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Research Article

Green Synthesis of Silver Nanoparticles Using Different Plant Materials and Their Antibacterial Activity

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Abstract

The green route of metal nanoparticles synthesis has received significant attention in recent years due to it's cost-effective, non-toxic and eco-friendly nature in comparison to other physical and chemical methods. This study reports on the synthesis of silver nanoparticles (Ag-NPs) from bio-reduction of 1mM aqueous silver nitrate ($AgNO_3$) by extracts prepared from three different plants namely, *Brassica oleracea* L. *var. italica Plenck* (Broccoli), *Capsicum annuum* L. (Chili) and *Parthenium hysterophorus* L. (Carrot grass). The synthesized Ag-NPs were characterized using UV- visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). Ag-NPs synthesized showed the surface plasmon resonance with the appearance of absorption peaks around the range of 410-430 nm. The possible biomolecules involved in the reduction and the stabilization of synthesized Ag-NPs were found to be alcoholic, phenolic, amine and carbonyl groups. SEM study revealed that Ag-NPs were spherical in shape with varied size about 10-40 nm. Besides, the analysis of antioxidant and antibacterial activities of Ag-NPs was carried out. The Ag-NPs synthesized using *B. oleracea* extract showed the higher antioxidant activity than Ag-NPs synthesized from both *C. annuum* & *P. hysterophorus* extracts. Ag-NPs exhibited good antibacterial activity against both Gram-negative (*Escherichia coli)* and Gram-positive (*Staphylococcus aureus)* bacteria. The higher antibacterial activity was shown by Ag-NPs synthesized from *P. hysterophorus* extract in comparison to Ag-NPs synthesized from both *C. annuum* & *B. oleracea* extracts. Hence, it can be concluded that Ag-NPs synthesized following the green route could be the source for potential antioxidant and antibacterial agents.

Keywords: silver nanoparticles; *Parthenium hysterophorus*; *Capsicum annuum*; *Brassica oleracea*; FTIR; Antioxidant; Antibacterial Activity

Introduction

Nanotechnology is emerging as a field of interest nowadays due to its applications in various sectors such as energy, drug delivery, medicine, electronic, optics etc. having huge potential to contribute to society. It is the science of manipulating matter at nanoscale level (1-100 nm) to create new materials having improved physical and chemical properties. The research on nanoscale materials for identifying new properties and applications seems to be

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exciting but the synthesis of these materials with controlled properties is still the challenge for nanoscience and nanotechnology (Dahl *et al*., 2007). Among the various metal nanoparticles, silver nanoparticles (Ag-NPs) have gained much interest due to their unique physical, chemical properties, and diverse applications. The different kinds of biomedical applications of silver nanoparticles have been reported such as diagnostic biological tags, biosensors, anti-fungal, antibacterial agents in apparel, anti-inflammatory, anti-permeability and anti-angiogenic activity (Chokriwal *et al*., 2014). The anticancer activity of silver nanoparticles against different cancerous cell lines have been found (Jeyaraj *et al*., 2013). Silver nanoparticles have been used in wound dressings and as an antimicrobial coating (Law *et al*., 2008) as well as in paints, plastics, cosmetics to thermal & electrical conductivity (Vijayaraghavan *et al*., 2012).

Nanoparticles synthesis can be carried out by three different approaches which are physical, chemical and biological methods. Although the rate of production of nanoparticles is faster using physical & chemical methods, these methods are costly and have several harmful effects on the environment. The chemicals used in these methods are usually flammable, toxic and hard to dispose-off in the environment (Kowshik *et al*., 2003). These methods lead to the formation of toxic chemicals on nanoparticles surface which makes them unsuitable to use in medical fields (Jain *et al*., 2010). Also, the synthesized Ag-NPs are not of expected purity due to sedimentation of their surfaces with chemicals and Ag-NPs with well-defined size are very difficult to prepare which requires the addition of capping agent to prevent particle aggregation (Malik *et al*., 2002). On the other hand, biological methods involve the synthesis of nanoparticles through the use of biological components such as plant extracts, bacteria, and fungi. But the use of plants instead of fungi and bacteria for nanoparticles synthesis seems to be very promising due to the lack of pathogenicity (Pantidos and Horsfall, 2014). Plants are mostly preferred as a reducing agent due to difficulties faced in other methods such as the requirement of highly aseptic conditions in case of fungi & bacteria if they are used as a reducing agent (Sithara *et al*., 2017). Green routes seem to deliver controlled particle size and shape which could be used for various biomedical applications (Gurunathan *et al*., 2014). Also, the routes involving plants mediated synthesis of nanoparticles are much faster compared to microorganisms (Ahmed *et al*., 2016). The main advantage of green synthesis of Ag-NPs is that the secondary metabolites, amino acids or proteins present in the reaction medium act as both reducing & capping agents preventing the particle aggregation. Furthermore, the green synthesis is cost-effective, non-toxic, eco-friendly and pollution-free (Zhang *et al*., 2016). Therefore, the green route of nanoparticles synthesis could be the best alternative for physical and chemical methods.

