

Synthesis of silver nanoparticles using *Aeschynomene indica* L. aqueous leaf extract and evaluation of its Antibacterial activity

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Abstract

Green nanoparticle synthesis methods have immense therapeutic applications due to their biocompatibility and lack of toxicity. We report the synthesis of silver nanoparticles (AgNPs) using the leaf extracts of traditional medicinal plant *Aeschynomene indica* (Fabaceae). The color change from yellow to brown and absorption peak at 447 nm indicated the synthesis of AgNPs. High-resolution transmission electron microscopy images exhibit polydisperse, spherical AgNPs in the size range of 10-24nm. X-ray diffraction studies confirmed the crystalline nature with a face-centered cubic (FCC) structure of the AgNPs. The stability of the nanoparticles was confirmed by dynamic light scattering and zeta potential studies. FTIR spectroscopy revealed the presence of compounds with functional groups such as primary amines, phenols, aldehyde/ketone, aromatic, and nitro groups, which may be responsible for the reduction of AgNPs. The antibacterial activity of plant extracts and synthesized AgNPs was confirmed by agar well diffusion method. The synthesized AgNPs showed potential antibacterial activity with a zone of inhibition of 14.5 mm on *Pseudomonas aeruginosa*; 14.2 mm on *Escherichia coli*; 11.5 mm on *Staphylococcus aureus*; and 10.3 mm on *Bacillus subtilis*. Also, the silver nanoparticles exhibited a 73.9% antioxidant activity confirmed by DPPH analysis. The antibacterial and antioxidant activities of AgNPs synthesized from leaf extracts of *A. indica* can be employed for biomedical applications.

Key words: *Aeschynomene indica*, DPPH, HR-TEM, AgNPs, antibacterial activity

Introduction

Currently, nanotechnology has been employed in biological systems to synthesize nanoparticles of biomedical importance (Almutairi, 2019; Nourmohammadi et al., 2018). Nanotechnology is the science of production and utilization of physical, chemical,

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and biological systems below 100nm (Ismail et al., 2019; Dubey and Singh, 2019). This particular area has great potential in countries that are considered rich in biodiversity (Ahmad et al., 2016; Bakhy, 2018). Bioactive compounds of traditional medicinal plants can be employed as agents to reduce the Ag ions. Compared to the chemical synthesis of nanoparticles, green chemistry (biocompounds of plants) can be employed to synthesize NPs with biocompatibility and less toxicity. Several strategies have been developed for the synthesis of AgNPs using the extracts of plants, which contain biomolecules along with secondary metabolites, which can participate in the biosynthesis of nanoparticles (Singh et al., 2016; Al-Bahrani et al., 2017; Ovais et al., 2016; Chokshi et al., 2016). At present, there is a great demand in the medical industry for AgNPs based on their size and similarity with biological molecules (Murugan et al., 2013).

The *Aeschynomene* genus belongs to the family Fabaceae that are marshy erect plants. Traditionally the plants belonging to this genus are used to treat body pains and swellings (Yoganarasimhan 2000; Wilson et al., 2007). *Aeschynomene* is comprised of more than a hundred species, which are mostly annual herbs or shrubs along with a few small trees (Padal, 2010). The aerial parts of *A. aspera* are used to treat general cold, cough, and fever whereas the dried shoot powder was traditionally used for clearing urinary troubles (Panda and Mishra, 2011). The aerial parts such as fruits, leaves, and flowers of *A. graniflora* are used to treat different ailments. The leaves are used as tonic, diuretic, laxative, and antipyretic. The flowers are used for headaches, dimness of vision, and to improve appetite (Joshi, 2008). The fruits laxative and also they are used as a to cure fever, bronchitis, anemia, colic, and jaundice. The roots of *A. aspera* are used in treating rheumatism, as expectorant and in painful swelling (Kirtikar and Basu, 2005). *Aeschynomene indica*, a traditional medicinal plant belonging to the family Fabaceae is found in most of the swampy areas. This plant has several common names depending on the location such as Tella-jeeluga (Telugu), Didhen, Phulan (Hindi), and Attunetti (Tamil). Several reports explained the importance of this plant in treating various medical ailments. This plant material has been exploited in Ayurveda for treating biliary calculi (Baskar and Chezhiyan, 2002) whereas in Siddha this plant is used to cure leprosy (Irfan Khan and Atiya Khanum, 2004). Along with different medical systems, some tribal people also utilized this plant for medical purposes. In Tamilnadu, Kani tribes used it as an antidote against snake bites along with other plant material, whereas, Chenchu tribes of Nalamalla hills used it for curing the ailments related to kidney and urinary tract (Sambamurthy, 2005). Also, Gujjar tribes of Uttar Pradesh used it as an external application along with Emblica fruit paste to treat skin eczema of

cattle (Gaur et al., 2010). In the traditional medicinal system, this plant is used to treat body pains and swellings (Yoganarasimhan, 2000; Wilson et al., 2007).

The phytochemical composition studies of *A. indica* showed the presence of alkaloids, flavonoids, phenols, steroids and glycosides from the aerial parts of the plant. Qualitative analysis of flavonoids revealed the presence of myricetin, kaempferol, apigenin, and orientin whereas, phenols include isochlorogenic acid, phloroglucinol, caffeic acid, salicylic acid, coumarin, p-hydroxybenzoic acid as the major phenolic compounds, and anthocyanins include delphinidin, malvidin, and peonidin. Antimicrobial activity studies with leaf extracts prepared in four different solvents such as cold water, hot water, alcohol, and methanol were tested against both Gram-negative and -positive bacteria. The bacteria selected for antimicrobial activity included *Bacillus subtilis*, and *Staphylococcus aureus* (Gram-positive), as well as *Pseudomonas aeruginosa* and *Escherichia coli* (Gram-negative). All the four extracts have shown great activity against the selected bacterial strains, which showed that leaf extracts can be used effectively against the pathogens belonging to the mentioned bacterial genus (Ashjaraan and Sheybani, 2019). The extracts have shown the activity more than the standard antibiotics such as gentamicin and ampicillin, which were used as controls in the experiment (Aruna et al., 2012(a)).

A. indica contains the compounds, which are antibacterial in nature and therefore the present work aimed to synthesize the AgNPs using the leaf extracts. The synthesized AgNPs were then characterized and tested for their antimicrobial activity.

Materials and Methods

Biological material collection and identification

A. indica grows well during the rainy season especially during the months of October to December in wet and waste lands. The plant is distributed mostly near the water basins and fields used for cultivation. The leaves of *A. indica* were collected from the agricultural fields near mamidigunta which is located between Chavatapalem and Kanupur villages of Venkatachalam Mandal, Nellore District, Andhra Pradesh, India.



