Synthesis of silver nanoparticles using *Nigella sativa* seed extracts and assessment of their antibacterial activity

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**ABSTRACT**

Silver nanoparticles were biosynthesized from *Nigella sativa* seed extracts using ethanol and chloroform. The antibacterial activity of silver nanoparticles against some drug-resistant bacteria has been established, but further study is needed to assess whether these particles could be an option for the treatment and prevention of drug-resistant microbial infections. Synthesized nanoparticles were characterized and screened for their antibacterial properties on resistant strains. The biosynthesized silver nanoparticles were characterized by UV-Visible, FTIR, Dynamic light scattering and Scanning Electron Microscope (SEM) analysis. The antibacterial action of biosynthesized silver nanoparticles was assessed by Microtitre Broth dilution process using Ciprofloxacin as standard, against resistant strains like *Pseudomonas aeruginosa*, *Clostridium difficile*, *Klebsiella pneumoniae* and *Streptococcus pyogenes*. The Silver nanoparticles obtained from chloroform extract of *Nigella sativa* seeds were more effective against *Pseudomonas aeruginosa*, *Clostridium difficile* and *Streptococcus pyogenes*; than ethanolic seed extracts at 120 μL. Our data propose that the silver nanoparticles are effective against a variety of drug-resistant bacteria, which makes them a potential candidate for use in pharmaceutical products that may help to treat drug-resistant pathogens in different clinical environments. The present study focuses on the ability of phytoconstituents capped with silver nitrate can be used to treat infections caused by resistant bacteria.

**INTRODUCTION**

Medicinal plants are used to treat diseases since prehistoric times. Herbal medicines are considered to be safe when compared to current allopathic medicines. *Nigella sativa* (Family Ranunculaceae) is considered as a miracle herb due to its historic and religious usage (Ahmad *et al*., 2013). The seeds of *N. sativa* have been broadly used for centuries in the treatment of several ailments (Abdallah, 2017). It possesses an extensive spectrum of activities like diuretic, anticancer, immunomodulatory, analgesic,antidiabetic, antimicrobial, etc. (Rajsekhar and Kuldeep, 2011). The seed is rich in many phytochemicals like Nigellone, dithymoquinone, thymoquinone, thymohydroquinone, nigellidine, beta-sitosterol, , nigellicimine, arachidonic acid, linoleic acid, linolenic acid etc. (Javed, 2012; Nallamuthu *et al*., 2013)
The infections produced by drug-resistant microorganisms result in significant increase in mortality, morbidity and cost related to prolonged treatments. Resistance of bacteria to antibiotics has increased in recent years due to the expansion of resistant strains. Few antimicrobial agents are exceedingly toxic and there is a necessity to find novel ways to formulate new kinds of safe and cost-effective compounds. Studies have revealed that antimicrobial nanoparticle formulations can be used as effective bactericidal materials.

Nanoparticles possess a higher surface to volume ratio with decreasing size. Definite surface area is pertinent for catalytic and anti-bacterial activity in silver nanoparticles. Using plants for nanoparticle synthesis can be preferred over other biological processes as it is a simple and cost-efficient way. In addition to their bactericidal activity and rapid antibacterial effect against a wide variety of drug-resistant bacteria, silver nanoparticles possess particular characteristics due to silver itself. This noble metal is effective against bacterial resistance (Liu et al., 2010) and is less toxic with minimal side effects. The bactericidal property of silver nanoparticles against multidrug-resistant bacteria can be used in conjunction with phytochemicals to overcome resistant microbial infections (Feng et al., 2000; Lara et al., 2010). The present study is aimed to prepare Silver nanoparticles with *Nigella sativa* seed extracts and to screen their Antibacterial activity on resistant strains.

**EXPERIMENTAL**

**Collection and authentication of plant part**

*Nigella sativa* seeds were purchased from a local market, Ananthapuramu, India, authenticated with voucher no. 0861. The seeds were shade dried at room temperature for 3-4 days and blended into a fine powder. The powder was stored in an airtight container for further use.

