ECO-FRIENDLY SYNTHESIS AND CHARACTERISATION OF SILVER NANOPARTICLES BY USING AQUEOUS EXTRACT OF FRESH LEAVES OF *LABLAB PERPUREUS* WITH BIOLOGICAL ACTIVITIES

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Abstract : This paper describing the synthesis of silver (Ag) nanoparticles from aqueous extract of fresh leaves of *Lablab perpureus.* This eco-friendly approach of synthesizing nanoparticles is green and cost-effective. The synthesized silver nanoparticles were characterized by various methods including ultraviolet–visible spectroscopy (UV–Vis), Fourier transform infrared (FTIR), Scanning electron microscopy (SEM), powder X-ray diffraction (XRD), and antimicrobial activity. UV–visible absorption of synthesized silver nanoparticles showed peak around 430 nm. The responsible biomolecules for the reduction of Ag^0 from Ag^+ and also the capping materials of silver nanoparticles were confirmed by FTIR . SEM image represents the morphology of the silver nanoparticles. The size of silver nanoparticles , crystalline nature and face centre cubic structure of nanoparticles confirmed by XRD. The biological activity of AgNPs against generally found bacteria and fungi was also analysed.

Keywords: ***Lablab perpureus*, Leaf extract, Silver nanoparticles, UV- spectroscopy, FTIR-spectroscopy, SEM, XRD, Biological activity.**

Introduction

Nanomaterials are principal requirement of the rapidly developing field of nanomedicine, bionanotechnology. Nanoparticles are used in clinical applications as it is known as the therapeutic engine in the infections, against microbes. The noble metal, silver nanoparticles have received considerable attention owing to their attractive physicochemical properties.

The nanoparticles can be synthesized three different methods such as chemical, physical and biological method. The chemical method involving toxic chemical and hazardous substances for health and environment. The nanoparticle ranging 1nm to 100 nm. New and improved properties showed by nanoparticles based on specific characteristics such as size and morphology. Nanocrystalline silver particles have found tremendous applications in the field of catalysis, micro-electronics (Jain et al., 2009), biological systems (Roy & Barik, 2010) and medicine due to their antimicrobial and antibacterial effects (Kamal et al. 2003). Silver nanoparticles are synthesized by the reduction of silver ions from Ag^+ to Ag^0 (Roy & Barik, 2010) using reducing agents which comes from plant extract (Dhanapal et al. 2015).

The use of environment friendly materials like plant leaf extract, for the synthesis of silver nanoparticles over chemical and physical method is cost effective, environment friendly, easily scaled up for large scale synthesis and no need to use of high pressure, energy, temperature and toxic chemicals. In medical industries silver and silver nanoparticles is used as topical ointments to prevent infection against burn and open wounds. Further these biologically synthesized nanoparticles were found highly toxic against different multi drug resistant human pathogens (Dhanapal et al. 2015).

Materials and methods

Plants and chemicals

AR-grade silver nitrate ($AgNO_3$) was purchased from Sigma-Aldrich Chemicals and fresh leaves of *Lablab perpureus* were collected from local area, Banasua, Cumilla, Bangladesh. The collected leaves washed with double-distilled water for several times for the experiments.

Fig 1. Leaves of *Lablab perpureus* plant.

Preparation of the extract

The fresh leaves cut into small pieces and sun dried then grinded to form powder. About 10 g of finely powder leaves were weighed separately and transferred into 250 ml beaker containing 100 ml distilled water and boiled for about 20 mins. Then the solution was incubated for 30 min. Further, the extract was filtered with Whatman no.1 filter paper, stored at 4^0 C and used for further experiments. Then this solution was used for the reduction of silver ions (Ag^+) to silver nanoparticles (Ag^o).

