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Research Article

Antibacterial activity of silver nanoparticles synthesized using *Amaranthus viridis* twig extract

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ABSTRACT

Development of nano biotechnology is generating interest of researchers toward ecofriendly, cost effective and biological synthesis of nanoparticles. In this study, synthesis of stable silver nanoparticles was done using *Amaranthus viridis* twig extract. These rapid biological synthesized silver nanoparticles were characterized with the help of UV–Vis spectrophotometer, Fourier transform infrared spectroscopy (FTIR), Scanning electron microscopy-Energy dispersive spectroscopy (SEM-EDX), Transmission Electron Microscopy (TEM) and X-Ray diffraction spectroscopy (XRD). Stability of reduced silver nanoparticles was analyzed using UV–Vis spectra shown that the absorption peak, occurring due to Surface Plasmon Resonance (SPR), exists at 423.5nm and their antibacterial activity was screened against both gram-negative and gram-positive bacteria. It was observed that *Amaranthus viridis* twig extract reduced silver ions into silver nanoparticles within 10 min of time. Thus, this method is rapid and eco-friendly. The TEM and SEM analysis inferred that the rapid biologically synthesized silver nanoparticles are spherical in shape and have an average size of 5-20nm. The antibacterial activity of silver nanoparticles against both gram positive and gram negative bacteria was performed in Luria–Bertani (LB) medium on solid agar plates and in liquid systems supplemented with different concentrations of nano-sized silver particles. These particles were shown to be an effective bactericide. Test for protein and nucleic acids leakage were performed to study the biocidal action of this nano sized particles. The results confirmed that the treated bacterial cells were damaged, showing leakage of proteins and nucleic acids into LB media. These nanoparticles, which can be prepared in a simple, rapid and cost-effective manner, are suitable for the formulation of new types of bactericidal materials.

Keywords: *Amaranthus viridis* twig extract; UV–Vis spectrophotometer; Fourier transform infrared spectroscopy (FTIR); Energy dispersive spectroscopy (EDX); Transmission electron microscopy (TEM); X-Ray diffraction spectroscopy (XRD); antibacterial activity; biocidal action

1. INTRODUCTION

Preparation of silver nanoparticles is one of the emerging techniques in Nanotechnology research. It gives the potential application to many fields. Silver nanoparticles having the effective inhibition on many microorganisms and different strains of bacteria. Nanoparticles from plant extracts having tremendous application in many ways of nanotechnology, Number of living organisms are already well-known to elaborate nanostructure composites such as Cyanobacteria (Mubarak Ali et al. 2011; Singaravelu et al. 2007), fungi (Jaidev and Narasimha 2010), actinomycetes, biomolecules and various plant materials such as Ebhlica officinalis, Medicago sativa, Avena sativa, Azardirachta indica, (Asmita J. et al. 2012; Thirumurugan A. et al. 2010), Tamarindus India (Ankamwar et al. 2005, Aloe Vera (Chandran et al. 2006), Coriandrum sativum (Sathyavathi et al. 2010), Muraya konigai (Laura Christensen et al. 2011) Parthenium hysterophorus (Ashok kumar 2012) Tritium vulgare, Ocimum sanctum (Tulsi), (Garima Singhal et al. 2011), Ocimum basillicum (Shivaranjani and Meenakshi Sundaram 2013), from roots (Dinesh et al. 2012) and gold nanoparticles also synthesized by biomolecules like honey Gold nanoparticles (Daizy Philip 2009) of different sizes, ranging from 1 nm to 8 nm and shapes including spherical, octahedral, sub-octahedral, decahedral multiple twinned, icosahedral multiple twinned, hydrophobisized gold nanoparticles (Gupta et al. 2010) Irregular shape, nanoprisms, tetrahedral, hexagonal platelets and nanorods (Ivan Moreno et al. 2013).

Rapid biological synthesis methods using biological extracts (Haverkamp and Marshall 2009) have shown a great potential in nanoparticle synthesis. However, understanding the mechanism of involvement of biomolecules is still now unclear. Nanostructured materials (Kamat P V 2002) showed many aspects of interesting characteristics, i.e., optical, catalytic, that greatly depends upon the size and shape of nanoparticles as

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an effect of quantum confinement of electrons. Metal nanoparticles are extensively used in many electromagnetic (Pradeep and Anshup 2009; Choi et al. 2007; Okuda et al. 2005), electro analytical and bioelectrochemical applications owing to their extraordinary electro catalytic activity. Although metal is a poor catalyst in bulk form, nano-sized particles can exhibit excellent catalytic activity due to their relative high surface area-to-volume ratio and their interface-dominated properties, are significantly differed from those of the bulk material.

