

BIOSYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL STUDIES OF AgNPs EXTRACT FROM BACOPA MONNIERA WHOLE PLANT

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Reliable and eco-friendly process for synthesis of metallic nanoparticles is an important step in the field of the nanotechnology. One of the options to achieve this objective is to use biological systems. Here we report the ethanolic extract of *B. monniera* to produce silver nanoparticles by reduction of AgNO₃. It was observed that the synthesis process was quite rapid and silver nanoparticles were formed within minutes of silver ion coming in contact with the plant filtrate. UV-visible spectrum of the aqueous medium containing silver ions demonstrated a peak at 436 nm corresponding to the plasmon absorbance of silver nanoparticles. X-Ray diffraction (XRD) analysis reveals the FCC structure of silver nanoparticles. Energy dispersive X-ray analysis (EDX) evidences the presence of silver nanoparticles in the aquatic solution of leaf extract. FTIR analysis of the nanoparticles indicated the presence of proteins, which may be acting as capping agents around the nanoparticles. From transmission electron microscopy (TEM) analysis, the size of the silver nanoparticles was measured (10-30 nm). Further the antimicrobial activity of synthesized particles showed effective inhibitory activity.

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1. Introduction

Nanotechnology is a sprouting interdisciplinary field of research interspersing material science, bionanoscience and technology. Remarkable advances are made in the field of biotechnology and nanotechnology to harness the benefit of life sciences, health care and industrial biotechnology [1-3]. Nanomaterials may provide solutions to technological and environmental challenges in the areas of solar energy conversion, catalysis, medicine and water treatment [4, 5]. This increasing demand must be accompanied by "green" synthesis methods. There is a constant interest in the synthesis of noble metal nanoparticles for their applications such as catalysis, electronics, optics, environmental and biotechnology [6-12]. A unique characteristic of these synthesized metal particles is that a change in the absorbance or wavelength gives a measure of the particle size, shape and interparticle properties [13, 14]. Nanoparticles offer a great possibility for biomedical applications, not only to deliver pharmaceuticals, but also to be used as novel diagnostic and therapeutic approaches [15]. Nanoparticles could reach a biological target of interest by having a small size. More over, functionalized, biocompatible and inert nanomaterials have potential applications in cancer diagnosis and therapy [16-20].

In view of the environmental sustenance, there is a need to develop eco friendly procedures to avoid toxic chemicals. Among noble-metal nanoparticles, silver have received

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considerable attention in the field of biology and medicine due to its attractive physicochemical properties. A number of approaches are available for the synthesis of silver nanoparticles. Recently, silver and silver nanoparticles are widely being applied to consumer products and medical uses. Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process.

Bacopa is referred to as one of the greatest multipurpose miracle herb of oriental medicine capable of improving memory and treating several neurological disorders, commonly known as Brahmi. It is extensively investigated for its pharmacological and therapeutic effects [21]. As an ayurvedic medicine it is used as a nerve tonic. Preclinical and clinical studies have shown that *Bacopa monniera* Improves memory and mental function [22]. It is thought to improve intelligence, memory and functioning of sense organs and it has been used to treat epilepsy, insomnia and asthma [23]. So far there is no report on the synthesis of nanoparticles by using *B.monnierea* whole plant extract. Thus, we report here the synthesis and antibacterial activity of Ag nanoparticles using *B.monnierea* whole plant extract.

2. Experimental details

2.1. Preparation of *Bacopa monniera* extract:

The whole plant of *B. monniera* was thoroughly washed with double distilled water and shade dried in dust free condition for one week at room temperature before being grinded to a fine powder. Finally powdered plant material (10g) was extracted with ethanol (100 ml). The mixture solution was left on constant magnetic stirring at room temperature for 24hrs. The extract was filtered and stored at 4°C for further experiments.

2.2. Synthesis of silver nanoparticles:

0.025M Aqueous solution of silver nitrate (AgNO_3) was prepared and used for the synthesis of silver nanoparticles. 4 ml of plant extract was added to 120 ml of aqueous solution of 0.025M silver nitrate for reduction into Ag^+ ions and kept at room temperature for one hour.

2.3. UV-Vis spectral analysis :

The bioreduction of pure Ag^+ ions was monitored by periodic sampling of aliquots (0.5) of the suspension, then diluting the samples with 5 ml deionized water and subsequently measuring UV-Vis spectra of the resulting diluents. UV-Vis spectroscopy analyses of silver nanoparticles produced were carried out as a function of bioreduction time at room temperature on UV-Vis spectrophotometer UV-2450(Shimadzu).