Fig 2. Leaf extract of *Lablab perpureus*

Synthesis of silver nanoparticles

Silver nitrate of 1mM concentration was prepared for nanoparticles synthesis.90 ml silver nitrate of 1mM concentration was mixed with 10 ml of leaves extract for reduction from Ag^+ ions to Ag^0 and kept at room temperature for some times until color change appeared. Formation of silver nitrate was confirmed by observing the change of color from light yellow to reddish brown. Then the solution was analyzed using UV–Vis spectrophotometer.

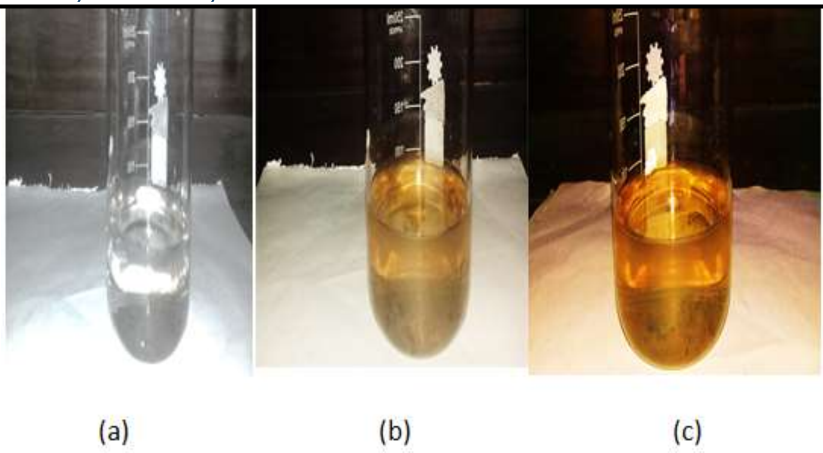

Fig.3 (a) 1mM $AgNO_3$ Solution (b) 1mM $AgNO_3$ with *Lablab perpureus* leaves extract (light brown) (c) 1Mm $AgNO_3$ with *Lablab perpureus* leaves extract after 4 hrs of incubation (raddish brown)

Characterization techniques

The formation of silver nanoparticles was formally confirmed by UV–Vis spectroscopy. The sample shows absorbance in the range of 200-800 nm, instrument naming UV–Vis spectrometer (Shimadzu-UV 1800) with distilled water as a reference (Anandalakshmi et al. 2016). For classifying biomolecules in the leaf extracts FTIR analysis was performed which results the responsible biomolecules for the reduction of metal ions and stabilization of nanoparticles. The FTIR analysis was done in the wavelength region 4000–400 cm^{-1} instrument naming Shimadzu IRAffinity-1S [5]. X-ray diffraction (XRD) was done by Explorer GNR, Wazed Miah Science Research Center (WMSRC), Jahangirnagar University, Dhaka Bangladesh, using monochromatic Cu ka radiation (k = 1.54187 Å) operated at 30 kV and 20 mA at a 2θ angle pattern. The scanning was done in the region of 20^0-50^0. The graphical image of XRD was obtained by using origin software to study crystalline nature and the Debye- Scherrer formula was used to study the nanoparticle size. The silver nanoparticles was further examined by SEM to study morphology and shape of the silver nanoparticles which was done by Institude of Fuel Research and Development (IFRD), Bangladesh Council of Scientific and Industrial research (BCSIR), Dhaka. The anti-bacterial activity against few common bacteria and fungi was done by Institute of Food Science and technology (IFST), Bangladesh Council of Scientific and Industrial research (BCSIR), Dhaka, followed by disk diffusion method.

Result and Discussion

UV-Visible spectra analysis

The Ag nanoparticles were formally confirmed by visual observation. Due to reduction of silver ion into silver nanoparticles the solution color was changed from light yellow to raddish brown [6]. The silver nanoparticles were confirmed by UV-vis. which result the absorption maximum at 430 nm and absorbance is decreased with wavelength increased (Priya et al.,2011). (Fig.4) .