The green synthesis of silver nanoparticles using leaves extract of *Olax scandens* (Mukherjee *et al*., 2014), *Nicotiana tobaccum* (Prasad *et al*., 2011), *Saraca indica* (Perugu *et al*., 2016), *Buddleja globosa* (Carmona *et al*., 2017) and widely available Indian plants (Banerjee *et al*., 2014) has been reported. Here, this study highlights on the synthesis of Ag-NPs through the bio-reduction of aqueous silver nitrate using extracts of three different plants namely, *Brassica oleracea* L. *var.italica Plenck* (Broccoli), *Capsicum annuum* L. (Chili) and *Parthenium hysterophorus* L. (Carrot grass). The selected plants have been reported to exhibit useful properties. Broccoli contains compounds called isothiocyanates such as sulforaphane & indole-3-carbinol which could possibly act as anticancer and antioxidant agents as well as help in detoxifying enzymes in the body (Caroling *et al*., 2013). *Capsicum* fruits were used as counter-irritant in lumbago, neuralgia, and rheumatic disorders since ages. The different types of antioxidant compounds are present in *Capsicum* fruits which act as an anti-aging factor (Jha and Prasad, 2011). *Parthenium hysterophorus* contains various compounds having medicinal values such as saponin, histamine, glucosides, and triterpenes. It was used as a folk remedy against ulcerated sores, facial neuralgia, anemia and fever (Kushwaha and Maurya, 2012). Here, we have reported on the synthesis of Ag-NPs, characterization of synthesized Ag-NPs and evaluation of their antioxidant & antibacterial activities.

Materials and Methods

Chemicals

Silver nitrate ($AgNO_3$) was purchased from Thermo Fisher Scientific Pvt. Ltd. India. Nutrient agar, nutrient broth, Mueller Hinton Agar (MHA) and antibiotics were purchased from Hi-media Laboratories Pvt. Ltd. Mumbai, India. Sterile deionized water was used throughout the experiments.

Plant Materials and Extracts Preparation

Capsicum annuum & *Brassica oleracea* were purchased from the local market and *Parthenium hysterophorus* was collected from Pepsicola, Kathmandu. The identification of plant species was done by Assistant Research Officer, Mr. Tirtha Raj Pandey, National Herbarium and Plant Laboratories, Lalitpur, Nepal. The fresh & healthy leaves of *P. hysterophorus*, fruits of *C. annuum* & florets of *B. oleracea* were washed with tap water followed by deionized water to remove dust particles and then dried at room temperature. 5 grams crushed sample of each plant were heated separately with 100 ml of deionized water in 250 ml beakers at 80ºC for 30 minutes. The prepared extracts were filtered through Whatman No. 1 filter papers and then stored at 4ºC until further use.

Synthesis and Purification of Silver Nanoparticles

The synthesis of silver nanoparticles was carried out by adding 10 ml extract of each plant into three separate conical flasks, each containing 90 ml of 1mM $AgNO_3$ solution. The conical flasks were wrapped with aluminum foil to avoid photo-activation of $AgNO_3$ and incubated at 60ºC in dark. The stirring of the reaction mixture was maintained for different time intervals. The color change of the reaction mixture was monitored periodically for 24 hours. The color change from light yellow to pale yellow and finally to reddish brown was observed indicating the formation of silver nanoparticles. The synthesized Ag-NPs were purified by centrifugation at 10,000 rpm for 30 minutes at 4ºC. The supernatants were discarded and pellets of colloidal silver were rinsed thrice with deionized water to remove organic impurities. At last, obtained Ag-NPs were dried in hot air oven at 60ºC and used for further experiments.