Fig 1: *Aeschynomene indica* plant with flowering-Natural habitat

The botanical identity of the plant was authenticated by Prof. N. Yasodamma, Department of Botany, Sri Venkateswara University (SVU), Tirupati, Andhra Pradesh, India. The Herbarium (VV 23) specimen of this plant was deposited in the Herbarium, Department of Botany, Sri Venkateswara University, Tirupati as per the standard methods (Jain and Rao, 1977).

Preparation of aqueous leaf extracts of *A. indica*

The leaves collected from the source were packed in a clean container and brought to the laboratory. After coming to the lab the leaves were washed several times with tap water and then washed with sterile distilled water to remove all the dust and unwanted visible particles. Then, the leaves were chopped into small pieces and dried at room temperature for 8-12 days to evaporate the residual moisture. The dried leaves were made into powder by using a kitchen blender. 10g of fine powder was taken into a 250 ml conical flask and added 100 ml of Milli Q distilled water and boiled for 30 min at 60°C. The mixture was then filtered with Whatman No.1 filter paper to separate aqueous leaf extract. The leaf extract collected was stored at room temperature for further use (Kokale, 1991).

Green synthesis of AgNPs with *A. indica* aqueous leaf extracts

Synthesis of AgNPs with leaf extracts of *A. indica* was carried out by mixing the aqueous solution of silver nitrate (2 mM) with aqueous leaf extracts of *A. indica* in the ratio of 9:1 (v/v). The mixture was stirred with a glass rod approximately for 10 min and then left at room temperature for an hour. Subsequently, the reaction mixture was observed for any color change during the incubation (Shankar et al., 2004). At the end of the incubation, the contents were centrifuged at 10,000 rpm and 25°C for 15 minutes to remove the presence of biological admixtures (Saifuddin et al., 2009). Finally, the AgNPs were collected and evaluated by UV-Visible Spectrophotometer set at wavelengths ranging from 225 nm to 800 nm with 25 nm intervals to record the absorption peaks of the samples.

Characterization of AgNPs

The synthesized AgNPs were characterized by using various techniques. The NPs were firstly characterized by UV-visible spectroscopy that is useful to analyze NPs. The reduction of Ag⁺ to Ag⁰ done by the leaf extract was evaluated by UV-visible spectroscopy (Nanodrop-8000 spectrophotometer) with a range of 200 to 800 nm. Dynamic light scattering (DLS) and Zeta potential of synthesized nanoparticles were analyzed to know the average size and stability of particles (Nanopartica analyzer, Horiba SZ 100, Japan). The functional groups of AgNPs were identified by FTIR (ALPHA interferometer, Bruker, Ettlingen, Karlsruhe, Germany) at the range of 4000-400cm⁻¹. The crystalline nature of AgNPs was confirmed by XRD, the powder X-ray diffraction pattern was recorded on a Bruker D8-Advance diffractometer using graphite monochromatic CuKα1 (1.5406Å) radiations. The precise morphology of the synthesized AgNPs was evaluated using High-Resolution Transmission Electron Microscopy (Model: FEI Tecnai, G² F20) at room temperature at an acceleration voltage of

200 kV. The sample composition and elemental contents were analyzed by using the energy dispersive analysis system of X-ray (EDAX).

DPPH method of antioxidant assay

DPPH (1, 1 - diphenyl - 2 - picryl hydrazyl) free radical scavenging method is one of the most widely used methods for measuring the antioxidant potential of the extract. This is the first approach to evaluate the antioxidant activity of an extract, a compound, or any other biological sources. To prepare the stock solution, 4mg of DPPH was dissolved in 100 ml methanol and stored at 20°C. 2ml of this solution was added into 1 ml of methanol solution containing test samples of *A. indica* leaf extract and *A. indica* leaf AgNPs at Different concentrations (50-250 µg/ml). Ascorbic acid was used as a reference standard (Sharma and Bhat, 1976).

$$\text{RSA (\%)} = [(Ac-As)/Ac] \times 100$$

where, *Ac* is the control absorbance, and *As* is the sample or standard absorbance.

Antibacterial activity

The antibacterial activity of methanolic and aqueous leaf extract and AgNPs of *A. indica* was tested against four bacterial strains of which 2 are Gram-positive and 2 are Gram-negative. Type strains, *Bacillus subtilis* NCIM 2476 and *Staphylococcus aureus* NCIM 2127 belong to Gram-positive whereas bacterial strains *Escherichia coli* NCIM2068 and *Pseudomonas aeruginosa* NCIM 5070 are Gram-negative. The simple disc diffusion method was used for measuring the antibacterial activity of different samples (Bauer et al., 1976). The method involves the spreading of each test organism (*Staphylococcus aureus* NCIM 2127; *Bacillus subtilis* NCIM 2476; *Pseudomonas aeruginosa* NCIM 5070; *Escherichia coli* NCIM2068) from their overnight broth cultures onto different nutrient agar plates. The sterile 5-mm discs of Whatman No.1 filter paper were put on the nutrient agar plates at equal distance. The filter paper discs were impregnated with the samples before placing them on an agar plate. 50 µl of 100 µg/ml concentrations of aqueous leaf extract, methanolic leaf extract and

AgNPs were applied on separate filter paper discs and used as test samples. For control purposes, ampicillin prepared in the same manner was applied to a separate filter paper disc. The nutrient agar plates were incubated at 37°C for 24h. After the incubation, the area of the zone of inhibition was measured in millimeters (mm).

Results and Discussion

Absorption spectra of AgNPs

Green synthesis of AgNPs was done by mixing silver nitrate solution with aqueous leaf extracts of *A. indica*. After mixing, the observation of color change from colorless silver nitrate to brown indicated the formation of AgNPs. The gradual change of color from light brown to dark brown during incubation for 1 hr indicated the complete reduction of AgNO₃ to AgNPs in the presence of aqueous leaf extracts of *A. indica* (Kaviya et al., 2011). After 1 hr incubation, there was no further change in the color intensity. The silver nitrate solution with no plant extract was used as the control, which showed no color change during the incubation under the same conditions. Initially, the formation of AgNPs was characterized by UV-Visible spectroscopy, a basic technique to monitor metal NPs in aqueous solutions. The absorption spectra revealed the bioreductive formation of AgNPs with a characteristic absorbance peak at 447 nm. The broadening of the peak indicated that the particles were polydispersed (Fig.2). Several reports have shown the time for synthesis of AgNPs in the range of minutes to hours and their absorption peaks in the range of 410 to 430 nm. Silver nanoparticles of *Sesbania grandiflora* aqueous leaf extract were synthesized within one hour with the absorption peak at 422 nm (Das et al., 2013). AgNPs of *Cicer arietinum* synthesized in two hours with an absorption peak at 418 nm (Agarwal et al., 2014). Synthesis of AgNPs of *Abrus precatorius* completed within 5 min, with an absorption peak in the range of 320 to 400 nm (Gaddala and Nataru, 2015). AgNPs of *Calliandra haematocephala* formed after 10 min with an absorption peak at 414 nm (Raja et al., 2017). *Buta monosperma* AgNPs were synthesized in 24h of incubation with an absorption peak around 400 nm (Sharma and Verma, 2017). The results obtained in the present study are consistent with earlier reports regarding the time for the synthesis of AgNPs and the UV-Visible absorption peaks.

