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# 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 aeuruginosa, Clostridium difficile* and *Streptococcus pyogenes*; than ethanolic seed extracts at 120 $\mu$L. 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.

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## 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 Rananculaceae) 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, thy-

moquinone, 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.

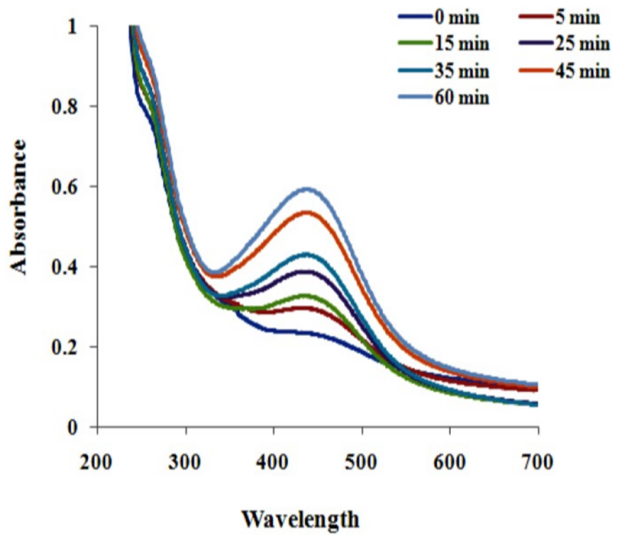



**Figure 1: UV –Visible spectra of *Nigella sativa* silver nanoparticles**

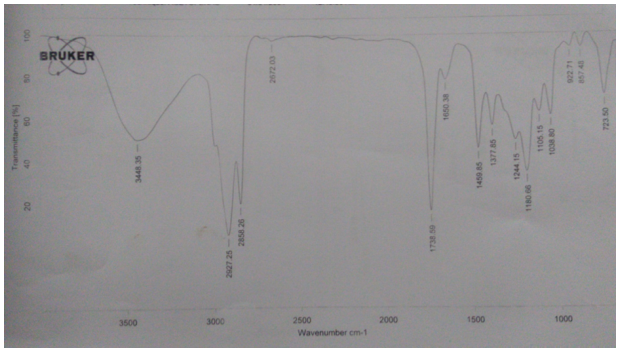



**Figure 2: FT-IR spectra of *N. Sativa* seed extract (ethanol)**

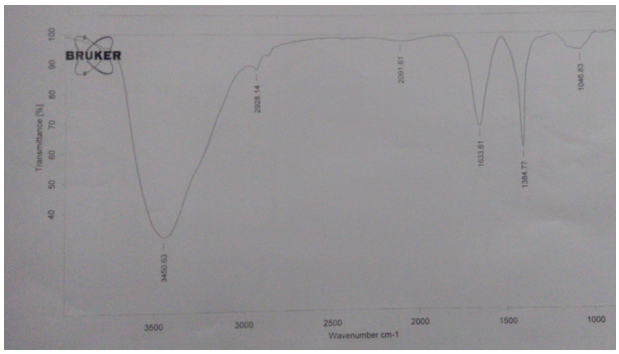



**Figure 3: FT-IR spectra of *N. Sativa* seed extract (ethanol) based silver nanoparticles**

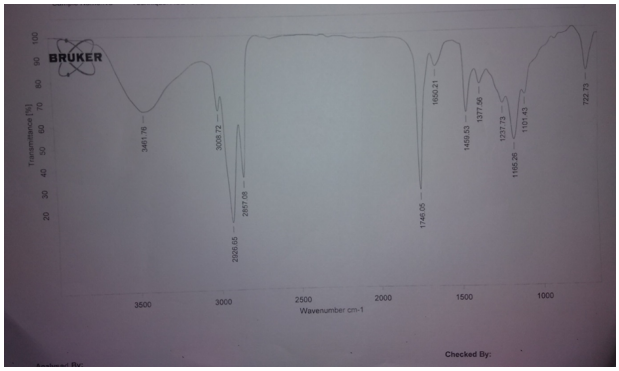



**Figure 4: FT-IR spectra of *N. Sativa* seed extract (chloroform)**

## 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 What-

**Table 1: Zeta size and Zeta potential of *N. Sativa* silver nanoparticles with Ethanol and Chloroform extract NES-*Nigella sativa* Ethanol Silver nanoparticles, NCS-*Nigella sativa* Chloroform 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 Chloroform extract**

