BIOSYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES USING FICUS BENGHALENSIS LEAF EXTRACT

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Abstract

The study demonstrates ecofriendly and reproducible synthesis of Silver nanoparticles (SNPs) employing Ficus benghalensis (F.B.) leaf extract). In this paper, we report stable and spherical SNPs of variable size ranging from 10-50 nm. The formation of SNP was monitored using ultraviolet-visible spectroscopy (UV-Vis) technique for surface Plasmon resonance and it is found at 435 nm and absorbance peak at 280 and 240 nm are also found and indicates the protein and hydroxyl anthraquinones capping. The kinetics of effect of time, pH, ionic strength, Concentration of leaf extract and temperature on the formation of SNP was studied using UV-Vis spectroscopy. Fluorescence spectroscopy and Fourier-transform infrared spectroscopy (FTIR) studies indicate the involvement of protein as a capping agent and Quinones as reducing agents. The morphology of the SNPs was determined AFM (Atomic Force Microscopy) and FESEM (Field emission Scanning electron microscopy) along with EDAX. X-ray diffraction (XRD) study was carried and found to be face centred cube (fcc).Thermal Gravimetric analysis (TGA) was performed to understand the thermal behaviour of biosynthesized silver nanoparticles. The results indicated that the proteins, which have amine groups, have played a significant role in stabilizing SNPs in the solutions.

Key Words: *Ficus benghalensis, Biosynthesis, Silver nanoparticles, Surface Plasmon Resonance, FESEM*

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1. INTRODUCTION

Nanotechnology [1] deals with the formation of objects less than 100 nanometers has generated considerable interest among researchers due to its profound impact on energy, chemical, electronics, space industries and drug/gene delivery [2]. Silver is well-known for its antimicrobial property since time immemorial and ancient Greeks and Romans took advantage of silver's antimicrobial effects and used silver particles to fight against infections. SNPs, a significant member of the noble metal nanoparticles, are excellent substrates for surface enhanced Raman scattering (SERS) [3] to probe single molecules [4].

The metal nanoparticles can be produced using different chemical and physical routes like chemical reduction, electrochemical method and radiation [5-7], but there is a growing need to develop eco-friendly and size control approach for synthesis of metal nanoparticle. So, biological resources have been extensively investigated for synthesis of noble metal nanoparticle which is a good way to fabricate various biocompatible nanostructures. Biological route of synthesis reduces the use or generation of hazardous substances to human health and the environment. Hence People have used variety of natural materials or it's by product to prepare noble metal nanoparticles, such as synthesis of nanoparticles using microorganism [8–10], enzyme [11-12], plant extract [13] and have been suggested as possible eco-friendly alternatives to chemical and physical methods. Using plant for nanoparticles synthesis can be advantageous over other biological processes by eliminating the elaborate process of maintaining cell cultures [13]. Recently silver nanoparticles have been synthesized using various natural products like, alfalfa sprouts, and chilli (*Capsicum annuum*), green tea (*Camellia sinensis*) and guava (*Psidium guajava*) leaf extract respectively [14-17].

In continuation with our efforts in search of synthesizing SNPs by biological route we have utilized *Ficus bengalensis* (banyan tree) leaf extracts for this purpose in present investigation. The effect of time, pH, $AgNO_3$ concentration, leaf extract and temperature was studied to understand synthesis rate and stabilization of SNP employing UV-visible spectroscopy. The shape and size of synthesized nanoparticles was understood by employing FESEM and AFM and elemental analysis is done using EDAX. Structure was determined from powder X-ray diffraction (XRD) technique and efforts have been made to understand the biomolecule involved in reduction and stabilization employing FTIR and Fluorescence spectroscopy techniques.

2. EXPERIMENTAL

2.1 Materials and methods

All chemicals procured from Hi-media unless otherwise stated and of analytical grade. All glassware were cleaned with soap solution followed by aqua regia (1 mL HNO3 (15.9 M): 3 mL HCl (12 M)) and rinsed with double-distilled water prior to use and dried in hot air oven. pH was

adjusted to the required value using 0.1N NaOH or 0.1 N HCl. The leaf of *Ficus benghalensis* procured locally from Gulbarga university campus, Gulbarga. (Karnataka), India.

