

Green synthesis and Characterization of Silver nanoparticles from *Nigella sativa* and its application against UTI causing Bacteria

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Abstract

Synthesis of silver nanoparticles using seeds of *Nigella sativa* as reducing agent was evaluated in this study. Homogenized extract of *N. sativa* exposed to sunlight gave a better synthesis of nanoparticles when compared to other methods. Antibacterial activity of nanoparticles was studied against urinary tract infection (UTI) causing bacteria by disc diffusion method. Further, the silver nanoparticles were characterized by UV, XRD, FTIR, SEM and EDAX. The findings suggest that silver nanoparticles from seeds of *N. sativa* may be effectively used against UTI causing bacteria.

Keywords: Silver nanoparticles, *Nigella sativa*, reducing agent, antibacterial activity, urinary tract infection.

Introduction

Nano, a scientific term used for determining the size of the particle (Albrecht *et al.*, 2006). Nanotechnology, a concept in the field of science and technology, in recent years, has also been likely to grow based on their demand, like other technologies. Nanoparticles are usually a cluster of atoms ranging between 1-100 nm in size and they exhibit new properties based on their size, distribution and morphology (Satyavani *et al.*, 2011). Many materials are synthesized in nano size for various applications including medicine, mechanical, biomedical and electronics (Kalimuthu *et al.*, 2008; Smitha *et al.*, 2008).

Metals are commonly used for synthesis of nanoparticles by chemical and biological methods. The chemical method usually involves use of chemicals for synthesis of nanoparticles which makes them certainly unsuitable against any application as it contains toxic compounds. Some chemical methods cannot avoid the use of chemicals, therefore use of noble metals like silver are into practice for synthesis of nanoparticles. An alternative, ecofriendly and advantageous approach to chemical method is the biological method. Synthesis of nanoparticle by biological method is through microbes like *Aspergillus flavus* (Vigneshwaran *et al.*, 2007), *Phoma exigua* (Karmakar *et al.*, 2010), *Pseudomonas* spp. (Silambarasan and Jayanthi, 2013) and plant sources such as *Chenopodium album* (Dwivedi and Gopal, 2010), *Acalypha indica* (Krishnaraj *et al.*, 2010), *Diopyros kaki* (Song and Kim, 2008), *Cynodon Dactylon* (Supraja *et al.*, 2013), *Glycyrrhiza glabra* (Dinesh *et al.*, 2012) *Nigella sativa* etc.

By modifying the shape and reducing the size to up to 100 nm, it is possible to increase the properties of the source material against various applications (Sivaranjani and Meenakshisundaram, 2013). Researchers in the field of nanotechnology are finding that metal nanoparticles have all kinds of previously unexpected benefits. They are usually prepared from noble metals, that is, silver, gold, platinum and palladium while silver nanoparticles (AgNPs) being most exploited (Roy and Barik, 2010), because of its wider range of application in medicine, electronics, energy saving, environment, textile, cosmetics, biomedical, etc. Though biological method is commonly adopted for the synthesis of silver nanoparticles, use of plant extracts is widely studied due to its advantages over others. A number of investigations had emphasized the antimicrobial effect of nanoparticles synthesized from plants and was found helpful in treatment against UTI causing bacteria usually *Escherichia coli* and *Staphylococcus aureus* showing zone of inhibition.

Among different plants, the seeds of *Nigella sativa* had shown to exhibit various medicinal properties such as antidiabetic (Hader *et al.*, 1993), anti-allergic (Kalus *et al.*, 2003), anti-inflammatory, antibacterial, antioxidant and anticancer activity (Bourgou *et al.*, 2012). But till date, up to our knowledge, there is no report on synthesis of silver nanoparticles from *Nigella sativa*. Hence, the present study was deliberately aimed with a simple and an effective approach of synthesizing silver nanoparticles using seeds of *Nigella sativa* as a reducing agent and to treat against UTI causing bacteria.

Materials and methods

Preparation of aqueous silver nitrate: Silver nitrate solution (1 mM) was prepared and stored in amber colored bottle.