| Volume spiked in $\mu$L | % 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 $\mu$g/ml | 21.36 | 38.25 | 20.14 | 19.23 |
| Cipro-5 $\mu$g/ml | 59.27 | 89.62 | 81.59 | 44.26 |
| Cipro-9 $\mu$g/ml | 79.05 | 98.52 | 95.27 | 76.54 |
| Cipro-13 $\mu$g/ml | 91.38 | 99.26 | 98.92 | 83.69 |
| Cipro-17 $\mu$g/ml | 98.91 | 99.58 | 99.08 | 89.48 |
| Cipro-21 $\mu$g/ml | 99.63 | 99.72 | 99.39 | 99.06 |
| Cipro-25 $\mu$g/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 |

mann 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.

### Chemicals and reagents

Silver nitrate ($AgNO_3$) 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 $AgNO_3$ 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 $AgNO_3$ 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 ($Ag^o$). The variations in both $AgNO_3$ 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* ethanolic 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

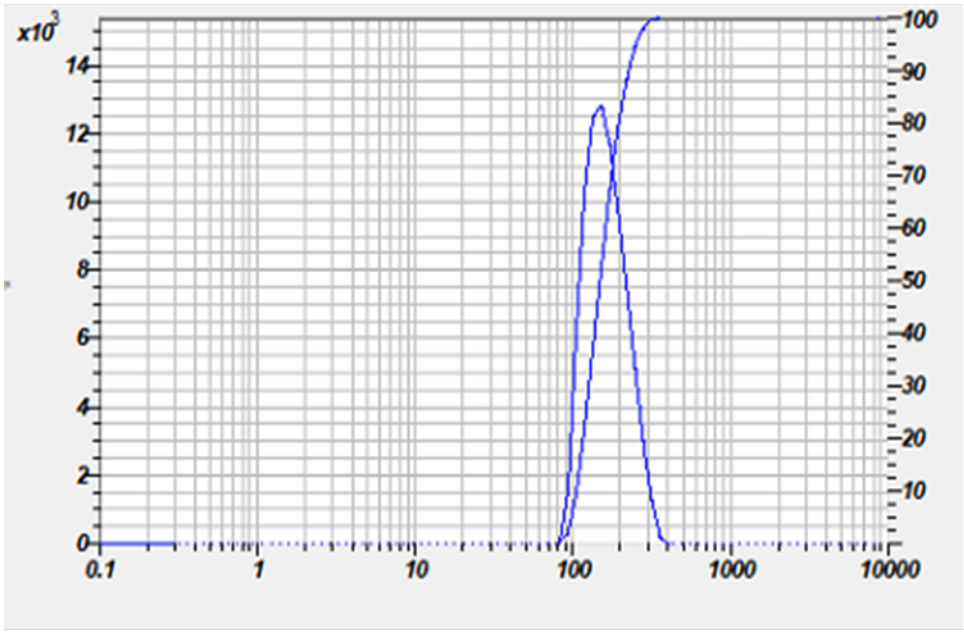
The size and Zeta potential of the synthesized nanoparticles was determined by using Zetasizer (Horiba SZ-100 Ver 2.20). The size dispersal of the nanoparticles was measured by Dynamic Light Scattering (DLS) (Vani and Navyashree, 2017). For AgNPs prepared from *N. sativa* chloroform extract, DLS analysis 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.

