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Green synthesis and antibacterial activity of silver nanoparticles from ripened fruit pulp of *Diospyros chloroxylon* Roxb

P.Shivakumar Singh[1] and G.M.Vidyasagar[2,*]

[1]Department of Botany, Palamuru University, Mahabubnagar-509001, Telangana, India.

[2*]Medicinal Plants and Microbiology Research Laboratory, Department of Post- Graduate Studies and Research in Botany, Gulbarga University, Gulbarga – 585 106, Karnataka, India.

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ABSTRACT

Biosynthesis of silver nanoparticles using *Diospyros chloroxylon* Roxb. ripened fruit pulp of extract was investigated and the effect of broth concentration in reduction mechanism and particle size is reported. The rapid reduction of silver (Ag+) ions was monitored using UV-visible spectrophotometer and showed formation of silver nanoparticles within 20 minutes. Transmission electron microscopy (TEM) showed that the synthesized silver nanoparticles are varied from 10-25 nm and have the varying in shapes like spherical, round uneven. Further the XRD analysis confirms the nano-crystalline phase of silver with FCC crystal structure. FTIR examinations confirms the Silver particles. The present study, it was found that the increasing broth concentration increases the rate of reduction and decreases the particle size.

1. Introduction

Silver nanoparticles (AgNPs) have become the spotlight of much research interest due to their extensive variety of applications [**1**]. Their ability to alter the physical, optical and the electronic properties of compounds [**2**] have found applications in various fields including electronic devices [**3**], chemical/biological sensing [**4**], and surface enhanced Raman spectroscopy [**5**]. In difference, a promising usage of AgNPs as antimicrobial agent is well known and has already found applications in antimicrobial paint coatings [**6**], textiles, water treatment, medical devices [**7**], and HIV prevention as well as treatment [**8**]. Traditional chemical methods of synthesizing silver nanoparticles include the use of ethylene glycol [**9, 10**], pyridine [**11**], and sodium borohydride [**12**]. A variety of plant (seed, ripened fruit pulp as well as tuber) extracts [**13-17**] for the synthesis of metal nanoparticles. Among them, plant ripened fruit pulp extract mediated biological process has been widely investigated due to their reasonably priced and simple protocol.

Therefore, there are no previous reports on the biosynthesis of AgNPs Via ripened fruit pulp of *Diospyros chloroxylon. So* the present report aimed to optimize the concentrations of ripened fruit pulp extracts and AgNPs concentrations at room temperature. The uncomplicated AgNPs were little cost and reproducible technique. Furthermore, the synthesized AgNPs were evaluated for antibacterial activity against pathogens.

2. Materials and Methods

2.1 Collection of material

Fresh ripened fruit pulp of *Diospyros chloroxylon* were collected from the Appayipally forests of Mahabubnagar District. Silver nitrate ($AgNO_3$) is procured from High Media Laboratories. Solutions were prepared with triply distilled water.

2.2 Preparation of the extract

25 g of ripened ripened fruit pulp was washed repeatedly with distilled water, so as to remove any organic impurities present on it and cut into fine pieces. The pieces of *Diospyros chloroxylon* ripened ripened fruit pulp of are then taken into 1000 ml beaker containing 500 ml double distilled water and were exposed to microwave for 180^0C to suppress the enzymes present in the solution. The raw extract obtained was filtered twice with Whatman filter paper No. 42 (pore size 0.45 μm and 0.22 μm sized). The resultant filtrate is nothing but extract of the *Diospyros chloroxylon* ripened fruit pulp used for the reduction of Ag^+ to Ag^0. The extract was treated with silver nitrate solution of concentration $10^{-3.}$

2.3 Synthesis of Silver nanoparticles from *Diospyros chloroxylon* ripened fruit pulp extract

The aqueous solution of 1mM silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 10 ml of *Diospyros chloroxylon* ripened fruit pulp extract was added into 490 ml of aqueous solution of 1 mM silver nitrate for reduction into Ag+ ions and kept for incubation for 50 min at room temperature. The filtrate acts as reducing and stabilizing agent for 1mM of $AgNO_3$.