**Preparation of plant material**

The powdered material was macerated in 250 ml of chloroform and ethanol for 48 hours with occasional stirring. The extract was filtered by Whatmann filter paper and dried under vacuum using a rota-evaporator. The oily substance was obtained after the complete evaporation of the solvents from the extract. The extracts were stored in a refrigerator.
Table 1: Zeta size and Zeta potential of *N. Sativa* silver nanoparticles with Ethanol and Chloroform extract NES-*Nigella sativa* Ethanol Silver nanoparticles

| S. No | Sample | Zeta size (nm) | Zeta potential (mv) |
|-------|--------|----------------|---------------------|
| 1.    | NES    | 158            | -8.9                |
| 2.    | NCS    | 195            | -18.8               |

Table 2: % growth inhibition of bacteria by *Nigella sativa* seed extracts nanoparticles Ethanol extract

| Volume spiked in µL | % growth inhibition of Pseudomonas aeruginosa | % growth inhibition of Klebsiella pneumoniae | % growth inhibition of Clostridium difficile | % growth inhibition of *Streptococcus* pyogenes |
|----------------------|-----------------------------------------------|--------------------------------------------|---------------------------------------------|-----------------------------------------------|
| NES @ 10             | 22.78                                         | 21.88                                      | 31.26                                       | 24.69                                         |
| NES @ 20             | 35.96                                         | 29.87                                      | 48.67                                       | 37.06                                         |
| NES @ 40             | 42.33                                         | 37.27                                      | 64.37                                       | 51.53                                         |
| NES @ 60             | 51.96                                         | 45.52                                      | 70.64                                       | 56.49                                         |
| NES @ 80             | 77.26                                         | 54.63                                      | 76.33                                       | 61.08                                         |
| NES @ 100            | 80.47                                         | 71.23                                      | 81.06                                       | 75.61                                         |
| NES @ 120            | 82.56                                         | 76.23                                      | 85.12                                       | 81.56                                         |
| NCS @ 10             | 32.36                                         | 11.56                                      | 16.21                                       | 12.04                                         |
| NCS @ 20             | 48.27                                         | 19.37                                      | 29.45                                       | 22.36                                         |
| NCS @ 40             | 62.34                                         | 28.33                                      | 42.76                                       | 36.93                                         |
| NCS @ 60             | 80.5                                          | 42.89                                      | 59.18                                       | 65.71                                         |
| NCS @ 80             | 84.31                                         | 53.75                                      | 71.47                                       | 80.47                                         |
| NCS @ 100            | 87.23                                         | 60.74                                      | 83.38                                       | 90.27                                         |
| NCS @ 120            | 92.41                                         | 65.23                                      | 89.36                                       | 95.43                                         |
| Cipro-1 µg/ml        | 21.36                                         | 38.25                                      | 20.14                                       | 19.23                                         |
| Cipro-5 µg/ml        | 59.27                                         | 89.62                                      | 81.59                                       | 44.26                                         |
| Cipro-9 µg/ml        | 79.05                                         | 98.52                                      | 95.27                                       | 76.54                                         |
| Cipro-13 µg/ml       | 91.38                                         | 99.26                                      | 98.92                                       | 83.69                                         |
| Cipro-17 µg/ml       | 98.91                                         | 99.58                                      | 99.08                                       | 89.48                                         |
| Cipro-21 µg/ml       | 99.63                                         | 99.72                                      | 99.39                                       | 99.06                                         |
| Cipro-25 µg/ml       | 99.84                                         | 99.69                                      | 99.78                                       | 99.47                                         |