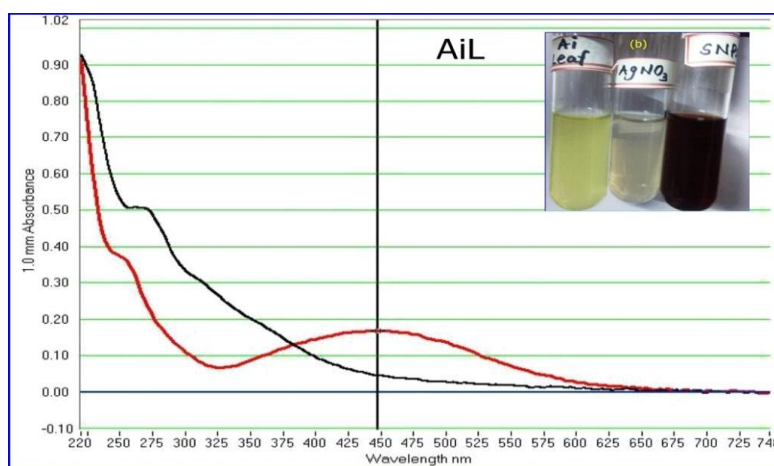


Figure 2: UV-Vis spectra of AgNPs synthesized by aqueous leaf extract of *A. indica*

Dynamic light scattering (DLS) Analysis

Synthesized nanoparticles of *A. indica* were added to 10 ml distilled water to identify the particle size with DLS by surpassing laser scattering instrumental conditions with dispersion medium viscosity of 0.893 mpas, at 25.1°C. Synthesized AgNPs exhibited the polydispersed type of particles with an average size of 17.2 nm

(Fig.3). The important observation found in the DLS experiment is related to the polydispersity index (PDI). It is the dimensionless index and its scale is such that values >0.7 show that the sample has a very broad size distribution. The polydispersity index of *A. indica* leaf AgNPs shows 0.352, which indicates that the sample has a very narrow size distribution of nanoparticles. The Z-average of *A. indica* is 3253.9 nm.

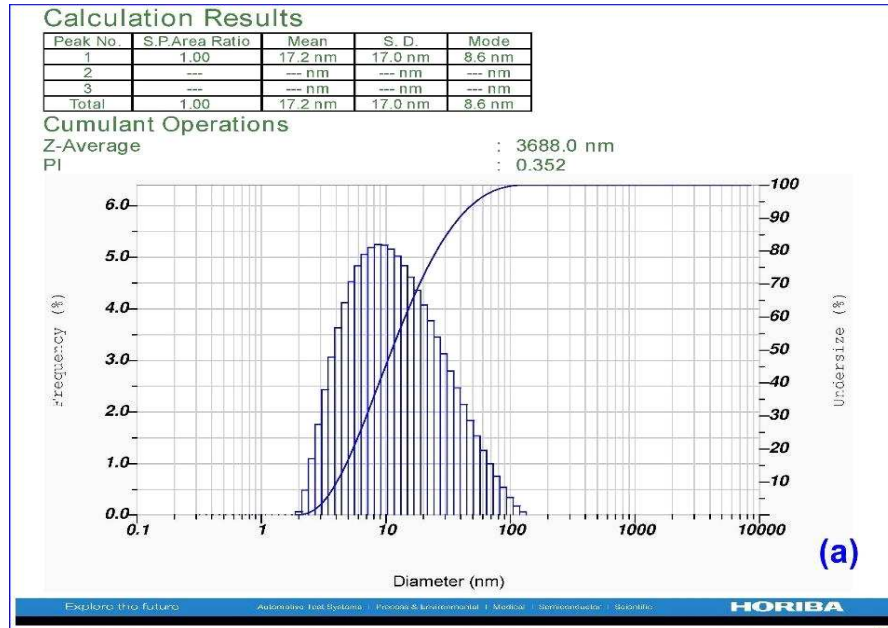


Figure 3: DLS analysis using leaf AgNPs of *A. indica*

Zeta potential Analysis

Zeta potential is an important tool to understand the state of the surface of nanoparticles and predict the long-term stability of the nanoparticles. In the present study, to know the Zeta potential, a few µl of dispersed nanoparticle solution was added to the electrode and the instrumental conditions like conductivity (0.430

Ms/cm to 2.272 Ms/cm), electrode voltage (3.3V) and temperature (25°C) were set. From the results, it is concluded that nanoparticles of *A. indica* leaf AgNPs have stability with Zeta potential of -25.6 mV (Fig.4). It was observed that the nanoparticles' surface was negatively charged and distributed in the medium. The negative value confirmed the repulsion among the particles and proved that they were very stable.

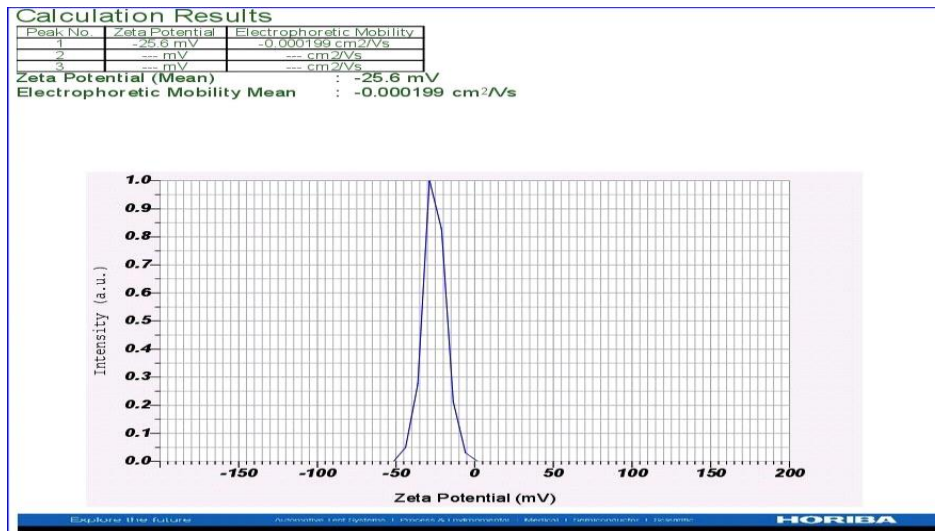


Figure 4: Zeta potential analysis using leaf AgNPs of *A. indica*

High-Resolution Transmission Electron Microscopy (HR-TEM) Analysis

HR-TEM was used to analyze the morphology and size of the silver nanoparticles. The selected area of electron diffraction (SAED) shows clear diffused concentric rings (Fig.6a), which are due to crystalline and polycrystalline spots. These results indicate that the synthesized nanoparticles were crystalline in nature. Figure.5 depicts the HR-TEM image of biosynthesized silver nanoparticles showing the lattice fringes quite clearly like projections of tunnels. 100 nm scales bar TEM image (Fig 6 b) signified the synthesized nanoparticles with a size range from 10 to 24 nm. The 5-nm scale bar image (Figs. 5 & 6 c) clearly showed a 0.238 nm size of 'd' space. The nanoparticles were spherical in shape (Fig. 6d). The average size of the nanoparticle was

