

Biofabrication of Silver nanoparticles by leaf extract of *Andrographis serpyllifolia* and their antimicrobial and antioxidant activity

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Abstract

Biofabrication of metallic nanoparticles have attracted the researchers of various disciplines for the past few decades, due to their wide range of applications in the various fields of electronics, textiles industries, nano medicine, biopharmacy and biotechnology. Keeping in view the above important applications, we have successfully biosynthesized the silver nanoparticles (AgNPs) using leaf extract of *Andrographis serpyllifolia*. These green silver nanoparticles are characterized by using different spectroscopic methods like ultra violet-visible spectroscopy (UV-Vis), Fourier transform-infrared spectroscopy (FTIR), transmission electron microscope (TEM), Energy-dispersive X-ray spectroscopy (EDX) and X-ray diffraction (XRD). The green As-AgNPs are characterized by spectral analysis by Nanodrop-UV-visible spectroscopy, the surface plasmon resonance peak of silver nanoparticles in colloidal solution, showed maximum absorption at 421 nm. Fourier transform-infrared spectroscopy results indicate the participation of N-H stretching of amides, carboxylic acids, ether groups and O-H hydroxyl groups and C-O stretching of alcohols are used in the formation of As-AgNPs. The XRD data indicate that As-AgNPs are the face-centered cubic structure in nature. The particle size analysis reveals that the biosynthesized As-AgNPs were spherical in shape and the average size is 6 ± 2 nm. The As-AgNPs are highly stable due the negative Zeta potential -27.2 mV indicates that the As-AgNPs are polydispersed in nature. The antimicrobial studies of As-AgNPs on different bacterial strains show effective antimicrobial activity when compared with the standard antibiotic. The biosynthesized As-AgNPs also showed excellent antioxidant activity by DPPH, Nitric oxide and Hydrogen peroxide method. Hence the biosynthesized silver nanoparticles can be useful for various biomedical applications.

Keywords: *Andrographis Serpyllifolia*; Antimicrobial Activity; Antioxidant Activity; As-AgNPs; Biofabrication; Spectral Characterization.

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INTRODUCTION

The combination of nanotechnology and green synthesis has created new possibilities for plant researchers, for the development of various biomedical applications through the synthesis of metallic nanoparticles. They are used for numerous applications such as the catalysis, electronics, optics, environmental and biotechnology [1-2]. The metals such as gold, silver and copper have been used extensively

for the synthesis of stable dispersion of nanoparticles, which are highly efficient in fields such as biological labeling, photonics, and surface-enhanced Raman scattering (SERS) detection [3-4]. Silver nanoparticles are also used in the various fields of bio-sensing, imaging, drug delivery, nanodevice fabrication and medicine [5-6]. The green synthesis of nanoparticles from plant extracts has drawn attention for its simple, rapid, stable, inexpensive and viable alternative method to chemical and physical methods.

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Recently, different parts of plant extracts have been extensively used for the synthesis of silver nanoparticles by using, *Andrographis paniculata* [7], *Cathranthus roseus* [8], *Ficus* fruit [9], *Coleus aromaticus* leaf [10] *Glycorhiza galbra* root [11] *Cassia alata* leaf [12] *Indigofera hirsuta* leaf [13] *Allamanda carthica* latex [14] synthetic humic substances [15] poly(*N* vinyl 2 pyrrolidone PVP, and also seed aqueous extract of *Olea europaea*. [16-17] *Prangos ferulaceae* leaves extract, *Cynanchum L* extract[18-19] have been used for the biosynthesis of metallic nanoparticles of different sizes and also studied their spectral properties and beneficial application.

It is a well-known that the silver ions and silver based compounds are highly toxic in nature to the microorganisms. This toxic nature depends upon the nanosized particles which are specific in size, shape and exhibits different physical and chemical properties [7-9]. Past studies have shown that high concentrations of nontoxic nanosized silver particles against different *Escherichia coli* bacteria exhibited good bactericidal activity [10, 16, 20]. Silver nanoparticles also exhibit antimicrobial properties against different bacterial species; current studies have been focusing on the physicochemical properties and concentrations of AgNPs, which ultimately inhibit *E.coli* [10], *Staphylococcus aureus* [16], *B. subtilis* and *K. mobilis Salmonella*, *Shigella* and *Proteus*. So the present study deals with the green synthesis of AgNPs using leaf extract of *Andrographis serpyllifolia*, to study antimicrobial and antioxidant activity.

