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# Biosynthesis of Silver Nanoparticles by Bamboo Leaves Extract and Their Antimicrobial Activity

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**Abstract**

The synthesis of nanoparticles from biological processes is evolving a new era of research interests in nanotechnology. Silver nanoparticles are usually synthesized by chemicals which are quite toxic and flammable in nature. This study deals with an environment friendly and biosynthesis process of antibacterial silver nanoparticles using bamboo leaves. The formation and characterisation of AgNPs were confirmed by UV-Vis spectroscopy, energy-dispersive spectroscopy (EDX), X-ray diffraction (XRD) and transmission electron microscope (TEM). The antimicrobial activities were carried out against *E. coli* and *S. aureus* strains by using disc diffusion method.

*Keywords*: Silver Nanoparticles; bamboo Leaves; Green Synthesis; Antibacterial

## 1 Introduction

Recently, metal nanoparticles have gained a lot of attention due to their unique chemical, optical, magnetic, mechanical, and electric magnetic properties. Thus metallic nanoparticles are used in different applications such as electronics, catalysis and photonic [1]. The silver metal has a great toxicity against a wide range of microorganisms, particularly; silver nanoparticle which has promising antimicrobial properties. Silver nanoparticles are found to be effective as anti-inflammatory, anti-angiogenesis, antiviral, anti-platelet activity and against cancer cells which makes them vital [2-7]. Accordingly, an environmental process for the synthesis of silver nanoparticles is important. Plant extract solutions and bio-organisms have been in spot light for their extreme ability to synthesis nanoparticles, including silver and gold nanoparticles.

Biosynthesis of silver nanoparticles has already been reported as clean, cost effective and non-toxic to environmental routes. Green synthesis offers improvement over synthetic, chemical or micro-organisms methods as it is cost effective, environmentally friendly and can easily be scaled up for large scale synthesis. The methods used for the synthesis of silver nanoparticles and toxic

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chemicals are used for the reduction process of substances such as citrates, $NaBH_4$, or ascorbates. Recently, green bio-reduction methods for the synthesis of silver nanoparticles were adapted by many researchers using plant extracts such as Macrotyloma uniflorum [8], Anacardium Mushroom extract [9], Coleus amboinicus lour [10], Medicago sativa [11], and Citrus sinensis peel [12] etc.

The main objectives of this study were to (i) to synthesize the silver nanoparticles using aqueous extract of bamboo leaves Phyllostachys aurea, (ii) to characterize the AgNPs by using UV–vis spectroscopy, (iii) to analyze antimicrobial properties against Gram-positive and Gram-negative bacteria.

# 2 Experimental

## 2.1 Materials

The bamboo leaves (Phyllostachys aurea) were collected from their trees available in the Xiasha campus of Zhejiang Sci-Tech University, Hangzhou, China. Silver nitrate ($AgNO_3$) was purchase from Strem Chemicals, Inc. (USA). Throughout the experiments distilled water was used.

## 2.2 Preparation of Bamboo Leaves Extract

To prepare the bamboo leaves extracts; 20 g of bamboo leaves were washed thoroughly with distilled water and dried for 24hrs at room temperature. The extract solution was prepared by boiling dried leaves in Erlenmeyer flash with 100 ml of distilled water for ten minutes at 100°C. Freshly prepared aqueous extract was used for synthesis. For this study no old extracts were used at any stage.

## 2.3 Synthesis of Silver Nanoparticles

In the experiment, 5 ml of fresh leaves extract was added to a conical flask containing 5 ml of 3 mM aqueous $AgNO_3$ solution heated at 65°C with continuous stirring. The silver ions were reduced to silver nanoparticles within few minutes by bamboo leaves extract. The quick conversion of solution color showed the formation of silver nanoparticles by observing color change from colorless to yellowish-brown color.

## 2.4 Antibacterial Assay

Silver nanoparticles biosynthesized from bamboo leaves extract were tested for antimicrobial activity by Kirby–Bauer method against pathogenic bacteria Escherichia coli (Gram-negative) and Staphylococcus aureus (Gram-positive). The pure bacteria cultures were sub cultured on nutrient agar media. Both strains were swabbed evenly onto the single plates using sterile glass rods. After incubation at 37°C for 24 hours, the levels of zone diameter inhibition of bacteria were measured.