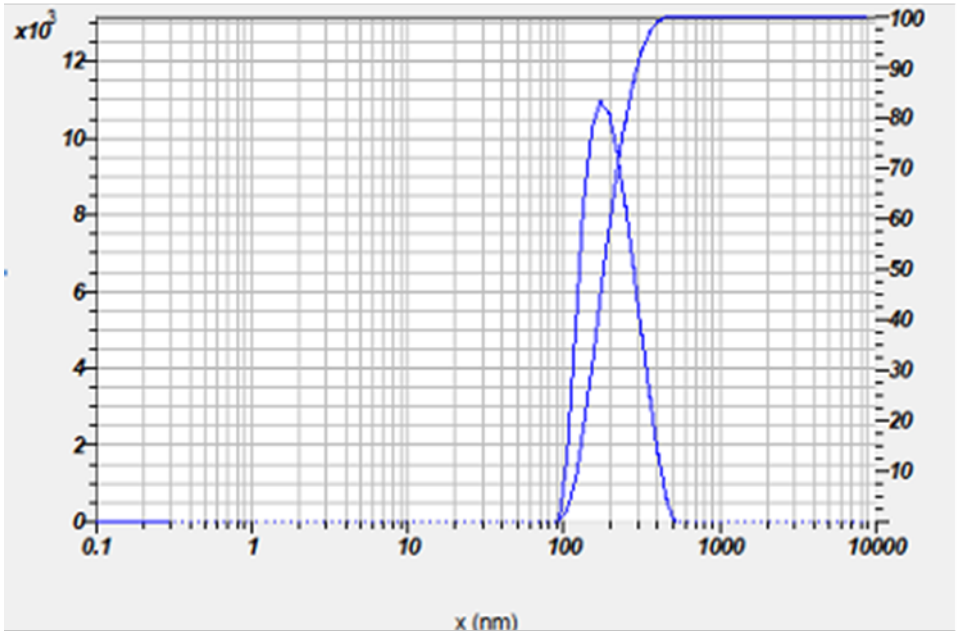
### SEM analysis

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.




(A)

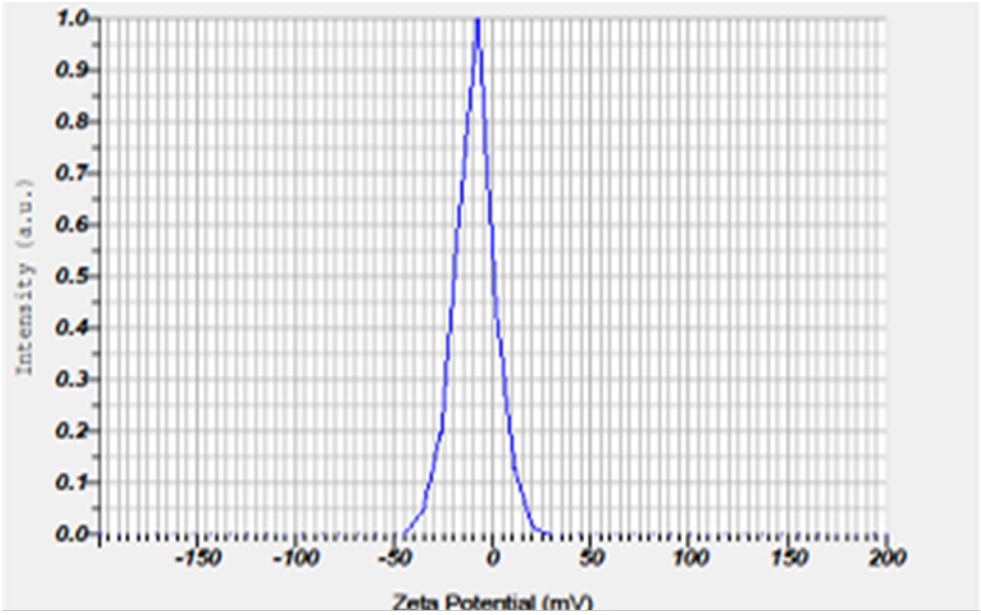



(B)

