PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF GREEN SYNTHESIZED SILVER NANOPARTICLES USING *NIGELLA SATIVA* EXTRACT

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ABSTRACT

Nanotechnology is one of the most active research fields at modern research of material science. Green mediated synthesis of nanoparticles is the present research in the limb of nanotechnology. The present work focuses on the synthesis of silver nanoparticles using different concentrations of *Nigella Sativa* (Kalonji seed) extract as capping agent and reducing agent. The biosynthesized nanoparticles were characterized by UV-VIS absorption spectrophotometer, Fourier-Transform infrared spectroscopy, X-Ray diffraction techniques. The FT-IR spectroscopy indicates flavonoids as a potential reducing agent. Crystalline structures of the synthesized silver nanoparticles are confirmed by X-Ray diffraction spectra. Green synthesized Ag NPs showed zone of inhibition against both Gram positive and Gram-negative bacteria.

Keywords— ***Antimicrobial activity, SEM, Silver Nanoparticles,Nigella Sativa seed Extract, XRD.***

1. **INTRODUCTION**

In everyday life Nanotechnology and Nanostructured materials play an important role in life sciences, research and development, as more and more products based on nanostructured materials are introduced into the market. Nanotechnology as the manipulation of matter with at least one dimension sized from 1 to 100 nanometres, i.e one billionth of metre or 10^{-9}m.The name Nano comes from the Greek word for dwarf and it is related to the Spanish word Nino [1]. Nanotechnology is a rapid growing field in science for creating and employing nano-sized particles [2]. It is the science and engineering involved in the design, synthesis, characterisation and application of materials and devices with one dimension is on the nanometre scale. Nanoscale materials having high surface area to volume ratio than the corresponding bulk materials [3].

Research has been established on advanced nanomaterials of noble metals like silver has a lot of interest among scientists through the past decades for its physicochemical properties such as size, shape, composition, stability, distribution and morphology, they have been studied for catalytic activity, optical properties, electronic properties, antimicrobial properties and magnetic properties [4-9] and its application in various fields like production of biomaterials, biochemistry, medical and pharmaceutical products, tooth pastes, optical receptors, biosensing etc [10-13]. A number of methods can be available for the synthesis of nanomaterials alike chemical reduction [14-16], electrochemical reduction [17], photochemical reduction [18], heat evaporation [19, 20] and so on. But in the above these methods toxic by-products will be produced which are hazardous and these methods are very expensive.

However, the simple and greener procedures will be beneficial for the synthesis of metal nanoparticles. In the phytochemical screening and green technology, the nanoparticles were synthesized using a one-step procedure, eco-friendly, less time consuming, low cost and no toxic by-products are formed [21, 22]. Plants give a better platform for the formation of nanoparticles as they do not have any toxic chemicals and it also contains natural capping agents. Among various plants, we have chosen *Nigella Sativa* seed extract for the present study due to it has several pharmacological effects like antibacterial, antifungal, anti-diabetic, antioxidant, anti-cancer, anti-inflammatory, anti-asthma and analgesic activities [23].

The present work focused on the syntheses of silver nanoparticles by green method using the *Nigella Sativa* seed extract and evaluation of antimicrobial activity against both Gram positive (*Staphylococcus aureus,Staphylococcusepidermidis*) and Gram negative (*Pseudomonas fluoresence*)bacteria. The synthesized silver nanoparticles were characterized by using UV-VIS, XRD, FT-IR, SEM and Phytochemical analysis.

2. Materials and Methods Materials

Analar grade silver nitrate was purchased from Merck. The young *Nigella Sativa* seeds were collected from nearby sattur, Tamil Nadu. For all experimental work, double distilled water and ethanol were used.

2.1. Preparation of Seed Extract

Fresh *Nigella Sativa* seeds were collected and then washed thoroughly with distilled water several times to remove the dust and dried under shade. The dried plants were cut into small pieces and ground to powder. 5g of *Nigella Sativa* plant powder was taken in a Soxhlet apparatus with ethanol used as a solvent and boiled at 78°C for 2 hours and then filtered using whatman No: 1 filter paper. Finally, the prepared extract solution was cooled at 4°C and stored for further synthesis of nanoparticles.

2.2. Preparation of 0.1 M Silver Nitrate Solutions

0.1 M silver nitrate solution was prepared by dissolving 1.69 g of $AgNO_3$ in 100 ml double distilled water, to avoiding the auto oxidation of Ag the prepared 0.1 M $AgNO_3$ solution was stored in Amber colored bottle.

