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Study of antimicrobial activities of green synthesized silver nanoparticles

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Green synthesis is the pollution free synthesis process, safe for the environment and cost-effective too. In the present paper we repot our studies on the green synthesis of silver nanoparticles using seed extracts of *Phaseolus vulgaris*, *Trachyspermum ammi* and *Trigonella Foenum-Graecum* and their applications for antimicrobial activity. The preliminary confirmation of the nanoparticles formation was done by visual observation then by UV-Visible absorption spectrum, which showed SPR peak in the range of 400–450 nm, which is characteristic peak of silver nanoparticles. To confirm the role of phytochemicals in the stabilization of silver nanoparticles as capping agents, the Fourier Transform Infrared Spectroscopy (FTIR) analysis carried out which show the presence of proteins with the synthesized silver nanoparticles. The plant extract was found to exhibit strong potential for rapid reduction of silver ions. The synthesized nanoparticles are then analyzed for their potency in medical applications for antimicrobial activity. Green synthesis of nanoparticles is proving to be more effective in medicinal applications than the bare metal nanoparticles since the biomass coatings on them act synergistically with the metal nanoparticles.

Keywords: Green synthesis, AgNPs, XRD, UV-Visible absorption spectroscopy, antimicrobial activity.

Introduction

Green synthesis of nanoparticles has been an exploring research area in recent years due to increased need to develop environmentally benign technologies in material synthesis. In the past few years silver nanoparticles (AgNPs) have gained demanding research interest due to their exceptional physicochemical characteristics including high surface area, catalytic activity, optical properties, electronic properties, antibacterial properties and magnetic properties[1–4]. These properties allow them to be suitable for application in medical field as antimicrobial agent, household appliances, textile, domestic water purification system, cosmetics and in electronics[5]. In view of the wide range of applications of AgNPs, various chemical and physical methods are used for commercial synthesis of these nanoparticles, but continuous use of these methods may cause a great risk to environment as well as for human beings because of the use of toxic chemicals[6]. Therefore need of some alternative method had gained the attention of the researchers, and hence opened the path of biosynthesis. The biosynthesis involves the synthesis of nanoparticles by using microbes or by plant extracts. The synthesis of silver nanoparticles using microbes is a slow process and requires time for culture preparation and then synthesis. In contrary to that the synthesis of silver nanoparticles using plant extracts is a very time efficient process. Various researchers have reported the synthesis of AgNPs using different plant extracts such as *Allium cepa*[7], *Ilex paraguariensis*[8], *Givotia moluccana*[9], Garlic, Turmeric, and Green tea[10] etc. The green synthesis has several advantages over the commercially available methods such as these are economically reasonable, ecologically innocuous, can be manageable for bulk synthesis without the need of

maintaining high pressure, energy consumption, high temperature and harmful chemicals. In the present work we have used three different plant (seed) extracts for the synthesis of AgNPs viz. *Phaseolus vulgaris*, *Trachyspermum ammi* and *Trigonella Foenum-Graecum.* Seeds of all of these three plants are easily available in the local market of Ajmer at reasonable price which made the synthesis process cost-effective, more feasible and less time consuming and all the three extracts show good potency for the synthesis of AgNPs. The phyto-chemicals present in these seed extracts act both as a reducing agent and the stabilizing agent (capping) in the synthesis of silver nanoparticles. The main phytochemicals involved are terpenoids, flavinoids, amids, carbonyl compounds and organic acids. It was suggested that phytochemicals play a key role in the reduction of silver ions and formation of AgNPs[11]. Since all the three plant seeds selected for the synthesis are edible and when used for synthesis of AgNPs the phyto-chemicals remains present as the capping agent with AgNPs will not be harmful.

From the ancient time silver has been used for many of the medical applications including antimicrobial applications. The nano size of silver enhances the antimicrobial potential of the AgNPs, due to the increase in the surface to volume ratio. The smaller size of the silver nanoparticles proves helpful in penetrating in the bacterial cell and which may causes structural changes in the cell membrane and results in the death of the cell[12].

Experimental

Materials: Silver nitrate (Grade LR 99.9% pure) used for the synthesis of nanoparticles was purchased from Global Instruments Pvt. Ltd., Ajmer, India, and the seeds of *Phaseolus vulgaris*, *Trachyspermum ammi* and *Trigonella Foenum-Graecum* were purchased from local market of Ajmer. Silver nitrate and powder of these seeds were used as precursor for the synthesis of AgNPs. All the glassware were washed thoroughly with doubly distilled water and dried in hot air oven.