2.4. XRD analysis:

X-ray diffraction (XRD) measurements of thin film of the bioreduced silver ions aqueous solution were drop coated onto glass slide and carried out on an INEL X-ray diffractometer. The diffraction pattern was recorded by $\text{Co-}\alpha_1$ radiation with λ of 1.78Å in the region of 2θ from 20° to 90° at 0.02°/ min. and the time constant was 2 sec.

2.5. Transmission Electron Microscopy(TEM) measurements:

The sample was first sonicated for 10 minutes. A drop of this solution was loaded on carbon coated copper grid and solvent was allowed to evaporate under Infrared light for 30 minutes. TEM measurements were performed on Philips Model CM 200 instrument operated at an accelerating voltage at 200KV.

2.6. EDAX measurements:

In order to carry out EDAX analysis, thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid and performed on ZIESS S-4500 SEM instrument equipped with a Thermo EDAX attachment.

2.7. FTIR analysis of dried residue after bioreduction:

To remove any free biomass residue or compound that is not capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 9000 rpm for 30 min. The obtained pellet was washed with ethanol for three times. There after the purified suspension was air dried to obtain dried powder. Finally the dried nanoparticles were analyzed by FTIR.

2.8. Antimicrobial activity

The antimicrobial activity of silver nanoparticles was evaluated against staphylococcus aureus, Escherichia coli, Klebsiella pneumonia and Bacillus subtilis by disc method. The 24 hrs old cultures were prepared in nutrient broth (composition (gm/L) peptone 15.0; yeast extract 3.0; sodium chloride 6.0; D (+) glucose (1.0).

Two replicas of respective microorganisms were prepared by spreading 100 μ l of revived culture on the nutrient agar plate (composition (gm/litre) peptone 15.0; yeast extract 3.0; sodium chloride 6.0; D (+) glucose 1.0; agar-agar (12.0) with the help of spreader. Discs were prepared by using Whatmann No.1 filter paper. Discs were placed on agar plates and sample of synthesized silver nanoparticles were placed on the Disc with the help of micropipette. The plates were incubated at 37°C overnight. Gentamycin disc was used as reference drug.

3. Results and discussion

Reduction of silver ions was visually evident from the colour change and was completed within two hours with a stable brown colour indicating the formation of silver nanoparticles as shown in Fig. 1. These nanoparticles were characterized using UV-Vis spectroscopy; it was shown that the age of the culture had a strong effect on the shape and size of the nanoparticles. Equivalent amounts of the suspension (0.5 ml) were diluted in a constant volume of de-ionized water (5 ml) and subsequently analyzed at room temperature. The progress of the reaction between metal ions and the leaf extracts were monitored by UV-Visible spectra of Ag nanoparticles in aqueous solution with different reaction times are shown in Fig.2. The UV-Vis spectra showed the appearance of a single and strong band absorption peaks centered at about 436 nm and 433 nm respectively, thus indicating that the nanoparticles are isotropic in shape and uniform in size. This band is called the surface plasmon resonance (SPR) [24]. There is significantly shift in the absorption peak of silver surface plasmon resonance suggesting the formation of smaller silver nanoparticles. This observation indicates the release of proteins into filtrate that suggests possible mechanisms for the reduction of silver ions present in the solution [25].



Fig. 1 Aqueous solution of $0.025M$ $AgNO_3$ with *B. monniera* extract before adding the leaf extract and after addition of extract at 1hr.