Amaranthus viridis is a native to India, and it is an erect, perennial broad leaf herb, growing up to 75 to 100cm high. The stems are green, often reddish and angled in cross section. Leaves and seeds are edible used as the vegetable. The tops are rich in calcium and iron. The plant is a good source of vitamins B and C and has anti-diabetic and anti-hyperlipidaemic potential (Ramdas Pandhare 2012) studies found it to be an excellent source of protein.

Using of plants in synthesis of nanoparticles is quite novel foremost to truly green chemistry, which provides advancement over chemical (Chaudhari et al. 2007) and physical method as it is cost-effective and environment friendly easily scaled up for large-scale synthesis and in this method, there is no need to use high pressure, energy, temperature and toxic chemicals. Nowadays, we are using bacteria, fungi for the synthesis of nanoparticles but use of leaf extracts to reduce the cost as well as we do not require any special culture preparation and isolation techniques. Polysaccharide (Carp et al. 2010; Travan et al. 2009). Inorganic oxides and composite materials also open the gates toward an alternative and less- toxic chemistry of Nanomaterials.

Here we report synthesis of silver nanoparticles, by the help of Amaranthus viridis twig extract. Through elaborate screening process involving the number of plants, we observed that Amaranthus viridis was a potential candidate for synthesis of silver nanoparticles. We studied the antibacterial property of Amaranthus viridis twig extract silver nanoparticles towards the different Gram negative bacteria like Escherichia coli, Pseudomonas putida and Klebsiella pneumoniae and Gram positive bacteria Staphylococcus aureus, Micrococcus luteus and Bacillus subtilis. Although, several previous reports have studied the antibacterial activity (Li W R et al. 2010; Schacht et al. 2012) of chemically synthesized silver nanoparticles but here we study the biologically (using Amaranthus viridis extract) synthesized silver nanoparticles. The pathogenic bacteria cultures were brought into broth culture and prepared for plates for disc diffusion method for antibacterial assay. Approximately, on well- spread bacterial LB agar plates and then plates were incubated at 37°C for 24 hours. The zone of inhibition was calculated and performed a test for protein and nucleic acid leakage into the broth due to cell wall lysis of bacteria.

2. MATERIALS AND METHODS

2.1 Collection of Amaranths Twigs

*Amaranthus viridis*Twigs (Figure 1A) were collected from the Local market, Andhra Pradesh, India. The twigs were rinsed with distilled water thrice followed by Milli Q water to remove the dust and other contaminants then dried at room temperature to remove the moisture for 2 hours.

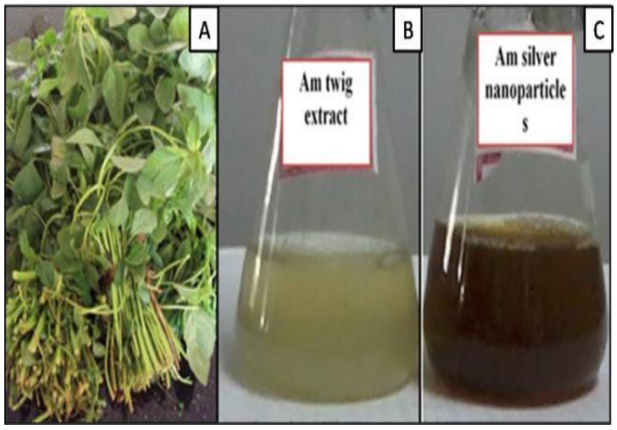

Figure 1: A. Plant Material, B. *Amaranthus viridis* Twig extract, C. Synthesized of silver

2.2 Preparation of Twig extract

5gms of green twigs were weighed and then sliced into small pieces. Then 30ml of Milli Q water was added and boiled for 10min at 50°C. After cooling the extract was filtered using whatman No.1 filter paper and stored at 4°C for further use (Figure 1B).

2.3 Preparation of 1mM $AgNO_3$ solutions

Accurate concentration of 1mM silver nitrate (Sigma grade, USA) can be prepared by dissolving 0.0421gms $AgNO_3$ in 250ml of Milli Q water and stored in an amber-colored bottle to prevent auto oxidation of silver.

