



Eco-Friendly Green Synthesis and Characterization of Silver Nanoparticles from Aqueous Extract of Solanum Nigrum and Its Antifungal Activity

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ABSTRACT

In the present study, the aqueous extract of Solanum Nigrum used to synthesize AgNPs by the reduction of aqueous silver metal ions. The synthesized silver nanoparticles were characterized by UV spectroscopy, FTIR, XRD, SEM and TEM image shows uniformly distributed spherical, hexagonal and triangular in shape nanoparticles. The antifungal effects of these nanoparticles were studied against Aspergillus fumigatus, Aspergillus nidulans, and Candida albicans. Transmission electron microscopy (TEM) measurements indicated that the newly formed nanoparticles were polydispersed with spherical, triangular, tetragonal, pentagonal, hexagonal and rod shapes. Crystalline nature of the nanoparticle in the face centered cubic (fcc) structure are confirmed by the peaks in the XRD pattern corresponding to (101), (98), (111) planes. Fourier Transform Infra-Red (FT-IR) spectroscopy analysis showed that the synthesized nanoparticles were capped with bimolecular compounds which are responsible for the reduction of silver ions. The present study indicates that Ag-NPs have considerable antifungal activity in comparison with standard antifungal drug, and hence further investigation for clinical applications is necessary.

KEY WORDS: Solanum Nigrum; green synthesis; silver nanoparticle.

INTRODUCTION:

Nanobiotechnology is the branch of science dealing between Nano science and biotechnology involving the application of biological systems for the production, manipulation and design of new functional nano sized materials with dimension ranging from 1-100 nanometer or one billionth. It combines biological methods with physical and chemical procedures to generate nano-sized particles with unique functions. The synthesis of nanoparticles using green technology is advantageous over chemical agents owing to their environmental anxieties.[1, 2] There is significant to produce inorganic nanoparticles as they supply greater material properties with effective

resourcefulness. Owing to their properties and significance over accessible chemical imaging drugs, inorganic nanoparticles have been studied as possible material for medical imaging along with for treating diseases. Silver nanoparticles have been using in medicine for antibacterial, antifungal, anti-viral, anti-inflammatory therapy and anticancer therapy.[3] Unique properties mostly depend on the size, shape and strange surface area to volume ratio with reducing size is more effective to high catalytic and antibacterial activity.[4]

Definite size organized metallic nanoparticles have been produced using physical and chemical methods. Though, these procedures utilize toxic chemicals as reducing agents,



organic solvents, or non-biodegradable stabilizing agents and are therefore possibly harmful to the environment and biological systems.[5] Consequently, there is a growing need to progress ecofriendly methods for nanoparticles synthesis without using toxic chemicals. At present biosynthesis of nanoparticles has been projected as a cost effective and eco-friendly alternative to physical and chemical methods.[6] Biosynthesis of nanoparticles using plant extracts is the significant method of green, eco-friendly production of nanoparticles and exploited to a vast extent since the plants with a choice of metabolites, are extensively distributed, easily available, and safe to handle.[7]

MATERIAL AND METHODS:

Plant Material

SolanumNigrum or Black nightshade is a common herb or short-lived perennial shrub, found in many wooded areas, as well as disturbed habitats. It reaches a height of 30 to 120 cm (12 to 48 in), leaves 4.0 to 7.5 cm (1.5 to 3.0 in) long and 2 to 5 cm (1.0 to 2.5 in) wide; ovate to heart-shaped, with wavy or large-toothed edges; both surfaces hairy or hairless; petiole 1 to 3 cm (0.5 to 1.0 in) long with a winged upper portion. The flowers have petals greenish to whitish, recurved when aged and surround prominent bright yellow anthers. The berry is mostly 6 to 8 mm (0.3 to 0.8 in) in diam., dull black or purple-black.[8] In India, another strain is found with berries that turn red when ripe.[9]

Collection and Preparation of Plant Materials

Fresh leaves of *Solanumnigrum* plant, free from contaminations were accumulated and washed 2-3 times with tap water and once with sterile water. After cleaning, it was dried in shade at room temperature for two weeks. The *Solanumnigrum* were initially rinsed thrice in distilled water and dried on paper toweling, and samples (25 g) were cut into fine pieces and

boiled with 100 ml of sterile distilled water for 5 min. The crude extract was passed through Whatman No.1 filter paper and the filtrates were stored at 4°C for further use.

Synthesis of Silver Nanoparticles

0.1mM of silver nitrate (AgNO_3) was prepared and utilized for the union of silver nanoparticles. 5mL of leaf concentrate of *Solanumnigrum* was added to 45mL of 0.1mM AgNO_3 for bioreduction process at room temperature.