Biosynthesis of Silver nanoparticles using *Ficus benghalensis*

The *Ficus benghalensis* (F.B) leaf extract is used for synthesis of silver nanoparticles in the present experiment. Fresh and green, leaves of (F.B) plant were collected from the Gulbarga University campus, Gulbarga. Prior to an experiment,10g of F.B. Leaves were put into conical flask containing 100 ml of distilled water and exposed for microwave irradiation for 3 min and the leaf extract filtrate was obtained by passing the above solution through Whatman filter paper no.1. The filtrate was collected and used for the subsequent reaction. In this typical experiment, 5 ml of the above filtrate was added to 95 ml of a 10^{-3} M aqueous $AgNO_3$ (Silver nitrate) and exposed for 3 min in microwave oven. Periodically aliquots of the reaction solution were removed and subjected to UV-Vis spectroscopy measurements for surface Plasmon resonance study of SNPs synthesis.

3. Characterization of silver nanoparticles

3.1 UV spectrometer measurements

Change in colour was visually observed in the silver nitrate solution incubated with *Ficus benghalensis* leaf extract. The reduction of precursor silver ions was monitored by sampling of aliquots (1 ml) at different time intervals. UV spectra were recorded using Lab India UV-Visible Spectrophotometer at 1 nm resolution.

3.2 Fluorescence Spectroscopy Analysis

Fluorescence intensity was measured with a Cary Varian Eclips (les Ulis, France) fluorescence spectrophotometer. With a 5 and 10 nm bandwidth for excitation (λ ex = 280 nm) and emission (λ em = 210 nm) was used.

3.3 X-ray Diffraction study (XRD)

X- ray diffraction (XRD) was performed using Rigaku, Ultima IV, X-ray diffractrometer (Japan). The scanning range (2Θ) was selected from 200 to 800 angles. The coherently diffracting crystallographic domain size (dXRD) of the silver nanoparticles was calculated from X-ray diffraction (XRD) line broadening after subtracting the contribution from the Cu Kα component (Rachinger correction) and correcting for the instrumental width. The integral line width was used in the Scherrer formula to calculated XRD of the (1 1 1) plane.

3.4 Thermal Gravimetric Analysis (TGA)

The freeze dried powder sample of SNPs synthesized from F.B. leaf extract was subjected to Thermal Gravometric analysis (TGA) using a LINSEIS STA PT 1600 (Germany) instrument over a temperature range of 30–800 C at a heating rate of 100C min^{-1} in the presence of N2 gas.

3.5 Field Emission Scanning Electron Microscopy of Silver nanoparticles

The morphology of the Silver nanoparticles were examined employing Field Emission Scanning Electron Microscopy (FESEM, FEI Nova nano 600, Netherlands), and the images were operated at 15 kV on a 0° tilt position.

3.6 Energy Dispersive X-ray Analysis (EDAX)

The EDAX spectrum was recorded using attachment to FESEM and showed strong signal of silver.

3.7 Atomic force Microscopy of Silver Nanoparticles

AFM Samples of Silver nanoparticles for atomic force microscopy (AFM) were prepared by solution casting onto silicon wafers (111) to make thin films. These films were analyzed in non-contact mode using a Pacific Nanotechnology Nano-R2 instrument.

3.8 FTIR spectroscopy analysis

The bio-transformed products of leaf extract filtrate were freeze-dried and the binding of Organic moiety to silver nanoparticles was analyzed by FTIR spectroscopy in transmission mode using a Nicolet iS5 spectrophotometer. Measurements were carried out in the range of 400 – 4000 cm^{-1} at 4 cm^{-1} resolution with 32 scans.