Andrographis serpyllifolia is commonly known as round leaf Kariyat, Aaku chandrika. This plant is a trailing herb auxiliary plant species which belongs to the *Acanthaceae* family, and mostly found in the southern India states of Andhra Pradesh, Tamil Nadu, Karnataka and Kerala. In Andhra Pradesh this plant was collected from the Talakona forest region of Chittoor district. *A.serpyllifolia* whole plant contains alkaloids, flavonoids, steroids, phenols, saponins, terpenoids, anthraquinones, sugar, glycosides, phycobalamin and tannins [21]. The bioactive phyto-constituents of *A.serpyllifolia* are associated to have important biomedical applications like antibacterial, anti diabetic, anticancer, anti ulcer, and anti inflammatory activities [22-23] It is also reported that in *A.serpyllifolia* have the presence of important chemical constituents like serpyllin,

apigenin 7,4'- dimethyl ether and tectochrysin compounds of high medicinal value [24-25]. Andrographolide (AG) has been reported as one of the potential bioactive component of *A. serpyllifolia* which is found to be responsible for several pharmacological and clinical activities [26-27]. *A.serpyllifolia* plant is used as traditional Indian herbal medicine for malaria and dysentery. The root extract of this plant is used to cure fever. The plant extract is used in treating wounds and also found effective in the treatment of jaundice. Aqueous and methanolic extracts of this plant showed anti-proliferative and antioxidant properties. *A. serpyllifolia* have excellent antimicrobial activity, found to be effective in the treatment of several microbial infections. [28-30] The root extracts like chloroform extract, methanolic extract exhibited higher anti-inflammatory activity both *in vitro* and *in vivo* conditions [31].

MATERIALS AND METHODS

Synthesis of silver nanoparticles

The *Andrographis serpyllifolia* plant leaves were collected from the shrubs growing in and around of Thalakona region of Tirupati and Chittoor district of Andhra Pradesh, India in the months of October and November. The leaves were collected and shade dried for a week at room temperature and then made into fine powder with the help of electric blender. The plant leaf extract was prepared, by adding 5g of finely powder leaves in a 500 ml Erlenmeyer flask with 250 ml of Milli Q ultra pure distilled water and the mixture was heated at 70°C for 30 min and then filtered through sterile muslin cloth followed by whatmann No.1 filter paper. This filtrate solution was used as source of extract for preparation of silver nanoparticles and was utilized in subsequent procedures. To the 5ml diluted filtrate, 10ml of 0.002(M) AgNO₃ was added and the sample was left at room temperature, until the color of solution changed from pale light green color to light brown and subsequently dark brown. The solution containing AgNPs was confirmed by the dark brown color (Fig. 1). So, in the current study the biosynthesis of silver nanoparticles (AgNPs) with *Andrographis serpyllifolia* (*A. serpyllifolia*) plant leaves extract was prepared without any external toxic chemicals which is popularly known as "Green synthesis" or "Green Method"

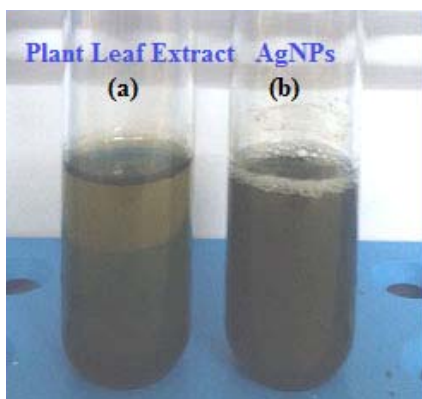


Fig. 1. (a) Aqueous Plant leaf extract of *Andrographis serpyllifolia* and (b) AgNPs solution after addition of extract to AgNO_3 solution.