Preparation of seed extract: Firstly the washing of all the three types of seeds was done with double distilled water followed by drying. Then, crushed with the help kitchen blender to get the dry fine powder of seeds. 1.0 g of powdered seed was soaked in 100 ml of double distilled water for 20 h and filtered and filtrate was centrifuged at 5000 rpm for 15 min.

Synthesis of AgNPs: A volume of 1 mM $AgNO_3$ solution was used for the synthesis of silver nanoparticles. The seed extract prepared was gradually added to 1 mM $AgNO_3$ solution in 1:10 ratio. The color of the resulting solution is the first indication of the formation of AgNPs. The appearance of red brown color confirms the formation of silver nanoparticles.

Characterization of AgNPs have been done by using various techniques as follows:

UV-Visible absorption spectroscopy: To observe the optical property of green synthesized silver nanoparticles, UV-Visible absorption spectroscopic studies have been done at room temperature between 200 and 800 nm ranges. The AgNPs were confirmed by measuring the wavelength of reaction mixture in the UV-Visible absorption spectrum.

FTIR spectroscopy: The aqueous seed extract and AgNPs were subjected to Fourier Transform Infrared (FTIR) spectroscopy in order to find possible bio-molecules responsible for reduction and stabilization of silver nanoparticles. The spectra were recorded in the range of 800–4000 cm^{-1}.

X-Ray diffraction measurements: X-Ray diffraction (XRD) measurement of the green synthesized silver nanoparticles was done on a Bruker D8 Advance X-ray diffractometer operating at a voltage of 40 kV and current of 20 mA with Cu K(α) radiation of 1.54187 nm wavelength. The scanning was done in the range of 20°θ to 80°θ.

Study of antimicrobial activity: The antimicrobial activity of the AgNPs synthesized by the seed extracts was determined against three bacterial strains *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumonia* as the mimimum inhibition concentration percentage using 10 μg/ml concentration of samples.

The disc diffusion method was employed to analyze the antimicrobial activity of green synthesized silver nanoparticles. In this method the Whatmann filter paper no. 1 sterile disc having diameter of 5 mm was impregnated with the samples of silver nanoparticles. Then these were placed on the nutrient agar plate at the temperature of 37°C for 24 h. After 24 h the inhibition zone around the dried impregnated discs were measured.

Results and discussion

UV-Visible absorption spectroscopy: The UV-Visible absorption spectra of colloidal AgNPs formed by all the three

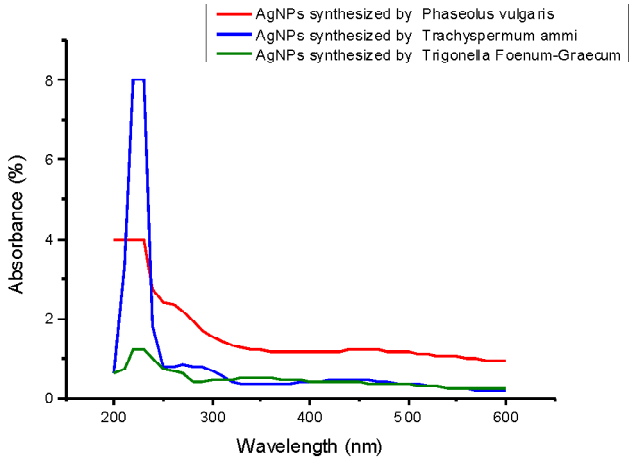

Fig. 1. UV-Vis absorption spectra of colloidal AgNPs synthesized by *Phaseolus vulgaris, Trachyspermum ammi and Trigonella Foenum-Graecum.*

plant extracts are shown in Fig. 1. Broad surface plasmon peaks in the range of 350–450 nm were observed in all the three spectra, which confirm the formation of AgNPs. The presence of an extra peak around 250 nm may be due to the formation of silver oxide nanoparticles or due to the amino acid residues in proteins of the extract. This is further an indication of the release of proteins in the colloidal solution and their possible role as capping agent[13].

