Green synthesis and characterization of silver nanoparticles using stalk extract of *Moringa oleifera* and its Antibacterial activity

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Abstract

Green synthesis of metal nanoparticles is a greatly developing area of research. Metallic nanoparticles have received enormous attention from chemists, physicists, biologists, and engineers who wish to use them for the development of a new-generation of nano devices. In this study, silver nanoparticles were biosynthesized from aqueous silver nitrate through a simple and eco-friendly route using *Moringa oleifera* stalk extracts, which acted as a reductant and stabilizer simultaneously. Characterizations of nanoparticles were done using different methods, which included UV-visble spectroscopy, FTIR, SEM and Antibacterial activity. The ultraviolet-visible spectrum of the aqueous medium containing silver nanoparticles showed an absorption peak at around 450 nm. The most needed outcome of this work will be the development of value-added products from *Moringa oleifera* for biomedical and nanotechnology-based industries. The present research opens a new avenue for the green synthesis of nanoparticles.

Key words

FTIR, *Moringa oleifera*, SEM, Silver Nanoparticles, UV-visble.

1. Introduction

Nanotechnology is a latest field of modern research dealing with synthesis, design and treatment of nano sized particles. Nanotechnology has gained remarkable importance in state of the art techniques for health care, food technology, cosmetics, surroundings health procedure, optics, chemical industries, biomedical, electronics, space commerce, energy science, drug-gene delivery, optoelectronics, catalysis, light emitters, nonlinear optical devices, single electron transistors, and photo electrochemical applications etc. Present, silver nanotechnology, is becoming popular due to its above wide applications and characteristic properties (e.g. size and shape dependent several property such as, optical, compelling and electrical properties), which can be

included into biosensor materials, antimicrobial applications, composite fibres, cosmetic products, cryogenic superconducting materials, and electronic components. Nanomaterials have a long list of applicability in improving human life and its location. 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 nano science and technology before the term 'nano' was even formed. Current science has immediately happening exploring nano science in the 21st century the wide applications have involved the consideration of scientists to produce them by diverse methods. The above substance methods are dreary, more time uncontrollable and expensive. The scientists, therefore, have now recently found easier ways of biological methods to prepare Ag nanoparticles through green nano route using plant extract intervention for compound reduction of AgNO3. The method allows undergoing highly controlled and hierarchical assembly. It also provides advantage of being cost effective and environment friendly. There is no need to use high heat, high weight or toxic. Silver nanoparticles are better than silver based compounds and silver ions kill microbes.

2. Material and methods:

Material

The stalk of *Moringa oleifera* is collected from Vengalapuram village, Tirupattur TK, Vellore district, Tamilnadu, India. AgNO3 was purchased from Avra synthesis private limited, Hyderabad.

Table :1 Systematic Position of *Moringa oleifera*

Kingdom	Plantae
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Rosids
Order:	Brassicales
Family:	Moringaceae
Genus:	*Moringa*
Species:	***M. oleifera***

Figure: 1. *Moringa oleifera* leaf &Stalk

Methods

Preparation of stalk extract

Moringa oleifera stalks were collected from the local area. All glass wares and the *Moringa oleifera* stalks were washed properly with double distilled water. 20g of *Moringa oleifera* stalks was weighed and added 100 ml of double distilled water in 250 ml Erlenmeyer flask and boiled for 10 minutes at 60°C. With the help of Whatman filter paper (NO.1), the boiled materials were filtered to get aqueous Stalk extract was used for the further process.

Synthesis of Silver Nanoparticles

1mM aqueous solution of silver nitrate was prepared for 100 ml of double distilled water. To this 5 ml of filtrate was added in 250 ml conical flask and kept for 24 hours incubation at 37ºC with intermittent shaking. After 24 hours the brown color development indicated the formation of silver nanoparticles. The separation of silver nanoparticles from the dispersion was carried out by centrifugation, a black precipitate was obtained that was dried overnight in a hot air oven at 37°C and used for the characterization studies.