calculated as 16.6 nm. The size measured by DLS is slightly greater than HR-TEM because the DLS measured the hydrodynamic diameter (Huang and El-Sayed 2010). The result analysis confirmed that both HR-TEM and XRD analysis showed a similar average size of nanoparticles. The slight differences in the average size of nanoparticles are due to the preparation of sample time and variable instrumental conditions. However apart minute differences, the size measured by DLS, HR-TEM, and XRD analysis showed nearly similar results (Table 2).

Table 1: Average size measured by DLS, HR-TEM, and XRD

DLS Analysis(nm)	HR-TEM(nm)	XRD(nm)
17.2	16.6	17

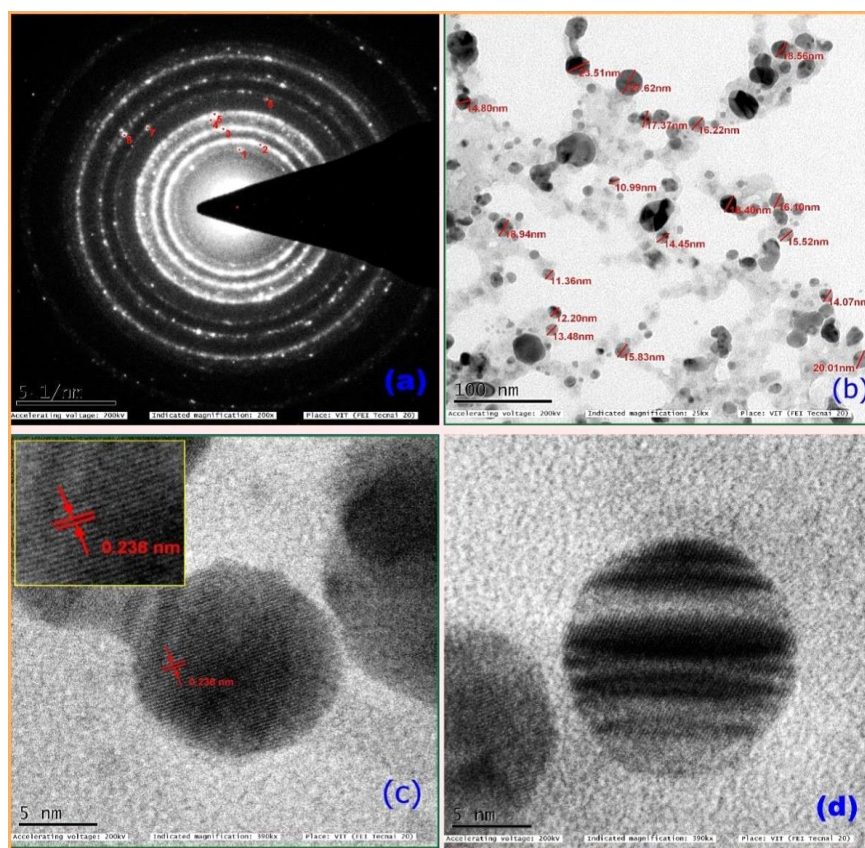


Fig. 5: HR-TEM Images of *A. indica* : (a) selected area diffraction pattern (SAED), (b) 100 nm scale bar image showing different sizes of nanoparticle sizes, (c) 5 nm scale bar image shows 0.238 nm of 'd' space, (d) 5 nm scale bar image shows spherical shaped nanoparticle.

Energy Dispersive X-ray Analysis (EDAX)

Figure 7 shows the energy-dispersive spectrum of the synthesized AgNPs, which illustrates the presence of Ag as the ingredient element. Generally, metallic AgNPs show a strong signal peak at 3keV, due to the surface plasmon resonance (Kaviya et al., 2011). The quantitative information of biosynthesized AgNPs is shown in Figure 7. The EDAX analysis demonstrated the presence of 10.69

% of strong silver, which shows an absorption peak at 3keV (Fig.7) along with different elements and their weight percentages like carbon 82.98%, Nitrogen 1.52%, and copper 4.81% (Table 3) without any contaminants. All the peaks of the silver are observed and assigned. Also, two strong signal peaks for Cu (Copper) and C (Carbon) appeared in EDAX data, which are due to the carbon-coated copper grid.

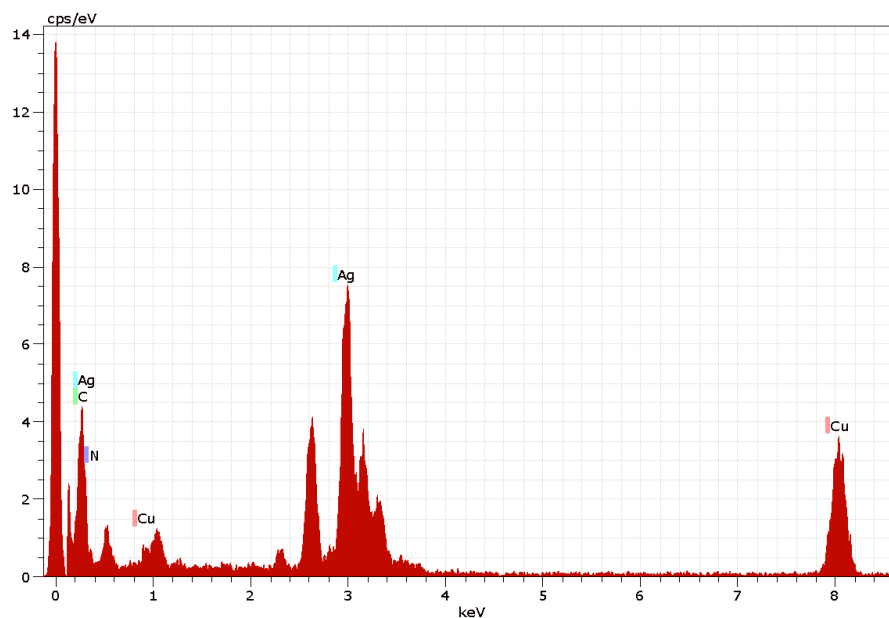


Figure 6: Energy dispersive X-ray analysis spectrum of AgNPs of *A. indica*.

