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Eco-Friendly Approach for Synthesising Silver Nanoparticles (SNPs) from an Exceptional Medicinal Plant *Bombax Ceiba* Bark Extract and its Anti-Bacterial Activity

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Abstract : Various divergent eco friendly methods of green mediated synthesis of nanoparticles are the present research trend in the limb of nanotechnology arena. Nowadays biologically synthesized silver nanoparticles are being widely used in the field of medicine. In the current study, green synthesis of silver nanoparticles (SNPs) was carried out by using a traditional medicinal plant, *Bombax ceiba* bark extract for the reduction of aqueous silver ions in a very short span period of time. The SNPs formation was physically visualised by colour change of the extract which was further confirmed by several techniques like UV-Vis spectroscopy, FTIR, and X-ray diffraction studies. In our study, green synthesized SNPs have showed a potential antimicrobial effects against infectious organisms such as *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumoniae* and *Staphylococcus aureus.* Based on the above results, it was inferred that the above plant extract can be efficiently used in the production of silver nanoparticles (SNPs) which could be employed in various fields of biomedical application.
Key words : Antimicrobial activity, medicinal plants,silver, nanoparticles, *Bombax ceiba.*

Introduction

Nano biotechnology is presently one of the most dynamic disciplines of research in contemporary material science wherein plants and different plant products are finding an imperative use in the synthesis of nanoparticles (NPs). In general, particles with a size less than 100 nm are referred to as NPs. Nanotechnology is the application of science to control matter at the molecular level[1]. Entirely novel and enhanced characteristics such as size; distribution and morphology have been revealed by these particles in comparison to the larger particles of the mass material that they have been prepared.

In recent years, green synthesis of metal nanoparticles is an interesting subject of nanoscience and nanobiotechnology. However, there is a growing attention for biosynthesis of nanoparticles using metal accumulating organisms. Among these biological entities, plant extracts are supremely suitable forlarge-scale biosynthesis of nanoparticles. Nanoparticles producedby plant extracts are much more stable and the rate of synthesis ismuch faster compared to microbe mediated synthesis.Moreover, thenanoparticles aremore various in shape and size in comparisonwith those produced by other organisms[2,3].Silver nanoparticles have drawn the attention of researchersbecause of their suitable applications in the fields ofelectronic, material science, and medicine[4,5].For instance, antimicrobial properties of SNPs have commercially being utilized in different fields of medicine,variousindustries, animal husbandry, packaging, accessories,cosmetics, health and military[6]. Henceforth, Silver nanoparticles will showa potential antimicrobial effect against infectious organisms such as *Escherichia coli*, *Bacillus subtilis*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Syphilis typhus*, and *Staphylococcus aureus*[7].

Bombax ***ceiba*** is commonly known as “silk cotton tree” and “semal” which belongs to the family Bombacaceae. *B. ceiba* is an important medicinal plant of tropical and subtropical regions of India. Its medicinal usage has been reported in the traditional systems of medicine such as *Ayurveda*, *Siddha* and *Unani* and has wide range of medicinal and pharmacological applications. Thus, in the present investigation production of silver nanoparticles (SNPs)using *Bombax ceiba* bark extract was achieved along with its significant antimicrobial activity. Further, these phyto-synthesized SNPs were tested for*their in-vitro* antibacterial activity and they have exhibitedpotential zone of inhibition against pathogenic organisms.

Experimental

Collection of Materials

Plant Material *Bombax ceiba* bark specimens were collected from a local district Bidar, Karnataka state. The plant was identified and authenticated at the Department of Botany, Gulbarga University, Kalaburagi. The collected specimens were dried at room temperature, and then grounded by using a conventional grinder which was stored at 4^{o}C for further utilization.Precursor $AgNO_3$ (Purity 99.98%) was used to synthesise silver nanoparticles was procured fromHIMEDIA Company, Bangalore. Further, all reagents purchased in this experimentation were of analytical grade and were used as and when required. All solutions were freshly prepared using double distilled water and kept in the dark to avoid any photochemical reactions. Glassware’s used in the above experimental procedures were cleaned in a fresh solution of HNO_3/HCl (3:1, v/v), washed thoroughly with double distilled water, and dried before use.