Characterization of Silver nanoparticles

The colour change in reaction mixture (metal ion solution + seaweed extract) was recorded through visual observation. The bioreduction of Ag ions in aqueous solution was monitored by periodic sampling of aliquots (0.5 ml) and subsequently measuring UV-Vis spectra (200 to 800nm) of the solution. UV-Vis spectra of these aliquots were monitored as a function of time of reaction on UV-Vis spectrophotometer.

To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 5000 rpm for 10 min. The supernatant was again centrifuged at 10000 rpm for 60 min and the pellet was obtained. This is followed by redispersion of the pellet of AgNPs into 1 ml of deionized water. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by FTIR Nicolet Avatar 660 (Nicolet, USA). Energy Dispersive X-ray Spectroscopy. In this study it was used to confirm that the nanoparticle suspension contains nothing but silver.

The Ag-NPs solution thus obtained was purified by repeated centrifugation at 5000 rpm for 20 min followed by re dispersion of the pellet of Ag-NPs in 10ml of deionized water. After freeze drying of the purified Ag- NPs, the structure and composition were analyzed by XRD and SEM. The dried mixture of Ag-NPs

was collected for the determination of the Ag-NPs formation by an X' Pert Pro x-ray diffractometer (PAN analytical BV, The Netherlands) operated at a voltage of 40kV and a current of 30mA with Cu K α radiation in a θ - 2 θ configuration. SEM analysis was done using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon 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 SEM grid were allowed to dry by putting it under a mercury lamp for 5 min. The structural characterization of the silver nanoparticles was carried out by Transmission Electron Microscopy (TEM). The sample was prepared by air-drying drops of diluted solutions of the preparations on carbon films supported by copper grids.[10]

Antifungal activity

Antifungal activity was observed using standard well diffusion method against human pathogenic fungal cultures: *Aspergillusfumigatus*, *Aspergillusnidulans*, *Canididaalbicans*. The Potato dextrose Agar (PDA) plates were prepared, sterilized and solidified, after solidification fungal cultures were swabbed on these plates. The sterile discs were dipped in silver nanoparticles solution (10mg/ml) and placed in the agar plate and kept for incubation for 7 days. After 7 days zone of inhibition was measured. [11]

RESULTS:

Green Synthesis of Silver Nanoparticles

SolanumNigrum was used for the synthesis of silver nanoparticles. Reduction of AgNO₃ was visually evident from the colour change of the reaction mixture. The color of the solution changed from pale yellow to brownish color shown in (Figure 1). Formation of silver nanoparticles by reduction of the aqueous silver during exposure to the extract of *SolanumNigrum* were confirmed by the formation of brownish colour after a week of synthesis which was confirmed by UV-Vis spectroscopy. The colour arises as a result of the excitation of surface plasmon vibration in the silver nanoparticles. This is due to the excitation of surface Plasmon resonance effect and the reduction of silver ions.[12]

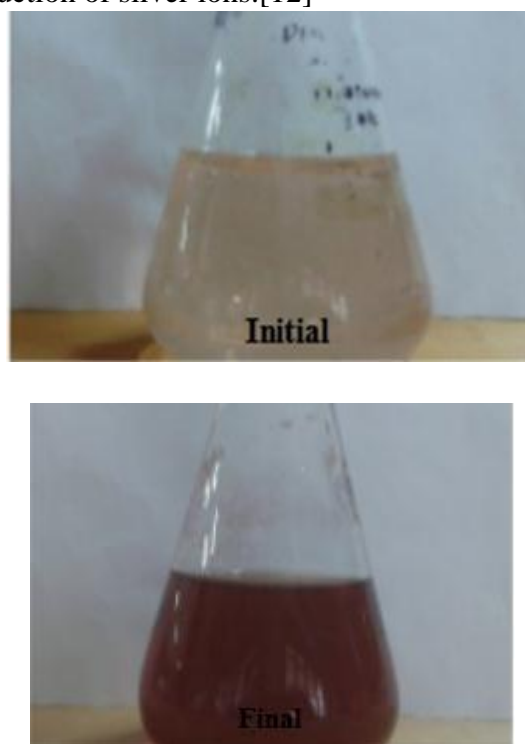


Fig.1. Initial and final visual observation of synthesis of silver nanoparticles from *Solanumnigrum*.