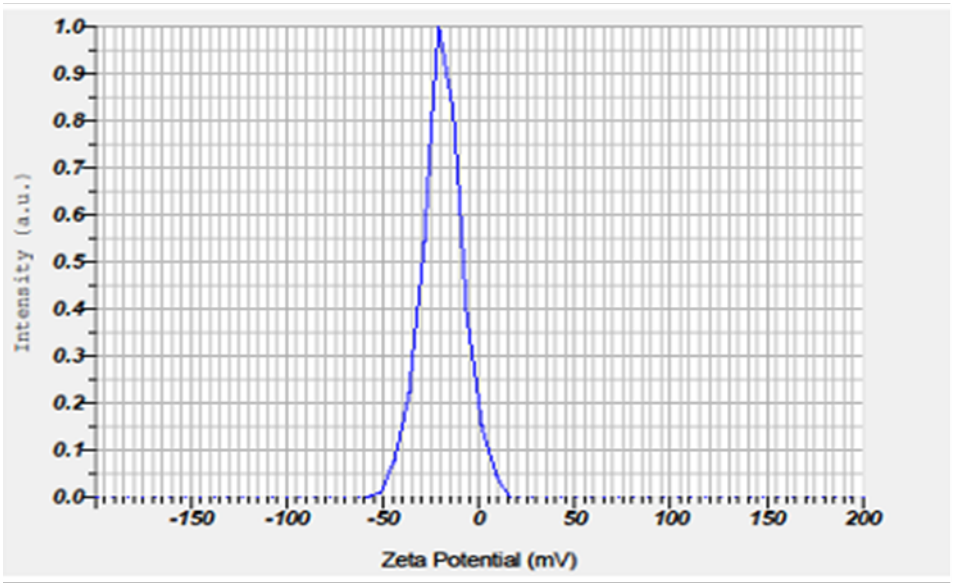
**Figure 5: Size distribution of *Nigella sativa* silver nanoparticles (A) Ethanolic extract (B) Chloroform extract**

### Antibacterial activity

The antibacterial activity was assessed by Microtitre Broth dilution method in sterile 96-well microtitre plates, Ciprofloxacin was used as standard (Sarker *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 $\mu$L. Four resistant bacterial Strains, namely Carbapenam resistant *Pseudomonas aeruginosa (Cr-P.a),* Cephalosporin resistant *Clostridium difficile (C.r-C.d)* Carbapenam resistant *Klebsiella pneumoniae (Cr-K.p)* and Macrolide resistant *Streptococcus pyogenes (Mr-S.p)* were cul-




(A)




(B)

**Figure 6: Zeta Potential of *Nigella sativa* silver nanoparticles (A) Ethanolic extract (B) Chloroform extract**

tured as per standard Protocol. An aliquot of 80 $\mu$l 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 $\mu$l of Mueller-Hinton (MH) broth, an aliquot of 20-$\mu$l of bacterial inoculum ($10^9$ CFU/ml) and a 5-$\mu$l 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$^o$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.

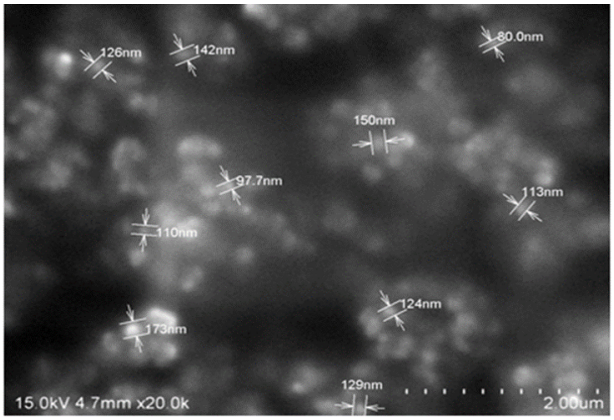



**Figure 7: SEM image of Nigella sativa-Chloroform silver nanoparticles**

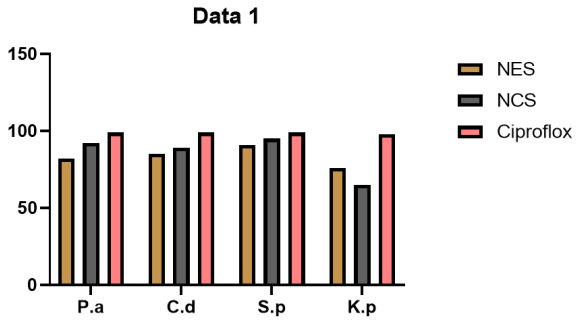



**Figure 8: Antibacterial activity of *N. Sativa* ethanol & chloroform extract-based silver nanoparticles at 120 $\mu$L concentration and Ciprofloxacin(25 $\mu$g/ml). P.a - Pseudomonas aeruginosa**
**C.d - Clostridium difficile**
**S.p - Streptococcus pyogenes**
**K.p - Klebsiella pneumoniae**

## 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 aeuruginosa, Clostridium difficile* & *Streptococcus pyogenes* than NES (*Nigella sativa* ethanolic extract-based silver nanoparticles) at 120 $\mu$L, 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.

## ACKNOWLEDGEMENT

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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.

### Funding

No financial assistance received from the funding agency.

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