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Biogenic Synthesis of Silver Nanoparticles using *Bauhinia Variegata* Bark Extract and its Antibacterial Efficacy

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Abstract: Biological entity is gaining significant importance due to its large area of medicinal applications. A green approach has been carried out due to its eco-friendly nature, cost-effective and very less toxic effect. 80% of the world populations preferred plant based ayurvedic preparations since very long time due its fewer side effects. Silver nanoparticles have numerous applications in medicinal domain. Here, in the present study a green rapid biogenic synthesis of Silver Nanoparticles using *Bauhinia variegata* plant demonstrated. Extract is made up of Bark of the plant. In this synthesis silver is reduced by bark extract which act as reducing agent as well as capping agent. During the reactions color change shows formation of silver nanoparticles at preliminary conformation. UV study reveals the final conformation for the formation of silver nanoparticles by Intense Surface Plasmon Resonance (SPR) band at 452 nm. The properties of the prepared silver nanoparticles characterized by Fourier Transform Infra-red spectroscopy (FTIR), UV-visible spectrophotometer, Transmission Electron Microscopy (TEM), X-ray Diffraction (XRD) and Scanning Electron Microscopy (SEM). Synthesized silver nanoparticles exhibited very effective Antibacterial activity against pathogenic bacteria.

Keywords: nanoparticles, *Bauhinia variegata* plant bark, reducing agent.

1 Introduction

About 80% of the world population using traditional medicinal plants as a primer health care. Every plant possess specific characteristic according to that it is used for the several treatments. In recent years, noble metal nanoparticles have been the subject of focused research due to their unique optical, electronic, mechanical, magnetic and chemical properties that are significantly different from those of bulk materials [1]. Among them Silver nanoparticles have been well recognized For combating bacterial drug resistance problems, biogenic silver nanoparticles can acts as effective and alternative bacteriostatic agents [2].

Chemically synthesized silver nanoparticles involve toxic chemical residues which show adverse effect in biomedical applications. Therefore biogenic synthesis by plant extract introduce selectively as alternative method for the synthesis of silver nanoparticles. It is evidently found that plant act as reducing agent as well as stabilizing agent and capping agent too. The biological synthetic approach for silver nanoparticles is advantageous over physicochemical method because it is simple, cost effective, environmental-friendly, and easy to scale up for mass production [3]. Using several methods, like Solution combustion method for Synthesized La_2O_3 nanoparticles using Propylene glycol and Glutaric acid as fuel and chelating agent [4]. Biosynthesis of nanoparticles is a sort of bottom-up approach, where the main reaction occurring is reduction [5-8].The advantage of using plant materials in nanoparticles synthesis is it does not need any elaborate processes such as intracellular synthesis, compound purification steps and the maintenance of microbial cell cultures [9].

This present demonstration study involves bark of Bauhinia variegata using double distilled water as a solvent and 1 mM AgNO3 solution. Bauhinia variegata shows anti-inflammatory [10], chemo protective [11] and hepatoprotectivity [12]. The plant containing major chemical constituents were to be found such as Tannins, Saponin, flavones, flavonol glycoside, triterpene, phenanthraquinone. Bauhinia variegata exhibited excellent antibacterial activity against pathogenic bacteria.

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2 Materials and Methods

2.1 Materials

Healthy bark of Bauhinia variegata was collected from The botanical garden of Hemchandracharya North Gujarat University (HNGU), Patan, Gujarat. Silver nitrate ($AgNO_3$) from Sigma-Aldrich. Bacterial culture purchased from MTCC Chandigarh.

2.2 Preparation of plant bark extract

Fresh bark of the plant sterilized with flowing water followed by double distilled water several times. Sample is kept for drying to remove water then cut into small pieces. Then 10gm of bark material (small pieces) was boiled with 100ml double distilled water at 60-70ºC for 20-30 min and cooled. Obtained extract was filtered by Whatman filter paper No.1 and stored at 4ºC in freeze for further use for the synthesis.

2.3 Biogenic synthesis of Silver Nanoparticles

Take 10ml of plant bark extract and add 40 ml 1mM $AgNO_3$ solution, put this mixture on magnetic stir with hot plate at 60ºC with constant stirring for 30min. At the starting point of the reaction we could observe the color brownish to dark brownish at different time intervals and the finally to blackish brown color (after 3 hours). **(Figure 1.)**. Color change reveals preliminary conformation of formation of silver nanoparticles. Reduction of Ag+ to Ag- was due to biomolecules present in plant which act as a reducing agent as well as capping agent. Finally, the confirm formation of silver nanoparticles was characterized by UV-visible spectral analysis and it showed peak at 452nm which conform the presence of silver nanoparticles.