Table 2: EDAX Analysis: Percentage of elemental composition in AgNPs of *A. indica*.

Element	Copper	Carbon	Nitrogen	Silver
Atomic Weight(%)	4.81	82.98	1.52	10.69

X-ray Diffraction (XRD) Analysis

The XRD pattern of the prepared sample of *A. indica* AgNPs was recorded by employing BRUKER D8 Advance X-ray Diffractometer using CuK radiation ($\lambda = 1.5406 \text{ \AA}$), 40Kv-40 mA, $2\theta/\theta$ scanning mode. Data was taken for the 2θ range of 10-90 degrees with a step of 0.049 degrees, step time 43.4s. XRD peaks at 2θ values of 38.291, 44.586, 64.640, and 77.620 degrees in the experimental diffractogram were due to Ag metal and corresponding to *hkl* values (111), (200), (220), and (311), respectively (Table 4). The diffractogram (Fig: 8) were compared with the standard powder diffractogram card of JCPDS, silver file

no.89-3722. The typical pattern of green-synthesized silver nanoparticles had a face-centered cubic structure (fcc). The peak intensity increased at (111) plane, indicating increased crystallinity. The average crystalline size *D* of AgNPs was estimated from the diffractogram using **Debye-Scherrer's** formula $D = K\lambda/\beta \cos\theta$. The average size of the crystalline nanoparticle was estimated at 17 nm. There were some more un-assigned peaks in the diffractogram at 23.688 θ , 28.166 θ , 32.450 θ , 46.533 θ , 55.100 θ , and 57.631 θ . These peaks were weaker than those of silver. This may be due to the bio-organic compounds that occur on the AgNPs surface.

Table 3: XRD results of AgNPs of *A. indica*

P.N O	2θ (θ)	$\cos \theta$	FWHM 2θ (θ)	d-spacing (\AA)	Crystalline Size ' <i>D</i> ' nm	<i>h k l</i> Identified From peak	$h^2+k^2+l^2$ from identified <i>hkl</i>
1	38.291	0.7848	0.5206	2.34871	16.8	111	3
2	44.586	0.7122	0.6172	2.03061	14.5	200	4
3	64.640	0.4283	0.6233	1.44076	15.7	220	8
4	77.620	0.2143	0.5506	1.22906	19.3	311	11

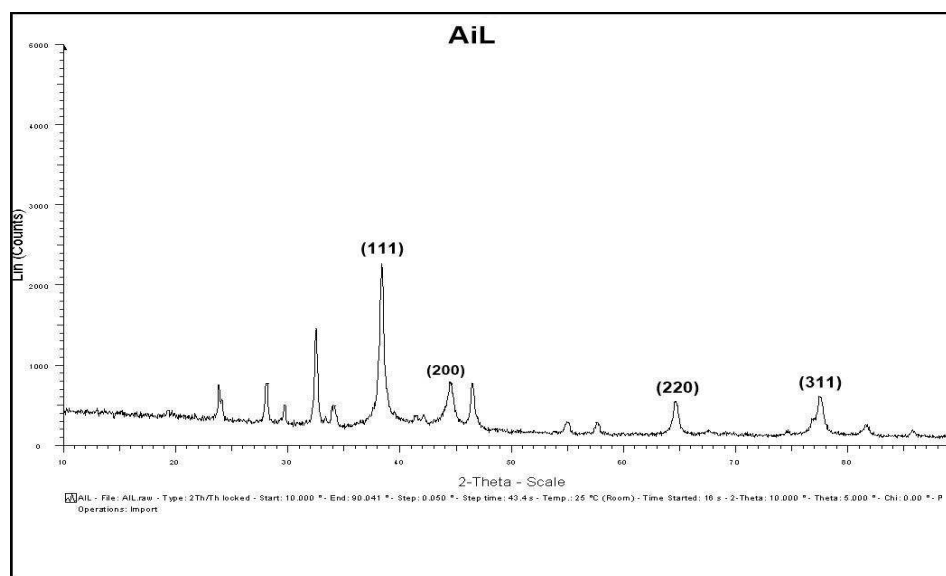


Figure 7: XRD patterns of synthesized AgNPs of *A. indica*

TEM and EDX analysis of *Sesbania grandiflora* AgNPs showed that the synthesized silver nanoparticles appeared as spherical in shape with approximately 25 nm size (Das, 2013). Similarly, SEM micrographs of AgNPs of *Cicer arietinum* confirmed the spherical shape of the synthesized AgNPs with a size of 40-60 nm (Agarwal, 2014). Several other studies in the area of AgNPs also reported the structure and size of the synthesized nanoparticles using the same techniques. AgNPs of *Abrus precatorius* were disk-shaped with an average size of 35-40nm (Gaddala and Nataru, 2015). AgNPs of *Calliandra haematocephala* also showed a spherical shape with 13.07 nm particle size (Raja et al., 2017). Synthesized AgNPs of *Pterocarpus santalinus* showed a spherical shape with 41 nm particle size (Gopinath et al., 2013). Though many of the AgNPs prepared from different plant extracts appeared as spherical in shape but they show variation with respect to size. AgNPs of *Pongamia pinnata* show spherical structure with 55 nm size whereas SNPs of *Buta monosperma* appeared as a spherical shape with 50 nm particle size (A. Sharma and R. Verma, 2017). In the present study, the analysis of AgNPs of *A. indica* by employing DLS, HR-TEM and EDX techniques revealed the structure of AgNPs as spherical with 16 – 17 nm particle size.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The functional groups of *A. indica* leaf extract were identified using FTIR spectroscopy in the range of 709-3724 cm^{-1} (Table 1). The FTIR spectrum (Fig. 5a) displays a number of absorption peaks 3724 & 3670 cm^{-1} with strong O-H molecule of water; 3535 cm^{-1} O-H stretch of alcohols; 3422 cm^{-1} N-H stretch of H-bonded amines; 3357 cm^{-1} N-H stretch of amides; 3271 cm^{-1} ($\text{C}\equiv\text{C}$) stretch of alkynes; 2935 cm^{-1} C-H stretch alkanes; 2599 cm^{-1} -SH thiol group; 2335 cm^{-1} -H-C=O aldehyde group; 1714 cm^{-1} -C=O ketone group; 1529 cm^{-1} - N-O asymmetric nitro compounds; 1389 cm^{-1} -C-H stretch of aldehyde group; 1061 cm^{-1} C-N- aliphatic amine compounds; 709 cm^{-1} - alkyl halide compounds. The functional groups of *A. indica* leaf AgNPs were identified using FT-IR spectroscopy in the range of 678 - 3320 cm^{-1} . Here, synthesized nanoparticles exhibit broad peaks at 3320 cm^{-1} - O-H stretch of alcohols/phenols; 2129 cm^{-1} - ($\text{C}\equiv\text{C}$) stretch of alkynes; 1637 cm^{-1} - assigned to amides; 734-617 cm^{-1} alkyl halide compounds. Most of the peaks that appeared in the *A. indica* leaf extract disappeared after the synthesis of nanoparticles (Fig.5b). The difference in peak areas confirmed the formation of nanoparticles. The FTIR analysis revealed that the proper mechanism for the formation of AgNPs may be due to the reduction of Ag^+ ions that occurs together with the oxidation of phenolic components (Klaus et al., 1999).