In the present Investigation,*Bombax ceiba* bark extract was used as a reducing agent for the development of silver nanoparticles (SNPs). 25g of bark powder was added containing 250mL deionised water in 500mL Erlenmeyer flask which was boiled further for around15min. Whatman filter paper No.1 was used for the filtration of boiled plant materials to prepare the aqueous bark extract, which was proceeded further for metal SNP synthesis.

Synthesis of silver nanoparticles

1ml of $AgNO_3$ (100mM) was added to the 10ml of plant extract sparingly and finally made the solution to 50 ml. This sample was additionally incubated in dark for 24 hours. After 24 hours,thephysical colour change of the above solution indicated the formation of silver nanoparticles (SNPs). This sample was further measured for its SPR (Surface plasma resonance) absorbance band using UV-Visible spectrophotometry.

Characterization of Silver nanoparticles

UV-Vis spectral analysis

The reduction in silver ions was monitored by measuring the UV-Vis spectroscopy of the reaction medium by diluting small aliquots of the reaction mixture. These dilutions were ten times diluted with Milli-Q water to avoid the error due to high optical density of reaction mixture at different intervals after 15 min. UV-Vis spectral analysis was done by using UV-Vis-NIR spectrophotometer (Ocean Optics).

Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) is an analytical technique which provides information on the vibrational and rotational modes of motion, of a molecule and has been classified as an important technique for identification and characterisation of nanoparticles reported earlier. Consequently, in this study, our phytosynthesised SNPs were analyzed under FT-IR investigation for the presence and conformation of different volatile metabolites along with capping substances during synthesis procedure.

X-Ray Diffractometry.

XRD measurements were recorded on Bruker D8 Advance, Germany. ForXRD measurements, the SN0Ps samples were dried in an oven at 60^{o}C, and this dried powder was further analyzed on XRD instrument for their phase structure and exact material identification, operating at 40 kV and a current of 30 mA at a scan rate of 0.388 min^{-1}.

Anti-bacterial activity

Anti-bacterial activity of our synthesised SNPs was determined by well diffusion method[8]. The zone of inhibition was evaluated against the pathogenic organisms like, *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Staphylococcus aureus.*The Bacterial cultures were procured from Department of Biotechnology, Gulbarga University, Kalaburgi. Initially, bacterial cultures were maintained on nutrient agar slants at 37^{0}C and were sub-cultured at regular intervals for further studies.The plates were then incubated for 24hours at 37°C.

Results

UV–visible spectroscopy analysis

The prepared SNPs were characterized by UV–visible spectroscopy. UV–visible spectroscopy is one of the most widely used and valuable technique for the observation of SNPs synthesis. The synthesized SNPs have displayed the characteristic surface plasmon resonance (SPR) band in the spectral range of 420nm after 24 and 48 hrs. The samples displayed an optical absorption band peak at 420 nm (Figure 1), which is a typical absorption for metallic Ag nanoclusters, due to the Surface Plasmon Resonance (SPR). Effect of the reaction time on SNPs synthesis was also evaluated with UV-Visible spectra and it was noted that with an increase in time the peak becomes sharper. The increase in intensity could be due to increasing number of nanoparticles formed as a result of reduction of silver ions presented in the aqueous solution. The weak absorption peak at 200 nm indicates the presence of several organic compounds which are known to interact with silver ions into solution and suggests a possible mechanism for the reduction of the metal ions presented in the solution.