Characterization

The bio-reduction of pure Ag^+ ions done with the leaf extract of *Andrographis serpyllifolia* was monitored periodically by sampling of the 1 μl and the optical absorbance of silver nanoparticles suspended in distilled water was recorded on UV-Vis Spectrophotometer (Nanodrop 8000 UV-Vis spectrometer) in 220–700nm wavelength range. The reaction solutions were carried out at room temperature on spectrophotometer at a resolution of 1 nm. The FT-IR was carried by using Bruker Tensor 27, Particle size and zeta potential measurement experiments were carried out by using a Nanopartica (Horiba Nanopartica SZ-100 instrument) EDX was performed by Oxford Inca Penta FeTX3 EDS instrument attached to Carl Zeiss EVO MA 15 Scanning Electron Microscope (200 kV) machine with a line resolution 2.32 (in \AA). These images were taken by drop coating AgNPs on an aluminum foil. Energy Dispersive Absorption Spectroscopy photograph of AgNPs were carried out by the SEM equipment, as mentioned above. In X-Ray diffractometry (XRD) study was conceded to prove the crystalline nature of the AgNPs using Ultima IV X-ray powder diffractometer (Rigaku Ltd, Tokyo, Japan) by means of $\text{Cu K}\alpha$ radiation source (Madras university, Chennai). TEM analysis was carried out, using 200 KV FEI-Tecnaï G2 20 S-TWIN High resolution on Transmission Electron Microscope (VIT, Vellore).

Antimicrobial activity

The antimicrobial activity of silver nanoparticles was evaluated against Gram positive; *Staphylococcus aureus*, *Lacto bacillus*, *Enterococcus* Gram negative *Escherichia coli*, by disc diffusion method. The

bacterial strains of microorganisms used for the antibacterial activities of silver nanoparticles were obtained from DST-PURSE Centre, S.V. University, Tirupati. All the bacterial strains maintained on nutrient agar and were sub cultured freshly and used for the studies. Bacterial cultures were prepared by transferring a single colony into a tube containing 10 ml nutrient broth and grown overnight at 37 °C. The overnight grown cultures were prepared in nutrient broth (Himedia, gm/L). The individual microorganisms were prepared by spreading 100 μl of culture on the nutrient agar plate with the help of spreader. Sterile discs were prepared by using Whatmann No.1 filter paper. The discs were placed on agar plates and different concentrations of sample of biosynthesized As-AgNPs were added on the disc with the help of micropipette. The plates were incubated at 37°C overnight and observed next day for zone of inhibition (ZIO) was measured and the results photographed and tabulated.

Antioxidant activity

The antioxidant activity of *Andrographis serpyllifolia* (A. *serpyllifolia*) plant leaves extract and biosynthesized As-AgNPs were evaluated *in vitro* by three methods. All the synthesized compounds showed good free radical scavenging activity against 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Nitric oxide (NO) and Hydrogen peroxide (H_2O_2) radicals. Ascorbic acid is used as a standard control.

DPPH radical scavenging activity

The hydrogen atom or electron donation ability of the compounds was measured from the bleaching of the purple colored methanol solution of (DPPH) [32]. The spectrophotometric assay uses the stable radical DPPH as a reagent. 1 ml of various concentrations of the test compounds (25, 50, 75, and 100 $\mu\text{g}/\text{mL}$) in methanol was added to 4 mL of 0.004% (w/v) methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was measured against blank at 517 nm. The percent of inhibition (%) of free radical production from DPPH was calculated by the following equation. All the experiments were conducted and radical scavenging activity values were calculated from the absorbance values using the following formula.

$$\text{Radical Scavenging Activity} = \frac{(\text{Ac} - \text{As})}{(\text{Ac})} \times 100$$

Where A_c is the absorbance of the control reaction (containing all reagents except the test compound) and A_s is the absorbance of the test compound. Tests were carried in triplicate.

Nitric oxide scavenging activity

Nitric oxide scavenging activity was measured by slightly modified methods [34]. Nitric oxide radicals (NO) were generated from sodium nitroprusside. 1 mL of sodium nitroprusside (10 mM) and 1.5 ml of phosphate buffer saline (0.2 M, pH 7.4) were added to different concentrations (25, 50, 75 and 100 $\mu\text{g}/\text{mL}$) of the test compounds and incubated for 150 min at 25°C and 1mL of the reaction mixture was treated with 1 mL of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore was measured at 546 nm.

Hydrogen peroxide scavenging activity

The H_2O_2 scavenging ability of the test compounds was determined according to the literature method [35]. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). 25, 50, 75 and 100 $\mu\text{g}/\text{mL}$ concentrations of the test compounds in 3.4 mL phosphate buffer were added to H_2O_2 solution (0.6 ml, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm.