2.4 Synthesis of *Amaranthus viridis*silver nanoparticles

For the synthesis of silver nanoparticles from *Amaranthus viridis*twig extract, To 5ml of extract, 20ml of 1mM $AgNO_3$ solution was added and further heated up to 100°C. The color change was observed, which stands as a preliminary confirmation for the formation of silver nanoparticles. Further the solution was centrifuged at 20000rpm for 30min. The separated nanoparticles settled at the bottom were collected and washed thrice with Milli Q water, then dried in an oven at 60°C for two hours. The stabilized powder forms of the nanoparticles were stored for further characterization.

2.5 Characterization of *Amaranthus viridis*silver nanoparticles (AmAgNPs)

An ELICO SL-159 UV-Vis spectrophotometer was used for the spectrometric analysis to confirm silver nanoparticles formation. The twig extract was used as reference blank. The purified suspension was oven dried

and the powder was subjected to FTIR spectroscopy analysis (Paragon 500, Perkin Elmer-RX1 spectrophotometer) in the diffuse reflectance mode at a resolution of $4cm^{-1}$ in KBr pellets. Further the size and shape of synthesized AgNPs were characterized by Scanning electron microscope (SEM) in Zeiss 700 Scanning electron microscope and Transmission electron microscope (TEM) in Philips model CM 200 instrument operated at an accelerating voltage at 200 kV and the confirmation the presence of elemental silver\signal was characterized by energy-dispersive X-ray microanalysis spectroscopy (EDX; Sigma) and X-Ray diffractometer operated at a voltage of 40 kV and a current of 30 mA with Cu Kα radiation.

2.6 Antibacterial activity using Disc Diffusion method

The antimicrobial activity of synthesized silver nanoparticles was determined using disc diffusion assay method. Luria Bertani media was prepared and poured into sterilized petriplates and then plates were spread with of *Escherichia coli, Pseudomonas putida, klebsilla pneumonia, Staphylococcus aureus, Micrococcus luteus and Bacillus subtilis* separately. Then sterile discs were kept and the samples were added at different concentrations to the disc and the plates were incubated at 37°C for 24 hours. Then zone of inhibition was measured.

2.7 Effect of concentration of silver nanoparticles on bacterial strains

Freshly grown bacterial cultures were inoculated in Luria-Bertani (LB) broth and are incubated overnight in the presence of 10, 40, 80µl/ml of Silver nanoparticles added in different flasks to observe the bacterial cell growth pattern in a shaker at 100 rpm at 37ºC. Total solution used in each flask is 2mL of broth and the growth rate is indexed by measuring optical density (OD) at 595nm. The readings obtained were plotted against concentrations of silver nanoparticles.

2.8 Time dependent treatment of bacterial strains with silver nanoparticles

The silver nanoparticles were suspended in distilled water to perform the time-dependent anti-bacterial study of Ag-nanoparticles with the bacterial strains. Freshly grown bacterial inoculums in Luria-Bertani (LB) broth in the presence of 40µl/ml of Silver nanoparticles added in different flasks to observe the bacterial cell growth pattern in a shaker at 100 rpm at 37ºC and incubated with varying time interval for same concentration. The growth rate is indexed by measuring optical density (OD) at 595nm. The readings obtained were plotted against different time intervals.

2.9 Cytoplasmic leakage analysis for silver nanoparticles treated bacterial cells

Ag-nanoparticles treated cells were centrifuged pellets discarded, and supernatant used to study protein and Nucleic acid leakage analysis.

2.9.1 Estimation of Nucleic acid leakage

The amount of nucleic acid (NA) released in the AgNPs treated cells was measured at 595 nm using UV spectrophotometer. For preliminary identification nucleic acid leakage by Diphenylamine test showed a positive results.

2.9.2 Estimation of Protein leakage

If bacterial cells burst, intracellular materials will be released outside. If concentration of the leaked protein is higher in the intracellular fluid of a sample, implies that the disruption rate of bacterial cells is higher. Protein leakage analysis of AgNPs treated cell was performed by Bradford's assay of protein determination. Supernatant was collected after centrifugation of the Ag-nanoparticles treated cells at 10000 rpm for 10 minutes. 200µl of a supernatant was mixed in 600 µL of Bradford's reagent. Optical density was measured at 595nmafter 10 minutes of incubation at room temperature.