Table 3: % growth inhibition of bacteria by *N. Sativa* seed extracts

| Conc. in mg/mL | % growth inhibition of Pseudomonas aeruginosa | % growth inhibition of Klebsiella pneumoniae | % growth inhibition of Clostridium difficile | % growth inhibition of *Streptococcus* pyogenes |
|----------------|-----------------------------------------------|--------------------------------------------|---------------------------------------------|-----------------------------------------------|
| NE @ 12.5      | 21.38                                         | 22.96                                      | 31.16                                       | 28.36                                         |
| NE @ 25        | 34.69                                         | 33.71                                      | 44.81                                       | 39.43                                         |
| NE @ 50        | 48.22                                         | 45.68                                      | 52.12                                       | 50.38                                         |
| NC @ 12.5      | 18.32                                         | 15.37                                      | 23.74                                       | 17.36                                         |
| NC @ 25        | 28.64                                         | 26.14                                      | 35.59                                       | 39.58                                         |
| NC @ 50        | 36.69                                         | 35.62                                      | 47.57                                       | 51.48                                         |
Chemicals and reagents
Silver nitrate (AgNO₃) was acquired from Sigma Aldrich. The other chemicals used were of analytical grade and received from Sigma Aldrich, India. Throughout the experiment, double distilled water was used.

Biosynthesis of Silver nanoparticles (Ag NPs)
One ml of extracts (chloroform and ethanol) was mixed with 10 ml of 2mM AgNO₃ solution in a 50 ml beaker. The preparation was kept in the dark overnight at room temperature. The color alteration from light yellow to brown determines the formation of silver nanoparticles (Singh et al., 2016). The obtained nanoparticles were centrifuged at 5000 rpm for 30 minutes. The solution was filtered by Whatmann filter paper and kept in the refrigerator for future use.

CHARACTERIZATION OF NANOPARTICLES
To confirm the formation of AgNPs in the extract, absorption studies of developed nanoparticles were carried out on a UV-visible spectrophotometer (LAB INDIA, UV-3092) for well-dispersed nanoparticles in the wavelength range 200-800 nm. The chemical composition of the synthesized AgNPs was studied by FTIR spectrometer. FTIR was taken for the N. Sativa seed extracts and for the silver nanoparticles prepared from Nigella sativa extract to identify the functional groups present in the seed extract, which are responsible for the reduction of AgNPs. The Size dispersal of the synthesized nanoparticles and zeta potential (ALHaj, 2010) was measured by Zetasizer (Horiba SZ-100). The detailed morphology of nanoparticles was established by Scanning electron microscopic (SEM) images.

UV-Visible spectroscopy
The reduction of AgNPs during exposure to seed extract could be detected by the color change. A color change from light yellow to brown was detected when the seed extracts containing silver nitrate solution was kept for overnight. It may be due to the addition of aqueous AgNO₃ solution into seed extract, that the Ag⁺ ions were attracted by the -O- group of biomolecules to form silver complex then after it was reduced silver (Ag⁰). The variations in both AgNO₃ and seed extract confirmed that the formation of NPs with the optimized concentrations exhibited superior plasmon resonance absorbance at 420 nm, as shown in Figure 1.

FTIR analysis
FTIR graph shows that the absorption bands at 3348 (O–H stretching, H–bonded of alcohols, phenols & N–H stretching of primary and secondary amines, amides), 2927 and 2858 (C-H stretching of alkanes). The -C=C- stretching and N–H bending of alkenes and primary amines is perceptible at 1650 cm⁻¹. C-C stretching of aromatics at 1459 cm⁻¹ and C-O stretching of alcohols, carboxylic acids, esters, ethers and C–N stretching of aliphatic amines at 1105 cm⁻¹. The prepared silver nanoparticles showed a shift of the absorption bands of 3386 to 3348, 2922 to 2912 and 1642 to 1651 cm⁻¹, 1442 to 1401 cm⁻¹ after bioreduction. The FTIR of Nigella sativa ethanol seed extract and ethanolic based nanoparticles is shown in Figure 4 and Figure 5 respectively. The vibrational bands due to -C= C- and –C= O are indicated flavonoids and alkaloids present in Nigella sativa seeds. So it is presumed that the biomolecules are accountable for capping, stabilization and reduction of Ag⁺ to AgNPs (Shankar et al., 2004). The FTIR graphs of Nigella sativa seed extracts and nanoparticles shown in Figures 2 and 3 and Figure 4.