RESULTS AND DISCUSSION

Ultra violet-visible spectroscopy of As-AgNPs

UV-visible spectroscopy is a straightforward technique for the detection and also for

confirming the formation of nanoparticles. The bio-reduction of Ag^+ ions by using the leaf extract of *Andrographis serpyllifolia* was monitored from time to time by sampling of the 1 μl aliquots and the optical absorbance was recorded on the Nanodrop 8000 UV-vis spectrophotometer in 220 – 700 nm wavelength range. The UV-visible spectra (Fig. 2) of the bio-reduced As-AgNPs solution showed an absorbance peak at 421 nm which is a characteristic surface plasmon resonance (SPR) peak of silver nanoparticles, which confirms their biosynthesis. The size and shape of the As-AgNPs reflects on the absorbance peak. [36] It well known that the SPR range of silver nanoparticles is between 390nm to 470nm, earlier reports on biosynthesized AgNPs also reveals the similar results that if the SPR of AgNPs is in-between 400nm to 450 nm range, it reveals that the AgNPs are small and spherical in shape, in the size range of 20nm to 100nm [37]. In the present study the SPR of As-AgNPs is 421nm, it indicates that the nanoparticles are small and spherical in shape this further confirmed by TEM analysis and Particle size analysis.

Fourier transform infra-red (FTIR) spectra analysis of As-AgNPs

The FTIR analysis was performed by using Bruker Tensor 27. The FTIR spectral analysis of As-AgNPs (Fig. 3) showed intensive peaks at 3844, 3735, 3292, 2921, 2351, 1640, 1528, 1408, 1242, 1030 and 534 cm^{-1} respectively. The sharp peaks at, 3844 cm^{-1} , 3292 cm^{-1} , 2921 cm^{-1} , 2351 cm^{-1} can be assigned to hydroxyl group of alkanes such as O-H and C-H stretching of the –alkenes group, 1640 cm^{-1} can be assigned to C=C stretching of

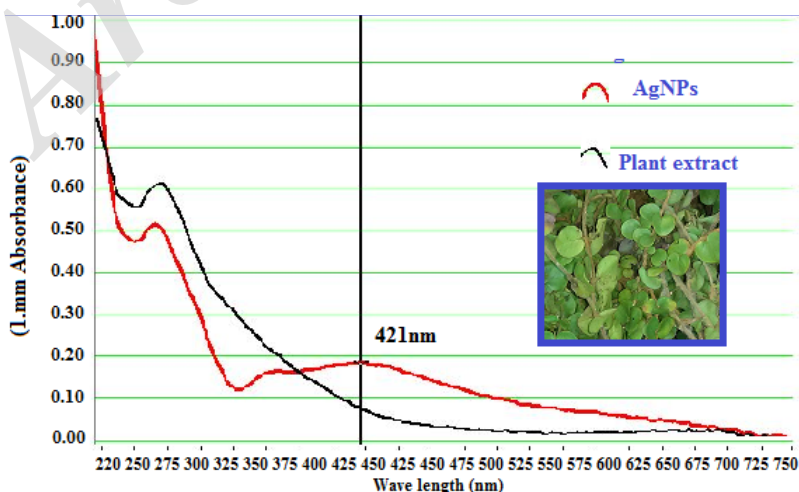


Fig. 2. UV-visible spectra of *Andrographis serpyllifolia* leaves extract and biosynthesized As-AgNPs with 0.002(M) silver nitrate.

the alkenes group, 1520 cm^{-1} , 1408 cm^{-1} can be assigned to Amide II stretching, 1242 cm^{-1} can be assigned to amide III stretching, 1030 cm^{-1} can be assigned to -C-O-C- stretching and -C=C- of the amide group.[15, 38] Therefore the FTIR analysis clearly revealed that the phytochemicals like amides, carboxylic acids, ether groups, hydroxyl groups and alcohol groups present in the leaf extract were mainly responsible for the reduction of silver ions (Ag^+) into nanoscale silver particles (Ag^0). From the above results, it may be concluded that Andrographolides are the major bioactive compounds responsible for capping and efficient stabilization of As-AgNPs by acting as capping agents [7].