2.4 Characterization

2.4.1 UV- Visible spectra analysis

The formation of AgNPs is verified by using UV–visible 5704SS ELICO spectrophotometer operated at with 1 nm resolution with optical length of 10 mm. UV–visible analysis of the reaction mixture was observed for a period of 300s.

2.4.2 X-ray-diffraction (XRD) analysis

This analysis carried out for crystallinity, films of colloidal AgNPs formed on Si(III) substrates by drop coating were used

Tele: 9449258812

E-mail address: gm vidyasagar@rediffmail.com

for X-ray-diffraction (XRD) study. The data were obtained using Ricago X-Ray Diffractrometer (Japan), operated at 30 kV and 20 mA electric power with Cu Ka(I = 1.54 A °).

2.4.3 Transmission Electron Microscopy (TEM) analysis

The transmission electron microscopy (TEM) images were obtained using Technai-20 Philips instrument operated at 190 keV. Sample for this analysis were prepared by Rapid Biosynthesis of Silver Nanoparticles Using *Diospyros chloroxylon* 109 coating of aqueous AgNPs drops on carbon coated copper grids, kept for 5 min; the extra solution was removed using blotting paper. The film of TEM grid is exposed to IR light for drying.

2.4.4 Atomic Force Microscope (AFM) analysis

The images of atomic force microscope (AFM) were collected under ambient conditions on a Veeco-Innova scanning probe microscope etched Si–nano probe tips (RTESPA-M) were used for the same.

2.4.5 Fourier Transforms Infra-Red Spectroscopy (FTIR) analysis

The powder sample of AgNPs was prepared by centrifuging the synthesized AgNPs solution at 10,000 rpm for 20 min. The solid residue formed is then washed with deionized water to remove any unattached biological moieties to the surface of the nanoparticles, which are not responsible for bio functionalization and capping. The resultant residue was then dried completely and the powder obtained was used for FTIR measurements carried out on a Nicolet iS5 FTIR with diamond ATR (Attenuated Reflectance Technique).

3. Antibacterial activity of AgNPs synthesized from *Diospyros chloroxylon* aqueous ripened fruit pulp extract.

3.1 Test microorganisms

Four bacterial species such as, *Candida albicans*, *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus* were used in the present study. All the tested strains were obtained from microbiology research laboratory, Gulbarga University, Karnataka, India. These test cultures were grown in PDA, nutrient broth (Himedia, M002) at 37ºC and maintained on nutrient and potato agar slants at 4ºC.

3.2 Agar-well diffusion method

The assay was conducted by agar well diffusion method. About 15 ml of potato dextrose agar medium was poured in the sterilized petri dishes and allowed to solidify. The bacterial strains were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 Mac Farland standards (108 CFU/ml). 1 ml of test strain was spread over the medium using a sterilized glass spreader. Using flamed sterile borer, wells of 4 mm diameter were punctured in the culture medium and required concentrations (80 *µl*, 60 *µl*, 40 *µl,* and 20 *µl*) of AgNPs solution were added to the wells.

Fig 1. *Diospyros chloroxylon* Roxb.

The plates thus prepared were left for diffusion of extracts into media for one hour in the refrigerator and then incubated at 37°C. After incubation for 48h, the plates were observed for zones of inhibition. The diameter of zone of inhibition was measured and expressed in millimeters. $AgNo_3$ solution and plant aqueous extract was used as negative control. The experiments were conducted in triplicates. The same method was followed for testing antibacterial activity using nutrient agar medium and incubated at 37°C for 18h.

3. Results and Discussion

Synthesis of Ag nanoparticles using *Diospyros chloroxylon* Roxb. ripened fruit pulp extract (Green synthesis)

For the synthesis of silver nanoparticles, 1 ml of ripened fruit pulp extract was added to 250 ml of 1mM $AgNO_3$ solution. The solution turned colourless to dark-brown within 15 minutes. Ag nanoparticles exhibit dark brownish colour in aqueous solution due to excitation of surface plasmon resonance. On mixing the extract with aqueous solution of the Ag ion complex, a change in the colour from colourless to dark brown was observed. It was due to the reduction of Ag+ which indicates the formation of Ag nanoparticles shown in Figure 2.