3. RESULTS AND DISCUSSION

The synthesis of silver nanoparticles is an advanced technique in modern nano biotechnology and is evolving as an important branch of nanotechnology. This study deals with the synthesis and characterization of silver nanoparticles using the twig extract of *Amaranths viridis.* Biologically synthesized silver nanoparticles were reddish brown in color. The color of the extract was changed from light yellowish to reddish brown after addition of $AgNO_3$ and on incubation for 5min at 100ºC. The coloration was due to the excitation of the surface plasmon vibration of the silver nanoparticles. Change in color after the reduction of Ag^+ to silver nanoparticles is shown in (Figure 1C). The reduction rate and formation of nanoparticles can be increased further by the increase in time. The bactericidal effect of AmAgNPs was measured by disc diffusion method.

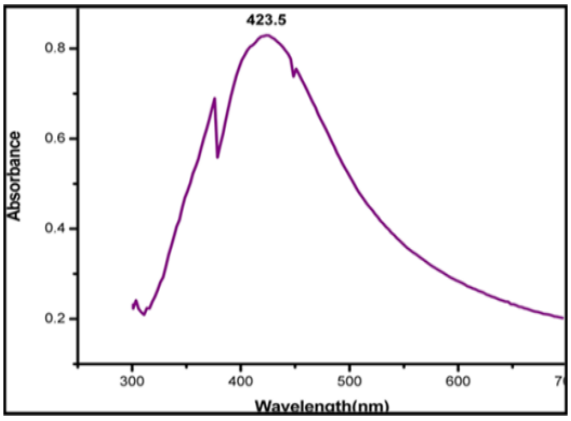

Figure 2: UV-Visible spectra of synthesized silver nanoparticles

3.1 UV-Vis spectrophotometer

The UV-Vis spectroscopy was the preliminary technique for the characterization of the silver nanopar-

ticles. The UV-Vis absorption was analyzed after centrifuging and redispensing the particles in deionized water, the maximum smooth and broad absorption peak was observed at 423.5nm (Figure 2).

3.2 FTIR analysis of silver nanoparticles

The FTIR spectrum indicates various functional groups present at different positions. IR spectroscopy study has confirmed that the carbonyl group of amino acid residues and peptides of proteins has a stronger ability to bind metal, so that the proteins could most possibly form a coat covering the metal nanoparticles (i.e. capping of AgNPs) to prevent the agglomeration of the particles, and thus, the nanoparticles are stabilized in the medium. The peaks in the region between 3423 to 2850 were assigned to O-H stretching of alcohol and phenol compounds and aldehyde–C-H- stretching of alkanes. The peaks in the region 1609 correspond to C=C medium weak stretching vibration of arenes, 1376 to 1025 correspond to N-H (bond) of primary and secondary amides and –C-N- stretching vibration of amines or –C-O- stretching of alcohols, ethers, carboxylic acids and anhydrides and peaks between658 and 775 were assigned to O-H (H-bonded), usually broad C-O variable weak bending vibrations of alcohols and phenols (Figure 3). FTIR analysis reveals the dual function of biological molecules possibly responsible for the reduction and stabilization of silver nanoparticles in the aqueous medium.

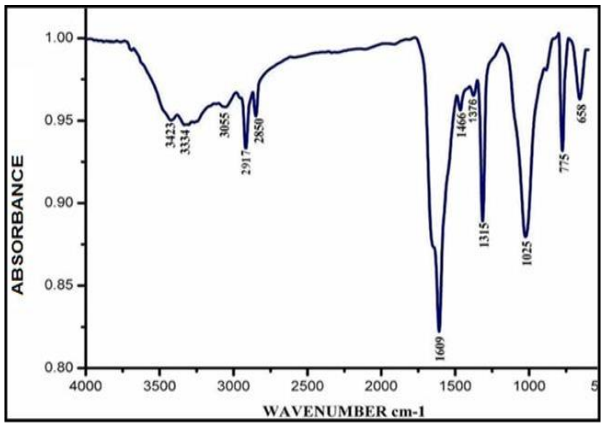

Figure 3: FTIR Spectrum of synthesized silver nanoparticles

3.3 SEM-EDX analysis

SEM technique was employed to visualize the size and shape of silver nanoparticles. SEM images were obtained for *Amaranthus viridis*twig extracts. The formation of silver nanoparticles as well as their morphological dimensions in the SEM analysis demonstrated that the average size was from 5-20nm with inter-particle distance. The shapes of the silver nanoparticles proved to be spherical (Figure 4A). EDX spectra recorded from the silver nanoparticles were shown in Figure 4. From EDX spectra, it is clear that silver nanoparticles reduced by *Amaranthus viridis*twig extract have the weight percentage of silver as 35.48% (Table 1).