Characterization of Silver Nanoparticles

UV-Visible Spectroscopy

The formation of silver nanoparticles was initially confirmed by a change in color. After color change, the further confirmation of the synthesized silver nanoparticles was done by scanning the absorption spectra within the range of 300-800 nm using LT-2802 double beam UV-visible spectrophotometer.

FTIR Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) analysis was done using IRPrestige-21 FTIR spectrometer (Shimadzu). The FTIR spectra were collected within the range of 250–5000 cm^{-1} in the transmittance mode at a spatial resolution of 4 cm^{-1}.

Scanning Electron Microscopy (SEM)

The size and shape of the synthesized silver nanoparticles were examined at various magnifications using Hitachi S-4800 scanning electron microscope operating at 10 kV.

DPPH Free Radical Scavenging Assay

The free radical scavenging activity of silver nanoparticles and extracts was evaluated according to the method described by Brand -Williams & colleagues (Brand-Williams *et al.*, 1995) with some modifications. DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a stable free radical which has the capacity to accept an electron or hydrogen radical from hydrogen donating groups. 0.1 mM DPPH solution was prepared in methanol and the solutions of Ag-NPs and extracts were adjusted to make different concentrations of 25, 50, 100 & 200 µg/ml. 1 ml of sample was added to 2 ml of DPPH solution and the mixtures were shaken vigorously by vortexing and allowed to stand at room temperature in the dark for 30 minutes. Then the absorbance was measured at 517 nm against methanol (blank) where ascorbic acid was used as the reference. The percentage inhibition of DPPH free radical was calculated by using following formula.

$$\text{Percentage inhibition} = \frac{100(\text{Ac} - \text{Ao})}{\text{Ac}}$$

Where A_c is the absorbance of the control reaction and A_o is the absorbance of the test samples & reference.

Antibacterial Assay

The antibacterial activity of the synthesized silver nanoparticles was studied by the agar well diffusion method. *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) were used as test strains to determine the antimicrobial activity. A pure culture of bacteria was maintained by growing a single colony in nutrient broth for overnight. 0.1 ml of overnight bacterial culture was spread uniformly on individual Mueller Hinton agar plates using sterile cotton swabs and the wells were made with the help of flamed cork borer having a diameter of 6 mm approximately. 80 µl of 100 µg/ml concentration of Ag-NPs & streptomycin (reference) were loaded in respective wells. A separate plate was maintained as a negative control (well loaded with deionized water) for both bacteria. Then these plates were incubated at 37º C for 24 hours in a bacteriological incubator and the total inhibition zone diameter which appeared as a clear area around the well was measured. Finally, the zone of inhibition (ZOI) was calculated by subtracting well diameter from the total inhibition zone diameter.

Results and Discussion

Visual Observation

The formation of silver nanoparticles was initially analyzed by observing the change in color. The color of the reaction mixture started to change within two hours of incubation, from light yellow to pale yellow and finally to reddish brown after completion of reaction as shown in Fig.1. The silver nitrate solution without extract (control) did not show any color change.

Fig. 1: silver nanoparticles formation resulting in color change.

UV-Visible Spectral Analysis

The color change of aqueous silver nitrate solution on addition of extract was observed. Furthermore, the increase in the intensity of color was detected with the passage of

time. This may be due to the reduction of silver ions into silver nanoparticles as well as excitation of surface Plasmon resonance in Ag-NPs (Mulvaney, 1996). The absorption peaks of synthesized Ag-NPs using extracts of *B. oleracea*, *C. annuum* & *P. hysterophorus* were obtained at 410 nm, 430 nm & 432 nm respectively (Fig. 2). The broadening of peaks was observed in case of *B. oleracea* and *C. annuum* as compared to *P. hysterophorus* which indicated the formation of polydispersed large nanoparticles (Aromal and Philip, 2012).

