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Biosynthesis and Validation of SNPs from *Nymphaea caerulea* Savigny

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ABSTRACT

Biosynthesis of nanoparticles is under explorations because of wide biomedical applications and research interest in nanotechnology. Silver nanoparticles have been recently used for a wide range of application including health.

The present study is aimed to synthesize silver nanoparticles from leaf extract of *Nymphaea caerulea*. Stable SNPs were produced after treating the extract with 1mM Ag $(NO_3)_2$ solution. A change of thick green color from dark brown was observed. The synthesized Ag NPs were monitored by UV-VIS spectroscopy, SEM, EDAX, AFM and FTIR for their size and shape.

The poly dispersed particles having a spherical shape with the range of diameter from 20 to 100 nm. These nanoparticles exhibited high antibacterial effect on the growth of *E. coli*.

It has been demonstrated that the leaf SNPs of *N. caerulea* are capable of producing silver nanoparticles having important advantage over conventional antibiotics.

Keywords: *Nymphaea caerulea*, Traditional medicinal plant, Silver nanoparticles, Antibacterial activity.

INTRODUCTION

Nanotechnology has wide range of applications in various areas including electronics, catalysis chemistry, energy and medicine. Nanobiotechnology is the most active areas of research in modern material science. The most important application of silver and SNPs is in medical industry such as tropical ointments to prevent infection against burn and open wounds[1]. Nanoparticles have been gaining a booming scientific interest due to their unique electronic, optical, mechanical, magnetic and chemical properties that are significantly different from those of bulk materials[2]. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology[3]. Antimicrobial capability of silver nanoparticles allows them to be suitably employed in numerous hold products such as textiles, food storage containers, home appliances and in medical

devices[4]. Lot of work had been carried out on synthesis of SNPs by using the leaves of terrestrial plants but in aquatic plants is scanty. Hence in the present study an attempt has been made for the synthesis of silver nanoparticles by reducing the silver ions present in the solution of silver nitrate with aqueous leaf extract of *N. caerulea*. Further these biologically synthesized nanoparticles were tested against different bacterial strains to evaluate their antimicrobial efficacy.

MATERIAL AND METHODS

Preparation of leaf extract[5]

N. caerulea Savigny, Genus: *Nymphaea*; belongs to the family nymphaeaceae with blue colored flower. Leaves were collected from Palakonda hills near Palakondaraya Temple, Kadapa District, Andhra Pradesh, India. The leaves were washed thoroughly thrice with distilled water and shade dried for 10 days. The fine powder was obtained from dried leaves by using kitchen blender. The leaf powder was sterilized at 121^{0}C for 5 min. 5 g of Powder was taken into a 250 ml conical flask and 100 ml of sterile distilled water was added and boiled for 15 min at 100^{0}C. Then the leaf extract was collected in a separate conical flask by a standard filtration method.

Development of silver nanoparticles[5]

60 mL aqueous solution of 1mM silver nitrate was reducing by 2.5 mL of leaf extract at room temperature for 10 min, resulting in a thick brown solution indicating the formation of silver SNPs nanoparticles.

UV-VIS spectroscopy

Synthesis of SNPs and metal concentration were measured using a Parkin-Elmer Lamda-45 UV-VIS spectrophotometer at 190-750 nm range.

FTIR

To remove any free biomass residue or compounds that are not the capping legend of the nanoparticles, the residual solution of 100 ml after the reaction was centrifuged at 5000 rpm for 10 min and the resulting suspension was re-dispersed in 10 ml sterile distilled water. The centrifuging and re-dispersing process was repeated three times. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by FTIR Nicolet Avatar 660 (Nicolet, USA).

SEM: (Scanning electron microscopy)

Scanning Electron Microscopic (SEM) analysis was done by 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 sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by using hot hair oven for 5 min.

