacterial activity against *Escherichia coli* (*E. coli*) (ampicillin resistant), *E. coli*, *S. aureus*, and *Salmonella typhi* (*S. typhi*) (multi-drug resistant). They reported that the effect was dose dependent and was more pronounced against gram-negative organisms than gram-positive ones. They found that the major mechanism through which Ag nanoparticles manifest antibacterial properties was either by anchoring or penetrating the bacterial cell wall, and modulating cellular signaling by dephosphorylating putative key peptide substrates on tyrosine residues<sup>[21]</sup>. The antibacterial efficacy of the biogenic Ag nanoparticles reported in the present study may be ascribed to the mechanism described above but it still remains to clarify the exact effect of the nanoparticles on important cellular metabolism like DNA, RNA and protein synthesis.

In conclusion, it has been demonstrated that the extract of *M. oleifera* leaf are capable of producing Ag nanoparticles extracellularly and the Ag nanoparticles are quite stable in solution. The formed silver nanoparticles showed considerable antimicrobial activity compared to the respective antibiotics. This biosynthesis silver nanoparticles prove to be potential candidates for medical applications where antimicrobial activity is essential.

### Conflict of interest statement

We declare that we have no conflict of interest.

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