# 3 Results and Discussion

Today nanomaterials are at the primary stage of fast developing nanotechnology phase. Nanomaterials are facilitating modern technology to deal with nano-sized objects, their unique properties especially size-dependent one makes them superior materials and essential in different human activities. Currently, nanomaterials are already being used in medical applications such as drug carriers, strong antibacterial, detection for pathogens/proteins and tissue engineering etc. There is some new development towards controlling the properties of nanomaterials, e.g. new method has been reported in which magnetic nanoparticles are driven to the tumour for drug release or just heating in order to destroy the surrounding tissues [13]. In wound healing management, a new therapeutic response has been developed in which drug is released accordingly to the type of wound, open or closed, large or small and drug is released at specific rates [14]. In tissue engineering, a wool keratin/Ha nano-composite has been reported in which cells showed improved feasibility ratio of organics and inorganics similar with those of natural bones [15]. For antibacterial properties different applications has been reported such as silver nanoparticles loaded surgical masks and surface modified cotton fibers by nano titanium dioxide with great antibacterial properties etc [16, 17].

## 3.1 Synthesis of Silver Nanoparticles from Bamboo Leaves

UV-Vis, EDX and TEM Studies confirmed the bioreduction of aqueous silver ions to silver nanoparticles which can easily be followed by UV-Vis spectrophotometer at first. UV-vis absorption spectrum was observed by Lambda 900 UV-vis spectrophotometer (Perkin Elmer, USA). The most important feature of optical absorbance spectra is surface Plasmon band among metal nanoparticles, which is due to the electron oscillations that collectively gather around the surface of metal particles. Bioreduction of silver ions to AgNPs mediated by bamboo leave extracts was observed by recording the absorption spectra. The yellowish brown color of silver nanoparticles became visible due to the excitation in surface Plasmon vibrations by absorbance in between 420 and 450 nm (Fig. 1).

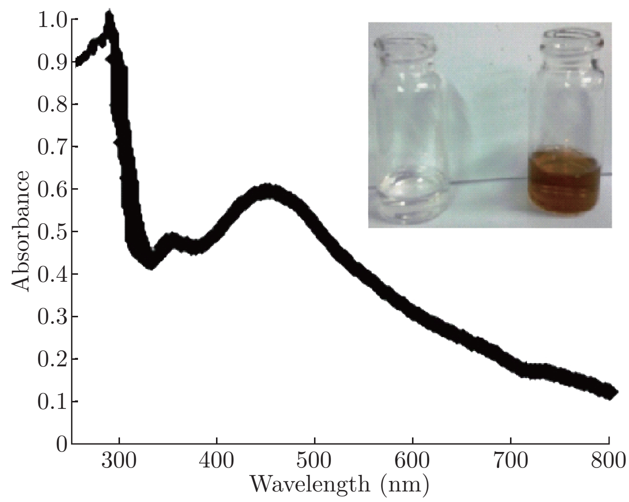



Fig. 1: Ultraviolet-visible spectra recorded as a purpose of reaction time of AgNO3 solution with bamboo leaves extract

The bamboo leaves (P. aurea) used in this investigation is a species of Phyllostachys genus native to China, commonly known as golden bamboo. In Chinese medicine, bamboos leaves are used for protecting the blood vessels of heart and brain, improving immune system, adjusting blood lipid and lower the blood viscidity. Particularly, bamboo leaves are also prescribed to be anti-viral, anti-bacterial and deodorization in nature. bamboo leaves are known to be rich in various phytochemicals like flavonoids, phenolic acids and lactones. Due to these diverse constituents of bamboo leaves bioreduction of silver nanoparticles became significantly possible (Fig. 2) [18]. bamboo leaves are also a common source of derivative compounds of phenolic acids, coumaric lactones and flavonoids like; homoorientin, orientin, isovitexin, vitexin, naringin-7-rhamnoglucoside, rutin, quercetin, luteolin, tricin, caffeic acid, chlorogenic acid and phydroxy coumaric acid [19, 20]. Flavonoids play an important role in the reduction process for biosynthesis AgNPs [21]. Accordingly, the high content source of flavonoids and phenolic acids in bamboo leaves extract (BLE) supports the potential bioreduction of $Ag^+$ to $Ag^o$.