Antimicrobial studies

The following bacterial strains were used in this study, viz., *Bacillus thuringiensis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia, Escherichia coli*, *Proteus vulgaris* and *Salmonella typhimurium*. Disc diffusion assay method was carried out by using standard protocol[6]. Overnight, bacterial cultures (100 µl) were spread over Muller Hinton Agar (Hi Media Laboratories Private Limited, Mumbai, India) plates with a sterile glass L-rod. 20 µl of each extract were applied to each filter paper disc, Whatman No. 1 (5 mm diemeter), and allowed to dry before being placed on the Agar media. Each extract was tested in triplicate and the plates were inoculated at 37^o C for 24 h after

incubation, the diameter of inhibition zones was measured with the help of MIC scale and the results were tabulated.

RESULTS AND DISCUSSION

The aquatic plant *N. caerulea* universally distributed and cultivating plant (Fig. 1a). In ancient Egypt, water lily preparations were taken to treat liver, relieve constipation, neutralize poison, and regulate the urinary system. It is an essential key to good health, great sex and rebirth. The petals were applied both externally and internally, often in the form of enemas[7]. *N. caerulea* contains apomorphine, a dopamine agonist, as well as nuciferine, nupharine, and nupharidine. The flower have also yielded a variety of alkaloids, including kaempferol, which has MAOI properties[8].

As the *N. caerulea* (Fig. 1a) leaf extract was mixed in the aqueous solution of the silver ion complex, it started to change the colour from thick brown (Fig. 1b) to dark green (Fig. 1c) due to reduction of silver ions which indicates the formation of silver nanoparticles. It is well known that silver nanoparticles exhibit thick green colour in aqueous solution due to excitation of surface plasmon vibrations of silver nanoparticles.

UV-VIS spectrum

Synthesis of SNPs had been confirmed by measuring the UV-VIS spectrum of the reaction media, The UV-VIS spectrum of colloidal solutions of *N. caerulea* has the characteristics absorbance peaks ranging from 260 to 381 nm (fig. 2). The broadening of peak indicated that the particles are poly-dispersed. The weak absorption peak at shorter wavelengths due to the presence of several organic compounds which are known to interact with silver ions. The same results were observed in *Boswellia ovalifoliolat*[9] and other medicinal plants[10].

SEM analysis

The SEM image (Fig. 3) showed relatively spherical shape silver nanoparticles formed with diameter range from 20 to 100 nm in *N. caerulea*. The same results were observed in plant extract of *Aloe vera*[11]; *Emblica officinalis*[12]; *Shorea tumbuggaia*[13]; *Svensonia hyderabadensis*[14]. *Catharanthus roseus*[15] Variation in shape and size of nanoparticles synthesized by biological system is common[16]. Variation in the morphology of silver nanoparticles from *Desmodium* species, where silver nanoparticles are spherical predominantly and also oval and elliptical. By altering the pH, strength of elements, plant source and incubation temperature of the nanoparticle synthesis reaction mixture, the synthesis methods, it is possible to create a wide range of different nanoparticles. Nanoparticles of various sizes and properties may be obtained by further tapping the plant bio-resources of diverse type in wild environment[17].

EDAX analysis

Analysis through Energy dispersive X-ray spectrometers (EDAX) confirmed the presence of elemental silver, which is a signal of silver nanoparticles (Fig. 4) the vertical axis displays the number of X-ray Counts while the horizontal axis displays energy in K eV. Identification lines for the major emission energies for silver (Ag) are displayed and these correspond with peaks in the spectrum, thus giving confidence that silver has been correctly identified. Table 1, shows the Ag L weight 6.80% and Ag L atomic number 1.39% along with O, K, Na and Ca.

AFM analysis

AFM analysis of SNPs were clearly distinguishable owing to their size difference of 20, 40, 80 and 140 nm. AFM image has given average size of 90 nm, SNPs had attached with one another in an area of 2 µm

with spherical shape (Fig. 5h), 3 D view shows (Fig. 5i)