FTIR Analysis

FTIR analysis of the biosynthesized silver nanoparticles was accomplished to find the probable biomolecules present in the extract which were involved in the reduction of silver ions and stabilization of the synthesized Ag-NPs (Fig. 3). The FTIR study of Ag-NPs showed the sharp peaks at 3278.99 cm^{-1}, 1635.64 cm^{-1}, 347.19 cm^{-1} (*C. annuum*), 3278.99 cm^{-1}, 1635.64 cm^{-1}, 331.76 cm^{-1} (*B. oleracea*) and 3278.99 cm^{-1}, 1635.64 cm^{-1}, 370.33 cm^{-1} (*P. hysterophorus*). The extracts showed the intense absorption peaks at 3271.27 cm^{-1}, 1635.64 cm^{-1}, 370.33 cm^{-1} (*C. annuum*), 3278.99 cm^{-1}, 1635.64 cm^{-1}, 362.62 cm^{-1} (*B. oleracea*) and 3278.99 cm^{-1}, 1635.64 cm^{-1}, 378.05 cm^{-1} (*P. hysterophorus*). The broad band at 3278.99 cm^{-1} represents the stretching vibrations of hydroxyl group (-OH) in alcohols and phenolic compounds and the band at 1635.64 cm^{-1} corresponds to the amide I bond of proteins arising due to carbonyl (C=O) stretch in proteins (Kong and Yu, 2007). From FTIR analysis, it can be concluded that the carbonyl group of amino acid residues and the proteins have the stronger affinity to bind metal ions specifying that the proteins could possibly aid in the capping of metal nanoparticles and hence stabilize the medium (Sathyavathi *et al*., 2010). Therefore, the presence of biomolecules in the medium could possibly perform the binary functions of reducing and stabilizing the Ag-NPs.

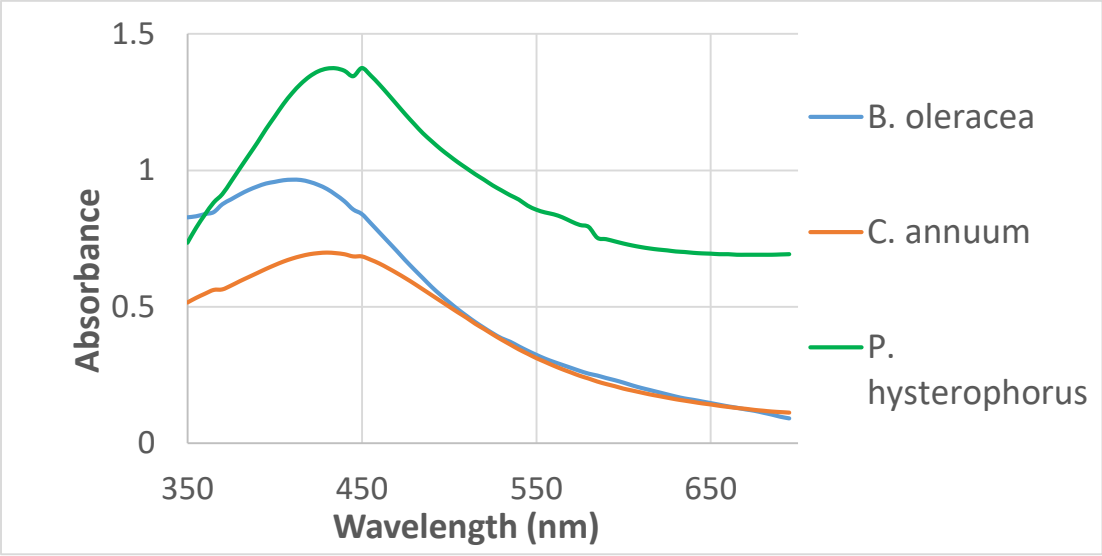

Fig. 2: UV-visible spectra of biosynthesized Ag-NPs using different plant extracts.