Characterization Studies

UV-Visible spectroscopy analysis

In order to study the formation of AgNPs, the UV-Visible **(**UV-vis) adsorption spectrophotometer was used. UV-visible spectral analysis was performed using Perkin Elmer lambda 35 model spectrophotometer. The UV-vis spectrum was recorded at room temperature using the quartz cuvette with a UV-visible spectra wavelength range 200-800 nm.

***FTIR* analysis**

FTIR measurement was carried out using Perkin Elmer Spectrum RX-1 FT-IR Spectrometer to investigate possible bio reducing agents present in the silver nanoparticles, after complete reduction of silver nitrate ions by Moringa oleifera stalk extract.

SEM *analysis*

SEM analysis was carried out using ZEISS EVO 18 SEM machine. SEM was used to record the micrograph images of synthesized Ag NPs. Thin films of the sample were prepared on a carbon coated copper grid by just dropping small amount of the sample on the grid and then the film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5min. The size and morphology of nanoparticles were examined.

Antibacterial Activity

The silver nanoparticles synthesized using *Moringa oleifera* stalk extract was tested for antimicrobial activity by well diffusion method against *Escherichia coli, Staphylococcus aureus* and *Salmonella typhi* and approximately 20ml of molten and cooled media (Mullar-Hinton agar) was poured into sterilized petri dishes. The plates were left at room temperature to solidify. The bacterial test organisms were grown in nutrient broth. 0.1ml of different pathogenic bacterial suspension was spread on Muller-Hinton agar plates. 5mg of synthesized silver nanoparticles was dissolved in 1ml of 5 % DMSO then poured into the well in different concentration then the plates were incubated at 37˚C for 24 hrs and the zone of inhibition was measured in mm.

3. RESULTS AND DISCUSSION:

Various methods have been employed for the synthesis of silver nanoparticles such as chemical and biological methods. Presently, synthesis of silver nanoparticles using plant material is getting more popular [10-12]. In this study, when we are adding the *Moringa oleifera* stalk extract to the aqueous solution of the 1mM silver nitrate the color of the reaction medium changed rapidly from colorless to brown. Similar results were shown by early workers [9]. The brown color indicated the formation of silver nanoparticles with the reduction of silver ion, whereas the control AgNO3 solution did not show any color change (Figure 2).

Figure 2: During the Color Changing

Figure 3, showed the UV-Vis spectrum of synthesized silver nanoparticles with the help of *Moringa oleifera* stalk extract as a reducing agent. Absorption bands were observed at 450nm after 24 hrs incubation. Similar results were shown by early workers [9]. Mostly, most of the papers were reported the wavelength range of AgNPs absorption peak is in the range of 440-460 nm. In our work, solution showed absorbance peak near at 450 nm as shown in Figure 3.