UV-Visible spectroscopy analysis:

In UV-V is spectra recorded for the response arrangement of decreased silver nitrate by leaf concentrate of *Solanumnigrum* (Figures 2). The most extreme absorbance top was seen 419nm.[13]

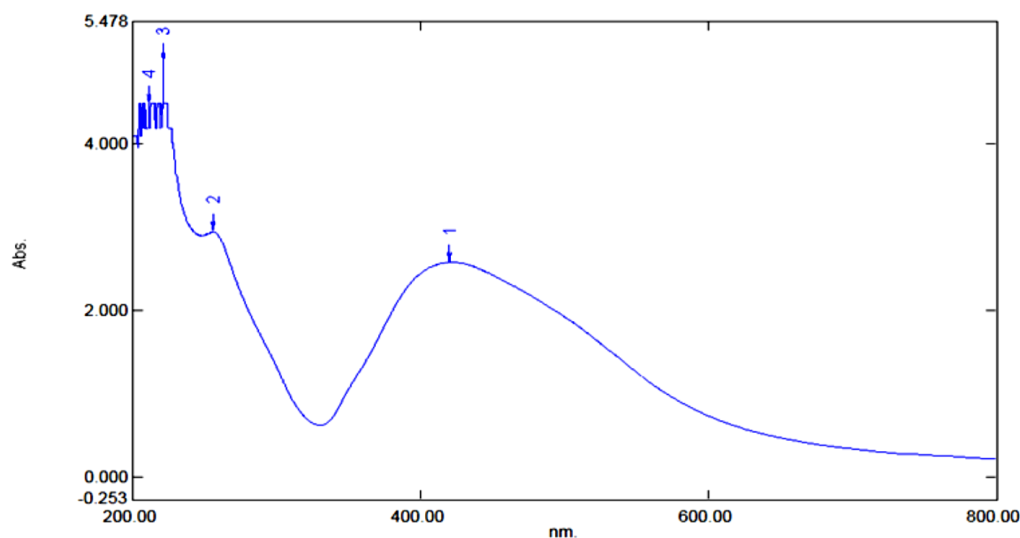


Figure 2: UV-VIS spectroscopy for silver nanoparticles incorporated utilizing *Solanumnigrum* leaves separates.

FTIR spectroscopy analysis

FTIR analysis was used for the characterization of the extract of *Solanumnigrum* and the resulting silver nanoparticles. FTIR absorption spectra of water soluble extract before and after reduction of Ag⁺ ions indications the capping ligand of the silver nanoparticles may be an aromatic compound or alkanes and carboxyl group exhibited in (Figure 3).

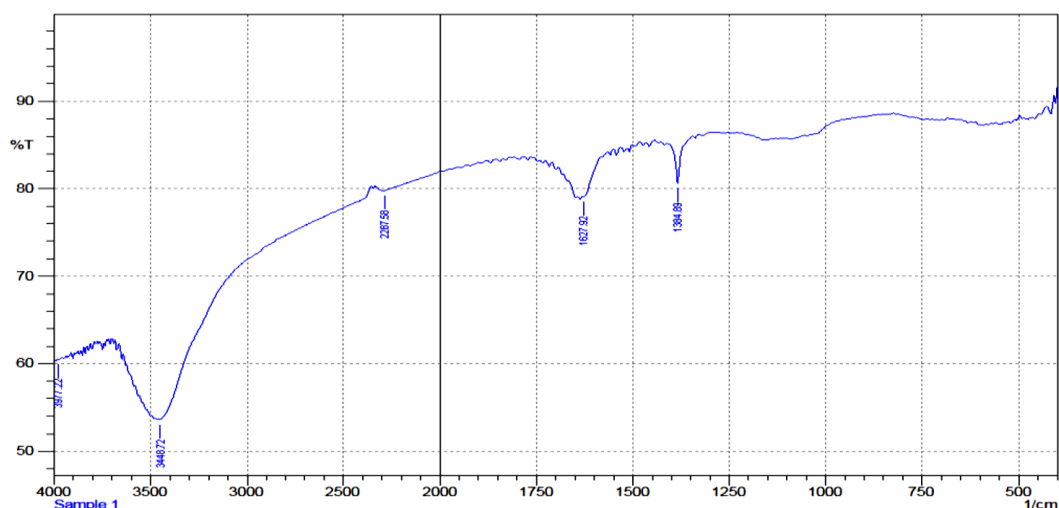


Figure 3: FTIR range of silver nanoparticles combined by utilizing the leaf concentrate of *Solanumnigrum*.