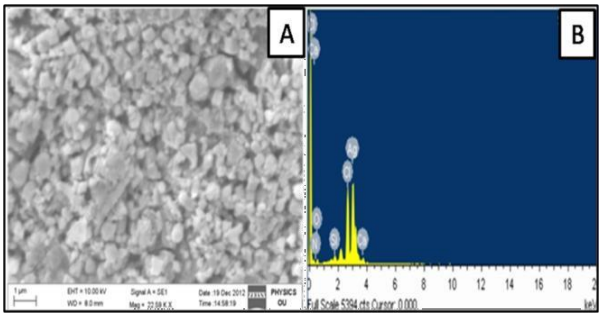

Figure 4: A.SEM analysis, B.EDX spectra of synthesized silver nanoparticles

3.4 TEM analysis

The silver nanoparticles synthesized by the help of *Amaranthus viridis*twig extracts were scanned using TEM from which we conclude that the average mean size of silver nanoparticles was in between 5-20nm (Figure 5B) and seems to be spherical in morphology as shown in (Figure 5A). Thus, the transmission electron microscopy gave a detailed descriptive image of the silver nanoparticles synthesized with their structural details and their size.

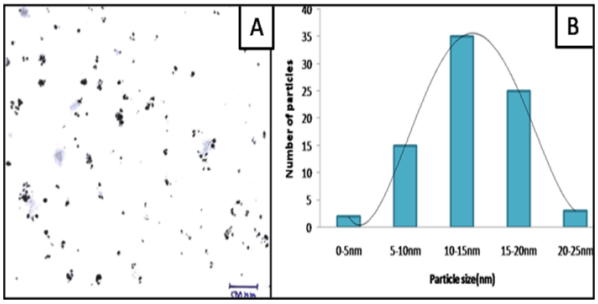

Figure 5: A.TEM analysis of silver nanoparticles, B.Particle size distributiom

3.5 XRD analysis

The Braggs reflections were observed in the XRD pattern at 2 θ= 38.76^0, 44.9^0, and 77.74^0, which indexed the planes 111, 200 and 311 of the cubic face-centered silver.. The lattice constant calculated from this pattern was a = 4.086Å and the data obtained was matched with the database of Joint Committee on Powder Diffraction Standards (JCPDS) file No. 04-0783. The average grain size of the silver nanoparticles formed during the bioreduction process was determined using Scherer's formula, d = (0.9×λ/ β *cos θ) and was estimated as 12nm (Figure 6). Hence XRD pattern thus clearly illustrated that the silver nanoparticles formed in this present synthesis are crystalline in nature. In addition to addition to the Bragg peaks representative of FCC silver nanoparticles, additional as yet unassigned peaks were also observed suggesting that the crystallization of bioorganic phase occur at the surface of the nanoparticles.

3.6 Antibacterial activity by disc diffusion technique

Antibacterial activity of biological synthesized silver nanoparticles against Gram negative (*Escherichia coli,*