The functional groups of SNPs were identified by FTIR spectra. The above FTIR spectra of the SNPs, representative spectra of obtained nanoparticles manifests absorption peaks located at about 3200 cm^{-1}, 2923 cm^{-1}, 2303 cm^{-1}, 1724 cm^{-1}, 1616 cm^{-1}, 1440 cm^{-1}, 1319 cm^{-1}, 1217 cm^{-1}, 1028cm^{-1}, 700cm^{-1} in the regian of 4000 cm^{-1} to 1000 cm^{-1}. The FTIR spectra revealed the presence of different functional groups like secondary alkyne (C=H bond), alkane (-C-H bond), arene (=C-H bond), aldehyde (C=O bond), Alkanyl (C=C bond) and alkane (-C=C- bond). Among them, the absorption peak at 1028 and 1217 cm^{-1} can be assigned an absorption peak C-O-C- or –C-O, also the peak at 1020 to 1250 cm^{-1}corespond to C-N stretching vibration of alipatic amines or to alcohols or phenols representing the presence of polyphenols[18]. The medium intense band at 1440 cm^{-1} arises from the C-N stretching mode of the aromatic amide group[19]. The spectra showed sharp and strong absorption band at 1616 cm^{-1} assigned to the stretching vibration of (N-H) primary amide group[20]. The Nitrile structure (C≡N bond), alkane (C-H bond) and –C (triple bond) C-H: C-H Stretch are strange shapes. That these biomolecules are responsible for capping and efficient stabilization of SNPs. The presence of reducing sugars in the extract could be responsible for the reduction of silver ions and formation of the nanoparticles [21].

The Antibacterial activity of silver nanoparticles was studied against various pathogenic bacteria of Gram positive and negative strains *B. subtilus*, *S. aureus*, *P. aeruginosa*, *K. pneumonia, E. coli*, *P. vulgaris* and *S. typhimurium* were using disc diffusion method. The comparing plant extract, Ag $(NO3)_2$ extract of SNPs along with streptomycin as standard were mentioned in 1, 2, 3 and 4. The diameter of inhibition zone around each disc with SNPs is represented in Table 2. The highest antimicrobial activity was observed against *Escherichia coli* (23 mm) followed by *Bacillus* (22 mm), *Proteus vulgaris* (15 mm), *Klebsiella pneumonia* (15 mm), *Salmonella typhimurium* (12 mm), *Pseudomonas aeuroginosa* (12 mm) and last was noticed against *Staphylo cocus* (8 mm). Silver has been used for its well known antibacterial properties since Roman time however the advances in generating SNPs have made possible a revival of the use silver as a powerful bactericide[22]. Many researchers used *E. coli* as a model for gram negative bacteria and proved that SNPs may be used as an antimicrobial agent.

CONCLUSION

Biologically synthesized SNPs by using *N. caerulea* leaf extract results an average size of 90 nm with spherical shape. The bio synthesis of silver nanoparticles is non-toxic and cost effective and thus remains to be an alternative method to the chemical reduction method. The results suggested that *N. caerulea* plays an important role in the reduction and stabilization of silver to silver nanoparticles. Present Study also concludes that the SNPs of *N. caerulea* showed potential antibacterial activity on both Gram positive and negative bacterial strains and should be explored for further antimicrobial applications.

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Table 1. EDAX spectrum of silver nanoparticles of *N. caerulea*

Element	Weight (%)	Atomic (%)
O K	41.66	57.44
Na K	10.39	9.97
Ca K	4.81	2.65
Ag L	6.80	1.39
Total	100	

Table 2. Antibacterial activity of *N. caerulea*

S. No.	Bacterial strains	Plant Extract	Ag $(NO_3)_2$	SNPs	Control (Streptomycin)
1	*Bacillus subtilis*	8.83±0.28 mm	10±0.6 mm	22±0.57 mm	36±1.2 mm
2	*E. coli*	7.5±0.28 mm	12±0.56 mm	23±0.44 mm	35±0.57 mm
3	*Klebsiella pneumonia*	10.5±0.28 mm	20±0.44 mm	15±0.28 mm	35±0.881 mm
4	*Salmonella*	8.16±0.16 mm	10±0.88 mm	12±0.28 mm	30±0.57 mm
5	*Proteus vulgaris*	7.5±0.28 mm	15±057 mm	15±1.2 mm	32±0.557 mm
6	*Pseudomonas aeruginosa*	7.5±0.60 mm	10±044 mm	12±0.6 mm	26±0.557 mm
7	*Staphylococcus*	6.5±0.28 mm	8±0.88 mm	8±0.8 mm	24±0.881 mm