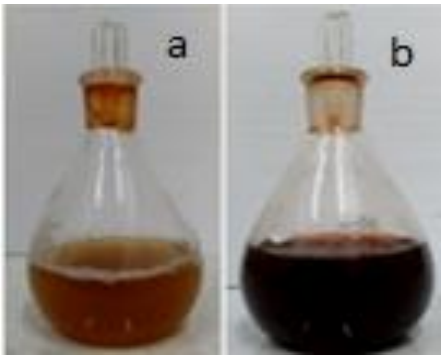

Figure. 1: (a) Plant extracts without $AgNO_3$ (b) plant extract + 1mM $AgNO_3$ solution

After color change, centrifugation was used to isolate silver nanoparticles from the reaction mixture. The mixture was centrifuged at 6000 rpm for 10-15 min. Pellets observed at the bottom of the centrifuge tube was purified two or three time with double distilled water then collected and dried at 60-70ºC in oven for 1-2 hour. Dry powder of silver nanoparticles kept for the antibacterial assay and instrumental analysis (FTIR, TEM, SEM and XRD).

2.4 Characterization

UV-visible spectra analysis (Shimadzu dual beam UV 1800) was performed at room temperature using wavelength of 800-200nm. FTIR analysis performed using Shimadzu (Range 400-4000cm-1) for the identification of the functional group present on surface of the silver nanoparticles. X-ray Diffraction (XRD) measurement was carried out using X-ray Diffractometer instrument. The average nanoparticles size calculated through the Debye Scherer's equation. SEM (Hitachi S-4500) analysis reveals morphology and average size of the silver nanoparticles. TEM analysis explains the size of the silver nanoparticles. The antibacterial activity of obtained silver nanoparticles was performed against *Bacillus subtilis (*MTCC 441), *Escherichia coli* (MTCC 1687), *Pseudomonas aeruginosa* (MTCC 1681) *and Stayphylococcus aureus* (MTCC 737)using Agar Well Diffusion method and Paper Strip method then zone of inhibition was measured successfully against all pathogenic bacteria.

3 Results and Discussion

3.1 UV-visible spectrophotometer

During experiment, Sample was subjected to UV-visible spectra for the spectrometric analysis to confirm the formation or presence of the silver nanoparticles. As like gold nanoparticles silver nanoparticles also known to exhibit a Plasmon absorption band in the visible region. Color change arises due to excitation of Surface Plasmon Resonance (SPR) of silver nanoparticles. The appearance of the blackish brown color was due to the excitation of the SPR, typical of AgNPs having λmax values in the visible range of 400-500 nm [13, 14]. SPR was observed at 452nm **(Figure 2.)** after completion of the reaction (blackish brown colored reaction mixture) which supports that plant extract posses strong reducing properties.

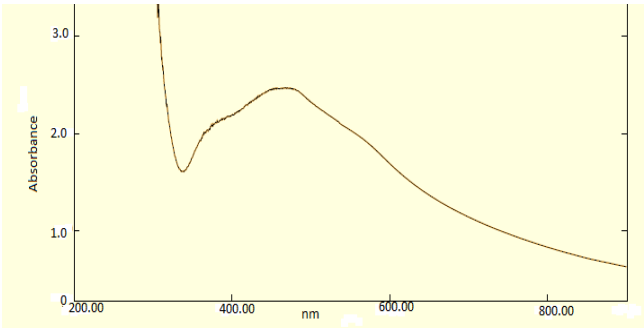

Figure. 2: UV-visible spectra of silver nanoparticles

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3.2 FTIR analysis - Fourier transforms infrared spectroscopy analysis

FT-IR analyses were performed using Shimadzu (Range 400-4000cm-1). FTIR measurements are carried out for the identification of biomolecules present on the surface of the synthesized silver nanoparticles **(Figure 3.)**. For the removal of free bio residues (free proteins, peptides etc.) or compound which is not capping agent of the nanoparticles, residual solution was centrifuged at 6,000 rpm for 15 min with sterile distilled water. Deposited pellets were isolated from bottom then dried it and finally analysed by FTIR spectroscopy. FTIR spectrum of AgNPs showed peak at 1022.27- 1480.11 cm^{-1}, 501.49 and 2372.41 represents corresponding amine (-NH-), Ag^{+} metal and hydroxyl group respectively.