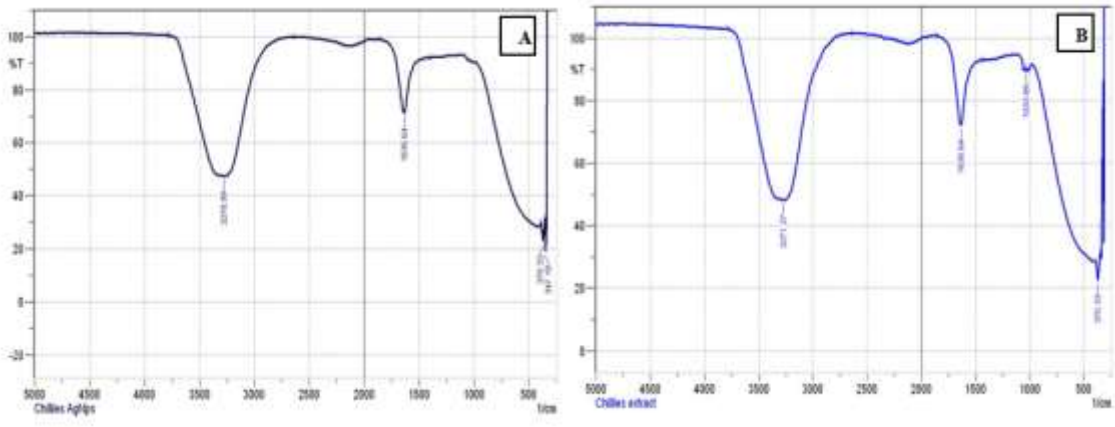

Fig. 3: FTIR spectra of silver nanoparticles (A) and extract (B).

SEM Analysis

The morphological characters and the structural analysis of the biosynthesized Ag-NPs were determined by the SEM micrographs at different magnifications. SEM is a surface imaging method which is capable of resolving particles of different sizes and shapes at the micro & nanoscale levels. Also, the morphology of particles can be analyzed using histogram obtained from images either by measuring & counting the particles manually or by using specific software (Fissan *et al*., 2014). From SEM analysis, silver nanoparticles were found to be highly aggregated spherical shape with varied size about 10-40 nm (Fig. 4).

Antioxidant Assay

The antioxidant activity of silver nanoparticles and extracts was evaluated and compared with ascorbic acid by the DPPH radical scavenging method (Table 1). The antioxidant activity of both silver nanoparticles and the extract was found to rise in a dose-dependent manner. The percentage inhibition of DPPH free radical was found to increase with an increase in the concentration of Ag-NPs and extract. The highest and the lowest percentage inhibition were observed in *P. hysterophorus* extract with 88.2 ± 0.07 % at 200 µg/ml and with 39.0 ± 0.06 % at 25 µg/ml in *C. annuum* extract respectively. Similarly, the Ag-NPs synthesized using *B. oleracea* extract showed the highest activity with 66.1 ± 0.11 % at 200 µg/ml and lowest activity by Ag-NPs synthesized using *C. annuum* with 24.0 ± 0.12 % at 25 µg/ml. The extract exhibited higher percentage inhibition than Ag-NPs for both *P. hysterophorus* and *C. annuum* whereas the opposite result was obtained in case of *B. oleracea* (i.e. higher percentage inhibition by Ag-NPs than extract). The antioxidant activity of synthesized Ag-NPs could possibly be due to functional groups attached to them which were derived from the extract (Bhakya *et al*., 2016). Therefore, this suggests that the variation in the antioxidant activity of extract and Ag-NPs may be due to the number of functional groups present in the extract and attached to synthesized Ag-NPs respectively.