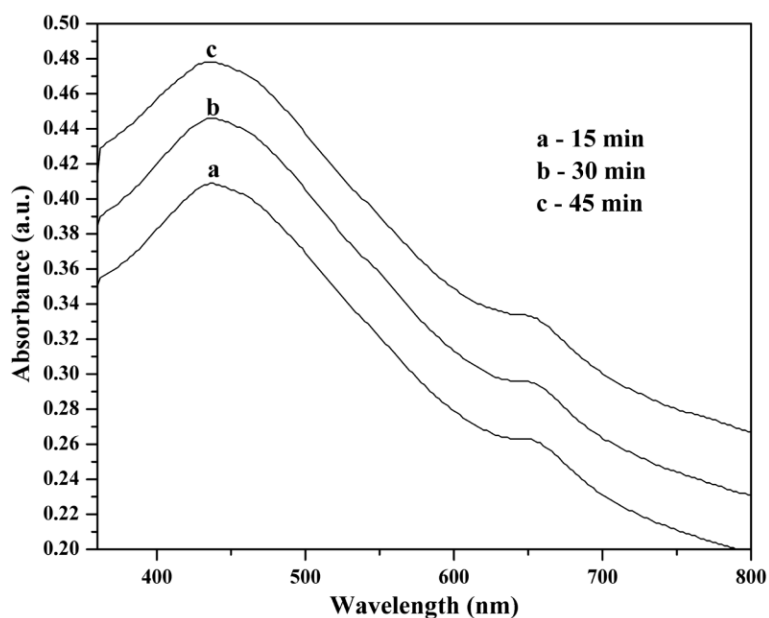


Fig. 2 UV-Vis absorption spectra of silver nanoparticles synthesized by exposure of *B. monniera* with $0.025M$ silver nitrate.

The distinct Bragg reflections corresponding to (111), (200) (220), (311), and sets of lattice planes were manifested in the X-ray diffraction patterns are shown in Fig.3. They may be indexed on the basis of face-centered cubic structure of silver. The obtained data was matched with the Joint Committee on Powder Diffraction Standards (JCPDS) file No.03-0921. XRD patterns were analyzed to determine peak intensity, position and full width at half maximum (FWHM) data was used with the Scherrer's formula to determine mean particle size. Scherrer's equation is given by $d=0.9\lambda/\beta\cos\theta$ where d is the mean diameter of the nanoparticles, λ is the wavelength of X-ray radiation source, β is the angular FWHM of the XRD peak at the diffraction angle θ [26] and the estimated mean size of the particle was 6 nm.

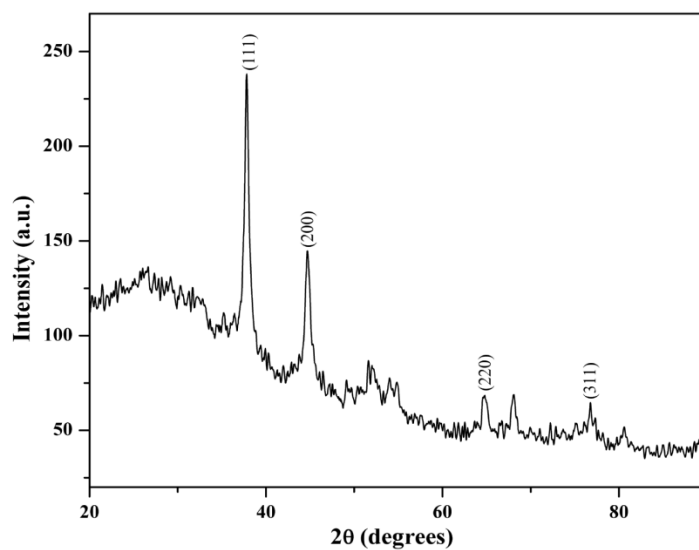


Fig. 3 XRD patterns of capped silver nanoparticles synthesized using *B. monniera* extract.

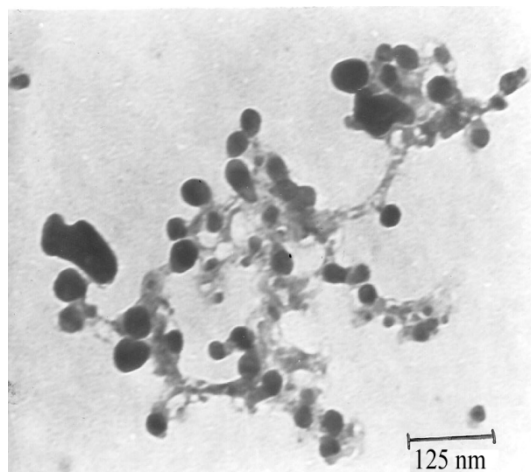


Fig. 4(a)

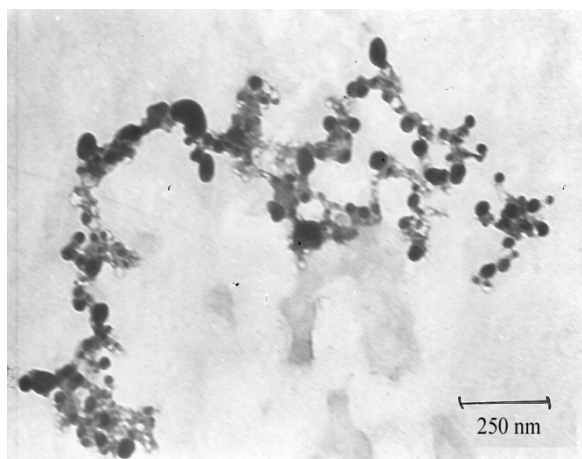


Fig. 4(b)

Fig. 4 TEM images and corresponding size distribution of silver nanoparticles obtained by reduction of AgNO_3 with *B. monniera*.