Preparation of extract from seeds by homogenization: The seeds of *Nigella sativa* were washed several times with deionized water. The extract used for the synthesis of silver nanoparticles was prepared by taking 20 g of thoroughly washed *Nigella sativa* seeds with 100 mL of distilled water. The suspension was homogenized and the homogenized suspension was centrifuged and the supernatant was collected. The extract obtained was filtered through Whatman No 1 filter paper. The filtrate was collected and stored at 4°C for further use.

Preparation of extract from seeds: *Nigella sativa* seeds were washed several times with deionized water. Twenty gram of coarsely ground *Nigella sativa* seeds were taken and boiled in 100 mL of double distilled water for 3 min and filtered through Whatman No 1 filter paper. The filtrate was collected and stored at 4°C for further use.

Optimization and synthesis of silver nanoparticles: Different concentrations of seed extracts (1 and 5 mL) were taken separately and to this 10 mL of 1 mM silver nitrate solution was added with constant stirring and exposed to different conditions like sunlight irradiation, UV irradiation, several short burst of microwave irradiation at a frequency of 2.45 GHz in a domestic microwave oven (National Model N N-GD 576M), at a power output of about 100 W in a cyclic mode (on 15s, off 15s) to prevent overheating as well as aggregation of metals, room temperature and direct boiling. The color change of the solution was checked periodically. The color change of the seed extract from yellow to dark brown indicated that the silver nanoparticles were synthesized from the seeds. Bioreduction of pure silver ions were monitored by measuring the UV-Vis spectrum of the reduction media after diluting a small aliquot of the sample in distilled water. UV visible spectroscopy was carried out on ELICO 150 UV visible spectrophotometer. Then the conical flasks were incubated at room temperature for 24 h. The contents were centrifuged at 10,000 rpm for 15 min. The same procedure was performed for both conventional and homogenization method of extracts.

Production and recovery of silver nanoparticles: Among various methods used, sunlight irradiation method was very effective and homogenized seeds extract had shown more synthesis of nanoparticles. Thus, homogenized seeds extract and sunlight exposure method was chosen for the bulk production of nanoparticles. For the bulk production of silver nanoparticles, 100 mL of 1 mM silver nitrate was added to the homogenized seeds extract in a conical flask and exposed to sunlight.

The solution consisting silver nanoparticles was subjected to centrifugation at 10,000 rpm for 15 min. The pellet formed was dissolved in 0.1 mL of toluene water and air dried.

Antimicrobial activity by disc diffusion method: The prepared nutrient agar was poured on to sterile petri plates and 17 h growing cultures of *E. coli* and *S. aureus* were swabbed on to the agar plates. Meanwhile, the sterile discs were impregnated with silver nanoparticle solution and a positive control drug and were placed inverted on the swabbed plate. Empty sterile disc was kept as negative control. The plates were incubated overnight at room temperature and the zone of inhibition was measured (Cruickshank, 1968).

Characterization of silver nanoparticles–UV-Vis spectral analysis: The reduction of pure Ag^{2+} ions was monitored by measuring the UV vis spectrum of the reduction media after diluting a small aliquot of the sample in distilled water. UV visible spectroscopy was carried out on ELICO 150 UV visible spectrophotometer.

XRD analysis: The silver nanoparticle solution was centrifuged at 2500 g for 20 min. The pellet was washed three times with 20 mL of deionized water. The dried mixture of silver nanoparticles was collected for the determination of formation of silver nanoparticles by X' Pert PRO X-ray diffractometer operated at a voltage of 40 kV and a current of 30 mA with copper potassium alpha radiation.

FTIR analysis: For FTIR measurements, silver nanoparticle solution was centrifuged at 20,000 rpm for 20 min. The pellet was washed three times with 20 mL of deionized water. The samples were dried and analyzed on IR-Prestige-21 (SHIMADZU) operating at a resolution of 2 cm^{-1} .

SEM and EDAX analysis: Scanning electron Microscopic (SEM) analysis was done using Hitachi S-4500 machine. Thin films of samples were prepared on a carbon coated copper grid by just dropping a very small amount of sample on the grid. Extra solution was removed using blotting paper and then films on SEM grid were allowed to dry by putting it under mercury lamp for 5 min.

Results and discussion

Optimization studies: Different optimization studies were carried out for the synthesis of silver nanoparticles from seeds of *Nigella sativa* extract. Among them, sunlight irradiation method and homogenized extract showed better production of silver nanoparticles as compared to other methods. The reaction medium confirmed the presence of the silver nanoparticles. The color of the reaction medium gradually changed to dark brown because of the surface plasmon resonance.