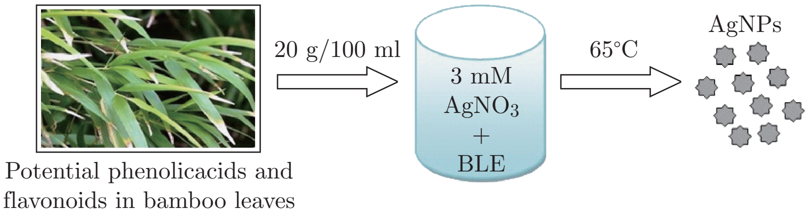



Fig. 2: Schematic illustration of synthesis mechanism of AgNPs from bamboo leaves extract (BLE) and 3mmol/L silver nitrate at 65°C

## 3.2 Characterization of Silver Nanoparticles

Fig. 3 shows the EDX spectra of AgNPs synthesized at 65°C. Strong signals from the silver atoms in the nanoparticles were observed. The presence of the elemental silver can be observed in the graph obtained from EDX analysis, which also supports the XRD results. This indicates the reduction of silver ions into elements of silver.

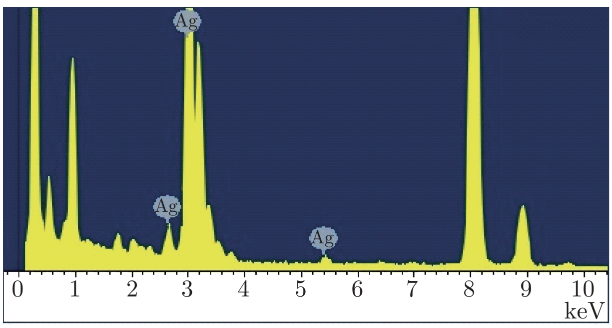



Fig. 3: EDX spectra of AgNPs

To investigate the crystalline nature and structure of AgNPs, the sample was analyzed on a ARL X'TRA X-ray powder diffractometer (XRD) (Thermo Electron Corp., USA) using a diffractometer equipped for $CuK_\alpha$ radiation ($\lambda = 1.5418$Å) in the $2\theta$ range of 0-85° with a step size of 0.04° and a scanning rate of 5.0°/min. The comparison of XRD spectrum results with standards confirmed the crystalline nature of silver nanoparticles formed peaks at $2\theta$ values corresponding to 111, 200, 220 and 311 of Bragg reflections for silver metal (Fig. 4). The crystallite size of AgNPs can be calculated by using Debye-Scherer's formula (1). The average size of synthesised AgNPs was found to be 13 ± 3.5 nm from XRD data and using Debye-Scherer equation.

$$D = K\lambda/\beta \cos\theta \tag{1}$$

Whereas, D is the crystal size of AgNPs, $\lambda$ is the wavelength of x-ray source used (1.541 Å), $\beta$ is the full width at half maximum of the diffraction peak, K is the constant of Debye-Scherer equation with value from 0.9 to 1 and $\theta$ is the Bragg angle [22]. The structural peaks present in XRD pattern and crystalline size suggests that the biosynthesised AgNPs by bamboo leaves are crystalline in nature.

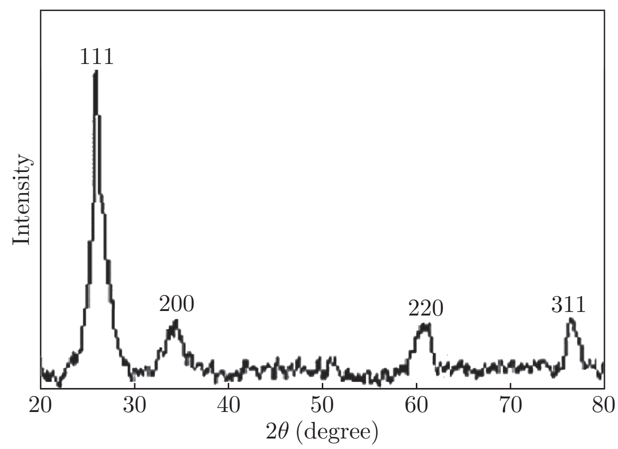



Fig. 4: X-ray diffraction pattern of biosynthesised AgNPs bamboo leaves extract

The silver nanoparticles samples were dispersed in distilled water under ultrasonic treatment, and then dropped onto the carbon-coated copper grids for the observation of TEM (JEM-1230, JEOL) at 80 KV. The TEM images of AgNPs are shown in (Fig. 5). From the images it is evident that the morphology of AgNPs are nearly spherical and some non-spherical in nature having size less than 100 nm. It is known that spherical as well as non-spherical (triangle or hexagonal) nanoparticles exhibits better physical properties if they are produced small in size, as the antibacterial properties of silver nanoparticles are size dependent.