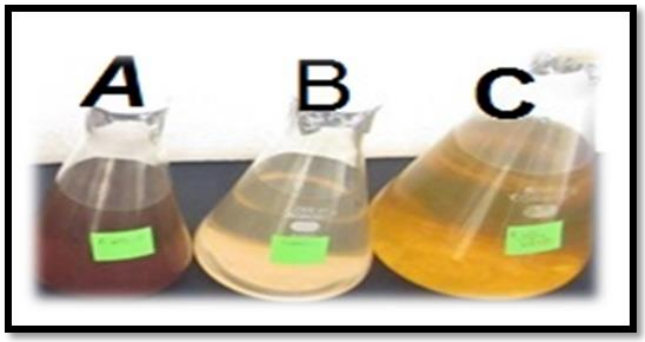


Fig 2. Synthesis of silver nanoparticles using *Diospyros chloroxylon* Roxb. ripened fruit pulp extract treating with AgNO3 solution at room temperature.
A) Formation of AgNPs, B) silver nitrate ($AgNO_3$) solution, C) *Diospyros chloroxylon* Roxb aqueous ripened fruit pulp extract.

UV-VIS spectra Analysis

A visible colour change from transparent to light brownish colour 15 min indicates the formation of silver nanoparticles which was confirmed by UV-visible analysis. Further, the colour change to dark brownish colour is due to increased concentration as well as growth of silver nanoparticles. After 30 minutes, there was no significant colour change, which is evidence for the completion of reduction reaction. (Figure 2)

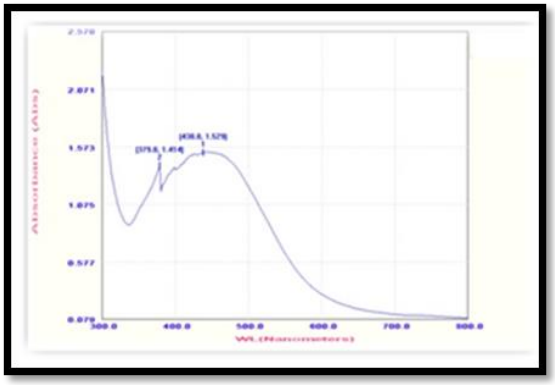


Fig 3. UV–visa spectrum of bio functionalized AgNPs showing surface plasmon peak at 440 nm.

UV–VIS spectroscopy analysis is an important technique to ascertain the formation and stability of metal nanoparticles in aqueous solution. Silver nanoparticles are known to exhibit a UV–Visible absorption maximum in the range of 400–500nm[24]. In this report the formation of AgNPs was initially confirmed using UV– Visible spectroscopy due to Surface Plasmon Resonance phenomenon-SPR[24]. The evidence of SPR was shown in Figure 3. One narrow absorption band was observed at 420-440nm which is a characteristic of mono dispersed AgNPs.

XRD-analysis

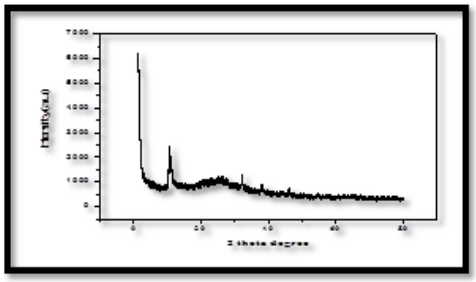


Fig 4. XRD patterns of bio functionalized AgNPs.

The XRD analysis of synthesized AgNPs using ripened fruit pulp of *Diospyros chloroxylon* was recorded and typical XRD pattern is shown in Fig.4. The peaks are indexed as (111), (220), (311) and plans of *Diospyros chloroxylon* silver by comparing[24] with JCPDS data. This may be due to the formation of crystalline bio-organic compounds/proteins that are present in the *Diospyros chloroxylon a* ripened fruit pulp broth. The detailed investigations on this crystalline phase existing with the silver nanocrystals are in progress.