FTIR analysis: In Fig. 2 FTIR peaks were observed in the range of 3310–3364 cm^{-1} shows O-H stretching vibration of the hydroxyl group[14], hydrogen bonded alcohols, phenols or N-H stretching of I and II amines and amides. The peak at 1651 cm^{-1} may be due to the C=O starching of primary amide of proteins. This is close to that reported for native proteins[15] which supports to the point that proteins are interacting with green synthesized silver nanoparticles as capping agent and stabilize them. It is also concluded from presence of this peak that the secondary structure of protein not affected during interaction with Ag ions or after binding with silver nanoparticles[16]. The presence of carbonyl and amino group of the proteins were confirmed by IR spectra. Due to the strong binding ability of carbonyl and amino groups with metal the proteins present in the seed extracts may act as a capping agent for silver nanoparticles which provide stability to the nanoparticles and prevent their agglomeration in the medium[17].

X-Ray diffraction measurements: XRD spectra (Fig. 3) showed strong diffraction peaks at 27.7846°, 32.2330°, 38.1169° and 46.2645 degrees of 2θ which corresponds to 110, 111, 200 and 211 crystal planes. These peaks corroborate with the standard Ag_2O (JCPDS 75-1532) and it was concluded that the synthesized nanoparticles were crystalline in nature having cubic shape. The average particle size of the synthesized silver nanoparticles was calculated by using the Debye-Scherrer equation[18,19] is 15.76 nm.

$$D = 0.94\ \lambda/\beta \cos\theta \qquad (1)$$

where λ is the wave length of X-ray (0.1541 nm), β is FWHM (full width at half maximum) in radians, θ is the diffraction angle and *D* is particle diameter size.

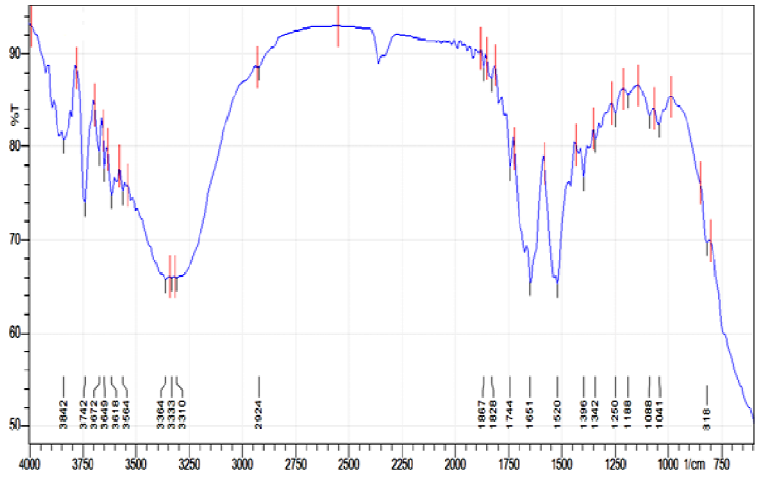

Fig. 2. FTIR spectra of biosynthesized nanoparticles by the plant extract of *Phaseolus vulgaris.*

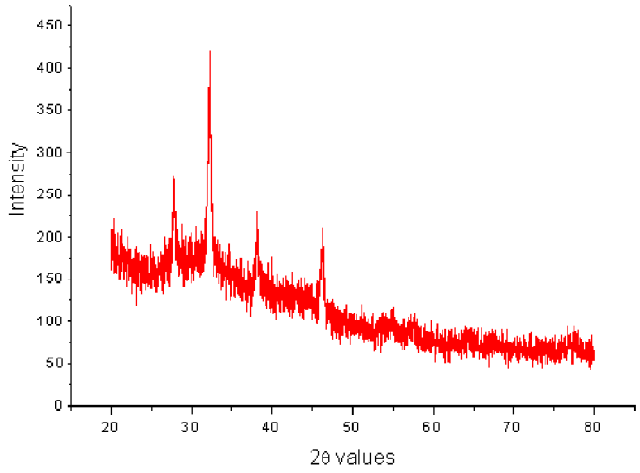

Fig. 3. Powder XRD pattern of the nanoparticles synthesized by aq. extract of *Phaseolus vulgaris.*