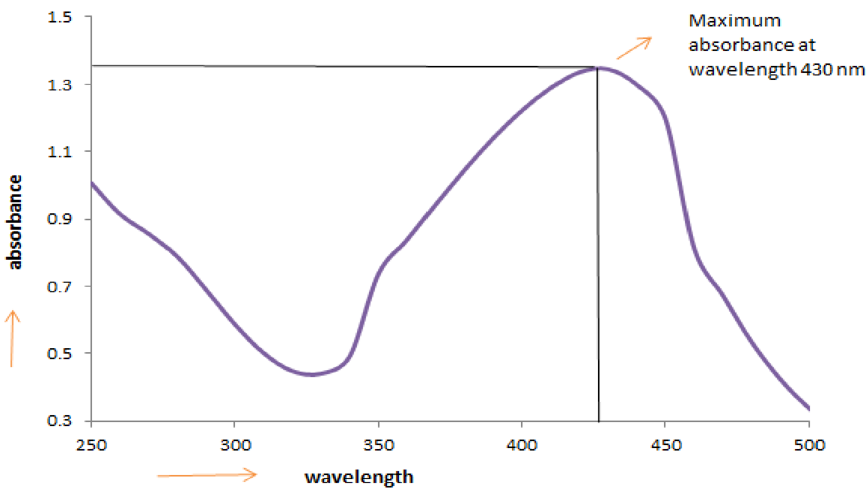

Fig. 4 UV spectrum of silver nanoparticles from *Lablab perpureus*

FTIR analysis

FTIR spectrum shows in (Fig. 5) which was found for the Ag nanoparticles synthesized from *Lablab perpureus* leaf extract. FTIR was carried out to determine the possible biomolecules which are responsible for capping and stabilizing of the metal nanoparticles synthesized by *Lablab perpureus* leaf extract. In spectrum , the broad peak at 3446 cm^{-1} is responsible for –OH stretching, the peaks near 2960cm^{-1} and 2825cm^{-1} are responsible for aldehydic C-H stretching. The peak at 1634 cm^{-1} is corresponding to primary amide arising due to the carbonyl stretch in proteins. The sharp peak at 1039 cm^{-1} is assigned to C-N stretching vibration of amine. The carbonyl group from amino acid residues and peptides of proteins was confirmed by FTIR which has stronger ability to bind the metal. So, a coat covering on the metal nanoparticles was formed possibly by protein to prevent agglomeration of the particles and stabilize the particles in the medium (Gole et al. 2001), (Narasimha et al. 2013). The proteins present over the silver nanoparticles surface act as capping agent, amino acid residues and peptides have strong ability to bind to silver particles (Narasimha et al. 2013), (Balaji et al. 2009).