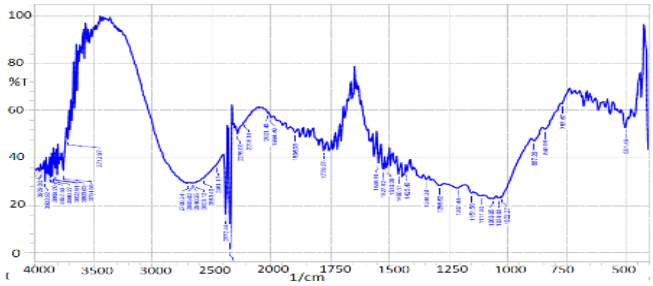

Figure. 3: FTIR of biologically synthesized silver nanoparticles

3.3 XRD – X-Ray Diffractometer

This measurement reveals the crystalline nature of silver nanoparticles. (**Figure 4)** Illustrate the XRD pattern of synthesized silver nanoparticles. The XRD spectra clearly indicate that the structure of the biologically synthesized silver nanoparticles are pure crystalline in nature. Spectra showed 3 peaks at 2θ values of 15.87, 28.16 and 39.68 correspond to the lattice plane are (101), (222) and (400) which indicate face centered cubic (FCC) silver nanoparticles. Other small peaks obtained due to crystallization of biomolecules present in bark extract of *Bauhinia variegata* which is much weaker than Ag indicate silver as a core material in conjunct. The average particle size calculated by Debye Scherrer formula ($D = 0.94k / \beta \cos\theta$, where, D= average crystalline size, *k*= X-ray wavelength, β= full width at half maximum and θ= diffraction angle) which shows the average size of the synthesized silver nanoparticles is 36 nm.

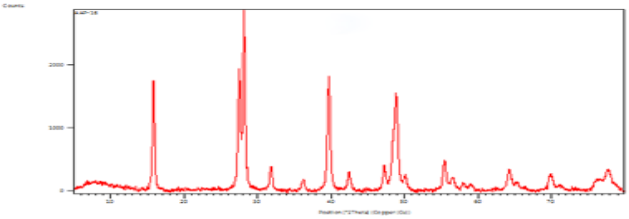

Figure. 4: XRD of biogenic silver nanoparticles

3.4 SEM - Scanning Electron Microscopy

The measurement by SEM (Hitachi S-4500 SEM) provides morphological features and average particle size details of the biogenic silver nanoparticles. SEM data revealed that the synthesized silver nanoparticles are spherical in shape and poly dispersed in solution. **(Figure 5.)**.

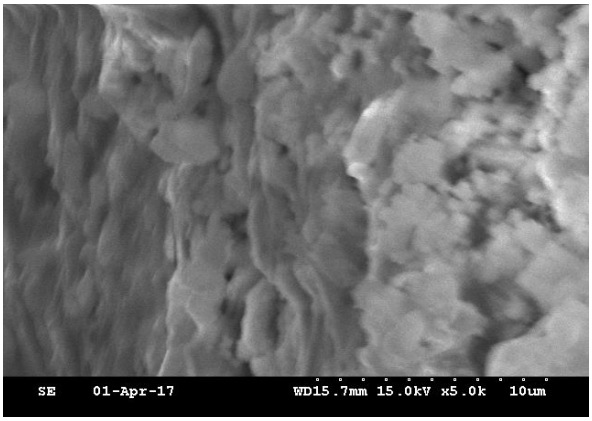

Figure. 5: SEM image of biogenic silver nanoparticles

3.5 TEM – Transmission Electron microscopy

A sample for Tem analysis prepared by putting drop of the reaction mixture on copper grid and solvent was allowed to evaporate. TEM analysis showed silver Nano-particles contain irregular spherical shape with 50 nm size (**Figure 6.).**