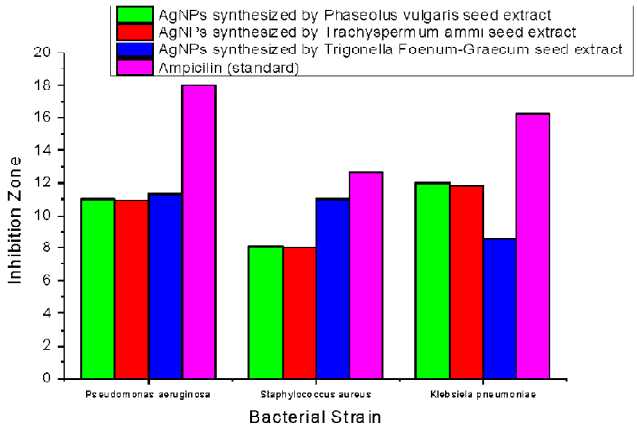

Fig. 5. Comparative antimicrobial activities of all the AgNPs synthesized by three different seed extracts and the Ampicilin (standard).

Antimicrobial activity: The results of the study of antimicrobial activity of synthesized AgNPs against three bacterial strains *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae* is depicted in Figs. 4 and 5 and in Table 1. All the synthesized nanoparticles are active against the bacterial strains and show different activity. Againt the *Pseudomonas aeruginosa* and *Staphylococcus aureus* the AgNPs synthesized by *Trachyspermum ammi* seed extract was most effective while for the *Klebsiella pneumoniae* the AgNPs synthesized by *Trigonella Foenum-Graecum* seed extract was show the maximum efficiency. The difference in the activity against the different bacterial strain may be due to the fact that different extracts contains different type and concentration of phytochemicals due to which size and shape along with the nature of the capping agent of silver nanoparticles may change which causes the variation in their activity. Some researchers also reported the antimicrobial activity of silver nanoparticles synthesized by microbes[20] but our results are more promising because it shows effective inhibition at much lower concentration of AgNPs for the above three bacterial strains. Since all these AgNPs were synthesized by plant extracts hence these are capped with the phytochemicals which makes them more suitable for the interaction with the bacterial cells. Silver is generally used as $AgNO_3$ for antimicrobial activity, but when it is used in the form of nanoparticles the surface area for microbes is increased to a great extend due to which very promising results are shown. Though researchers are still working to know the exact mechanism for the antimicrobial activity shown by AgNPs but it has been hypothesized that AgNPs may cause cell-lysis or inhibit the cell transduction[6] which results in the bacterial death.

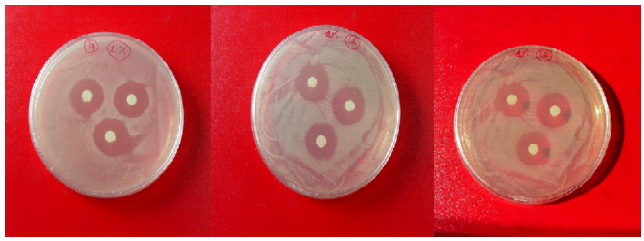

Fig. 4. Study of microbial activity of green synthesized AgNPs.

Table 1. Results of antimicrobial studies of green synthesized AgNPs in minimum inhibition concentration percentage

Sr. No.	Compounds	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Klebsiella pneumoniae*
1.	AgNPs synthesized by *Phaseolus vulgaris* seed extract	11.00±0.57	8.10±0.16	12.00±1.15
2.	AgNPs synthesized by *Trachyspermum ammi* seed extract	10.94±0.48	8.04±0.10	11.88±0.70
3.	AgNPs synthesized by *Trigonella Foenum-Graecum* seed extract	11.33±0.66	11.00±0.57	8.58±0.29
4.	Ampicilin (standard)	18.0±0.21	12.66±0.50	16.26±0.30

Conclusions

In past few years various methods including chemical synthesis, physical synthesis and synthesis by microbes have been employed for the synthesis of silver nanoparticles but they were found to be expensive and not the environmental friendly. In contrary to this the plant extracts are an excellent choice for the synthesis of AgNPs as they are easily available, non toxic in nature and provide fast and cost-effective synthesis option. All the three selected plant seed extracts are effective to reduce the silver ions and formation of stable AgNPs. The AgNPs capped with the phyto-chemicals can easily penetrate the bacterial cell and inhibit their growth hence show the good antimicrobial potential. The phyto-chemicals helps to improve the susceptibility of the cells for AgNPs and due to nano size the increases surface area improves the penetration of silver into the bacterial cell.

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