Table: 4. FTIR analysis: Identified functional groups from *A. indica* leaf aqueous extract and AgNPs

S. No.	Identified Frequency (cm^{-1}) from <i>A. Indica</i> leaf aqueousextract	Functional group Stretch/ bending	Identified functional group
1	3724 & 3670	O-H	Water
2	3535	O-H	Alcohols
3	3422	N-H	Amines
4	3357	N-H	Amides
5	3271	$\text{C}\equiv\text{C}$	Alkynes
6	2935	C-H	Alkane
7	2599	S-H	Thiol
8	2335	C-H	Aldehyde
9	1714	C=O	Ketone

10	1529	N-O	Nitro compounds
11	1389	C-H	Aldehyde
12	1061	C-N	Aliphatic amines
13	709	CH ₃ -X	Alkyl halides
	Identified Frequency (cm⁻¹) from <i>A. indica</i> AgNPs	Functional group Stretch/bending	Identified functional group
1	3320	O-H	Alcohols/Phenols
2	2127	C≡C	Mono substituted Alkyl groups
3	1637	C=O-NH ₂	Amide
4	678	C-H-X	Alkyl halides

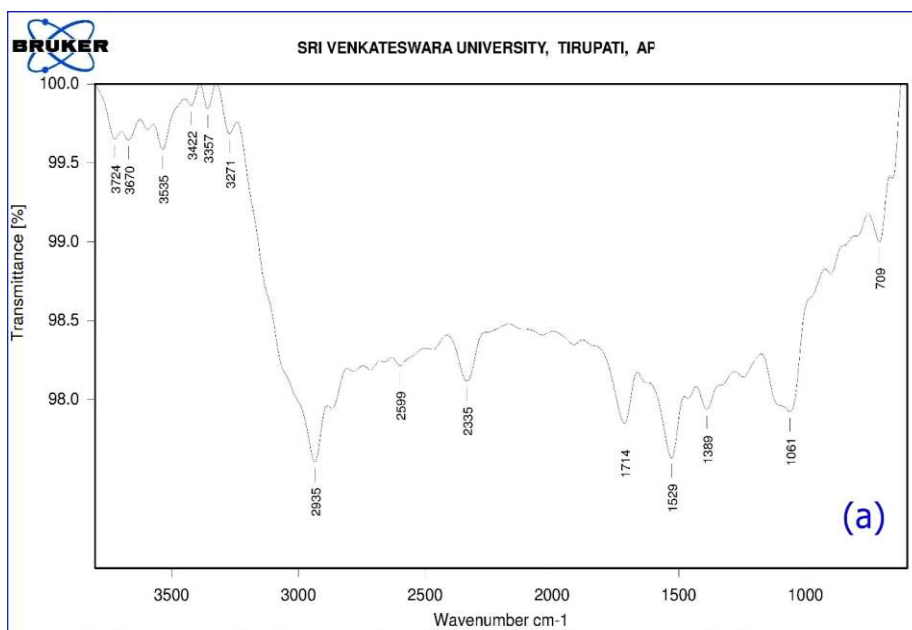


Figure 8: (a) FTIR Spectrum of *A. indica* leaf aqueous extract

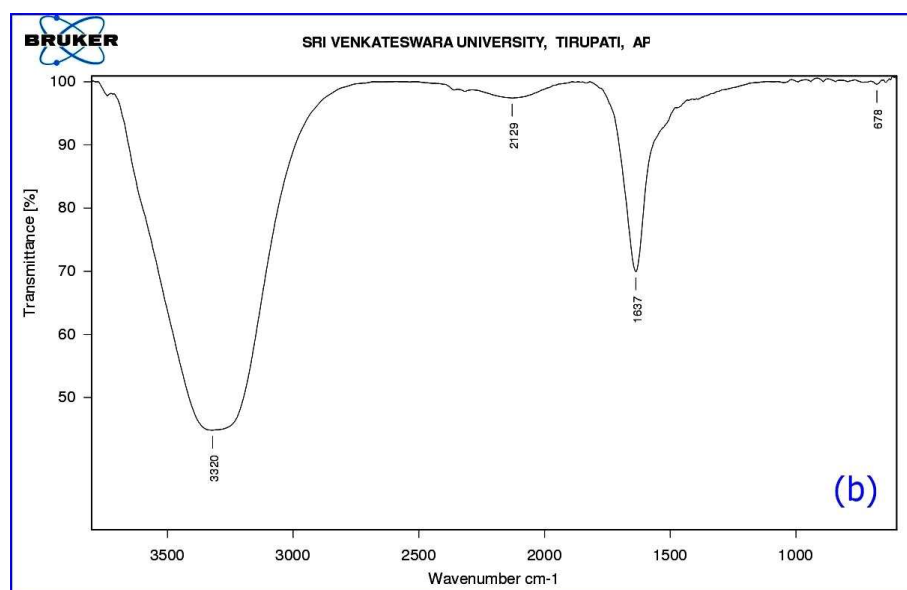


Figure 8: (b) FTIR Spectrum of synthesized AgNPs.

FT-IR analysis is a powerful tool to reveal the phytochemical composition. Several reports employed FTIR analysis to describe the phytochemical constituents of plant extracts. Indirectly this composition analysis of both plant extracts and synthesized AgNPs using plant extracts gives evidence for components that are responsible for the reduction of silver ions. Plant extracts of *Sesbania grandiflora* showed the presence of major compounds responsible for biological synthesis as secondary amines, carboxylic acids and alcohols (Das et al., 2013). FTIR analysis of *Cicer arietinum* revealed the presence of primary phenols, amines, alcohols, ketones, carboxylic acids, and aldehydes as the major compounds (Agarwal et al., 2014). Phytochemical composition of *Abrus precatorius* obtained through FT-IR shows the presence of phenolic compounds, alcohols, and amide bond carbonyl proteins, (Gaddala and Nataru, 2015). Also, FTIR analysis of AgNPs of *Pterocarpus santalinus* revealed the presence of glycosides flavonoids, triterpenoids, phenols, tannins, saponins, and steroids (Gopinath et al., 2013). Likewise, the FTIR analysis of *A. indica* showed the presence of water, alcohols, amines, amides, alkynes, alkanes, thiol group, aldehyde group, ketone group, nitro compounds as major compounds, whereas, the analysis of AgNPs prepared using *A. indica* aqueous leaf extract exhibited the presence of less number of functional groups including alcohols,

phenols, monosubstituted alkyl groups, amides and alkyl halides which may be responsible for the reduction of Ag^+ ions.