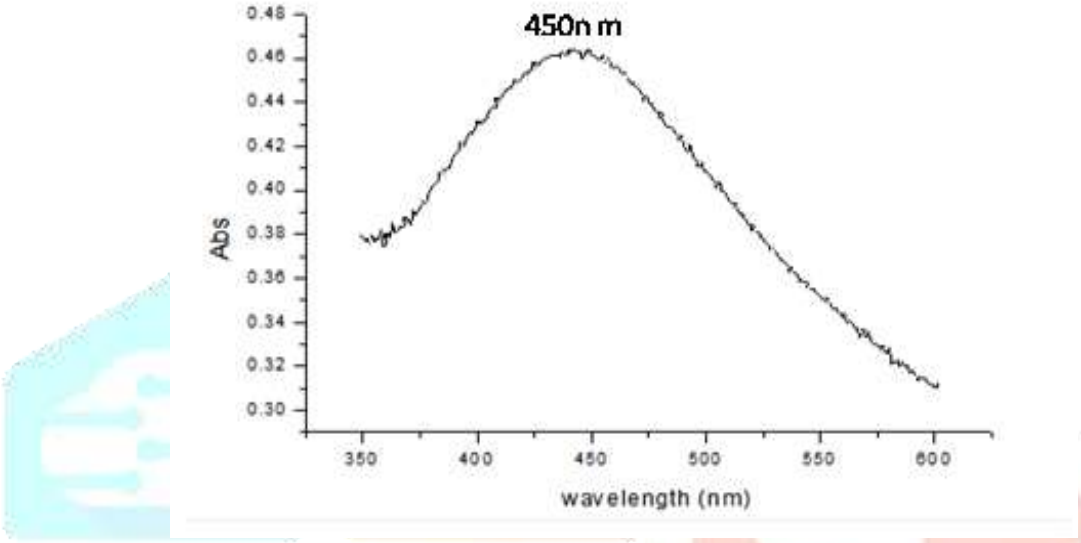

Figure 3: UV –Vis Absorption Spectra of AgNPs

The FTIR spectrum recorded from the biologically synthesized Ag nanoparticles is shown in Figure.3. In FTIR analysis bands are observed at 3299.79 cm^{-1},1233.89 cm^{-1},2851.32 cm^{-1}, 2090.72 cm^{-1}, 1649cm^{-1},1454cm^{-1},1384.67 cm^{-1}, 1233.89 cm^{-1}, 1077.28 cm^{-1}, 1030.78 cm^{-1}, 829.77 cm^{-1}, 719.16 cm^{-1}, 700.32 cm^{-1}, 658.48 cm^{-1} and 611.70 cm^{-1}. The bands are observed at 3299.79 cm^{-1}, 1030.78 cm^{-1}, 1077.28 cm-1, 1649 cm^{-1} and 1233.89 cm^{-1} corresponds to N-H bending and C-N stretching of secondary amide and aliphatic amine. 829.77cm^{-1}, 719.16 cm^{-1}, 700.32 cm^{-1}, 658.48 cm^{-1} and 611.70 cm^{-1} denotes Aromatic C-H. The FT-IR spectra of silver nanoparticles confirmed the presence of amines which have the ability to form a layer covering the silver nanoparticles to prevent their agglomeration and these molecules can act both as reducing and capping agent in the synthesis of silver nanoparticles.

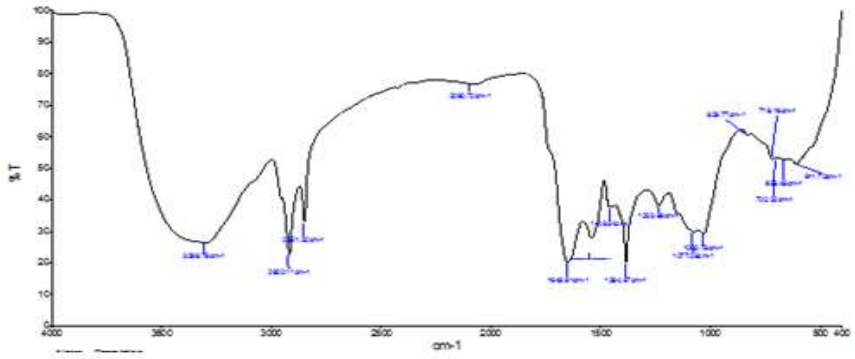

Figure 4: FTIR Analysis of AgNPs

Morphology study of green synthesized silver nanoparticles was carried out with the help of scanning electron microscopy (SEM). Images of the surface morphology of the biosynthesized nanoparticles were clearly indicated that bio synthesized AgNPs were roughly spherical in shape and uniformly distributed and accumulation was found in AgNPs as shown in Figure 4. It can also be observed that few nanoparticles have slightly deviated from their shape from spherical, may be due to the presence of different group of natural chemicals which helps in reducing and stabilizing the nanoparticles during their initial stage and the average size of AgNPs was obtained of 79 nm.

