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RESEARCH ARTICLE

# SILVER NANOPARTICLES OF *MORINGA OLEIFERA* – GREEN SYNTHESIS, CHARACTERISATION AND ITS ANTIMICROBIAL EFFICACY

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**ABSTRACT:**

The traditional medicinal plant *Moringa oleifera* is an integral part of the Indian diet and has notable beneficial effects in its leaves, stems, flowers, roots, bark and seeds. It has reported properties like antimicrobial, anti inflammatory, ant diabetic, anti oxidative, anti tumorogenic amongst many other properties. In the present study we have devised a new green method of synthesis of silver nanoparticles to evaluate its antimicrobial efficacy using the aqueous plant extract as the reductant as well as the stabilizer. A cold method of synthesis of silver nanoparticles using silver nitrate solution is performed. After the synthesis step, the nanoparticles are characterised using UV VIS spectroscopy, scanning electron microscopy and transmission electron microscopy. The results show the incorporation of the silver ions in the extract and also the reduction of the particle size to the nano range. The antimicrobial potential of these nanoparticles synthesized is tested against various gram positive and gram negative strains of bacteria keeping streptomycin as the standard positive control antibiotic. The antibiotic assay is performed using agar well diffusion method and comparable results are obtained in comparison to the standard antibiotic. It is seen that the nanoparticles have good antibacterial efficacy against the tested strains. Hence nanoparticles of *Moringa oleifera*_aqueous extracts can be used as a potential alternative to traditional antibiotics using this non toxic safe way of green synthesis.

**Keywords:** *Moringa oleifera,* silver nanoparticles, antimicrobial

## INTRODUCTION

Nanotechnology is now creating a growing sense of excitement in life sciences especially biomedical devices and biotechnology[1]. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Metal nanoparticles have received significant attention in recent years owing to their unique properties and practical applications[2,3]. Traditionally, the chemical and physical methods used to synthesize silver nanoparticles are expensive and often raise questions of environmental risk because of involving the use of toxic, hazardous chemicals[4]. Also, majority of the currently prevailing synthetic methods are usually dependent on the use of organic solvents because of hydrophobicity of the capping agents used[5]. Recently, the search for cleaner methods of synthesis has ushered in developing bio-inspired approaches. Bio-inspired methods are advantageous compared to other synthetic methods as, they are economical and restrict the use of toxic chemicals as well as high pressure, energy and temperatures[6]. Nanoparticles may be synthesized either intracellularly or extracellularly employing yeast, fungi bacteria or plant materials which have been found to have diverse applications.

The prospect of exploiting natural resources for metal nanoparticle synthesis is a competent and environmentally benign approach. Green synthesis of nanoparticles is an eco-friendly approach which might pave the way for researchers across the globe to explore the potential of different herbs in order to synthesize nanoparticles. In recent times, several groups have been reported to achieve success in the synthesis of Au, Ag and Pd nanoparticles obtained from extracts of plant parts, e.g., geranium leaves, lemongrass, neem leaves, aloe vera and others[7-11]. These researchers have not only been able to synthesize nanoparticles but also obtained particles of exotic shapes and morphologies. The impressive success in this field has opened up avenues to develop “greener” methods of synthesizing metal nanoparticles with perfect structural properties using mild starting materials.

The silver nanoparticles have various important applications in several ways historical; silver has been known to have a disinfecting effect and has been found in applications ranging from traditional medicines to culinary items. It has been reported that silver nanoparticles(SNPs) are non-toxic to humans and most effective against bacteria, virus and other eukaryotic micro-organisms at low concentrations and without any side effects [12.] Plant extracts have shown large prospects in silver nanoparticle (AgNP) synthesis.

*Moringa oleifera* (*M. oleifera*) (Family: Moringaceae, English name: drumstick tree) has been reported to be essentially used as an ingredient of the Indian diet since ages. It is cultivated almost all over India and its leaves and fruits are traditionally used as vegetables[13]. Almost all parts of the plant have been utilized in the traditional system of medicine. The plant leaves have also been reported for its antitumor, cardioprotective, hypotensive, wound and eye healing properties[14].

In the present study, synthesis of AgNPs in cold condition has been reported, reducing the silver ions present in the silver nitrate solution by the aqueous extract of *M. oleifera* leaves. Further, these biologically synthesized nanoparticles were found to be considerably sensitive to different pathogenic bacterial strains tested.