DPPH Antioxidant Analysis

The aqueous leaf extract and synthesized AgNPs of *A. indica* showed excellent antioxidant potential when compared to standard ascorbic acid by the DPPH scavenging assay method (Figure 9). Different concentrations from 50 to 250 $\mu\text{g/ml}$ of *A. indica* aqueous leaf extract and *A. indica* AgNPs were used in the assay. Similarly, the positive control, Ascorbic acid, was taken at the same concentration for comparison (Table 5; Fig: 9). Antioxidant activity increased in a dose-dependent manner showing high percentage activity in the order of *A. indica* AgNPs (73.98) > Ascorbic acid (72.26) > *A. indica* aqueous leaf extract (59.86). From the results, it is concluded that AgNPs of *A. indica* have high antioxidant potential when compared with that of ascorbic acid and aqueous leaf extract alone. The DPPH radical scavenging activity of AgNPs reported previously on *Azadirachta indica* supported the results of this study (Lalitha et al., 2013).

Table 5: DPPH antioxidant Activity *A. indica* leaf aqueous extract and AgNPs

Concentration ($\mu\text{g/ml}$)	AiL aqueous leaf extract (% of activity)	AiL - AgNPs (% of activity)	Ascorbic acid (% of activity)
50	27.09	34.72	42.27 \pm 0.26
100	40.46	48.82	52.43 \pm 0.07
150	49.06	56.96	61.44 \pm 0.56
200	52.22	64.08	66.54 \pm 0.67
250	59.86	73.98	72.26 \pm 0.67

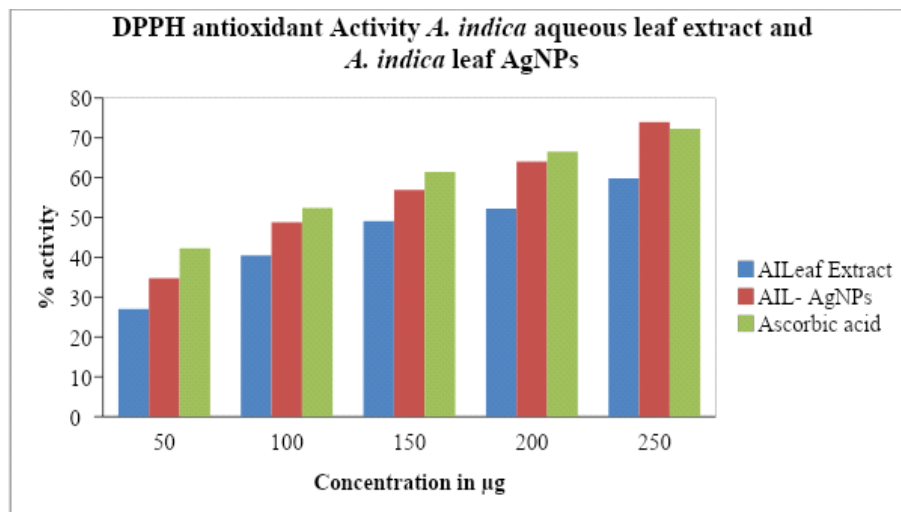


Fig 9: Antioxidant efficacy bar graph of *A. indica* aqueous leaf extract, AgNPs of *A. indica* and Ascorbic acid using DPPH radical scavenging assay.

Antibacterial activity:

In the present study, the biologically synthesized AgNPs of *A. indica* showed antibacterial activity against bacterial strains

belonging such as *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Figs.10 & 11, Table 6). The antibacterial activity result was expressed in terms of the inhibition zone around the sample application. The

results clearly showed that the AgNPs synthesized using *A. indica* aqueous leaf extract possess the highest antibacterial activity compared to *A. indica* aqueous leaf extract and *A. indica* methanol leaf extract. The results of AgNPs of *A. indica* are near to the values of standard antibiotic ampicillin, which was used as the positive control. The zone of inhibition shown by the AgNPs of *A. indica* on different bacterial strains are in the range of *P. aeruginosa* 14.5 mm, *E. coli* 14.2 mm, *S. aureus* 11.5 mm and *B. subtilis* 10.3 mm. The antibacterial activity is stronger with respect to Gram-negative compared to Gram-positive bacteria. The higher antibacterial activity of *A. indica* AgNPs is due to its smaller size with a large surface area, which is in accordance with other reports

showing the antibacterial activity with AgNPs (Wang et al., 2007). Numerous reports show the antibacterial potential of AgNPs against Gram-positive and -negative bacteria with different intensities. AgNPs of *C. arietinum* are effective against *E.coli* (Agarwal et al., 2014) whereas the antibacterial activity of *S. grandiflora* AgNPs is effective against both Gram-negative and -positive bacteria (Das et al., 2013). Similarly, AgNPs of *A. precatorius* and *C. ternatea* are effective against both Gram-negative and -positive bacteria (Gaddala and Nataru, 2015). AgNPs of *C. haematocephala* exhibit antibacterial activity equal to that of standard antibiotic chloramphenicol against Gram-negative bacteria *E. coli*.

Table 6: Antibacterial activity of AgNPs of *A. indica* and their extracts

Sample/ Test organism	<i>Bacillus subtilis</i>	<i>E. coli</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>
AiLE	8.4 ± 0.06**	5.1 ± 0.07**	5.3 ± 0.31**	—
AiLM	9.3 ± 0.34**	12.3 ± 0.46**	9.1 ± 0.18**	10.6 ± 0.08**
AiNPs	10.3 ± 0.11**	14.2 ± 0.46**	11.5 ± 0.27**	14.5 ± 0.33**
Control Ampicillin	25.3 ± 0.57	17.2 ± 0.11	25.0 ± 0.13	27.1 ± 0.56

All the data are expressed as mean ± SEM: **p<0.01, * p<0.05 as compared to the control group, n=4: (One -way ANOVA followed by Dunnett's test).