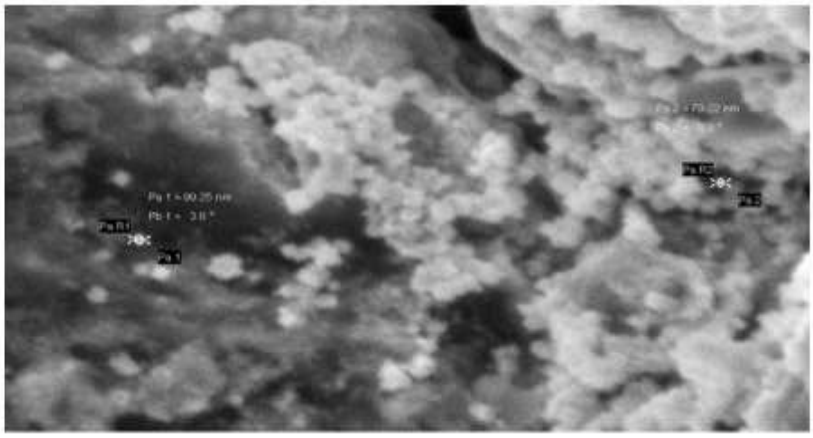

Figure 5: SEM Analysis of AgNPs

The antimicrobial activity of synthesized silver nanoparticles was determined against two Gram negative bacteria and one Gram positive bacteria such as Escherichia coli and *Salmonella typhi and Staphylococcus aureus* by well diffusion method showed in Table:2. Silver nanoparticles exhibited antibacterial activity against *Escherichia coli, Staphylococcus aureus* and *Salmonella typhi* as it showed a clear inhibition zone at concentration of 150µl contains 750µg of silver nanoparticles. This reported point out that silver nanoparticles synthesized from stalk extract of *Moringa oleifera* showed potential antimicrobial activity against pathogenic bacteria.

Table 2: Antibacterial activity of biologically synthesized Silver Nanoparticles

S. No	Organisms	Zone of inhibition in 50µl (mm)	Zone of inhibition in75µl (mm)	Zone of inhibition in100µl (mm)	Zone of inhibitin in 125µl (mm)	Zone of inhibition in150µl (mm)
1.	Escherichia coli	Nil	Nil	Nil	12mm	17mm
2.	*Staphylococcus aureus*	Nil	Nil	15mm	23mm	38mm
3.	*Salmonella typhi*	Nil	Nil	30mm	13mm	32mm

Conclusion:

In this work we have reported the new and simple method for the green synthesis of silver nanoparticles which offers a valuable contribution in the area of green synthesis and nanotechnology without adding different physical and chemical steps. *Moringa olifera* stalk extract were prepared and successfully employed for the development of silver nanoparticles with spherical shapes. The formation of silver nanoparticles using *Moringa olifera* stalk extract was confirmed by UV-Visible spectroscopy, FTIR, and SEM. In UV- Visible spectroscopy, the biosynthesized silver nanoparticles showed an absorption peak at 450 nm. The FT-IR spectra of silver nanoparticles confirmed the presence of amines which have the ability to form a layer covering the silver nanoparticles to prevent their agglomeration. This shows the unique character of biological molecules that can act both as reducing and capping agent in the synthesis of silver. The morphology and size of the silver nanoparticles were examined by SEM analysis. All the silver nanoparticles are found in average size of 79nm. Present investigation also performed that the synthesized silver nanoparticles are exhibited toxic effect on bacterial pathogens Antibacterial studies of silver nanoparticles was highly effective with different inhibition zone capability against two Gram negative and one Gram positive bacteria. Silver nanoparticles showed high antibacterial activity against *Salmonella thyphi Escherichia coli* and *Staphylococcus aureus*. The plant mediated methods of synthesizing nanoparticles have proved to be one of the best methods till date. This is due to its environment-friendly nature.

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