4. RESULTS AND DISCUSSIONS

UV-Vis spectroscopy analysis

The detailed study of biosynthesis and characterization of silver nanoparticles using *Ficus benghalensis* was carried out and is reported in this paper. An UV-Vis Spectroscopy is one of the important techniques to study the formation of metal nanoparticles, provided Surface Plasmon resonance exits for the metal. Fig.1. shows sample tubes containing (A) $AgNO_3$ solution, (B) *Ficus benghalensis* leaf extract and (c) $AgNO_3$ solution after 10 min of reaction time with leaf extract. It is observed that the colour of silver nitrate solution changed from colourless to brown with increasing intensity with incubation time, indicating formation of silver nanoparticles. It is well known that silver nanoparticles exhibit yellowish brown colour in water and this colour arise due surface Plasmon vibration of metal nanoparticles [18].

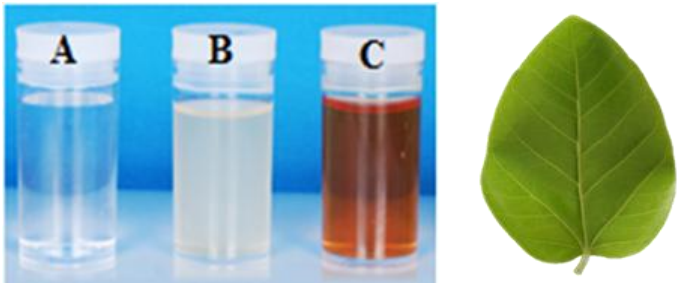

Fig.1 Sample tubes containing Silver nitrate solution (1 mM) (A) Aqueous leaf extract of *Ficus benghalensis* (B) Silver nitrate solution (1mM) after 10 min of reaction with leaf extract (C). *Ficus benghalensis* Leaf at the extreme right used for SNPs synthesis.

Fig.2 Shows the absorption spectra of the *Ficus benghalensis* Leaf extract and the peaks are typical for absorptions of proteins and Quinones. The leaf extract shows prominent absorption peak in UV region at 210 nm was assigned to absorption of peptide bonds and absorption at 280 nm indicated the presence of aromatic amino acids like tyrosine, tryptophan, or phenylalanine residues in the proteins, which are known to interact with silver ions, anthraquinones show intense quinonoid bands in a range from 260 to 290 nm and hydroxyl anthraquinones show an absorption band between 220 and 240 nm [19 (a,b,c)].

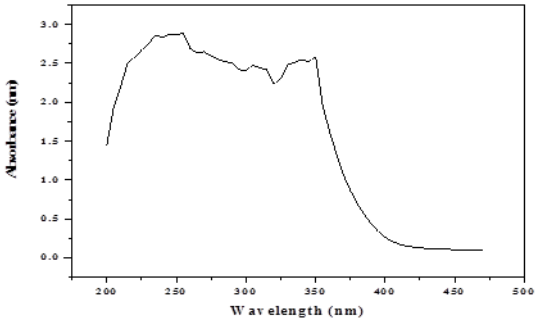

Fig.2 Absorption Spectra of leaf extract of *Ficus benghalensis* showing presence of proteins. The absorption maxima at 280 nm arise due to electronic excitations in tyrosine and tryptophan residues of proteins.

Fig.3 Shows the UV-Vis spectra recorded from F.B. reaction vessel at different time intervals of reaction. The bio-transformed products were simultaneously characterized by UV-Vis spectroscopy. The absorption spectra of nanoparticles showed maximum absorption at 430- 435 nm with steadily increased in intensity as a function of time of reaction without any shift in the peak. This indicates the presence of silver nanoparticles due to the excitation of surface Plasmons resonance. After 24 h of incubation period, no further increase in color intensity was found this indicates complete reduction silver ions.

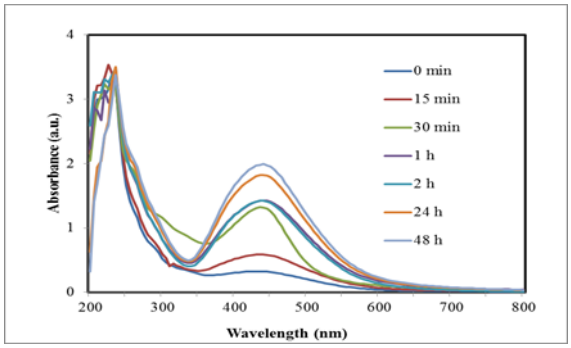

Fig.3 UV-Vis. Spectra recorded as a function of time with aqueous solution of 1mM $AgNO_3$ and *Ficus benghalensis* leaf extract. Reaction Time is indicated in the inset.