(a) **(b)** **(c)**

Figure 1. (a) *N. caerulea,* (b) Aqueous leaf extract, (c) color change of leaf extract

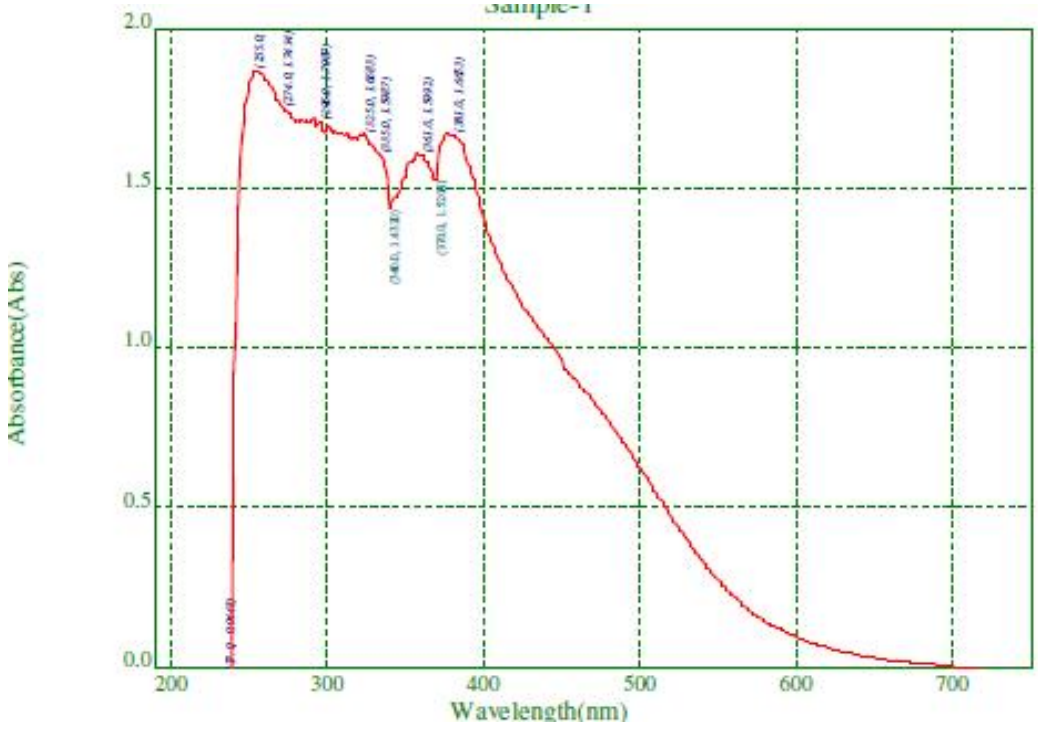


Figure 2. UV-Vis spectroscopy of synthesized silver nanoparticles from *N. caerulea*