Particle size and Zeta potential analysis of As-AgNPs

The particle size of the biosynthesized As-AgNPs is detected by the intensity and laser diffraction method using the biosynthesized colloidal solution in which the As-AgNPs are poly-dispersed in mixture solution. The distribution of As-AgNPs are in the range of 1nm to 12 nm in size with and the average size of synthesized As-AgNPs was found to be 6.1 nm (Fig. 4) with and PI value of 0.437 (poly disperse index). Further the zeta potential analysis of As-AgNPs was detected to be -27.2 mV , due to its high negative zeta potential it prevent the As- AgNPs from agglomeration in the medium, leading to long term stability, because

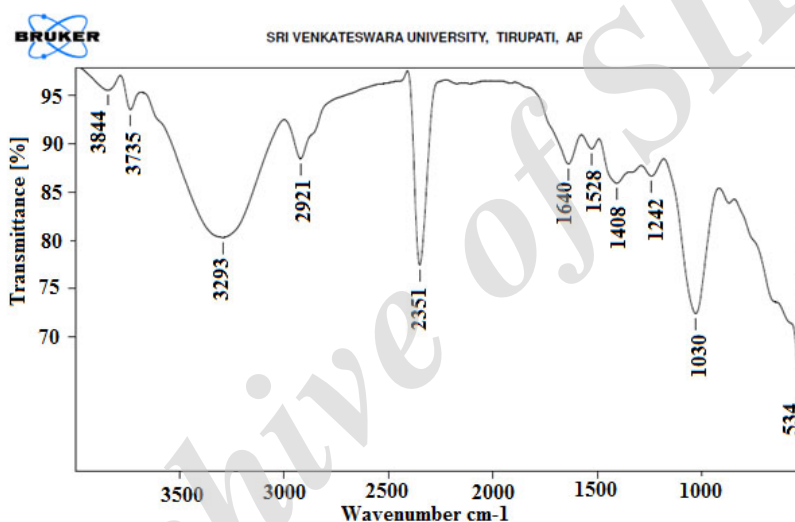


Fig. 3 FTIR analysis biosynthesized As-AgNPs by leaf extract of *Andrographis serpyllifolia*.

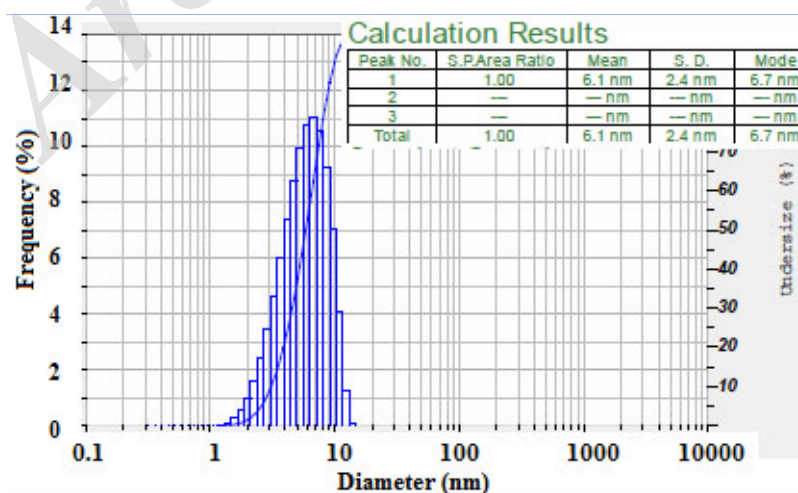


Fig. 4. Particle size distribution curve for As- AgNPs.

of the electrostatic repulsive force between the As-AgNPs. The zeta potential of As-AgNPs of *Andrographis serpyllifolia* leaf extract was found to be -27.2 mV (Fig. 5) which is similar to earlier reports [7-9, 16]

Energy dispersive X-Ray spectroscopy (EDX) of As-AgNPs

The EDX analysis were performed with the help of the Oxford Inca Penta FeTX3 EDS instrument attached to Carl Zeiss EVO MA 15 scanning electron microscope (200kV) with a line resolution of 2.32 (in Å). The EDX data revealed that, very strong signal to silver and weak signals to other elements

indicates the complete reduction of silver ions to elemental silver that is As-AgNPs (Fig. 6). The results are similar to earlier reports [10-13, 16]

X-Ray diffraction analysis (XRD) of As-AgNPs

XRD is an advanced spectroscopic technique used to evaluate the crystalline nature of biosynthesized As-AgNPs. The XRD pattern obtained revealed that As-AgNPs are face centered cubic structures in nature (Fig. 7) and that five characteristic diffraction peaks indexed to (111), (220), (311), (420), (422) and (220) planes of face centered cubic crystal lattice of Silver (JCPDS card No.01-1167).[13, 39-40].