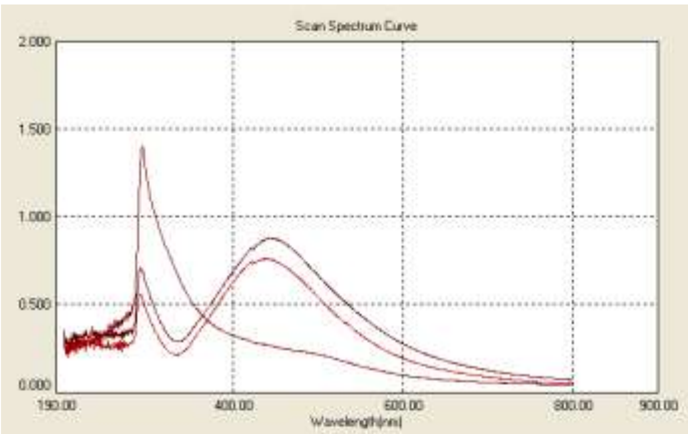

Fig 1: UV-Visible spectrum of *Bombax ceiba* bark extract observed at 420nm after 24 and 48 hrs

XRD analysis of SNPs

The XRD technique was used to determine and ascertain the crystal structure of eco-friendly synthesized stable SNPs. In the present study the Figure2clearly shows four well-defined characteristic peaks at scattering angles (2h) of 38.19, 45.27, 64.26, 77.28 corresponding to the (111), (200), (220), and (311) sets of lattice planes which may be indexed as the band for face-centered cubic (fcc) crystal structure of SNPs. The peak corresponding to the (111) is more intense than the other planes. The broadening of these peaks are mostly due to the effect of nano-sized particles. The width of the (111) peak was employed to calculate the average nanoparticles size using the Scherrer formula with an applied geometric factor of 0.97. It was found that the calculated average particle size was about 8.04 nm. Two small insignificant impurity peaks were observed at 35° and 85° which are attributed to the presence of other organic sub- stances in culture supernatant. The X-ray diffraction peaks were found to be broad around their bases indicating that the silver particles are in nano sizes. The peak broadening at half maximum intensity of the X-ray diffraction lines is due to a reduction in crystallite size, flattening and micro-strains within the diffracting domains.

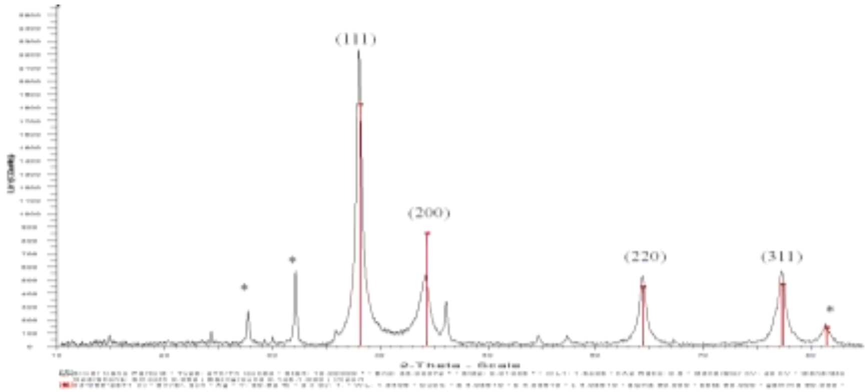

Fig 2: X-ray diffraction patterns of SNPs of *Bombax ceiba* bark extract

FTIR analysis of SNPs

Fourier transform infrared spectra of nanoparticles were recorded to provide an evidence for the interaction of functional groups involved in the reduction of Ag NO3 and the capping of subsequently formed SNPs. The FTIR spectra of SNPs are shown in Fig.3. The FTIR spectra has exhibited a characteristic stretching frequencies at 3665,3259,1754,1602,1475,1374 it suggest the bonds O-H stretch free hydroxyl, O-H stretch, C=O stretch, C-C stretch, C-H bend, C-H rock with functional groups alcohols, phenols carboxylic acid, ester, saturated aliphatic, aromatics, alkanes respectively. Therefore, the synthesized nanoparticles were surrounded by proteins and phenols having functional groups. From the analysis of FTIR studies it can be confirmed that the carbonyl groups 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.