The typical TEM micrographs of the synthesized Ag nanoparticles are presented in Fig.4. It is observed that most of the Ag nanoparticles were spherical in shape in addition there are agglomerated silver nanoparticles in places, thereby indicating possible sedimentation at a later time. There is a variation in particle sizes and the average size estimated was 22 nm. According to the size distribution shown in (Fig.5.), most of the nanoparticles ranged from 5 to 30 nm in size. More particles smaller than 10 nm. The energy dispersive X-ray analysis (EDX) reveals strong signal in the silver region and confirms the formation of silver nanoparticles (Fig.6). The typical optical absorption peak at 3 KeV confirms the metallic nanoparticles due to surface plasmon resonance [27]. The other elemental signals were recorded, possibly may be due to elements from enzymes or proteins present with in the *B. monniera*.

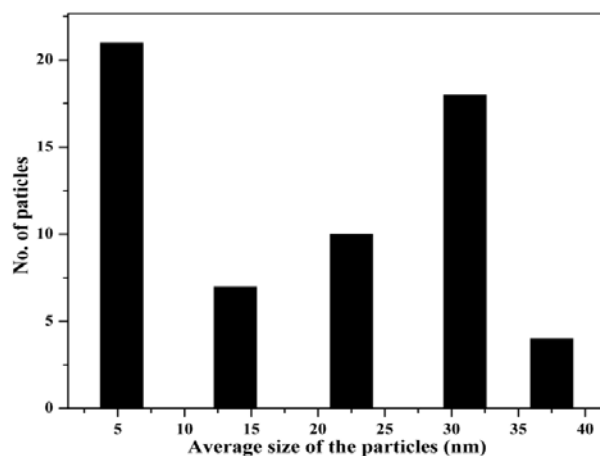


Fig. 5 The percentage distribution of different size of silver nanoparticles.

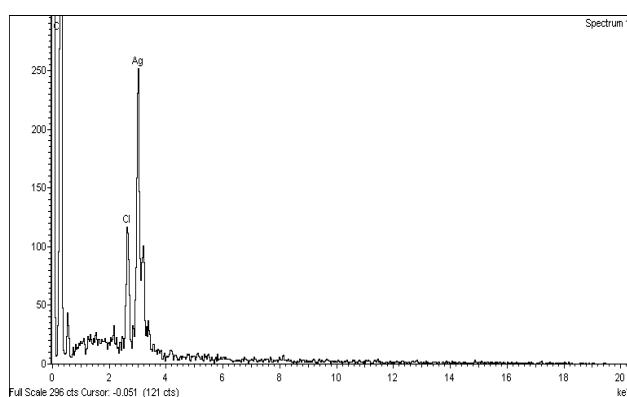


Fig. 6 The EDX spectrum for silver nanoparticles. Strong signals from the atoms in nanoparticles are visible with the signals for Cl.

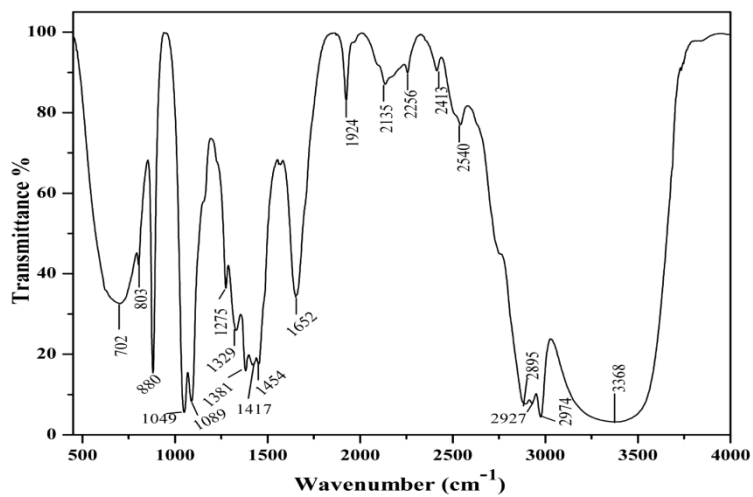


Fig. 7 FTIR spectra of the silver nanoparticles synthesized by the reduction of silver nitrate with the *B. monniera* extract.