Particle size and Zeta potential
The size and Zeta potential of the synthesized nanoparticles was determined by using Zetasizer (Horiba SZ-100 Ver 2.20). The size dispersal of the nanoparticles was measured by Dynamic Light Scattering (DLS) (Vani and Navashree, 2017). For AgNPs prepared from N. sativa chloroform extract, DLS analysis showed nanoparticles with an average diameter of 158 nm, with a Polydispersity Index (PDI) of 0.309, while for AgNPs derived from Nigella sativa ethanol extract, the mean diameter calculated was 190 nm with a Polydispersity Index of 0.321, as shown in Table 1 and Figure 5. In this AgNPs system, the zeta potential for AgNPs prepared from N. sativa chloroform extract was -18.8 mV. In the case of AgNPs prepared from N. sativa ethanol extract, the zeta potential was -8.9 mV as shown in Figure 6. Zeta potential values of nanoparticles in the range +30 mV or below -30 mV are considered electrostatically stable. The stabilization of nanoparticles is due to electrostatic interactions and steric hindrance provided by biomolecules.

SEM analysis
Scanning electron Microscopy confirmed morphology & size details of Silver nanoparticles. The experimental result showed that the diameter of the prepared nanoparticle (chloroform extract) with an average size of about 158 nm, as shown in Figure 7.

Antibacterial activity
The antibacterial activity was assessed by Microtitre Broth dilution method in sterile 96-well microtitre plates, Ciprofloxacin was used as standard (Sarker Singh et al., 2010).
et al., 2007). Solutions of each compound *Nigella sativa* ethanol seed extract (NE), *Nigella sativa* chloroform seed extract (NC), *Nigella sativa* ethanolic seed extract-based silver nanoparticles (NES) & *Nigella sativa* chloroform seed extract-based silver nanoparticles (NCS) were used at appropriate concentrations. Each Source solution (NE and NC) was diluted to obtain the final concentrations of 12.5, 25 and 50 mg/ml. NES and NCS (Silver nanoparticles) were diluted to obtain the final concentrations of 10, 20, 40, 60, 80, 100 and 120 μL. Four resistant bacterial Strains, namely Carbapenam resistant *Pseudomonas aeruginosa* (Cr-P.a), Cephalosporin resistant *Clostridium difficile* (Cr-C.d) Carbapenam resistant *Klebsiella pneumoniae* (Cr-K.p) and Macrolide resistant *Streptococcus pyogenes* (Mr-S.p) were cultured as per standard Protocol. An aliquot of 80 μl of each dilution of a formulation was released to a well on a 96-welled (12 x microtitre plate, along with an aliquot of 95 μl of Mueller-Hinton (MH) broth, an
Figure 6: Zeta Potential of *Nigella sativa* silver nanoparticles (A) Ethanolic extract (B) Chloroform extract

aliquot of 20-μl of bacterial inoculum (10^9 CFU/ml) and a 5-μl aliquot of 0.5% of 2,3,5-triphenyl tetrazolium chloride (TTC). The above contents were transferred into a well; the microplate was incubated at 37°C for 18 h. The formation of pink colouration due to TTC in a well showed bacterial growth, and the absence of the color was taken as mean inhibition of bacterial growth. The microplate first well was control without extract and the second well contains Ciprofloxacin as a positive control (Salem *et al.*, 2015). The Minimum inhibitory concentration value was noted at the well, where no color was exhibited. 90% of growth inhibition was considered as MIC of the compound as per standard protocol.

RESULTS AND DISCUSSION

Antibacterial activity

The antibacterial action of *N. Sativa* seed extracts with ethanol and chloroform was compared with that of silver nanoparticles synthesized form the
same extracts. Ciprofloxacin was used as a standard drug. The antimicrobial applications of AgNPs is due to their stability and the presence of medicinally important thymoquinone as capping agents (Sangeetha et al., 2014). The results are presented in Table 2. The seed extracts showed minimal antibacterial activity, given in Table 3. The antibacterial action of prepared silver nanoparticles NCS (Nigella sativa chloroform extract-based silver nanoparticles) were more effective against Pseudomonas aeruginosa, Clostridium difficile & Streptococcus pyogenes than NES (Nigella sativa ethanolic extract-based silver nanoparticles) at 120 μL, shown in Figure 8.