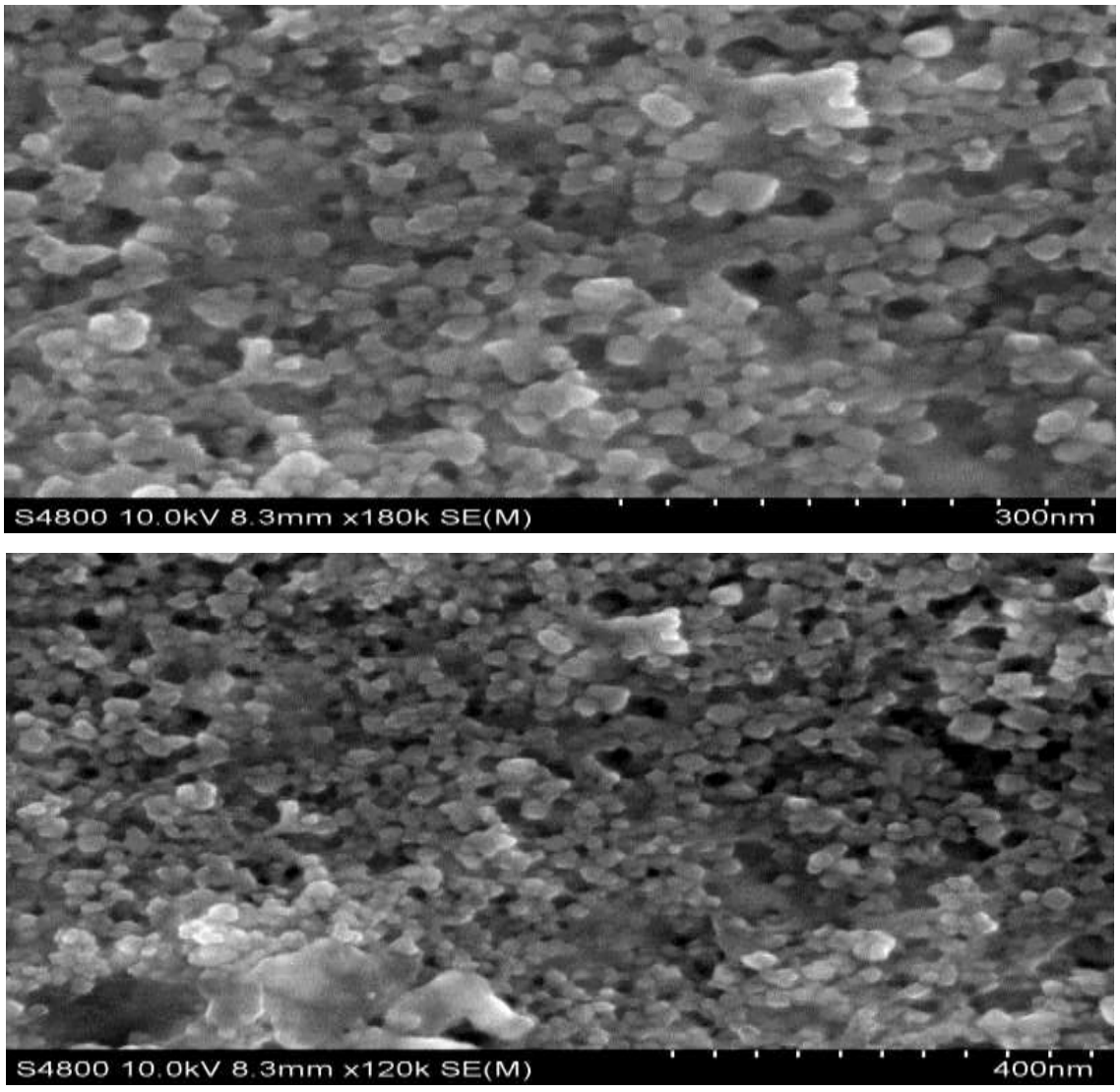

Fig. 4: SEM images of Ag-NPs synthesized from *P. hysterophorus.*

Antibacterial Assay

The Ag-NPs synthesized showed the bactericidal effect against both Gram-positive & Gram-negative bacteria (Table 2). The maximum and the minimum zone of inhibition were observed in *S. aureus* with 15.5 ± 0.08 mm & 8.8 ± 0.06 mm by Ag-NPs synthesized from *P. hysterophorus* & *C. annuum* extracts respectively. Likewise, the inhibition zone was larger in *S. aureus* than *E. coli* for Ag-NPs synthesized from *P. hysterophorus* extract whereas opposite result was observed for Ag-NPs synthesized from *C. annuum* & *B. oleracea* extracts (i.e. larger inhibition zone in *E. coli* than *S. aureus*). The extracts did not show any prominent zone of inhibition in comparison to synthesized Ag-NPs (Fig. 5). No inhibition zone was observed in the case of negative control. The difference between the susceptibility of Gram-positive & Gram-negative bacteria towards silver nanoparticles was reported. This is probable due to the difference in the cell wall structure between Gram-positive & Gram-negative bacteria since the difference in structural features of bacterial species and their interaction with silver nanoparticles could lead to differential susceptibility (Kora *et al*., 2010). Furthermore, the antibacterial action of silver nanoparticles synthesized using different plant materials may differ due to variations in shape & size of silver nanoparticles, bacterial inoculum size, exposure time and nutrient media used at the time of antibacterial assay (Raut *et al*., 2014)

Table 1: Antioxidant activity of extracts, Ag-NPs, and ascorbic acid.