The role of protein on the stability of SNPs was studied by changing the pH of reaction mixture. Fig.4 shows that the SPR band of the SNPs at different pH (i.e. pH 5–10). The protein-capped SNPs were stable at slighly basic, neutral and slightly acidic conditions. However, decreasing the pH below 6.0, the absorbance decreases. We also observed high absorbance and narrow peaks at higher pH was observed (pH 10), The appearance of more than one peak is likely due to the formation of nanoparticles of various shapes and sizes. The absorption peak are observed at 405,415,430,440,450 nm which may be due to the formation of monodispersed and smaller sized SNPs and Further, the gradual red-shift of the absorption peaks suggests the formation of larger particles with increase with time [20, 21]. It was observed that the synthesized SNPs are stable at pH 7-9. And pH 8 was found to be most stable hence it was used for synthesis. The formation of SNPs occurs rapidly, in neutral and basic pH which is evident from observation and this may be due to the ionization of the phenolic group present in the leaf extract of F.B. [22]. At higher pH, however, the large number of functional groups available for silver binding facilitated large number of silver nanoparticles to bind and form a large number of nanoparticles with smaller diameters. pH induced aggregation of the nanoconjugate arises from protonation carboxylate groups, of protein at pKa ~ 5, with consequent reduction in electrostatic repulsion [23]. Additionally, the formation of intermolecular H-bonding between protonated functional groups of surface-conjugated protein molecules of different particles could also contribute to aggregation or agglomaration [24]. These results suggest that an electrostatic mechanism is the main stabilization factor for SNPs.

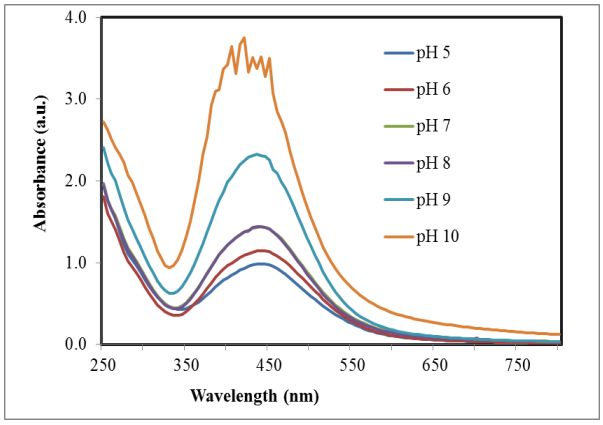

Fig.4 UV-Vis. Spectra recorded at different pH of reaction medium after 30 min of reaction time.

Fig.5 shows the effect of different ionic strength of silver nitrate. With the increase of ionic concentration of $AgNO_3$ from 1 to 6 mM the intensity of SPR band first increased, attaining maximum with 4 mM and then decreased. The λ max decreased first and then increased. The decrease in absorbance at the beginning of reaction indicates that the amount of reducing agent is more at the beginning as compare to the ionic strength of Silver nitrate solution. At higher concentrations of $AgNO_3$ indicates that an concentration of silver nitrate ion solution increases and the amount of reducing agents is utilized so the formation of nanoparticles reduced. Very stable nanoparticles are found to be at 1mM concentration hence this concentration is used for further study. Dubey et. al also reported the decreasing trend in the formation of silver nanoparticles with the increase of silver ion concentration [25].

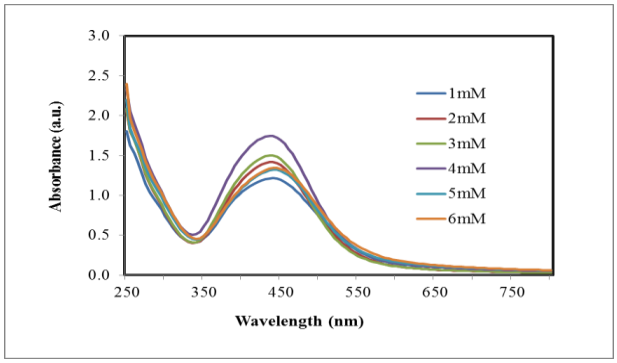

Fig.5 UV-Vis. spectra recorded as a different concentration of aqueous solution of AgNO3 (1 mM – 6 mM) after 30 min of reaction time.