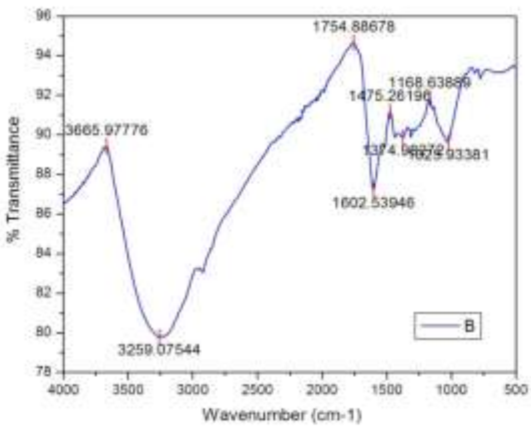

Fig3: FTIR analysis SNPs of *Bombax ceiba* bark extract

Toxicity studies on pathogen will opens a door for nanotechnology applications in medicine. Biological synthesis of metal NPs is a traditional method and the use of plant extracts has a new awareness for the control of disease, besides being safe and no phytotoxic effects[7]. The biologically synthesized silver nanoparticles using medicinal plants were found to be highly toxic against different pathogenic bacteria species. The SNPs of *bombax ceiba* bark extract have shown highest antibacterial activity as observed against *Staphylococcus aureus* followed by *E. coli* and *salmonella typhi, Klebsiella pnemoniya* species shown in Table 1.

Table-1: Antimicrobial activity SNPs of *Bombax ceiba* bark extract

S. No.	Bacterial species	Inhibition zone (mm)		
		Control	SNPs	Ag(NO3)
1	*Escherichia coli*	7	9	10
2	*Salmonella typhi*	9	7	10
3	*Klebsiella pneumoniae*	6	11	12
4	*Staphylococcu saureus*	8	10	14

Discussion

The present study reports the green synthesis of SNPs using *bombax ceiba* bark extract, as its reducing agent. While there have been numerous methods on synthesizing nanoparticles, most of the methods use expensive chemicals and therefore are not cost effective. Moreover, the residues produced are hazardous and toxic. This will result in pollution which could lead to disastrous effects on our environment. Recently the usage of flowery and plant extracts has become an interest due to its clean and simpler approaches. The XRD peaks revealed that the structure corresponds to face-centered cubic crystal of Ag. In this study, pure Ag crystallite was obtained, while some studies reported the presence of peaks corresponding to unassigned peaks[10], weak peaks[11], oxide of Ag[12], or incomplete peaks[13]. unassigned peaks were probably observed due to the fact that the crystallization of bioorganic phase occurs on the surface of the nanoparticles[14], which in our case does not occur. The absorption peak in UV-vis test in the range of 420–450 nm confirmed the formation of Ag. The sizes of the AgNPs are in the range of other reports[15,16].

SNPs are most notably known for their antibacterial properties. The widespread cases of multidrug resistant bacteria against the standard antibiotics have led researches to potentially incorporate SNPs and other nonmaterial's as ingredient to boost the antibiotic effects. There have been several proposed mechanisms on how SNPs work as antibacterial although the exact mechanism is still unknown. Several reports suggested that the SNPs could produce Ag ions which will damage the cell membrane, interrupt the metabolic activity, and subsequently lead to denaturation of protein and finally cell death[17,18,19,20].

Conclusion

The present study included the bio-reduction of silver ions through medicinal plants extracts and testing for their antimicrobial activity. The aqueous silver ions exposed to the extracts, the synthesis of silver nanoparticles were confirmed by the change of colour of plant extracts. These environmentally benign silver nanoparticles were further confirmed by using UV-Vis spectroscopy. The results indicated that silver nanoparticles have a good antimicrobial activityagainst different microorganisms. The plant material responsible for the reduction and stabilization of NPs needs further study including extraction and identification of the compounds present in the extract.

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