## MATERIALS AND METHODS:

**Plant material and preparation of the extract:** Fresh leaves of *Moringa oleifera* was collected from North 24 Parganas district of west Bengal, India and washed with distilled water thrice. A hot water extract of the leaves was prepared by taking 5g of leaves in 100 mL of distilled water and boiled in Erlenmeyer's Flask for 5 minutes. The extract has a light green colour. The clear extract is obtained by standard filtration method. The extract is stored at $4^0$C for further use and to be used in one week[15].

**Synthesis of Silver Nanoparticles**: 190mL of 1mM silver nitrate solution was added slowly to 10mL of extract with continuous stirring. The mixture was stirred in a magnetic stirrer at room temperature for 4 hours till the colour turns to reddish brown[15,16]. The mixture was centrifuged at 5000 rpm for 20 minutes at room temperature. The supernatant was discarded, pellet re-dispersed in distilled water and lyophilized to dryness and stored at room temperature for further use.

**UV Vis Spectra Analysis:** UV-Vis spectrum of the reaction medium was performed to determine the reduction of pure $Ag^+$ ions at 4 h after diluting the sample (AgNPs) with distilled water. UV-Vis spectral analysis was performed by using Varian makes carry 50 Bio UV-VIS spectrophotometer.

**Scanning Electron Microscopic Analysis:** SEM analysis of the lyophilized AgNPs was performed in, model JSM6360 JEOL make (UK) equipped with an electron probe microanalyzer system. The particles were coated with a gold coating in order to have a good conductivity.

**Transmission Electron Microscope Analysis**: AgNPs was dispersed in double distilled water. A drop of thin dispersion is placed on a "staining mat". Carbon coated copper grid is inserted into the drop with the coated side upwards. After about ten minutes, the grid is removed and air dried. Then screened in JEM 2100 HRTEM JEOL make (JAPAN) Transmission Electron Microscope.

**Micro-organism culture:** Pure bacterial cultures of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli* were grown overnight at 37°C ± 0.5°C, pH 7.4 in a shaker incubator in nutrient broth (NB). Their sensitivity to the reference drug, Streptomycin was also checked.

**Antimicrobial Assay:** Antimicrobial activities of the synthesized Ag nanoparticles were determined, using the agar well diffusion assay method[17]. Fresh inoculom (50µl) of each culture were spread on to NB agar plates. Two wells containning 30 µl of AgNPs and two wells containing 30 µl of the standard antibiotic, Streptomycin were created in each plate. The plates containing the test organism and Ag nanoparticles along with the standard antibiotic 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.

## RESULTS AND DISCUSSION:

UV–Vis spectroscopy is an important technique to establish the formation and stability of metal nanoparticles in aqueous solution[18].The relationship between UV-visible radiation absorbance characteristics and the absorbate's size and shape is well-known. Consequently, shape and size of nanoparticles in aqueous suspension can be assessed by UV-visible absorbance studies[18].

Reduction of silver ions present in the aqueous solution of silver complex during the reaction with the ingredients present in the *Moringa oleifera* leaf extract have been seen by the UV-Vis spectrograph and has been recorded as a function of time with water as reference. Maximum absorbance was seen at 412 nm, indicating that the formation of spherical silver nanoparticles in majority or anisotropic particles whose appearance and ratio increases with time.

It is well known that silver nanoparticles exhibit reddish–brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles[19].

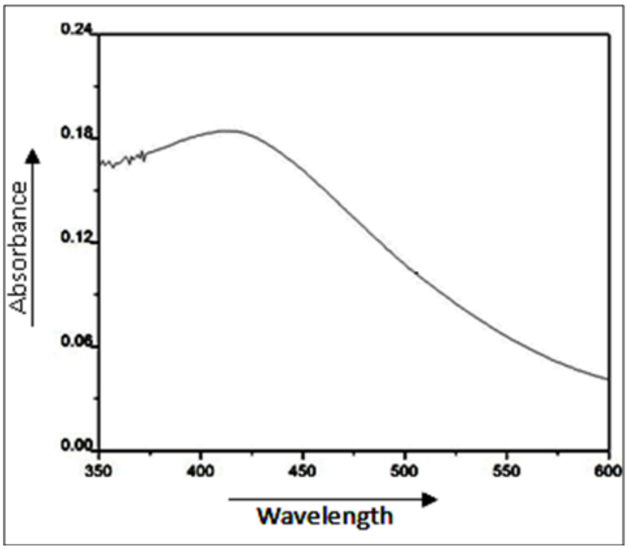


Fig 1: UV Vis spectra of diluted solution of AgNPs at 4hr

The appearances of reddish-brown colour in the reaction vessels suggest the formation of silver nanoparticles.[8,20,21]. (Fig-1).