2.3. Synthesis of Silver Nanoparticles

About 0.1 M of aqueous solution of silver nitrate was prepared and used for the synthesis of silver nanoparticles. Then seed extract of *Nigella Sativa* with various concentrations such as 10 and 20 ml was added separately to the 10 ml of 0.1 M silver nitrate solution at room temperature. After 20 minutes, the solution was turned from yellow to dark brown color indicating that the formation of silver nanoparticles [24-27].

2.4. Characterisation of Silver Nanoparticles

The synthesized silver nanoparticles were characterized by UV- visible spectrophotometer in the range of 200 nm to 800 nm. The size of the nanoparticles was calculated from the XRD studies. Morphology of synthesized silver nanoparticles was characterized by SEM analysis. The sample has been placed in an evacuated chamber and scanned in a controlled pattern by an electron beam. The synthesized nanoparticles were recorded FT IR in the range of 4000 -500 cm^{-1} to study the functional group present in plant extract.

2.5. AntimicrobialStudies

An agent that kills microorganisms or stops their growth is known as antimicrobial agent. Antimicrobial medicines have been grouped according to the microorganisms they act primarily against.

The antimicrobial medicines that are used to treat infection are known as antimicrobial chemotherapy. The important classes of antimicrobial agents are as follows,

- Disinfectants,
- Antiseptics,
- Antibiotics.

The term "antibiotic" described only those formulations derived from living micro-organisms but are now also applied to synthetic antimicrobials, alike sulphonamides, fluoroquinolones. Antibacterial agents have been further subdivided into bactericidal agents (kill bacteria) and bacteriostatic agents(slow down / stall bacterial growth)[28-32].

3. Results and Discussion

3.1 UV – VisibleDiffuse Reflectance Spectra Analysis

The addition of *Nigella Sativa* seed extract to 0.1M silver nitrate solution results the appearance of yellowish-brown colour solution after 20 minutes indicating the formation of silver nanoparticles. The synthesized Ag NPs for different plant extract concentrations (10 and 20 ml) showed the surface palsmon resonance band at 393 nm to 304 nm. The UV-Visible spectra of *Nigella Sativa* seed extract (a) and the synthesized Ag NPs with 10 ml seed extract (b), 20 ml seed extract (c) were shown in Figure .1. It shows that the peak is blue shifted when increasing in plant extract concentrations. This observed blue shift is due to the reduction of Ag NPs size. The absorbance of Ag NPs increases when increasing the plant extract concentrations. As the concentration of plant extract is increased, size of the particles decreased due to a greater number of bio molecules available, which acts as reducing agents [33, 34].