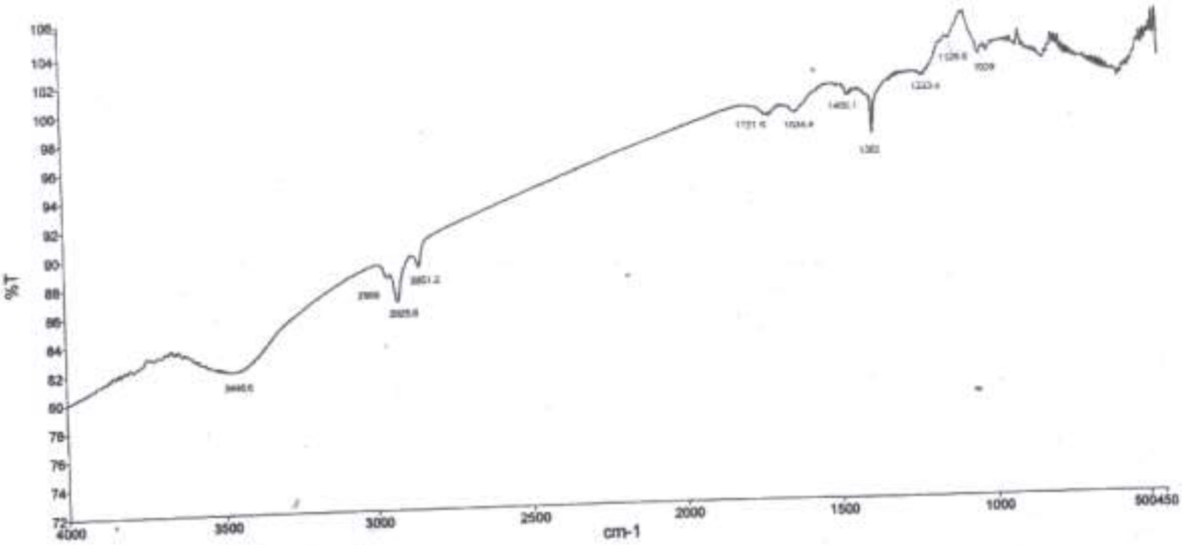

Fig.5 FTIR spectrum of synthesized silver nanoparticles from *Lablab perpureus* leaf extract.

SEM analysis

The presence of silver nanoparticles was finally confirmed by scanning electron microscopic (SEM) shows in (Fig.6). SEM images show that relatively spherical and uniform Ag nanoparticles formed with diameter of 2 to 7 nm (confirmed by XRD). The synthesized nanoparticles from plant extract was combined on the surface due to the interactions such as hydrogen bond and electrostatic interactions between the bio-organic capping molecules and bound to the Ag nanoparticles [6]. The nanoparticles are not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent (Priya et al.,2011), (Song & Kim 2008).