Figure 3. SEM image of SNPs of *N. caerulea*

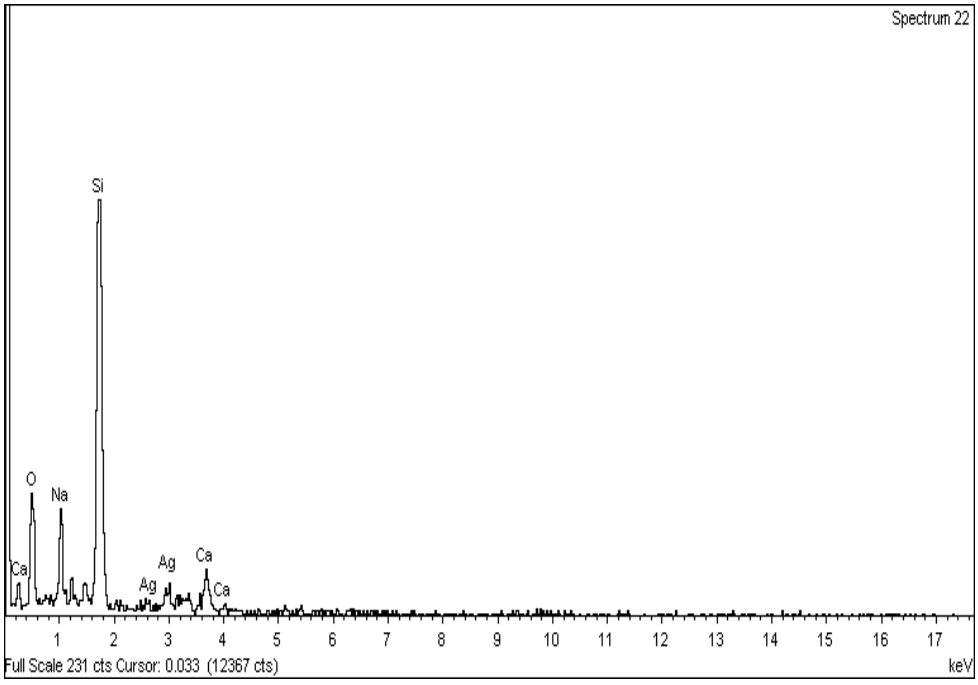
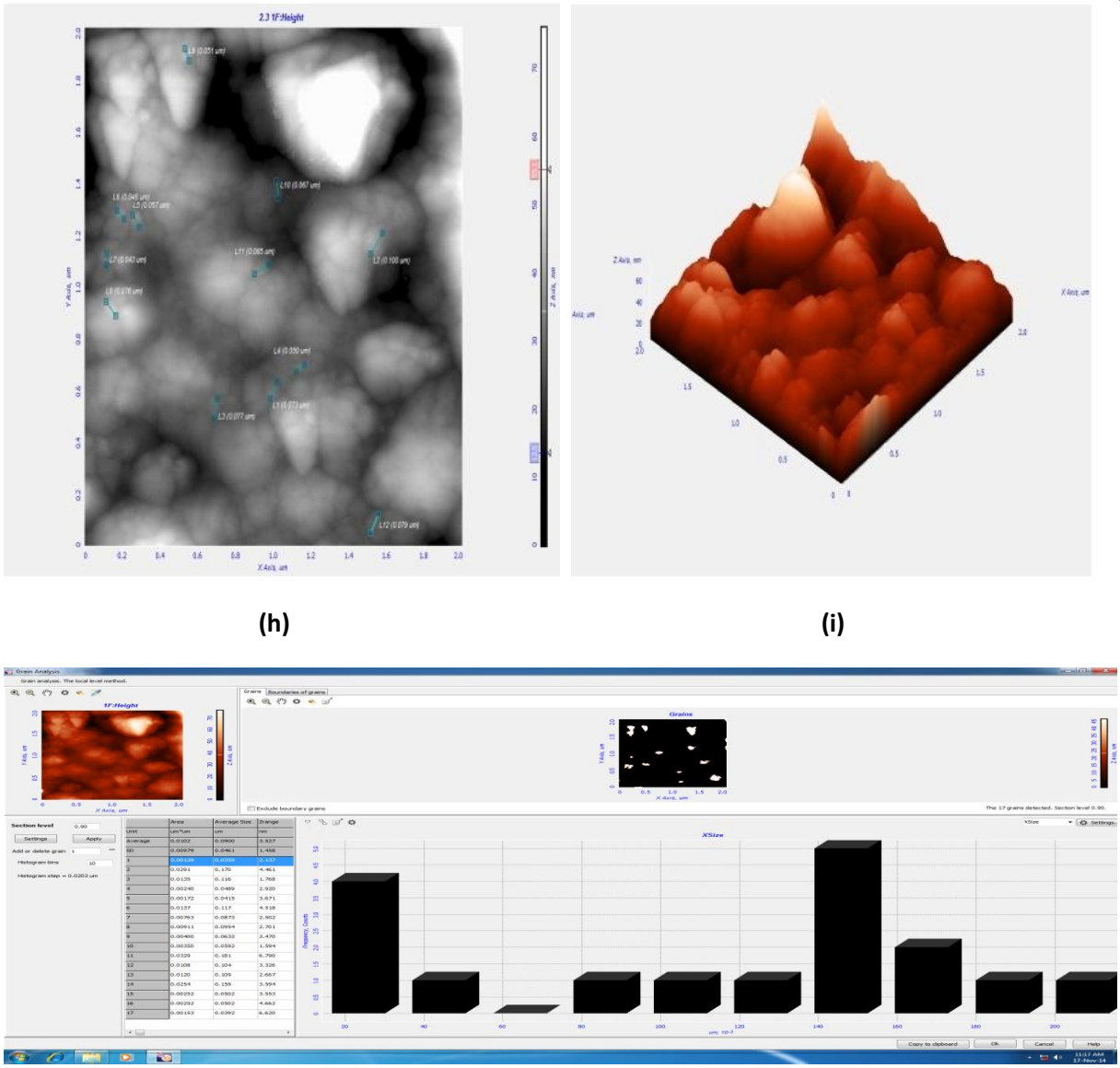