Fig.6 show the effect of leaf extract was assessed by changing the volume of leaf extract (1 to 3 ml) to silver nitrate (20 ml) solution. With increasing ratio of leaf extract, the intensity of the SPR band increased, it was observed that with higher volumes of leaf extract the particles were not stable and agglomeration was observed. The λ max was slightly red shifted (430, 430 and 440 nm in 0.5, 1 and 2 ml extract, respectively) with the increase of volume of extract. The peak are broader at lower amount of leaf extract and sharp peak are observed with increase with concentration.

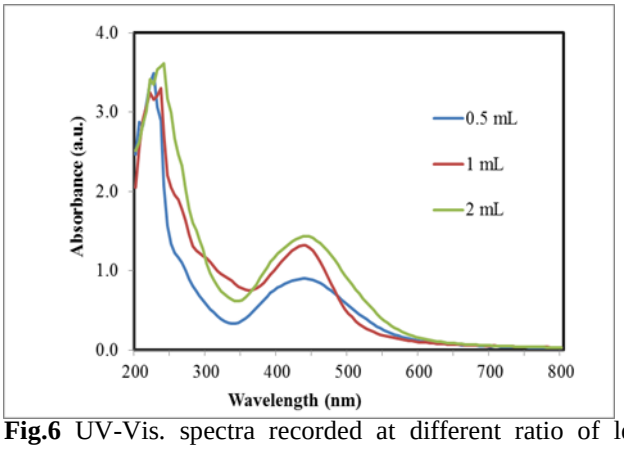

Fig.6 UV-Vis. spectra recorded at different ratio of leaf extract to 20 ml of 1mM $AgNO_3$ after 30 min of reaction time.

The effect of temperature on the formation of SNPs was shown in Fig.7. The absorption spectra obtained from different reaction temperature ie. (40 - 100^0 C) at pH 8 was shown. The SPR peak observed at 430 425 420 and 415nm for 40, 60, 80 and 100^0C respectively. These peaks are characteristic Plasmon band for silver nanoparticles [26].The increase in reaction temperature, shows sharp narrow peaks at lower wavelength regions (420 nm at 100^0C), which indicates the formation of SNPs, whereas, with decresing reaction temperature, the peaks observed at higher wavelength region (420 nm at 100^0C, 430 nm at 40^0C) which, clearly indicates increase in SNPs size. With the increase of temperature the intensity of SPR band increased and with sharpness and the formation of nanoparticle is rapid. Agglomeration was observed at higher temperature within 48 h and at lower temperature formation was slow but no agglomeration was observed almost for 16-20 weeks.

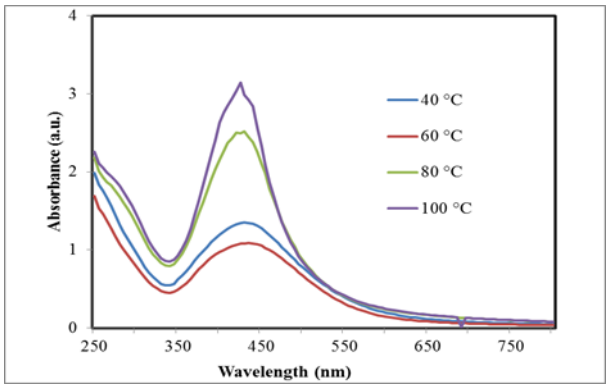

Fig.7 Absorption spectra at different temperature (40 ^{0}C, 60 ^{0}C, 80 ^{0}C, 100 ^{0}C) of reaction medium after 30 min of reaction time.