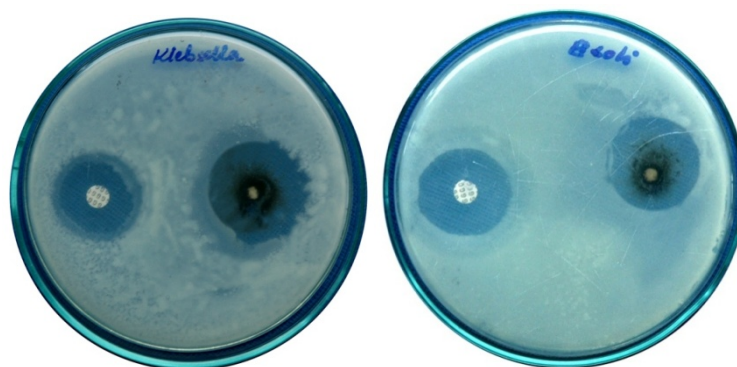


Fig. 8 Antimicrobial activities of Ag nanoparticles by using *B.monnierra* extract and 0.025M AgNO₃ Gentamycin as standard.

The FTIR spectra of *B. monnierra*, leaf extract sample containing silver nanoparticles are depicted in Fig. 7. The band at 3368 cm⁻¹ corresponds to O-H stretching H-bonded alcohols and phenols. The peak at 2927 cm⁻¹ corresponds to O-H stretch carboxylic acids. The assignment at 1652 cm⁻¹ corresponds to N-H bend primary amines. The peak at 1381 cm⁻¹ corresponds to C-N stretching of aromatic amine group and the bands observed at 1089, 1042, 1059 cm⁻¹ corresponds to C-N stretching alcohols, carboxylic acids, ethers and esters. Therefore the synthesized nanoparticles were surrounded by proteins and metabolites such as terpenoids having functional groups of alcohols, ketones, aldehydes and carboxylic acids. From the analysis of FTIR studies we confirmed that the carbonyl group from the amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly from the metal nanoparticles (i.e., capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium [28]. Further the nanoparticles synthesis by green route was found highly toxic against 4 bacterial species at a concentration of 10 µl Ag nanoparticles, revealed higher antibacterial activity against *Bacillus subtilis*, where as intermediated activity was revealed against *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae* (Fig.8). The inhibitory activities in culture media of the Ag nanoparticles reported in Table 1 were comparable with reference drug viz. Gentamycin.

Table 1 Inhibitory activity of silver nanoparticles on bacteria.

S. No.	Organism	Zone of inhibition(cm) Ref. drug Gentamycin	Nanoparticle
1	<i>Staphylococcus aureas</i>	1.3	1.2
2	<i>Bacillus subtilis</i>	1.4	1.5
3	<i>Escherichia coli</i>	1.2	1.2
4	<i>Klebsiella pneumoniae</i>	1.2	1.3

All values represented in the table are average of results of two separately conducted experiments.

The antimicrobial activity of silver has been recognized by clinicians for over 100 years [29]. It is only in last few decades mode of action of silver as an antimicrobial agent has been studied. Raut Rajesh et al. [30] investigated the antibacterial activity of phytosynthesised silver nanoparticles against *Staphylococcus aureas*, *Escherichia coli*, *P. aeruginosa* and *K. pneumonia*. Similarly, Kim et al. [31] reported antimicrobial activity of silver nanoparticles against *E. coli* and *S. aureus*. The effect was dose dependent.

The silver nanoparticles also exhibited the antibacterial activity against both gram-positive and gram-negative and formed the zone of inhibition of diameters 1.2, 1.5, 1.2 and 1.3 cm, respectively.

4. Conclusions

The silver nanoparticles of average size ≈ 10 nm have been synthesized using whole plant of *B. monniera*.

The characterizations from UV-Vis, TEM, support the stability of the biosynthesized nanoparticles.

The FCC structural analysis conformed by the XRD. The EDX analysis strongly suggests the formation of silver nanoparticles.

The silver nanoparticles using *B. monniera* proved excellent antimicrobial activity.

These silver nanoparticles may be used in food and pharmaceuticals industries.

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