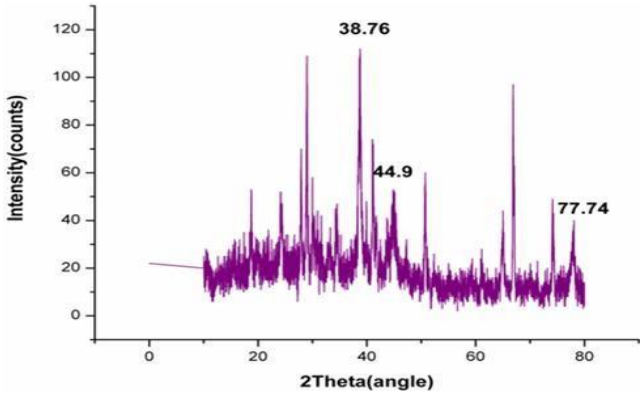

Figure 6: XRD analysis silver nanoparticles

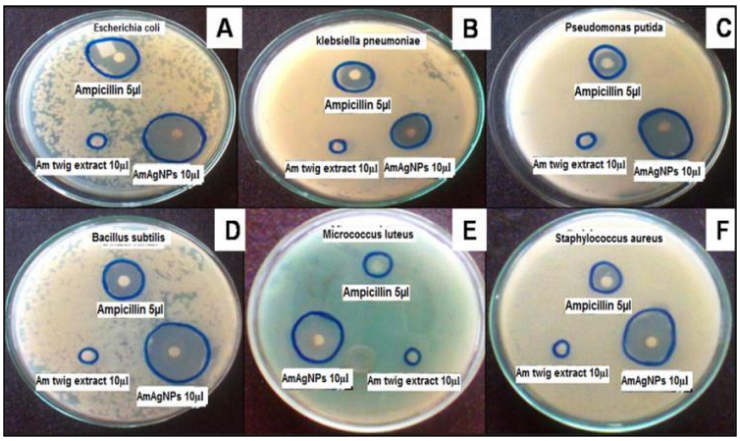

Figure 7: Antibacterial activity of silver nanoparticles

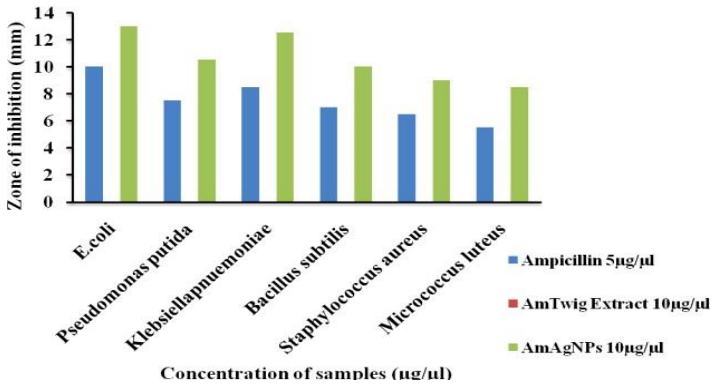

Figure 8: Zone of inhibition for antibacterial activity

Pseudomonas putida and Klebsiella pneumonia) and Gram positive (*Staphylococcus aureus, Micrococcus luteus and Bacillus subtilis)* bacteria revealed an antibacterial activity (Figure 7). The zone of inhibition of silver nanoparticles was measured (Figure 8 and Table 2). The results indicated that silver nanoparticles synthesized from *Amaranthus viridis*twig extract showed effective antibacterial activity both in Gram negative and Gram-positive bacteria compared with ampicillin.

3.7 Effect of concentration of silver nanoparticles on bacterial strains

The growth of the bacterial strains decreased with the increase in the concentration of silver nanoparticles. It was measured at 595nm in UV-Vis spectrophotometer. It revealed that as the concentration of silver nanoparticles increases the growth of the bacterial cells decreases due to biocidal action (Figure 9).

3.8 Time dependent treatment of bacterial strains with silver nanoparticles

In bacterial cells, the OD values decreased as the incubation time increased for treated cells while the OD value of untreated cells increased as the incubation time is increased (Fig.10). It showed that as the incubation time of treated bacterial cells increases the biocidal action will increase.

3.9 Cytoplasmic leakage analysis for silver nanoparticle treated bacterial cells

The amount of Nucleic acid and protein released from the cell increased as the amount of Ag-nanoparticles