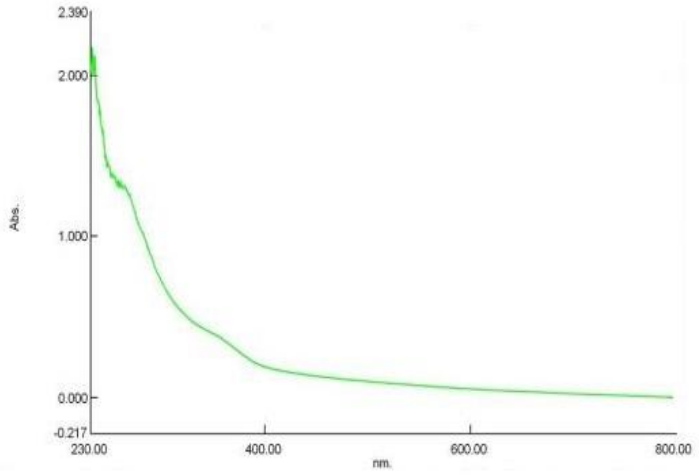

Figure: 1 (a) UV-VISIBLE Spectrum of *Nigella Sativa* Seed Extract

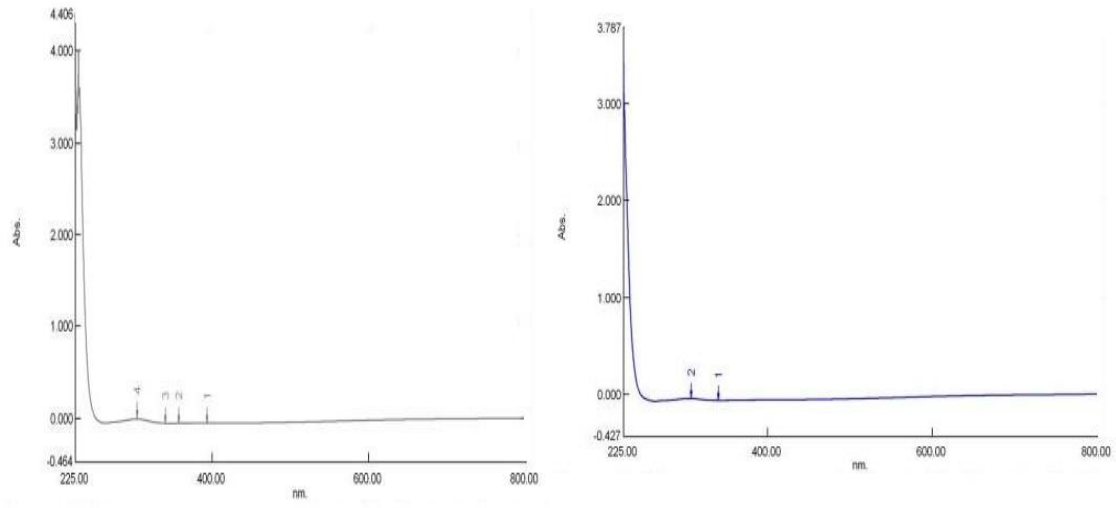

Figure: 1 (b & c) UV-VISIBLE Spectrum of Ag NPs with 10 & 20ml Seed Extract respectively

3.2. FT-IR Spectroscopy

The FT-IR spectrum of the extract gives information about the functional groups involved in the reduction of the Ag^+ ions. The FT-IR study identifies the minerals that capped on Ag NPs and some possible bio molecules from *Nigella Sativa* seed extract are changes from Ag^+ to Ag. The FT-IR spectra of an ethanolic seed extract and synthesized Ag NPs with 10 ml seed extract (b), 20 ml seed extract (c) were shown in Figure. 2. Table .1 shows the FT-IR peak values for bio molecules present in the *Nigella Sativa* seed extract and the synthesized Ag NPsusing different concentrations of the *Nigella Sativa* seed extract. The peak at 3247.9 cm^{-1} in *Nigella Sativa* seed extract correspond to H-bonded OH group of phenolic compounds which is shifted to 3568.06 cm^{-1} in synthesized Ag NPs with 10 ml seed extract and disappeared in Ag NPs with 20 ml seed extract. So, the corresponding phenoliccompounds involved in the reduction of Ag^+ ions into Ag NPs [35-40].