Fig 8(a) Shows the fluorescence emission spectra of the leaf extract of *Ficus benghalensis* .The presence of peptide found found in UV-Vis analysis was analyzed by emission spectra by excitation wavelength at 210 nm correspond to peptide bond was analyzed and emission band was observed at 453 nm and 528 nm. Fig.8.(b) Presence of

tryptophan/tyrosine residues in proteins release in the leaf extract was studied and the excitation wavelength was 280 nm, in close to maximal optical transitions of the tryptophan and tyrosine. An emission band centred at 443 and 561 nm was observed. The nature of the emission band indicates that the proteins may be bound to the nanoparticle surface [27].

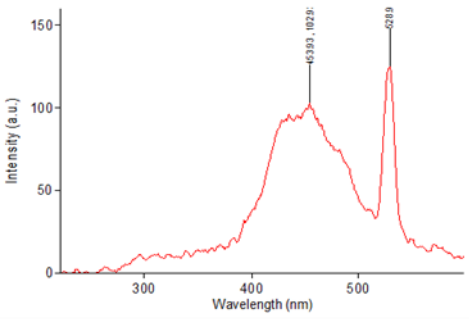

Fig.8 (a) Fluorescence emission spectra of *Ficus benghalensis* leaf extract. Fluorescence emission was monitored at 453 nm and 528 nm following excitation at 210nm (10 nm slit width).

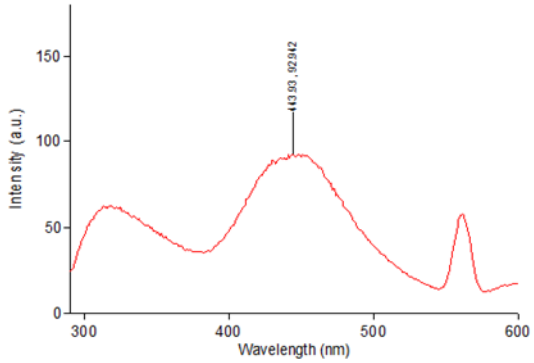

Fig.8 (b) Fluorescence emission spectra at 443 and 561 nm following excitation at 280nm (5 and 10 nm slit width).

Fig.9 Shows XRD patterns of silver, prominent Bragg reflections at 2 Θ values of 38.23, 44.28, 64.39 and 77.48 were observed , which correspond to Bragg reflections of face-centred cubic (fcc) silver nanoparticles. Which are in agreement with JCPDS File No. 87-0720. Meanwhile, the mean particle size of these silver nanoparticles was estimated from the Debye–Scherrer equation by determining the width of the (111) Bragg reflection. The equation uses the reference peak width at angle Θ, where λ is the X-ray wavelength (1.5418 Å), β1/2 is the width of the XRD peak at half height and K is a shape factor. The calculated average particle size of the silver was found to be 3 nm, which was also in line with the observation of the FESEM and AFM results discussed later.

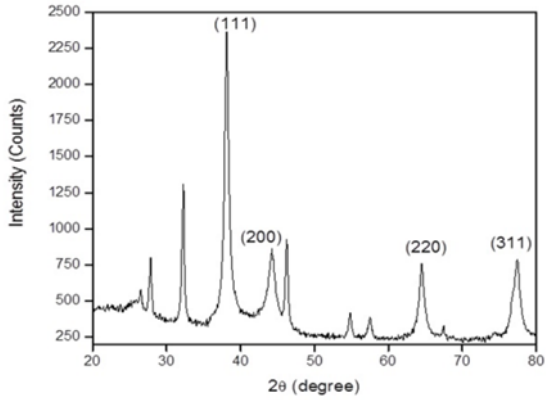

Fig.9 XRD patterns of silver nanoparticles synthesized by *Ficus benghalensis* leaf extract

Fig.10 Shows TGA trace of SNPs plotted temperature versus % weight loss. It shows two step weight loss and total weight loss of 32% was observed and 68% of residue is remained in the alumina crucible. The first step of the trace i.e. 30^0C to 100^0C is due to the evaporation of water molecule adsorbed on the surface of SNPs. The second step weight loss between 100^0C to 460^0C is due to the loss of organic capping over the SNPs. Suggests that the metallic core surrounded by biomolecules which is later explained in FESEM and EDAX results. The residue remained after heating is pure silver microstructures.