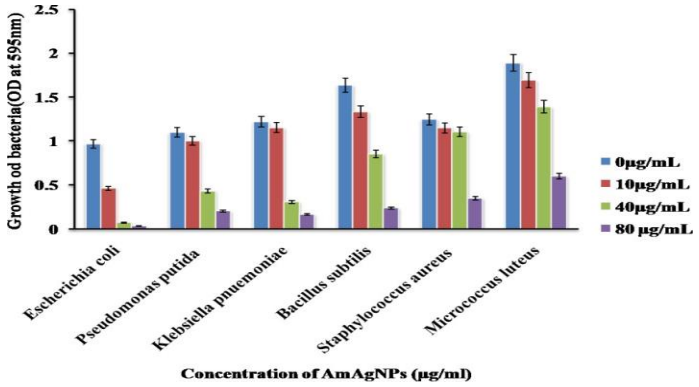

Figure 9: Effect of concentration of silver nanoparticles on bacterial strains

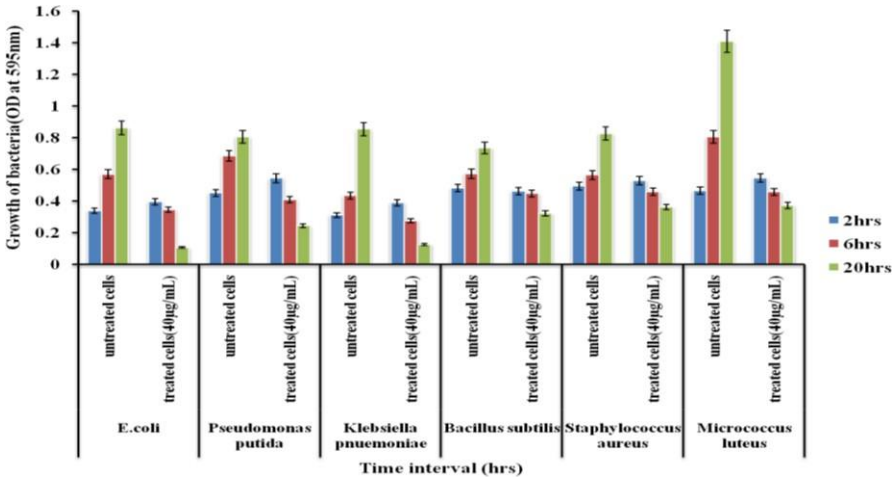

Figure 10: Nucleic acid leakage from bacteria by silver nanoparticles

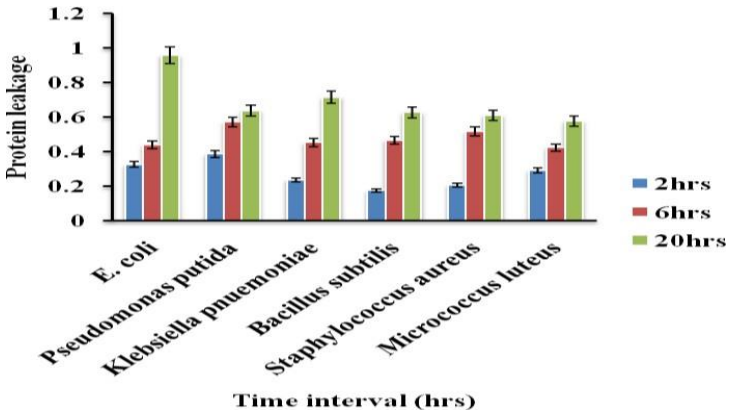

Figure 11: Protein leakage from bacteria by silver nanoparticles

Table 1:The composition of silver nanoparticles synthesized from *Amaranthus viridis*Twig extract

Element	Weight%	Atomic%
O K	8.58	30.31
Si K	1.12	2.26
Cl K	21.32	33.99
Ca K	2.69	3.80
Ag L	67.73	35.48
Totals	100.00	

increased, These results indicated that most of the nanoparticles treated cells were ghost cells from which intracellular materials were released into the cell suspension due to the lysis of the cell wall of bacteria.

3.9.1 Estimation of Nucleic acid leakage

As the time interval increased the amount of nucleic acid leakage increased (Figure 11), which is measured by Diphenyl amine method.

Table 2: Zone of inhibition by Disc diffusion method

Name of the Organism	Zone of inhibition in mm		
	Ampicillin 5µg/µl	AmTwig Extract 10µg/µl	AmAgNPs 10µg/µl
Escherichia coli	10	Not determined	13
Pseudomonas putida	7.5	Not determined	10.5
Klebsiellapnuemoniae	8.5	Not determined	12.5
Bacillus subtilis	7	Not determined	10
Staphylococcus aureus	6.5	Not determined	9
Micrococcus luteus	5.5	Not determined	8.5

3.9.2 Estimation of Protein leakage

As the time interval increased the amount of protein leakage increased (Figure 12) which is measured by Bradford Assay.

CONCLUSION

In this study, silver nanoparticles which were synthesized from *Amaranthus viridis*twig extract showed potential antibacterial activity against Gram negative and Gram-positive bacterial strains. Thus, it is investigated that the silver nanoparticles synthesized from *Amaranthus viridis*twig extract seem to be a promising and effective antibacterial agent against bacterial strains. This biological approach to the synthesis of silver nanoparticles is highly essential effort being addressed in nanomedicine because of its varied advantages. Plant extract being very eco friendly and cost effective can be used for the large-scale synthesis of silver nanoparticles in nanotechnology processing industries.

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