Silver nitrate is used as reducing agent as silver has distinctive properties such as good conductivity, catalytic and chemical stability. The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol. Research reports indicate that there are three different routes for the reduction of silver in plant extracts. The secondary metabolites present in plant systems may be responsible for the reduction of silver and synthesis of nanoparticles. The second biogenic route is the energy (or) electron released during glycolysis (photosynthesis) for conversion of NAD to NADH led to transformation of $Ag(NO_3)_2$ to form nanoparticles and the another mechanism is releasing of an electron when formation of ascorbate radicals from ascorbate reduces the silver ions[22].

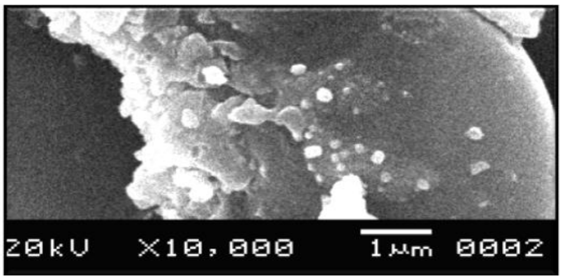


Fig 2: Nanoparticles of *Moringa oleifera* as observed under SEM

SEM analysis shows that *Moringa oleifera* leaf extract has shown the ability to synthesize silver nanoparticles which are roughly spherical in shape(Fig 2). This SEM analysis was performed just after the synthesis of the nanoparticles.

The silver nanoparticles synthesized with the help of *Moringa* leaf extract were scanned using TEM from which we can conclude that roughly the average mean size of Ag nanoparticles was 30 nm and seems to be spherical in morphology as shown in Fig 3(A,B,C). The Ag nanoparticles seem to have formed nanoclusters and are aggregated together. The nanoparticles were seen to be stable and having the same configuration even after 6 months of synthesis as this TEM analysis was done after 6 months of synthesis.

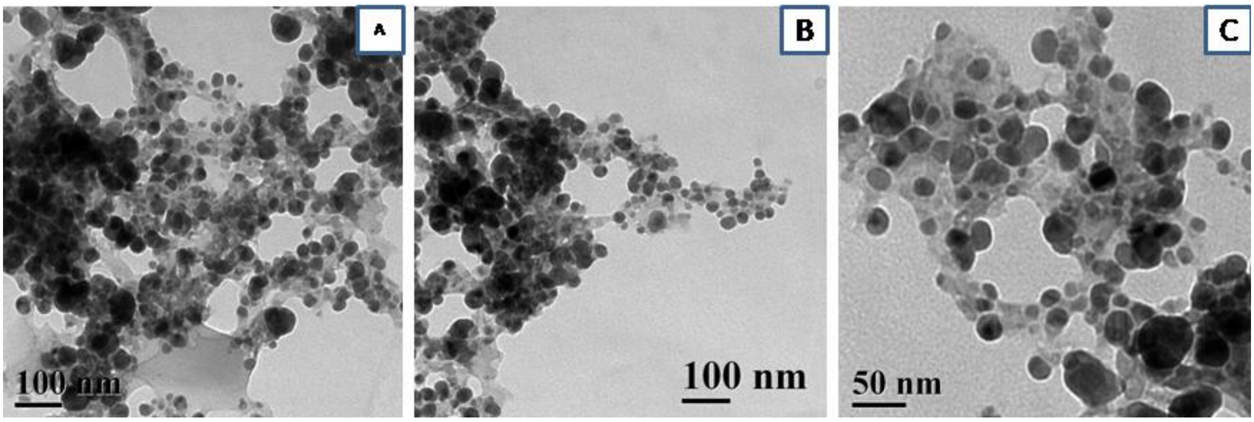


Fig 3(A,B,C): Nanoparticles of Moringa oleifera as observed under TEM.