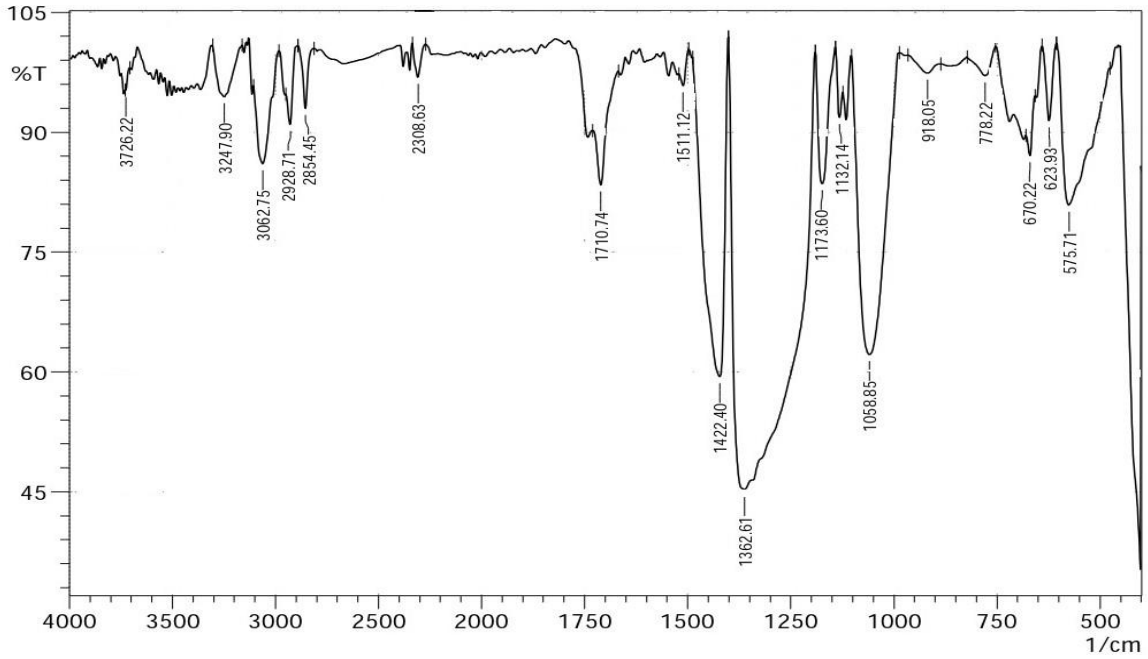

Figure:2 (a) FT-IR Spectrum of *Nigella Sativa* Seed Extract

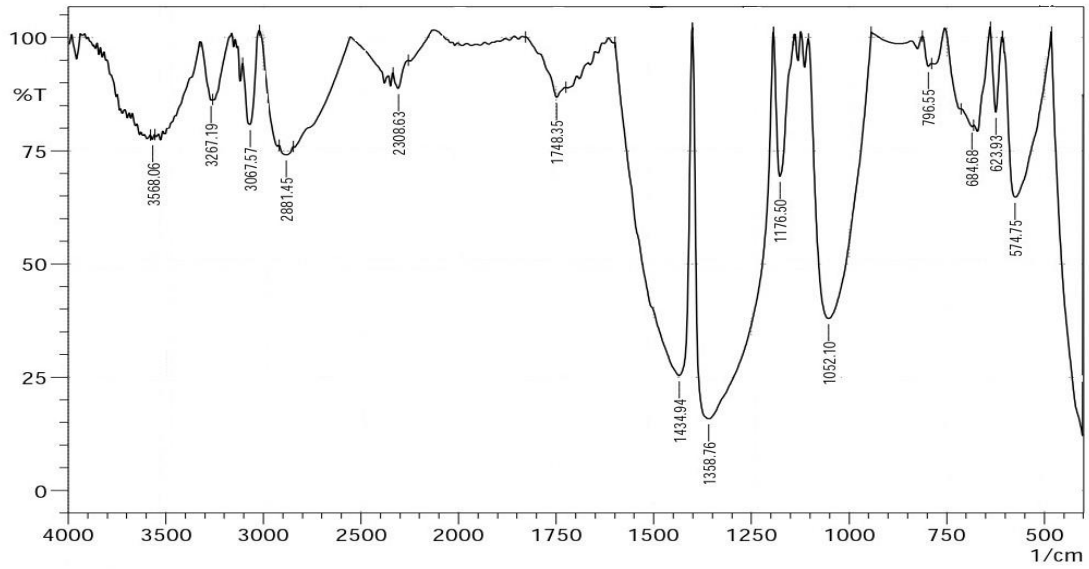

Figure: 2 (b) FT-IR Spectrum of Ag NPs with 10 ml Seed Extract

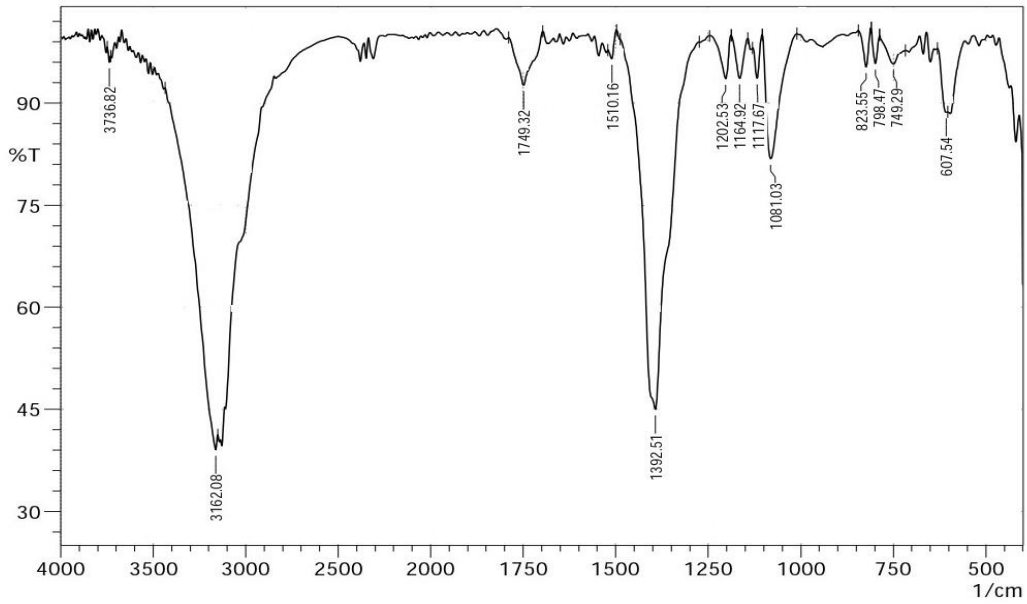

Figure: 2 (c) FT-IR Spectrum of Ag NPs with 20 ml Seed Extract

Functional Group	Peak Values (cm^{-1})		
	Seed Extract	**Ag NPs (10ml Seed Extract)**	**Ag NPs (10ml Seed Extract)**
C – Br Str	575.71	574.75	-
C – Cl str	623.93 to 670.22	623.93 to 684.68	607.54
C – H Str	778.22	796.55 to 798.47	749.29
C = O Str	1058.85 to 1173.6 (acid flavanones) 1511.2 (aromatic acids) 1710.74 (carbonyls)	1052.1 to 1176.5 (acid / flavanones) 1748.35 (esters)	1081.03 to 1392.51 (acid / flavanones) 1749.32 (esters)
C – O Str	918.05 (epoxides)	-	-
C – H Str	2854.45 (aldehydes) 2928.71 (alkanes)	2926.78 (alkanes)	-
C = C Str	1422.4 (aromatic hydrocarbons)	1434.94 (aromatic hydrocarbons)	1488.94 (aromatic hydrocarbons)
= C – H Str	3062.75 (aromatic alkene)	3067.57 (aromatic alkene)	-
NO_2 Str	1362.61 (nitro compounds)	1358.76 (nitro compounds)	1392.51 (nitro compounds)
N – H Str	-	-	3162.82 (hydrogen bonded amides)
-OH Str	3247.9 (H – bonded OH) 3726.22 (free OH)	3267.9 (H – bonded OH) 3568.06 (free OH)	3247.9 (H – bonded OH) 3726.22 (free OH)

Table 1 FT-IR peak values of bio molecules

3.3 X- Ray Diffraction Analysis