The absorbance band was observed at 1384.88 (cm⁻¹) and 1627.92 (cm⁻¹) assigned to the C-H and C=C stretch alkanes group respectively. The band at 2207.58 (cm⁻¹) corresponding to the S-H bend

Mercaptans group. The band seen at $3448.72 \text{ (cm}^{-1}\text{)}$ corresponding to the O-H stretch Carboxylic acids group. The result revealed that the capping ligand of the silver nanoparticles may be an aromatic compound or alkanes. From the analysis of FTIR studies we confirmed that the carbonyl group from the amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly from the metal nanoparticles (i.e., capping of silver nanoparticles) to prevent accumulation and thereby stabilize the medium. This recommends that the biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium. [14]

X-Ray Diffraction analysis

X-ray diffraction analysis of the synthesized silver nanoparticles was observed from 10° to 90° at 2θ angles in the XRD spectrum. The intensive peaks were observed at 32.00 , 27.49 and 31.14 in the spectrum with their corresponding lattice plane values (101), (98) and (111) respectively which indicates the synthesized silver nanoparticles were crystalline in nature in the reaction mixture is represented in the (Figure 4).

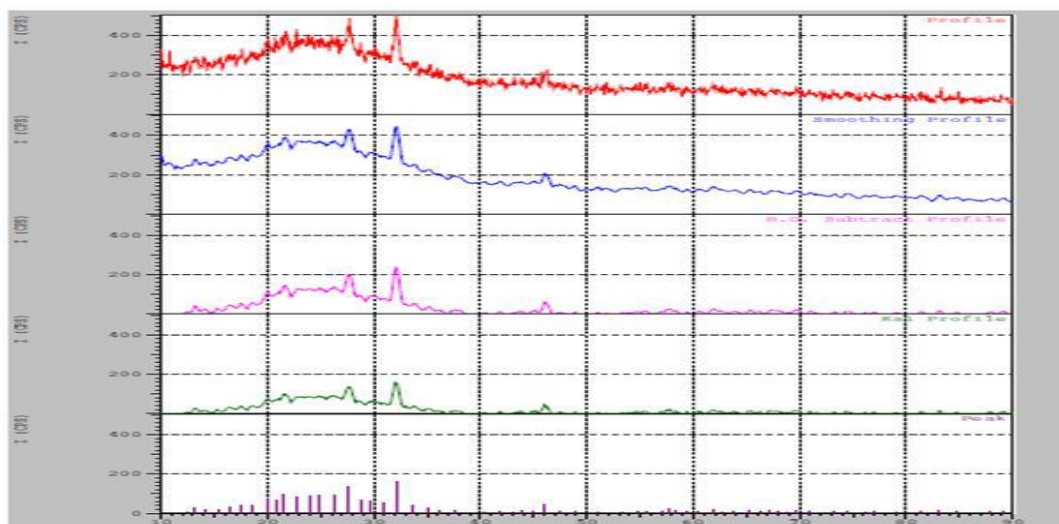


Figure 4: XRD example of silver nanoparticles combined utilizing *Solanum nigrum* leaf additional.

The XRD pattern showed intense peaks in the whole spectrum of 2θ values ranging from 10 - 80 . The intense peaks observed in the spectrum agree to the Bragg's reflection of the silver nanocrystals reported in the literature. The typical XRD pattern revealed that the samples contains (crystalline structures) of the silver nanoparticles.[15]

Scanning electron microscopy (SEM)

The SEM image showing the high density Ag-NPs synthesized by the *Solanum nigrum* further confirmed the development of silver nanostructures.

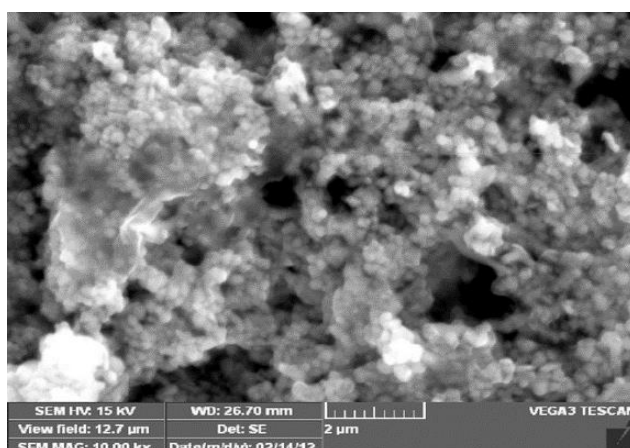


Figure 5: SEM micrograph of silver nanoparticles incorporated by utilizing the leaf concentrate of *Solanumnigrum*.