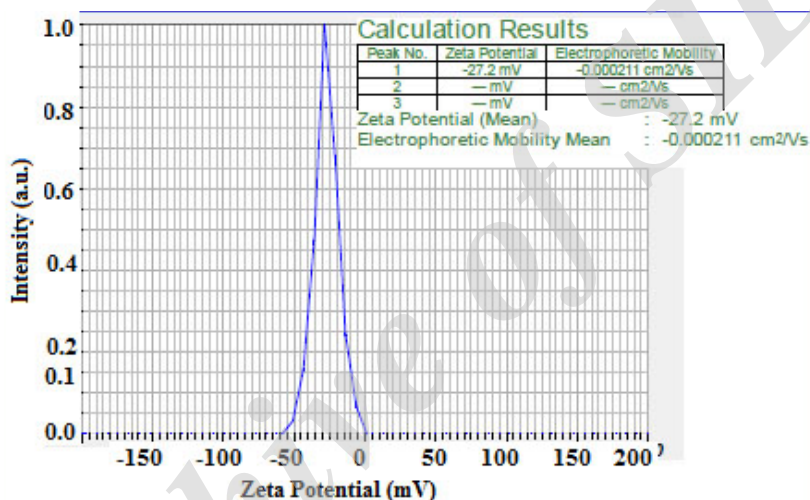


Fig. 5. Zeta potential of biosynthesized As-AgNPs.

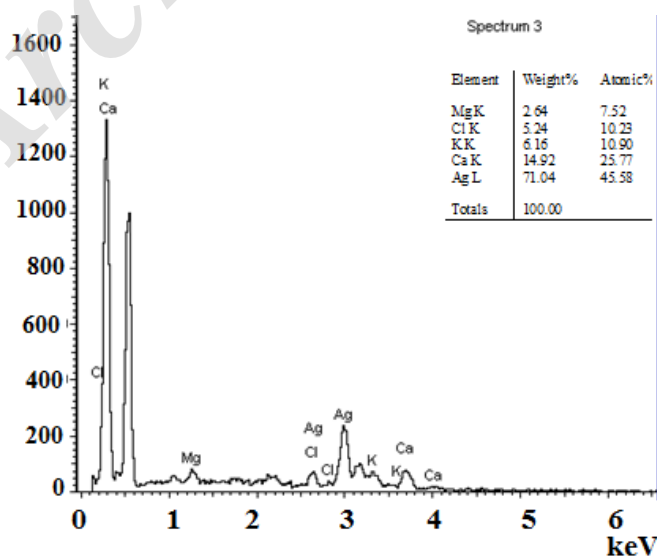


Fig. 6. EDX spectrum of biosynthesized As-AgNPs.

Transmission electron microscopy (TEM) analysis of As-AgNPs

TEM analysis was performed to determine the shape and size of the biosynthesized As-AgNPs. TEM analysis was carried out using 200 kV FEI-Tecnaï G@ 20 S-TWIN High resolution TEM (VIT, Vellore). The TEM image analysis also revealed that the As-AgNPs are roughly spherical in shape the As-AgNPs size is in-between 2 nm to 20 nm with an average size of 6 ± 2 nm (Fig. 8), the results are similar to particle size analysis. The SAED pattern analysis also reveals that the particles have different planes of cubic crystal lattice which is already confirmed by XRD analysis.

Biomedical Applications of As-AgNPs synthesized from *Andrographis serpyllifolia*

The antimicrobial activity and the antioxidant activity were performed from the As-AgNPs

biosynthesized from leaf extract of *Andrographis serpyllifolia*.

Antimicrobial activity of As-AgNPs

To reveal the biomedical importance of the As-AgNPs biosynthesized from *Andrographis serpyllifolia*, antimicrobial activity was performed against four bacterial species viz. Gram positive *Staphylococcus aureus*, *Lactobacillus*, *Enterococcus* and Gram negative *Escherichia coli*, by the Kirby – Bauer disc diffusion method. The synthesized As-AgNPs from *Andrographis serpyllifolia* exhibited excellent antibacterial activity against *Lactobacillus* and *Staphylococcus aureus* and zone of inhibition (ZOI) of diameters 8 mm, 12 mm, 14 mm and 20 mm and 7mm, 12 mm, 14mm and 16mm respectively. The As-AgNPs showed a very good antibacterial activity against both Gram +ve and Gram –ve bacteria (Fig. 9).