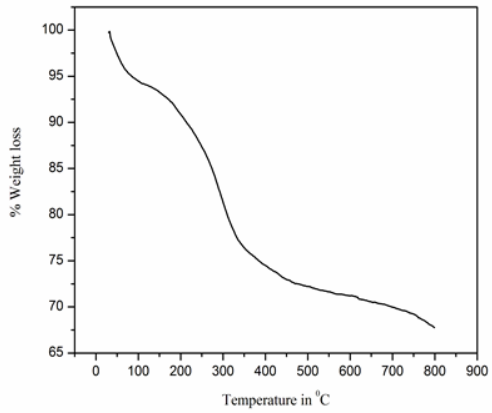

Fig.10 TGA spectra of silver nanoparticles

FESEM technique was used to visualize the size and shape of the SNP. Fig.11shows the FESEM images of the SNPs shows, a thin organic shell covered on each individual SNPs, which may be responsible for reducing Ag+ ions a and helps in inter particle binding with nanoparticle. Most of the silver nanoparticles are spherical in shape, and are in the range of 10–60 nm in size indicating polydispersity. A few aggregations of SNPs are also observed in some places, indicating agglomeration at a later time (after 16 weeks).

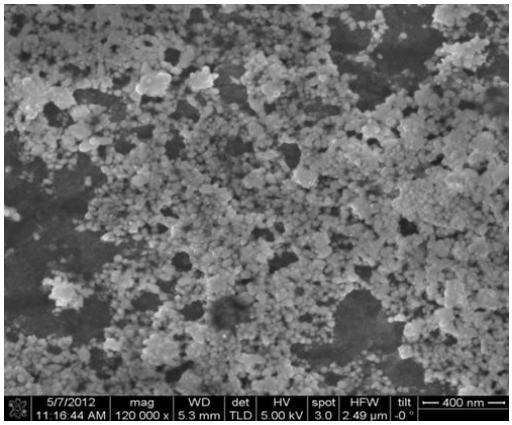

Fig.11 FESEM micrographs showing uniformly distributed silver nanoparticles

A particle size distribution histogram determined from the FESEM microscopy is shown in Fig.12 from this figure, it is observed that the particle are of different sizes, almost 28% of the particles are in the range of 1-10 nm and 20% in 11-20 nm range and 17% in 21-30 nm ranges. The SNPs size distribution obtained from 1,002 nanoparticles is shown in Fig.12 this histogram infers that the particles are distributed in a very narrow range with almost 90% of the particles are in 45 ± 5 nm. 8% of the particles are in the range above 51 nm.

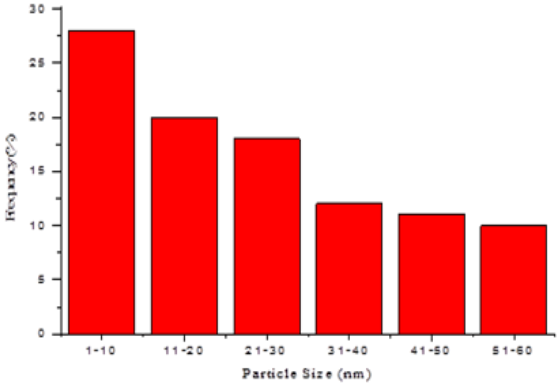

Fig.12 Particle size distribution histogram of silver nanoparticles from Field emission electron microscope

The result of EDAX gives a clear idea about the elements present in the biosynthesized nanoparticles. The EDAX profile of phyto-capped SNPs shows strong signal of the Ag atoms indicates the crystalline property as shown in Fig.13. The optical absorption peak is at 3 keV, which is typical for the absorption of metallic silver nanocrystallites [28]. Other than this, weak signals for C and O are observed which may originate from the biomolecules capped to the surface of the SNPs. Together with TEM images, Shankar et al.and Raghunandan et al [14, 18] reported that nanoparticles synthesized using plant extracts are surrounded by a thin layer of some capping organic material from plant leaf broth. The appearance of Al in figure is because of the aluminum grid base used for the analysis. Mg indicate the presence of proteins as a capping material on the surface of silver.