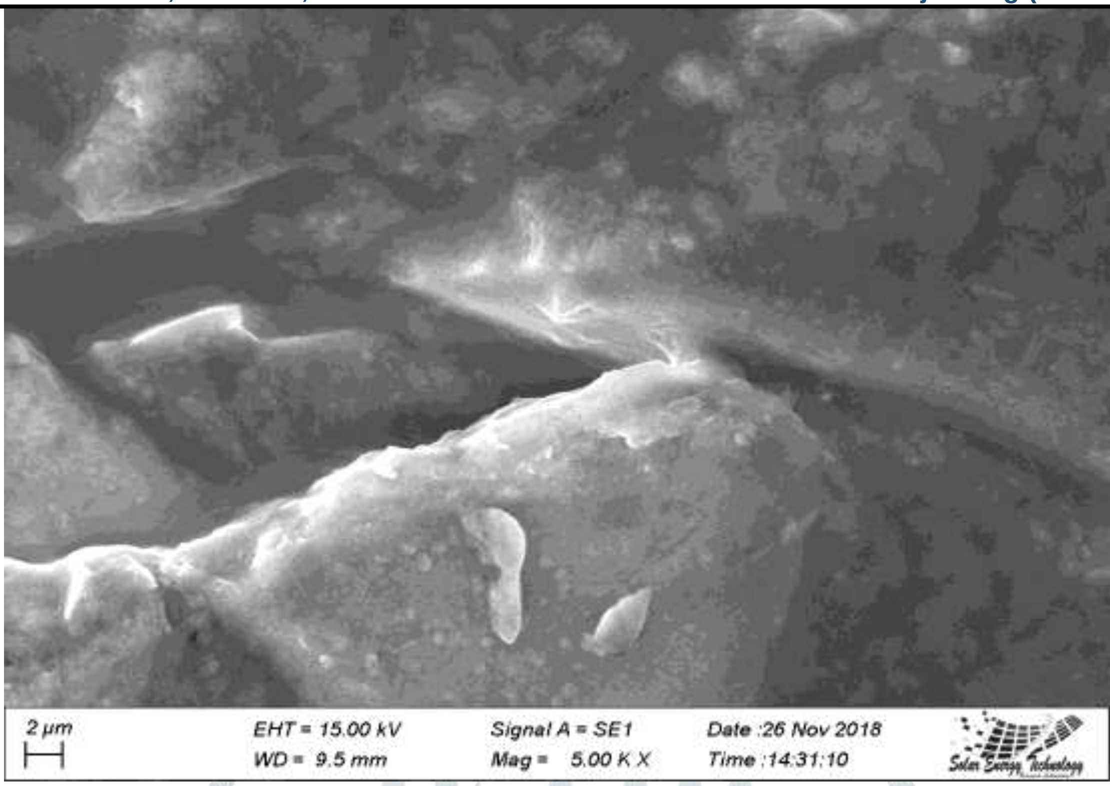

Fig. 6 SEM image of synthesized silver nanoparticles from *Lablab perpureus*

XRD analysis

The nanoparticles which synthesized from *Lablab perpureus* plant extracts were confirmed by characteristic peaks observed in XRD image (Fig. 7). The peaks was drawn using origin software. The XRD showed four intense peaks in Bragg angle of 2θ values ranging from 20 to 50 degree. The average size of the nanoparticles synthesized by extract was 4nm with size ranging from 2 to 7 nm. The particle sizes of the samples were calculated using the Debye-Scherrer formula. The Bragg reflections corresponding to the (111), (200) sets of lattice planes are observed which may be indexed for the face centered cubic (fcc) structures of silver. The peaks were observed also suggesting that the crystallization of bio-organic phase occurs on the surface of the silver nanoparticles (Priya et al.,2011), (Sathyavathi et al. 2010).

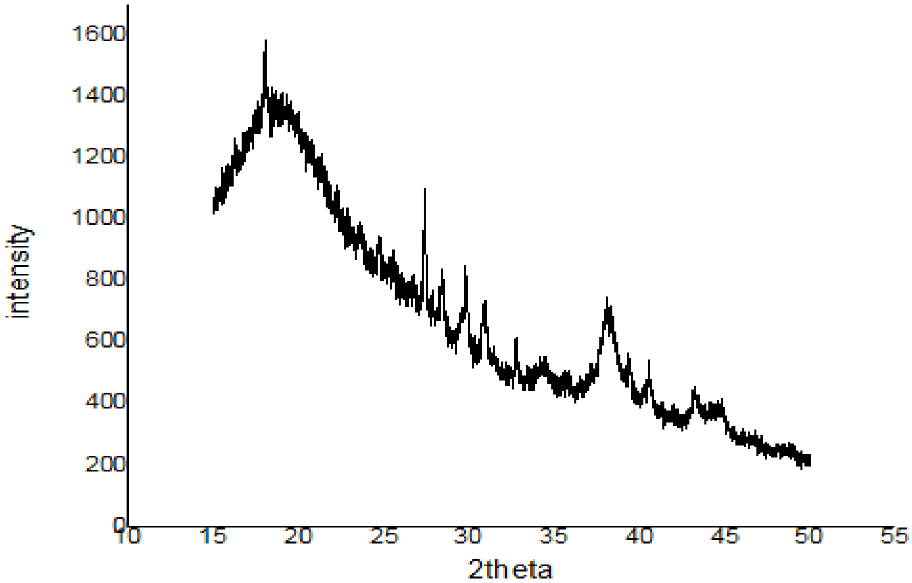

Fig.7 XRD spectrum of synthesized silver nanoparticles

Table1. XRD spectrum analysis data by using Debye-Scherrer formula

SL. No.	2θ	Cosθ	Sinθ	FWHM degree	β radian	Crystalline size 'D' nm
1	27.414	0.97152	0.23695	1.55102	0.02707	4.98021
2	29.774	0.96643	0.25691	1	0.01745	7.68396
3	30.904	0.96385	0.26643	2.04925	0.03576	3.73963
4	32.794	0.95932	0.28229	3.72	0.06492	2.05039
5	38.114	0.94519	0.32650	1.80083	0.03143	4.17312

Anti-bacterial assay:

The antibacterial assays were done on bacterial organisms like *Escherichia coli, Staphylococcus aureus,* and *Salmonella sp* by standard disc diffusion method.