SN	Concentration (µg/ml)	Percentage inhibition of DPPH free radical						
		Ascorbic acid	Extract			Ag-NPs		
			P. hysterophorus	*C. annuum*	*B. oleracea*	*P. hysterophorus*	*C. annuum*	*B. oleracea*
1.	25	88.5 ± 0.07	71.1 ± 0.10	39.0 ± 0.06	47.2 ± 0.16	43.2 ± 0.18	24.0 ± 0.12	55.4 ± 0.10
2.	50	96.8 ± 0.05	76.7 ± 0.08	43.4 ± 0.11	48.5 ± 0.11	51.6 ± 0.16	36.9 ± 0.14	60.6 ± 0.06
3.	100	97.2 ± 0.08	84.2 ± 0.14	44.5 ± 0.10	52.9 ± 0.12	55.1 ± 0.15	41.1 ± 0.16	64.6 ± 0.14
4.	200	97.9 ± 0.06	88.2 ± 0.07	49.8 ± 0.12	59.0 ± 0.17	61.7 ± 0.19	43.7 ± 0.15	66.1 ± 0.11

Data represent mean ± S.D. of triplicate experiments.

Table 2: Antibacterial activity of biosynthesized Ag-NPs.

Pathogenic bacteria	Zone of inhibition(mm)			
	Ag-NPs			Streptomycin (100µg/ml)
	P. hysterophorus	*C. annuum*	*B. oleracea*	
E. coli	15.1 ± 0.09	10.8 ± 0.08	14.6 ± 0.07	16.5 ± 0.05
S. aureus	15.5 ± 0.08	8.8 ± 0.06	9.0 ± 0.09	16.6 ± 0.06

Data represent mean ± S.D. of triplicate experiments.

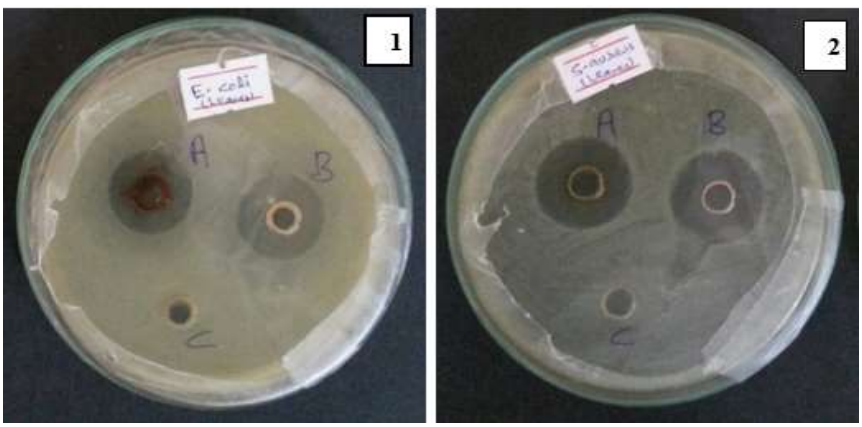

Fig. 5: Antibacterial activity of biosynthesized Ag-NPs: (1) *E. coli* and (2) *S. aureus* – Ag-NPs (A), streptomycin (B) & extract (C).

Conclusion

The synthesis of metal nanoparticles following green route has gained a lot of attention in recent years due to its eco-friendly and non-toxic nature. Among the various biological entities, plant materials have been chosen as a primary interest since they contain different bioactive compounds which act as both reducing & stabilizing agents in the reaction medium. The synthesis of silver nanoparticles (Ag-NPs) from bio-reduction of silver ions using the extracts of *P. hysterophorous*, *C. annuum* and *B. oleracea* was carried out. The synthesized silver nanoparticles were characterized with the help of UV-visible spectroscopy, FTIR and SEM analysis. The characteristic absorption spectra were determined using UV-visible spectrophotometer and the analysis of SEM images revealed that Ag-NPs were spherical in shape with varied size. FTIR analysis confirmed the presence of biomolecules in the extract responsible for the reduction and stabilization of Ag-NPs. Also, the Ag-NPs showed the appreciable amount of antioxidant and antibacterial activities. These biosynthesized Ag-NPs exhibited good antibacterial activity against both Gram-positive and Gram-negative bacteria. Therefore, due to ease of production, stability, efficient antioxidant & antimicrobial property, biosynthesized silver nanoparticles could be exploited in pharmaceutical and medical fields.

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