TEM analysis

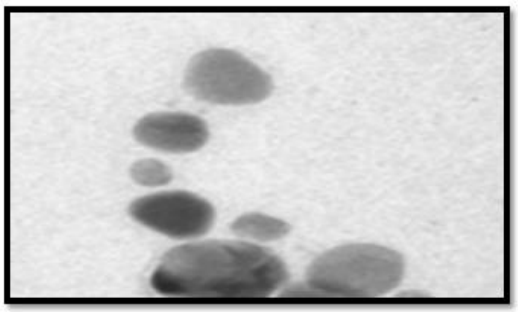


Fig 5 A. TEM image of bio functionalized AgNPs.

Transmission electron microscopic analysis carried out at IIT Mumbai revealed the particle size ranges from 18 to 54 nano-meters and shape from spherical to irregular. Of the total particles, 40% particles were of 18 nm, 20% were of 25-35 nm and remaining 40% particles were of 35 to 54 nm size.

FTIR analysis

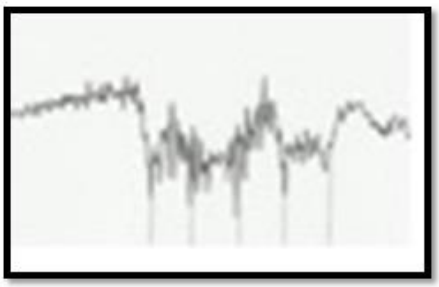


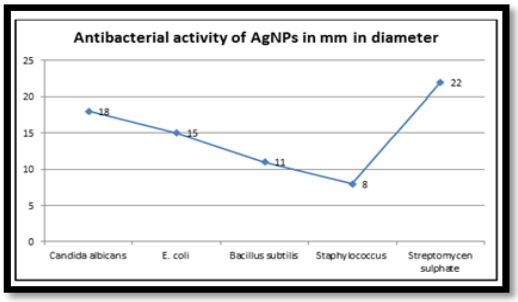
Fig 6. FTIR spectrum of bio functionalized AgNPs.

FTIR measurement was carried out to identify the possible bio molecules in *Diospyros chloroxylon* ripened fruit pulp extract responsible for capping leading to efficient stabilization of the AgNPs (fig.6). The IR spectrum of silver nanoparticles manifests prominent absorption band located 1616, 44 cm−1. The strong band at 1650.53 cm−1 may result from the N-H stretching vibration and can be assigned as absorption bands of C=H, -O-H, -S-H, -N=C=N, -C=O and -S=O stretching vibration. These are derived from water soluble compounds such as, flavonoids, alkaloids and polyphenols present in leaves. Biological components are known to interact with metal salts via these functional groups and mediate their reduction to nanoparticles.

Antibacterial activity of AgNPs of *Diospyros chloroxylon* ripened fruit pulp aqueous extract

The AgNPs of *Diospyros chloroxylon* ripened fruit pulp aqueous extract at 80 μl/well showed (graph 1) maximum antibacterial activity against *Candida albicans* (18.00 mm), followed *E. coli* (14.00mm), 11.00mm *Bacillus subtilis* and minimum of activity was observed against *Staphylococcus* 08.00 mm. Whatever antibacterial activity was observed in the study That was directly proportional to the concentration of AgNPs.

The streptomycin sulphate was used as standards (negative controls) against pathogens showed the inhibition zones of 22.00 mm to 23.00mm, respectively.



Graph 1. Antibacterial activity of AgNPs of *Diospyros chloroxylon* ripened fruit pulp aqueous extract in mm in diameter.

Conclusion

Diospyros chloroxylon ripened fruit pulp shown exceptional natural ecofriendly resource for synthesis of silver nanoparticles and the resulted AgNPs are shown the potential antibacterial activity against pathogens.

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