Figure 4. EDAX spectrum of silver nanoparticles of *N. caerulea*



(h) **(i)**

(j)

Figure 5. (h) SNPs synthesized structure (i) 3D view of AFM (j) AFM analysis of SNPs synthesized from the leaves of *N. caerulea*

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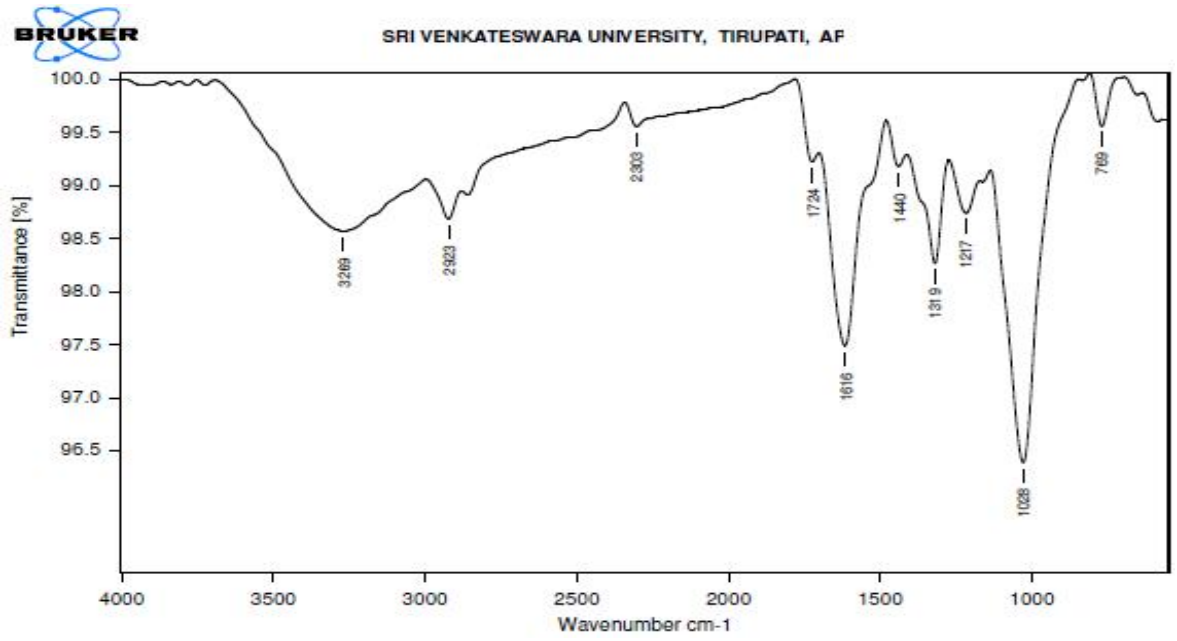