In XRD analysis, the green synthesized silver nanoparticles by employing *Nigella Sativa* seed extract was demonstrated and confirmed. The diffraction peaks are observed with 2Θ values of 19˚78`, 29˚83`, 31˚97`, 35˚51`, 38˚18`, 44˚39` and 77˚51` they are indexed to (100), (110), (110), (110), (111), (200) and (310) crystal lattice planes of cubic structure respectively. The high intensity peak was observed at 19 ˚ 76' for Ag NPs with 10 ml seed extract and 35 ˚ 67' for Ag NPs with 20 ml seed extract. These sharp and high intense peaks are confirmed that the synthesized nanoparticles were composed of pure crystalline Ag. The Ag NPs size is calculated in the range of 1.96 to 2.35nm shown in Table 2. Figure .3 indicates the XRD pattern of the synthesized Ag NPs with 10 ml NS seed extract (a), Ag NPs with 20 ml NS seed extract (b) [41-43].

Table 2. **Average Size of the synthesized Ag NPs**

Synthesized Ag NPs	Average size (nm)
Ag NPs with 10ml seed extract	2.35
Ag NPs with 20ml seed extract	1.96

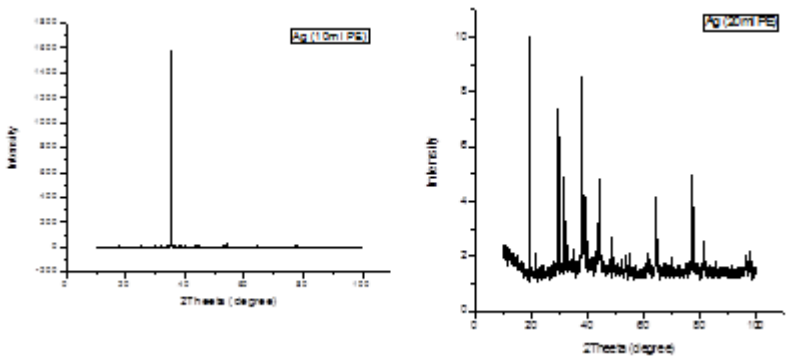

Figure: 3 XRD pattern of the Ag NPs with 10 & 20ml seed extract

3.4. Scanning Electron Microscopy

Morphology of synthesized silver nanoparticles was characterised by SEM analysis. The sample has been placed in an evacuated chamber and scanned in a controlled pattern by an electron beam. Interaction of the electron beam with specimen produces the SEM images of Ag NPs are shown in Figure 4. It can be view that the Ag NPs formed well dispersed and evenly distributed in all direction [44, 45].