The color change was observed after adding silver nitrate solution to the homogenized extract. The visible range of UV spectra for silver is between 400-600 nm.

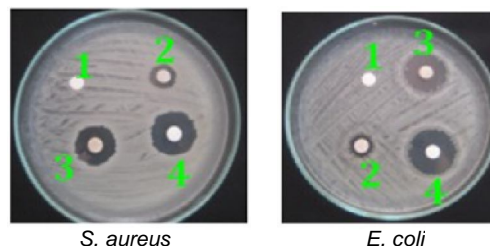
Antimicrobial activity: Antibacterial activity was studied for UTI causing bacteria namely *E. coli* and *S. aureus*. This study was carried out for silver nanoparticles of *N. sativa* and was compared with a standard antibiotic. The results depict (Fig. 1) that silver nanoparticles are efficient when compared with antibiotic giving a zone of inhibition of 10 mm and 12 mm against *E. coli* and *S. aureus* respectively. The exact inhibition mechanism was not clearly known as it inhibits the organism at varying concentrations. Thus, the silver nanoparticles of *N. sativa* seeds significantly inhibits the pathogens, however, further investigation is required for understanding its mechanism better.

Characterization of silver nanoparticles–UV-Visible spectroscopy: An absorption peak between 430-460 nm confirms the presence of silver nanoparticles. The samples obtained through different synthesis process were observed in the UV-Visible spectra analysis. The maximum absorption spectrum is obtained for silver nanoparticles that are synthesized using *Nigella sativa* seeds extract under sunlight. A similar pattern was observed by Zarchi *et al.* (2011), where the synthesis of silver nanoparticles was done using ethanolic extracts of *Andrachnea chordifolia* by sunlight irradiation.

XRD analysis: XRD analysis showed four distinct diffraction peaks at 29.18° , 34.18° , 39.45° 47.18° and can be indexed the angle values of (111), (200), (220), (240) crystalline planes of cubic Ag. This analysis revealed that nanoparticles are orthorhombic crystals. The high peaks in the analysis indicate the active silver composition with the indexing (Fig. 2). Thus, the XRD confirms the crystalline nature of the silver nanoparticles and from the angle value it is clear that the compound is stable.

FTIR analysis: FTIR measurement was carried out to identify possible biomolecules of *Nigella sativa* seeds extract responsible for the formation and stabilization of nanoparticles (Fig. 3). The linkage at 3413 and 1560 cm^{-1} confirms the N-H stretching and deformation, the peaks at 2922 and 2852 cm^{-1} confirms the presence of the CH_3 and CH_2 stretching, the carbonyl stretch was confirmed by the peak formation at 1653 cm^{-1} . The coupled C-O stretch and the O-H deformation were confirmed at the peaks at 1405 and 1045 cm^{-1} . The peaks formed at the 605 cm^{-1} confirms the C-S stretch in the analysis. The overall observation confirms the presence of protein in samples of silver nanoparticles along with amino acids for its stability and to protect it from further changes. The silver nanoparticles that are obtained through biological process were more stable when compared to chemical reduction method.

Fig. 1. Antibacterial activity of silver nanoparticles from *N. sativa*.



1–Control; 2–Nanoparticle; 3–Antibiotic; 4–Nanoparticle + Antibiotic.

Fig. 2. XRD spectrum of silver nanoparticle from *N. sativa*.

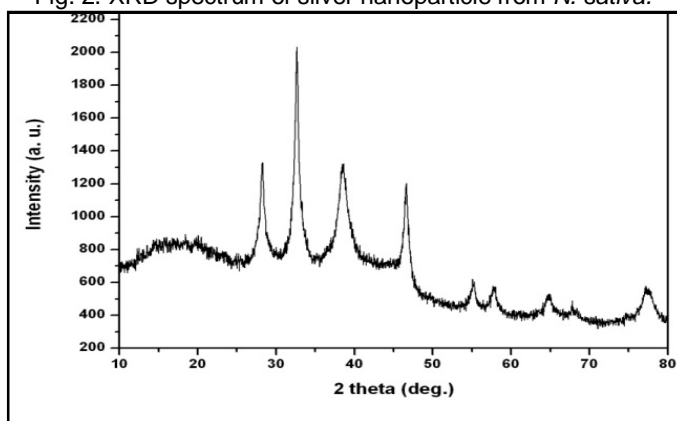


Fig. 3. FTIR spectrum of silver nanoparticle from *N. sativa*.