AiL – *A. indica* L; E – Aqueous leaf extract; M – Methanol; C – Control; NPs – AgNPs

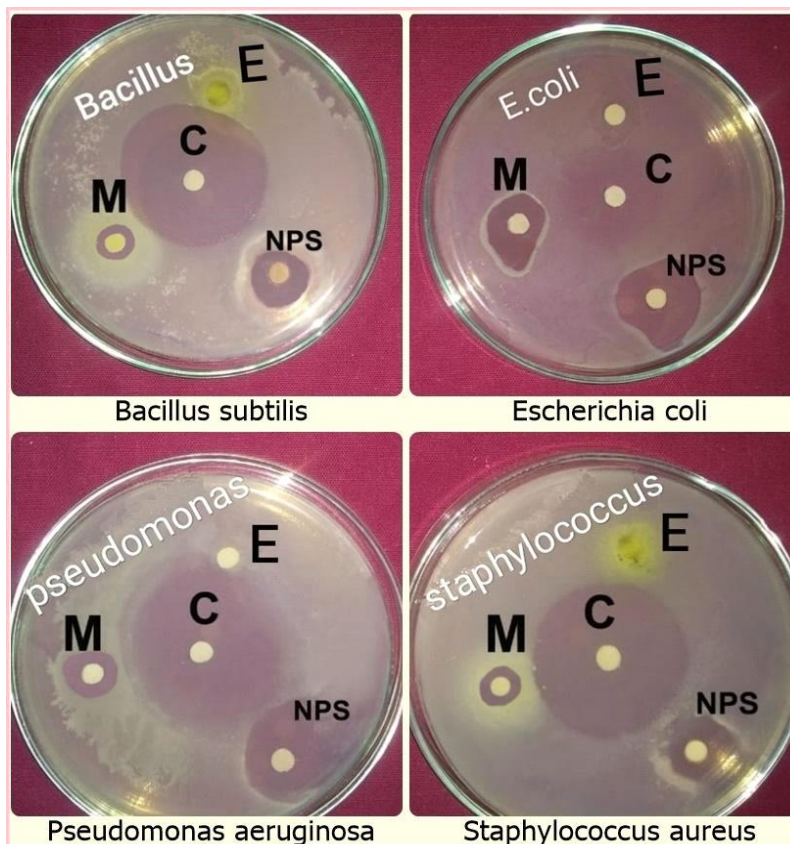


Fig 10: Antibacterial activity of *A. indica* aqueous leaf extract, AgNPs, *A. indica* methanol extract against different bacterial strains

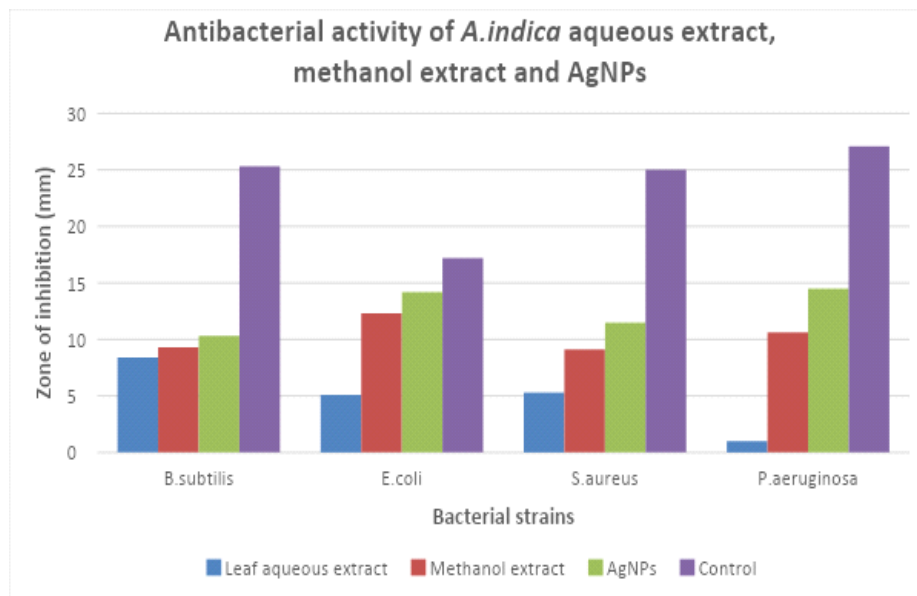


Figure 11: Zone of inhibition of different extracts of *A. indica* on bacteria strains

Conclusion

Nanoparticles have many applications especially in the field of medicine both in the diagnosis and management of diseases. Silver nanoparticles have immense applications in various industries as they possess antibacterial activity. AgNPs prepared from several plant extracts are widely used in the preparation of antibacterial ointments and creams specifically to prevent burn and wound infections (Mondal et al., 2011). Numerous reports are available on the use of plant extracts in preparing AgNPs, such as *C. arietinum*, *S. grandiflora*, *A. precatorius*, *C. ternatea*, and *C. haematocephala* plant extracts (Agarwal et al., 2014; Das et al., 2013; Gaddala and Nataru, 2015). The bioreduction of metal ions to zero-valance metal NPs is mainly due to the reducing capacity of different biomolecules present in the plant extracts. These biomolecules include reducing sugars, proteins, phenolic compounds, terpenoids, and amines. Also, the plant extracts contain some natural capping agents, which prevent the aggregation of nanoparticles and hence control the size of the particle (Vivekanandhan et al., 2014).

The present work explains the green synthesis of AgNPs of *A. indica* aqueous leaf extract for the first time. Preliminary information about the formation of AgNPs has come from the visual color change observation and from the UV-Visible absorption spectrum. The particle size and stability of AgNPs were studied by using DLS and Zeta potential analysis. From DLS, the particle size was measured as 17.2 nm whereas -25.6 mV of negative Zeta potential value indicates the stability of nanoparticles. FTIR analysis has given a clear picture of the involvement of *A. indica* phytoconstituents in the bioreduction, stabilization, and capping of AgNPs. Several studies are presented on the capping and stabilization of silver nanoparticles through plant extracts. Phytochemicals such as quinines, phenolic compounds, terpenoids, and quercetin are popularly well-known as

stabilizing and capping agents during AgNPs synthesis. In the current study, we reported that the nitro, phenolic, aromatic, and aldehyde/ketone compounds of the *A. indica* extract might have participated in the bioreduction and stabilization of AgNPs. HR-TEM analysis revealed that the synthesized NPs have a spherical shape with an average size of 16.6 nm. EDAX analysis confirmed the composition of elements. The face-centered cubic (fcc) structure and 17-nm average size of the particles was revealed by XRD analysis. According to the comparative analysis of DLS, HR-TEM, and XRD, it is quite clearly confirmed that the average size of synthesized silver nanoparticles of *A. indica* was 17 nm. The green synthesized AgNPs are considered a good source of antioxidants by DPPH scavenging assay.

The antibacterial potential of green synthesized AgNPs of *A. indica* prepared from aqueous leaf extracts clearly demonstrated enhanced activity as compared with plant extract alone. This paves the way for employing these AgNPs as effective antibacterial agents against drug-resistant pathogenic bacteria. These AgNPs can also be employed in preparing solutions for external applications.

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