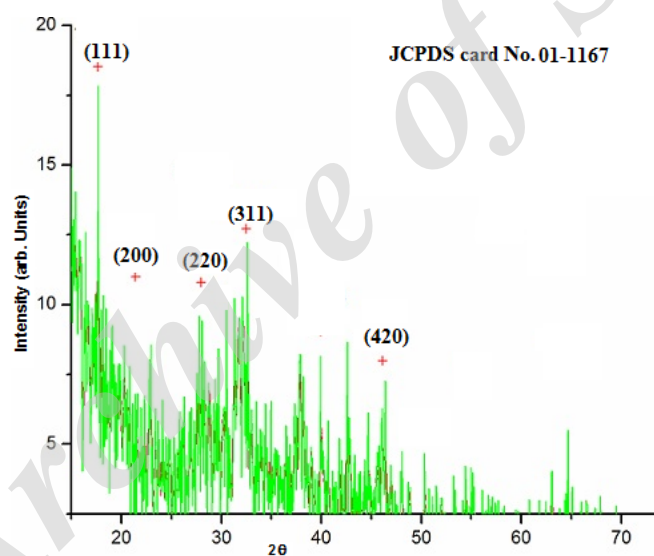


Fig. 7. XRD analysis of As-AgNPs.

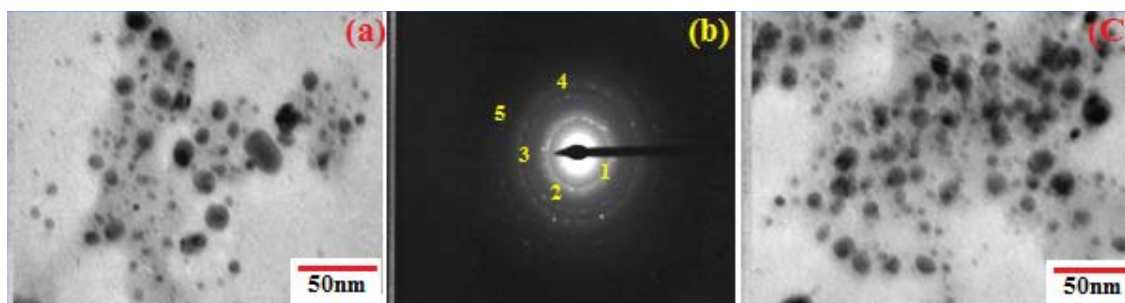


Fig. 8. TEM micrographs of biosynthesized As-AgNPs (a) As-AgNPs at 50nm scale, (b) SAED pattern of As-AgNPs, (c) As-AgNPs at 50nm scale.

The As-AgNPs exhibited almost moderate activity with that of Amoxyclav (Table 1). The results were similar to the earlier reports [7-10, 16-17]

Antioxidant activity of biosynthesized As-AgNPs

In the present study we determined the free radical scavenging activity that is the measurement of the antioxidant levels in leaf extracts of

Andrographis serpyllifolia and biosynthesized As-AgNPs, by different method like DPPH, H₂O₂ and NO antioxidant activity. Plants are usually rich in polyphenols. In the present study, the *Andrographis serpyllifolia* plant has a very high content of Andrographolides which are supposed to poses various medical and pharmaceutical functions. These important plant components give

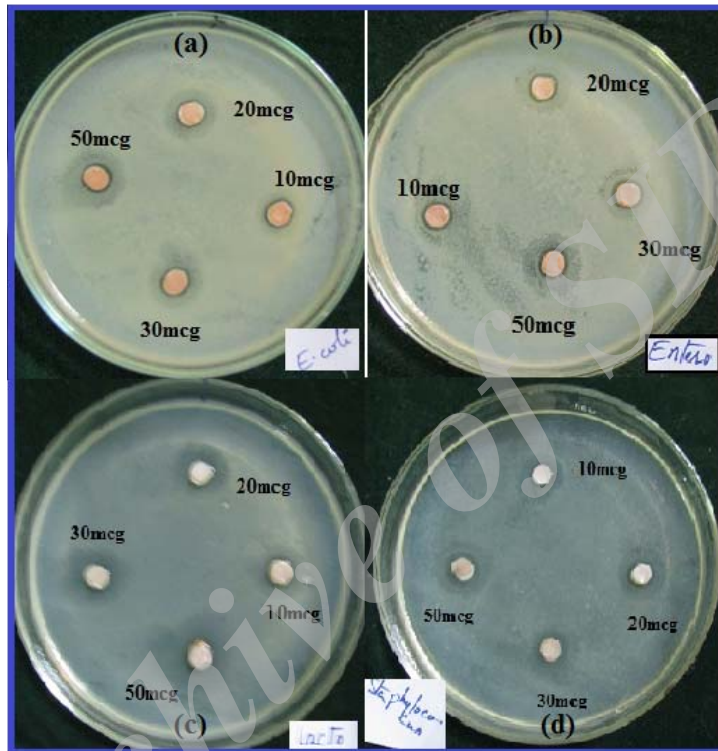


Fig. 9. Antimicrobial activity of biosynthesized As- gNPs against Gram +ve and Gram -ve bacteria *Escherichia coli*, (b) *Enterococcus*, (c) *Lactobacillus*, (d) *Staphylococcus aureus*.