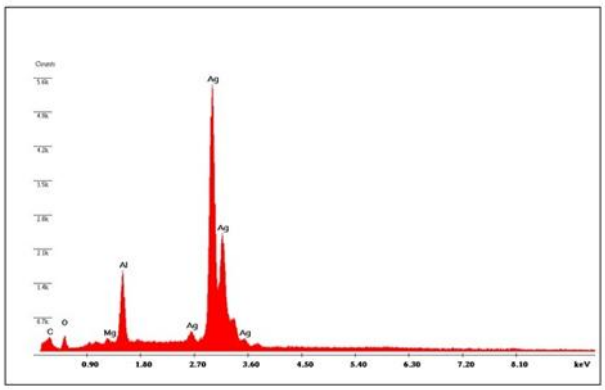

Fig.13 EDAX graph showing the presence of elemental silver.

Atomic force microscopy (AFM) was used to observe the sample's surface morphology and roughness. Figure 14 shows spherical particles with grains sized 20-75 nm in diameter with mean size of about 35 nm, which was much greater than that of estimated values from FESEM images. This magnification is attributed to the convolution of true particle size with that of the AFM tip and also on the preparation of samples for AFM. Wider scans covering a few micrometers yielded a root mean square (RMS) roughness of 10 nm with a maximum peak-to-valley distance of 65 nm.

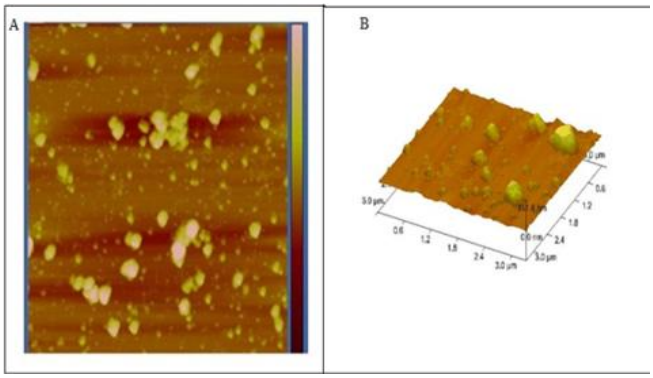

Fig.14 AFM image of silver nanoparticles (A) lateral micrographs showing uniformly distributed nanoparticles and some agglomeration (B) 3D image of silver nanoparticle.

The major factor responsible for bioreduction of silver ions (Ag+) to SNPs (Ag0) present in the F.B. leaf extract.which was identified using FTIR spectroscopy. The IR spectrum of freeze dried sample of SNP showed the distinct peak in the range of 1640 and 1540 cm−1 Which represents amide I and amide II of proteins and arises due to carbonyl stretch and –N–H stretch vibrations in the amide linkages shown in Fig.15. Carbonyl group of amino acid residues and peptides of proteins has the stronger ability to bind metal, so proteins could most possible organic moiety for stabilizing the SNP in the medium. [10] The IR bands of 1348 cm−1 were due to the strong stretching vibrations of C- N aromatic amine.

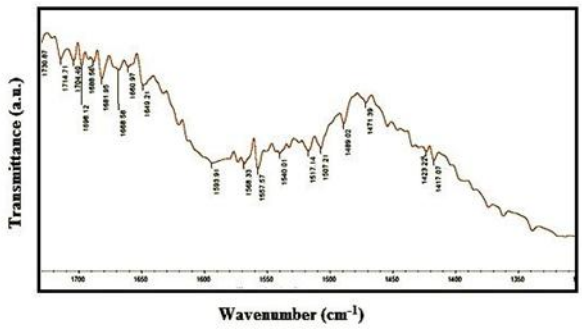

Fig.15 FTIR spectra of freeze-dried powder of SNPs

CONCLUSIONS

In conclusion, the present method of synthesis of SNPs is a rapid, green, and economic. Reduction is accomplished probably due to phytochemicals such as Quinones and protein capping might helped in stabilization of SNPs. Efforts are underway in our laboratory to purify and characterize the proteins and Quinones to understand their mode of action and possible interactions with silver nanoparticles. As the SNPs synthesized are biocompatible and economic as they are investigated for agricultural and medical applications this study is under progress.

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