The SEM micrographs of nanoparticles obtained in the filtrate showed that Ag-NPs are crystalline structure are also shown in (Figure 5), with an average size of about 267.1-485.7nm. It is clear from the SEM that the air dried silver particles from the bio reduced colloidal suspensions measured 364.5nm in size. The SEM micrographs of nanoparticles obtained in the filtrate showed that Ag-NPs are spherical shaped, well distributed without aggregation in solution.[16]

Transmission Electron Microscopy (TEM)

The TEM image showing the high density Ag-NPs synthesized by the *Solanumnigrum* further confirmed the development of silver nanostructures. A transmission electron microscope was engaged to analyze the structure of silver nanoparticles that were formed. The morphology and size of the particles is determined by the TEM images and they are shown in (Figure 6).

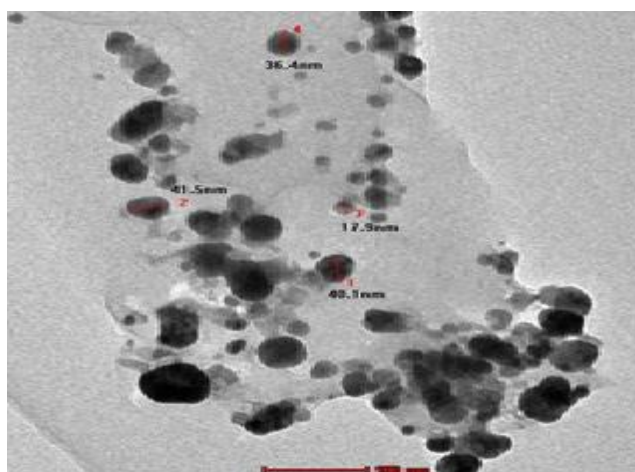


Fig.6. TEM micrograph of silver nanoparticles synthesized from *Solanumnigrum*.

The particles formed were spherical, hexagonal and triangular in shape. The nanotriangles formed where shown to have high surface area. The nanoparticles formed were in the range of 12-46 nm in size.[17]

Antifungal activity of the silver nanoparticles

The antifungal activity of the silver nanoparticles synthesized from *Solanumnigrum* was investigated against the following pathogenic fungi: *Aspergillusfumigatus*, *Aspergillusnidulans*, *Canididaalbican* by well diffusion method, the zone of inhibition around the well was measured and is represented in (Figure 7).



Fig. 7. Antifungal activity of silver nanoparticles synthesized by the reduction of silver nitrate with the *Solanumnigrum* extract against selected fungal pathogens.

Further the nanoparticles synthesis by green method by using *Solanumnigrum* extract was found highly active against tested fungal species at a concentration of 50µl of synthesized Ag nanoparticles. The results showed higher antifungal activity against *Aspergillusfumigatus* (23.4 vs. 21.2) and *Aspergillusnidulans*, (19.2 vs. 17.3), whereas moderate activity was revealed against *Canididaalbican* (21.15 vs. 17.0), when compared with standard antifungal agent Clotrimazole. The evaluation of antibiotic resistant pathogenic fungi has stimulated the search for effective antifungal agent from alternative sources. Many studies have shown the antimicrobial effects of Ag-NPs.[18] However, only limited studies supports the effects of Ag-NPs against fungal pathogens. Thus the silver nanoparticles synthesized from *Solanumnigrum* seem to be promising and effective antifungal agent against the pathogenic fungal strains.

Conclusion

A basic need in the field of nanotechnology is the advancement of a dependable and eco-accommodating procedure for union of metallic nanoparticles. Nanoparticles are being seen as basic building pieces of nanotechnology. Silver nanoparticles assume a significant part in the field of science and drug because of their alluring physiochemical properties. In the present study, we have shown that utilization of a characteristic,

minimal effort organic diminishing specialists and *Solanumnigrum* leaves concentrates can deliver metal nanostructures, through effective green nanochemistry procedure, evading the vicinity of dangerous solvents and waste. The biosynthesized silver nanoparticles utilizing *Solanumnigrum* leave remove turned out to be brilliant against nosocomial pathogens furthermore contrasted with the way that the silver nanoparticles of *Solanumnigrum* against fungal pathogens. The present study



demonstrated a straightforward, quick, and practical course to combine silver nanoparticles. The utilization of *Solanumnigrum* has the additional point of interest this plant can be utilized by nanotechnology handling commercial enterprises. Arranged nanoparticles can be utilized as bactericidal and, in wound recuperating, water decontamination, furthermore in the field of pharmaceutical because of these applications, this technique is possibly energizing for the huge scale union of nanoparticles.

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