

SILVER NANOPARTICLES OF MORINGA OLEIFERA GREEN SYNTHESIS CHARACTERISATION AND ITS ANTIMICROBIAL POTENCY

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INTRODUCTION

Nanotechnology is now creating a growing sense of excitement in life sciences especially biomedical devices and biotechnology1. 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 applications2,3. 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 chemicals4. Also, majority of the currently prevailing synthetic methods are usually dependent on the use of organic solvents because of hydrophobicity of the capping agents used5. 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 temperatures6. 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 others7-11. 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 *Copyright* © 2017, Scholarly Research Journal for Interdisciplinary Studies

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 vegetables13. Almost all parts of the plant have been utilized in the traditional system of medicine. The plant leaves have also beenreported for its antitumor, cardioprotective, hypotensive, wound and eye healing properties14. 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 40C for further use and to be used in one week15.

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 brown15,16. 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 $37^{\circ}C \pm 0.5^{\circ}C$, 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 method17. Fresh inoculom (50µl) of each culture were spread on to NB agar plates. Two wells containing 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 studies18. 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 nanoparticles19.

The appearances of reddish-brown colour in the reaction vessels suggest the formation of silver nanoparticles.

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.

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