CONCLUSIONS

The present work reported a simple method of biosynthesis of silver nanoparticles from the seed extracts of Nigella sativa. The synthesized nanoparticles effectively controlled the growth of resistant bacterial strains like Pseudomonas aeruginosa, Clostridium difficile & Streptococcus pyogenes. Thus silver nanoparticles with plant extracts can be commended as effective broad-spectrum bactericidal agents against resistant strains of bacteria.

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Conflict of Interest

The authors have no conflict of interest.

Ethical issues

This work didn't involve any animals or human subjects. So, there is no Ethical issue.

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REFERENCES

Abdallah, E. 2017. Black Seed (Nigella sativa) As Antimicrobial Drug: A Mini-Review. Novel Approaches in Drug Designing and Development, 3:55603–55603.

Ahmad, A., Husain, A., Mujeeb, M., Khan, S. A., Najmi, A. K., Siddique, N. A., Damanhouri, Z. A., Anwar, F. 2013. A review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pacific Journal of Tropical Biomedicine, 3(5):337–352.

ALHaj 2010. Characterization of Nigella Sativa L. Essential Oil-Loaded Solid Lipid Nanoparticles. American Journal of Pharmacology and Toxicology, 5(1):52–57.

Feng, Q. L., Wu, J., Chen, G. Q., Cui, F. Z., Kim, T. N., Kim, J. O. 2000. A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. Journal of Biomedical Materials Research, 52(4):662–668.

Javed, S. 2012. Nutritional, phytochemical potential and pharmacological evaluation of Nigella Sativa (Kalonji) and Trachyspermum Ammi (Ajwain). Journal of Medicinal Plants Research, 6(5).

Lara, H. H., Ayala-Núñez, N. V., del Carmen Ixtapan Turrent, L., Padilla, C. R. 2010. Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria. World Journal of Microbiology and Biotechnology, 26(4):615–621.

Liu, J., Sonshine, D. A., Shervani, S., Hurt, R. H. 2010. Controlled Release of Biologically Active Silver
from Nanosilver Surfaces. *ACS Nano*, 4(11):6903–6913.

Nallamuthu, I., Parthasarathi, A., Khanum, F. 2013. Thymoquinone-loaded PLGA nanoparticles: antioxidant and anti-microbial properties. *International Current Pharmaceutical Journal*, 2(12):202–207.

Rajsekhar, S., Kuldeep, B. 2011. Pharmacognosy and Pharmacology of Nigella sativa - A review. *International Research Journal of Pharmacy*, 2:36–39.

Salem, W., Leitner, D. R., Zingl, F. G., Schratter, G., Prassl, R., Goessler, W., Reidl, J., Schild, S. 2015. Antibacterial activity of silver and zinc nanoparticles against Vibrio cholerae and enterotoxic Escherichia coli. *International Journal of Medical Microbiology*, 305(1):85–95.

Sangeetha, J., Sandhya, J., Philip, J. 2014. Biosynthesis and Functionalization of Silver Nanoparticles Using Nigella sativa, Dioscorea alata and Ferva asafoetida. *Science of Advanced Materials*, 6(8):1681–1690.

Sarker, S. D., Nahar, L., Kumarasamy, Y. 2007. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. *Methods*, 42(4):321–324.

Shankar, S. S., Rai, A., Ahmad, A., Sastry, M. 2004. Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (Azadirachta indica) leaf broth. *Journal of Colloid and Interface Science*, 275(2):496–502.

Singh, P., Kim, Y. J., Zhang, D., Yang, D. C. 2016. Biological Synthesis of Nanoparticles from Plants and Microorganisms. *Trends in Biotechnology*, 34(7):588–599.

Vani, L. R., Navyashree, H. T. 2017. Synthesis and Characterization of Silver Nanoparticles from Malvastrum coromandelianum L. Leaf Extract and Their Antibacterial Assay. *Int J Eng Sci Comp*, 7:14988–14992.