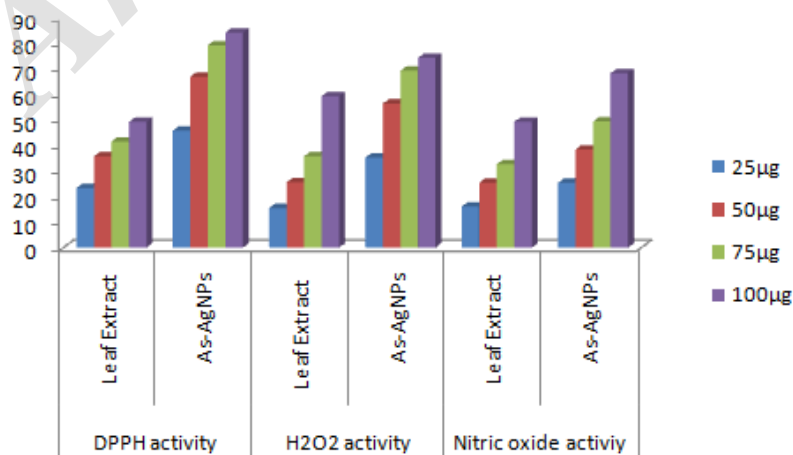


Fig. 10. Antioxidant activity of biosynthesized As- AgNPs.

Table 1. Antimicrobial activity of biosynthesized As-AgNPs.

S.No.	Tested Bacterial Species	10mcg	20mcg	30mcg	50mcg
1	<i>Staphylococcus aureus</i>	7mm	12mm	14mm	16mm
2	<i>Lactobacillus</i>	8mm	12mm	14mm	20mm
3	<i>Enterococcus</i>	5mm	9mm	11mm	18mm
4	<i>Escherichia coli</i>	6mm	10mm	12mm	18mm
5	Amoxyclav antibiotic (30mcg)			22 mm	

Values are expressed as diameter of zone of inhibition (ZOI).

Table 2. Antioxidant activity of biosynthesized As-AgNPs.

S.No	Concentration	DPPH activity		H ₂ O ₂ activity		Nitric oxide activity	
		Leaf Extract	As-AgNPs	Leaf Extract	As-AgNPs	Leaf Extract	As-AgNPs
1	25µg	23.42	45.84	15.49	35.24	16.14	25.42
2	50µg	35.81	66.94	25.61	56.44	25.41	38.45
3	75µg	41.54	79.20	35.84	69.28	32.67	49.40
4	100µg	49.24	84.17	59.34	74.31	49.24	68.27

up hydrogen atoms from their hydroxyl groups to free radicals and form stable phenoxyl radicals. The results revealed that both plant leaf extract and biosynthesized As-AgNPs have excellent antioxidant activity. DPPH method proves to be the more efficient method when compared with H₂O₂ and NO antioxidant activity [32]. The previous reports on plant mediated humic acids also have very good antioxidant activity [33]. The antioxidant activity was more with bioinspired As-AgNPs when compared with plant leaf extract. (Fig. 10) and (Table 2).

SUMMARY AND CONCLUSION

The biofabricated silver nanoparticles (AgNPs) using leaf extract of *Andrographis serpyllifolia* where characterized by using different spectroscopic methods. The biosynthesized green As-AgNPs were spherical in shape and the average size was depicted to be 6 ± 2 nm. The As-AgNPs are highly stable due the negative Zeta potential indicating that the As-AgNPs are polydispersed in nature. Further the antimicrobial study of As-AgNPs on different bacterial strains indicates effective antimicrobial activity, As-AgNPs also showed excellent antioxidant activity by DPPH, Nitric oxide and Hydrogen peroxide method. Therefore the biosynthesized As-AgNPs can be valuable for a variety of biomedical applications.

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CONFLICT OF INTEREST

All Authors don't have any conflict of interest.

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