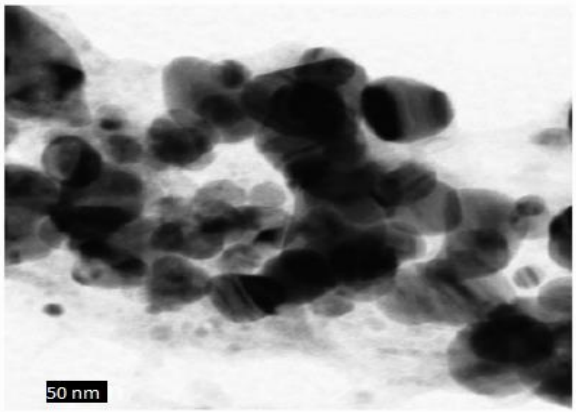

Figure. 6: TEM image of biogenic silver nanoparticles

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3.6 Antibacterial assay

The antibacterial screening of the synthesized silver nanoparticles performed by both agar well diffusion method and paper strip method against *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 1687), *Pseudomonas aeruginosa* (MTCC 1681) *and Stayphylococcus aureus* (MTCC 737). Same process applied for plant extract for comparison. Fresh overnight culture of each strain swabbed uniformly by cotton on plates containing sterile Luria Bertani agar and 4 wells (diameter size- 6 mm) were prepared using cup borer. 50 µL of sample nanoparticles pour into each well and commercial disc of gentamicin was used as positive control. Incubated it for 24 hr at 37°C, after that around the well diameter of inhibition zone was observed in millimeter **(Figure 7) (Table 1)**. Inhibition zone of bacterial growth is due to inhibitory compounds from the tested sample. We concluded that agar well diffusion method exhibited good antibacterial activity in which *Bacillus subtilis* showed excellent zone of inhibition and *Stayphylococcus aureus* showed weak results compare to all bacteria. Plant extract didn't give any results. Experiments with each strain performed three times for good results.

Table 1. Antibacterial activity of biogenic silver nanoparticles

Bacteria	Zone of Inhibition			Average zone of inhibition
	1	2	3	
B. subtilis	21 mm	22 mm	22 mm	22 mm
E. coli	12 mm	10 mm	12 mm	12 mm
P. aeruginosa	16 mm	14 mm	16 mm	16 mm
S. aureus	8 mm	8 mm	7 mm	8 mm

4 Conclusion

The biogenic synthesis of silver nanoparticles performed using bark material of the *Bauhinia variegata* plant without involving any toxic chemicals. In this reduction reaction metal ions were reduced (Ag^+ to Ag^o) very rapidly and reaction was finally completed within 3hours to produce silver nanoparticles. Different plants will take different time to complete the reaction due to different properties they have. The characterization of synthesized silver nanoparticles elucidated by microscopic and spectroscopic techniques which includes SEM, FTIR, TEM, XRD and UV-visible confirms the formation of silver nanoparticles. Synthesized silver nanoparticles showed high stability even after six months at ordinary room temperature. They exhibited an excellent antibacterial activity against mentioned all pathogenic bacteria. So it can concluded that biologically synthesized silver nanoparticles using *Bauhinia variegata* can be used as environment-friendly with low cost and excellent antibacterial activity.

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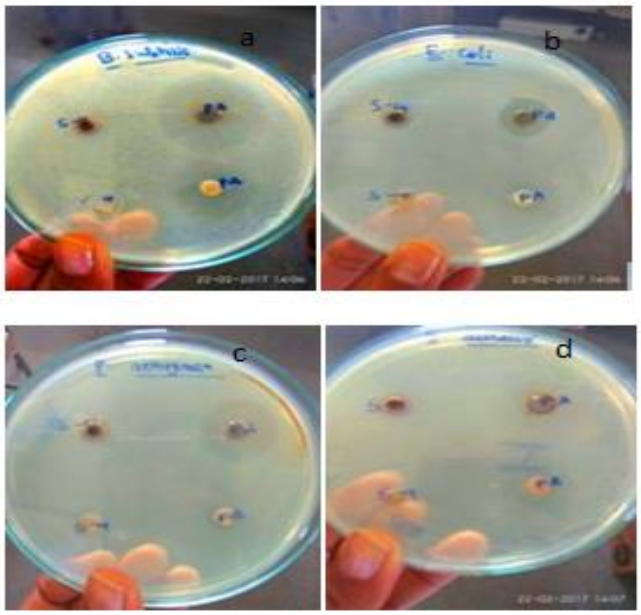

Figure. 7: Antibacterial activity of synthesized silver nanoparticles(a) *Bacillus subtilis* (b) *Escherichia coli* (c) *Pseudomonas aeruginosa* (d) *Stayphylococcus aureus* and S4=plant extract and PA=biogenic silver nanoparticles

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