Figure.4 shows different magnification of SEM images of the green synthesized Ag NPs using *NigellaSativa* plant extract. It shows that the synthesized Ag NPsare in spherical shape. Furthermore, the particle size is observed to be 1.96 to 2.35 nm.

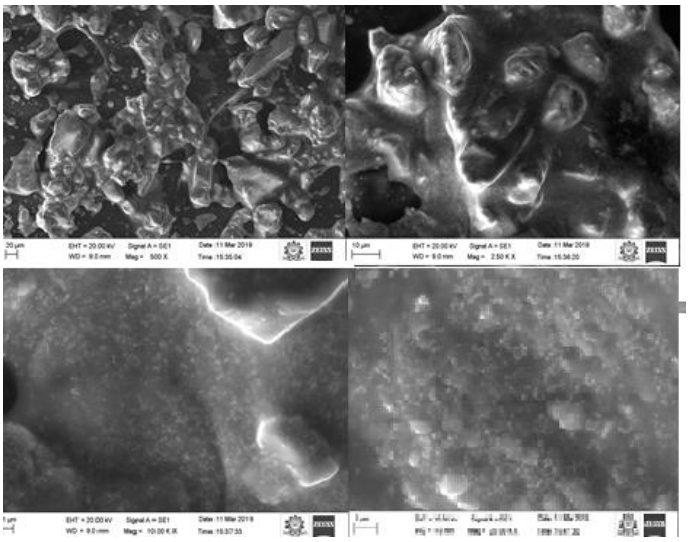

Figure: 4 SEM images of green synthesized Ag NPs using *Nigella Sativa* Seed Extract

3.5. Phytochemical Analysis:

Phytoconstituents in an aqueous extract of *Nigella Sativa* seed were analysed [46, 47]. Phytoconstituents results were given in Table.3.

Phytoconstituents	**Indication**
Alkaloids	+
Carbohydrates and Reducing sugars	+
Steroids	+
Proteins	+
Tannins	+
Phenolic compounds	+
Flavonoids	+
Gums and Mucilage	-
Glycosides	+
Saponins	+
Triterpenoids	+
Fixed Oils and fats	-
Volatile oils	+
Lignin	-
Present	+
Absent	-

Table 3 Phytochemical Analysis of *NigellaSativa* Seed Extract

3.6 Antimicrobial Activity

The plant extract and mediated silver nanoparticles were tested for respective antimicrobial activities towards both gram positive and gram-negative bacterial strains showing the Zone of Inhibitions given in the table 4. The maximum zone of inhibition was measured on the synthesized Ag NPs using 20ml of *Nigella Sativa* seed extract [48-51].

Sample	**Pathogen used**	**Zone of Inhibition**	
		50µl	**100 µl**
SP1	S.aureus	12mm	14mm
	P.fluoresence	10mm	15mm
	S.epi	10mm	15mm
SP2	S.aureus	14mm	17mm
	P.fluoresence	15mm	18mm
	S.epi	15mm	17mm

Table 4 ZOI of synthesized Ag NPs using 10 & 20 ml*NigellaSativa* **Seed Extract**

4. Conclusion

Silver nanoparticles were synthesized using *NigellaSativa* seed extract by eco-friendly green method. Theformation of Ag NPs was confirmed by UV-VIS absorption spectroscopic analysis. The functional group present in the plant extract has been confirmed by FT-IR. The polyphenols of plant extract were mainly responsible for the reduction of Ag^{+} ion to Ag NPs. The synthesized Ag NPs were analyzed using FT-IR, UV-VIS, XRD & SEM. XRD pattern of Ag NPs confirms the synthesized particles are in cubic crystalline structure and the sizes of Ag NPs are in the range of 1.96 to 2.35 nm. SEM analyses shows the synthesized Ag NPs is in spherical shape. Ag NPs were effectively utilized for the antimicrobial activity study. The maximum zone of inhibition was found to be more in gram negative bacteria when compared to gram positive bacteria. The *NigellaSativa* plant may be effectively utilized for the product ofAg NPs with economically for many pharmaceutical applications.

5. References

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