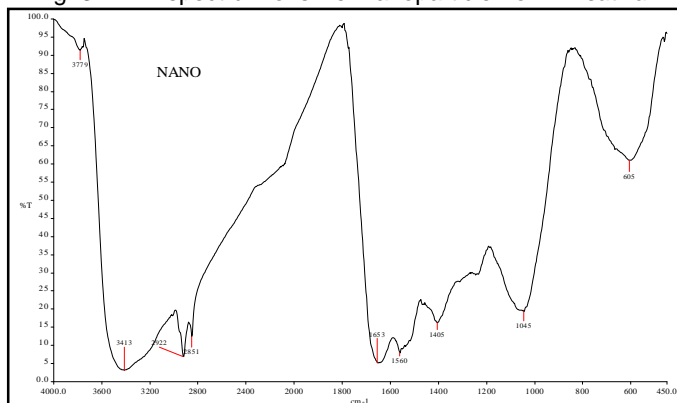
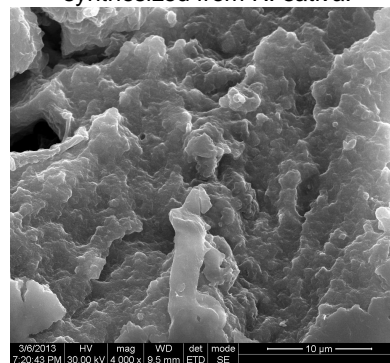


Fig. 4. SEM image of silver nanoparticle synthesized from *N. sativa*.



The silver nanoparticles are more biocompatible as they are synthesized from the natural sources and also have the stability for the further biocontrol studies and the medicinal application (Parida *et al.*, 2011).

SEM and EDAX analysis: The results of SEM analysis (Fig. 4) show that the silver nanoparticles synthesized are of uniform size distributed evenly and cylindrical in shape. Thus, the silver nanoparticles synthesized are on equal distribution throughout the process and also the size and stability of nanoparticles are uniform on the process. Researchers have reported the synthesis of nanoparticles from various plant sources. The reports shows that the silver nanoparticles obtained from various sources are mostly spherical in shape and different sizes (Shankar *et al.*, 2005; Kasthuri *et al.*, 2009; Nestor *et al.*, 2008). The results of EDAX help in finding the elemental composition of the silver nanoparticles (Fig. 5). The analysis helps in confirmation of the presence of the silver nanoparticles in the sample on a varying range between 1.5-4 nm.

Conclusion

In conclusion, the bioreduction of silver ions using seeds of *Nigella sativa* as reducing agent has been illustrated. From the present study, it is clear that the silver nanoparticles synthesized through the green route using seeds of *N. sativa* can inhibit the organisms causing urinary tract infection providing a significant zone of inhibition. Thus, the silver nanoparticles from seeds of *N. sativa* may be used in the management of urinary tract infection causing bacteria.

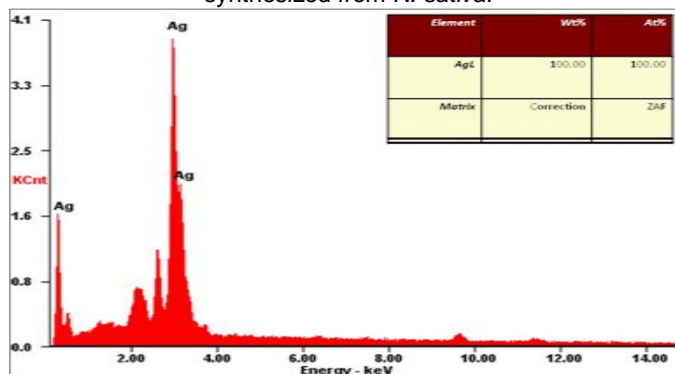
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Fig. 5. SEM-EDAX image of silver nanoparticle synthesized from *N. sativa*.



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