Figure 6. FTIR spectra of *N. caerulea* aqueous leaf extract

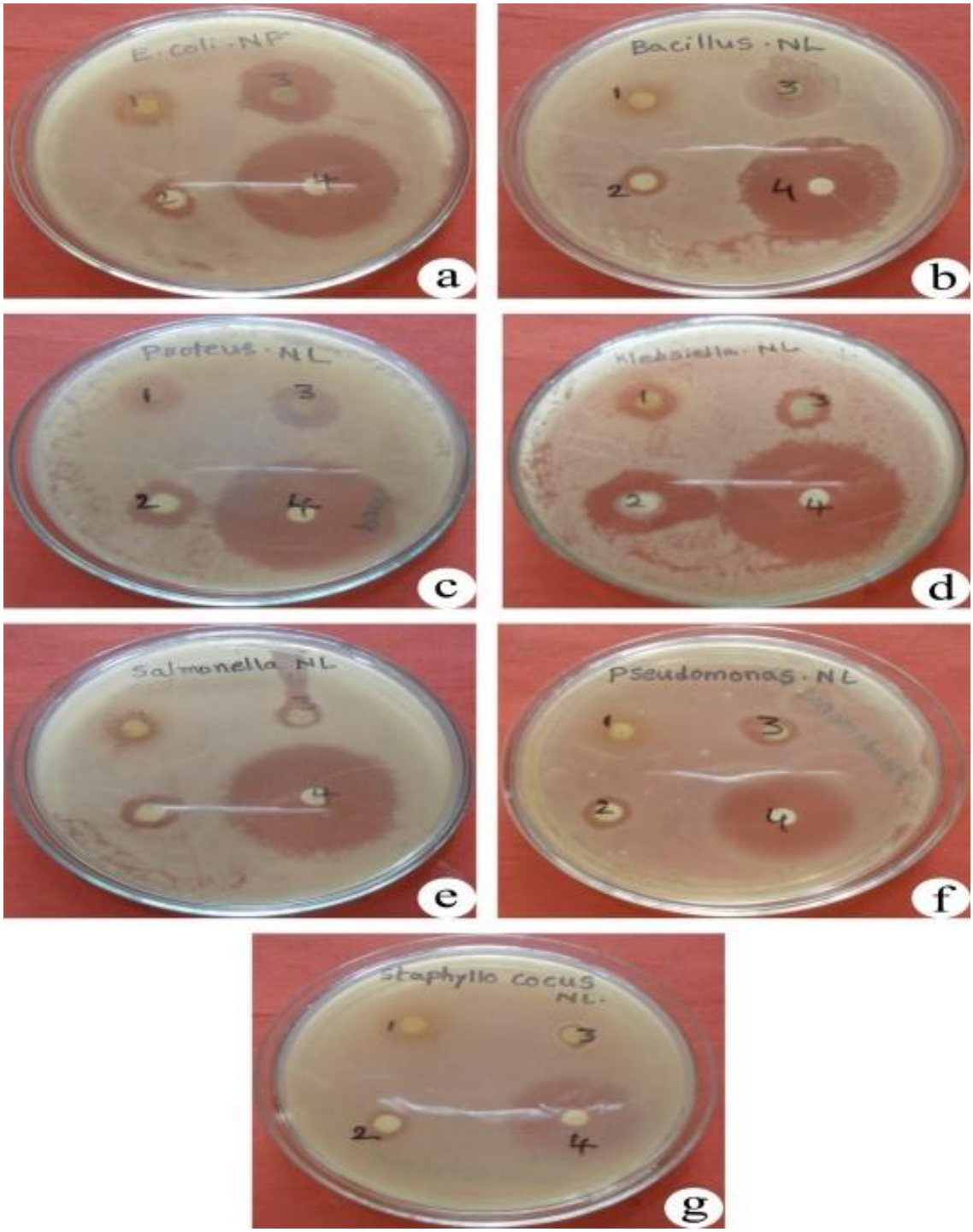

Figure 7. Antibacterial activity of silver nanoparticles (SNPs) against pathogenic bacteria. (a) *E. coli,* (b) *B. subtilis,* (c) *P. vulgaris,* (d) *K. pneumonia*, (e) *S. typhimurium*, (f) *P. aeuroginosa and* (g) *S. cocus,* (1) Plant extract; (2) $Ag(NO3)_2$; (3) SNP's and (4) Streptomycin