The sample silver nanoparticles prepared shows diameter of inhibition zone against *E.Coli* 9 mm, *Salmonella sp.* 7mm and *Staphylococcus aureus* 6mm (The results in Figures 8,9 and 10). So, the antibacterial effect of silver nanoparticles was more pronounced against gram (-) than gram (+) bacteria. A comparative analysis was done on the basis of this concept (table 2).

Table 2. Comparison of activities of Silver Nanoparticles on Gram (-) and Gram (+) bacteria

Bacteria	*Salmonella sp.*	*Escherichia coli*	*Staphylococcus aureus*
Variety of bacteria	Gram (+)	Gram (-)	Gram (+)
Zone Of Inhibition (mm)	7	9	6

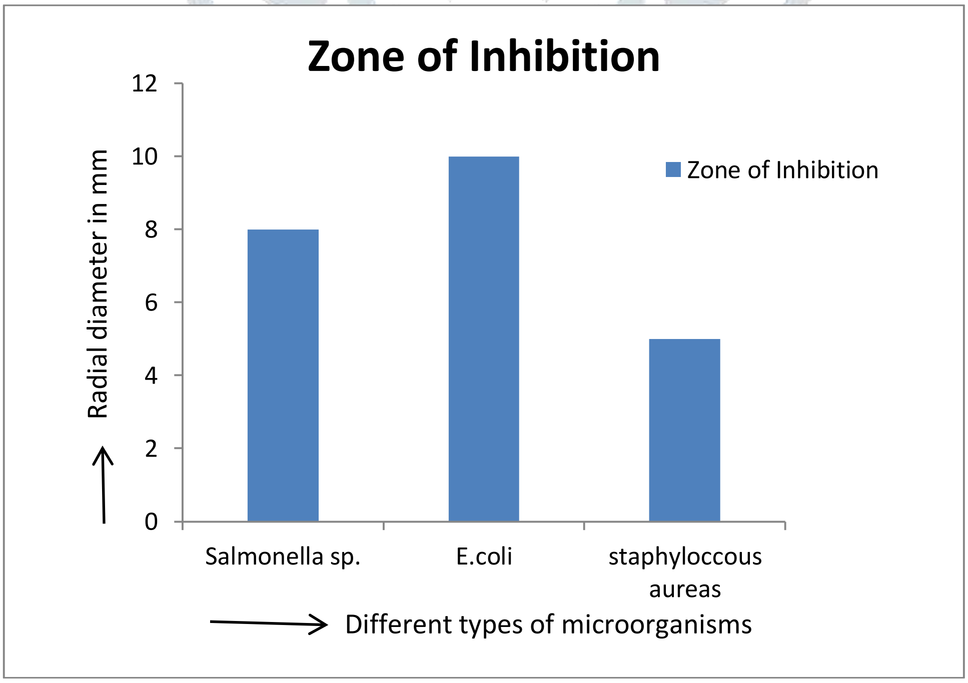

Fig.8 Histogram shows radial diameter of zone of inhibition against different types of micro-organisms in case of *Lablab perpureus*

Conclusion:

This method of synthesizing nanoparticles provide a nontoxic, ecofriendly and efficient route at room temperature condition. In this environmental friendly procedure no need to use high pressure, energy, temperature and toxic chemicals etc. Based on this study, some other nanoparticles may be prepared in future. In nanotechnology, this is a significant advancement to synthesize silver nanoparticles. The synthesized silver nanoparticles are in spherical shape with average particle size of 4 nm. Their characterizations have been successfully done using UV, FTIR, XRD and SEM. The analysis result of antimicrobial effect of silver nanoparticles was more pronounced against gram (-) bacteria than gram (+) bacteria. It also results that the nanoparticles are inhibited the growth of fungi. This synthesized silver nanoparticles can be useful in food industries, cosmetic industries, medicines, water treatment etc.

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