AgNPs were studied for antimicrobial activity against pathogenic microorganisms by using standard zone of inhibition (ZOI) microbiology assay, with a well size of 5 mm diameter and 30 µL of AgNPs against all the test organisms. The ZOI of the standard antibiotic, streptomycin is also shown for comparison (10 mg/mL concentration).

The Ag nanoparticles synthesized showed inhibition zone against all test organisms. The ZOI of AgNP for *Escherichia coli* (16mm) was greater than that of the standard antibiotic streptomycin (15mm). (Table 1) (Fig 4). In all other cases we can see that the antibacterial efficacy of AgNPs on *Pseudomonas aeruginosa* and *Staphylococcus aureus* is 80% and 70% in case of *Klebsiella pneumoniae* compared to the standard. In these cases it can be said that maybe with increase in the concentration of the silver nitrate solution used the antibacterial potency may also increase.

**Table 1:** Zone of Inhibition of AgNPs synthesised from *M. oleifera* leaf extract

| Bacterial strains | Zone of inhibition(AgNPs) | Zone of inhibition (Standard Drug) |
|---|---|---|
| *Staphylococcus aureus* | 15 | 18 |
| *Escherichia coli* | 16 | 15 |
| *Pseudomonas aeruginosa* | 13 | 16 |
| *Klebsiella pneumonia* | 9 | 13 |

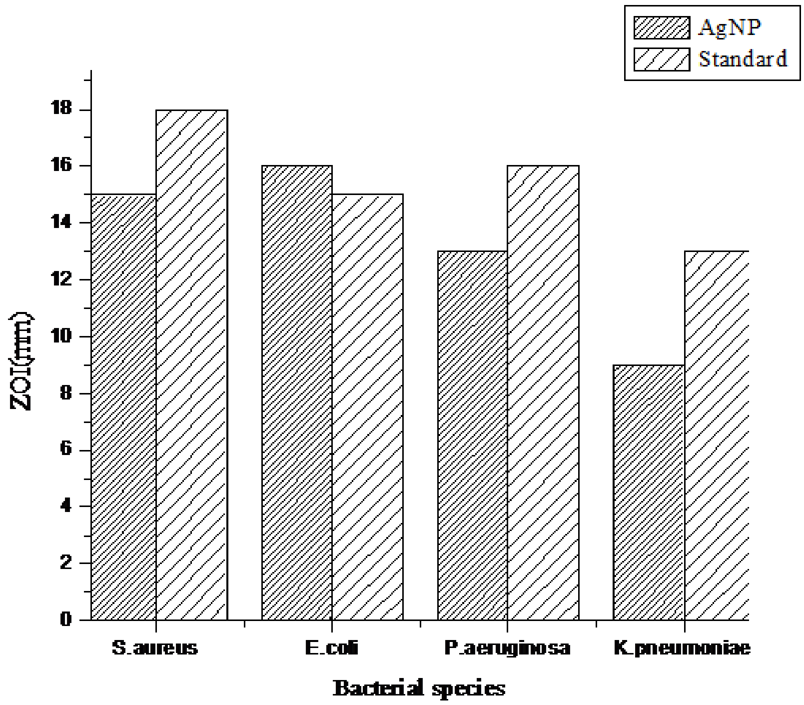


Fig 4: Antibacterial assay of AgNPs

$AgNO_3$ which is readily soluble in water has been exploited as an antiseptic agent for many decades. The exact mechanism of the antibacterial effect of silver ions is not totally understood. Research reports reveal 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 negatively-charged cell membrane of microorganism and positively-charged nanoparticles [23-25].

When the antibacterial activity against *E. coli* (ampicillin resistant), and *S. aureus* (multi-drug resistant) was studied, it was 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 AgNPs 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[26].

## CONCLUSION

The present study presents a non-toxic as well as eco-friendly procedure for synthesizing AgNPs. This technique gives us a simple and efficient way for the synthesis of nanoparticles with good antibacterial properties. Antibiotic resistance by the pathogenic bacteria has been observed since last decade; hence, the researchers are focusing on the development of new antibacterial agents which can overcome such resistance. Hence, our current study proves to be an important step in this direction.

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