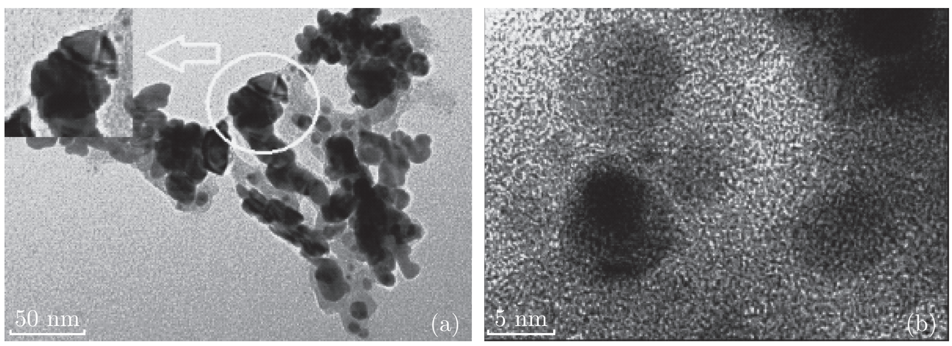



Fig. 5: TEM micrograph of the silver nanoparticles: (a) The scale bar correspond to 50 nm, (insert: formation of non-spherical silver nanoparticles), (b) The scale bar correspond to 5 nm

## 3.3 Antibacterial Activity

Silver is said to be a universal antimicrobial substance for centuries. Though, silver ions or salts have limited usefulness as an antimicrobial agent. Such as, the interfering effects of salts and antimicrobial mechanism of continuous release of enough concentration of Ag ions from the metal form. This kind of limitation can be overcome by using silver nanoparticles. However, to use silver against microorganisms, it is essential to prepare it with environmentally friendly and cost-effective methods. Besides, it is also important to enhance the antimicrobial effects of silver ions [23].

In this study, the antibacterial effects of the bioreduced silver nanoparticles from bamboo leaves were investigated against two bacterial strains, *S. aureus* / ATCC-25923 (gram-positive) and *E. coli* / ATCC-25922 (gram-negative) using the disc diffusion method. The agar petri dishes for antibacterial activity were prepared by boiling 12.5 gm of LB (Luria-Bertani) agar (10 gm of Tryptone, 5 gm of Yeast Extract and 10 gm of NaCl) in 100 ml of water till it melted down completely. The agar solution was inoculated for 30min. After inoculation, the bacterial strains (*E. coli* and *S. aureus*) were poured with different concentrations of AgNPs (20, 40, 60 and 80 $\mu$g/mL) in agar plates and spread evenly. The prepared agar plates with bacterial strains and AgNPs were then kept at 37°C for 24 hours.

For the antimicrobial evaluation of synthesized AgNPs, the MIC and MBC method was used. In this study, the lowest concentration of AgNPs 20 $\mu$g/mL shows measureable cell growth on agar plates counting $1 \times 10^8$ CFU/mL (Fig. 6). The cell growth gradually decreased to none as the concentrations increased, ranging from $1 \times 10^7$ to $1 \times 10^3$ CFU/mL representing bactericidal properties of AgNPs. In MIC/MBC study, the lowest concentration of AgNPs 20 $\mu$g/mL was found to be effective in killing the bacteria up to $1 \times 10^7$ CFU/mL concentrations, as there was no cell growth seen on plating after incubation at 37°C for 24 hours. The analysis of MIC/MBC study states that these ratios are responsible for inclusive killing of bacteria up to mentioned concentrations. Fig. 7 shows the inhibition of two challenge bacterias by AgNPs extracted from bamboo leave extracts. It was evident that around the paper soaked in AgNPs with different concentrations has a significant inhibition zone against *E. coli* and *S. aureus*.

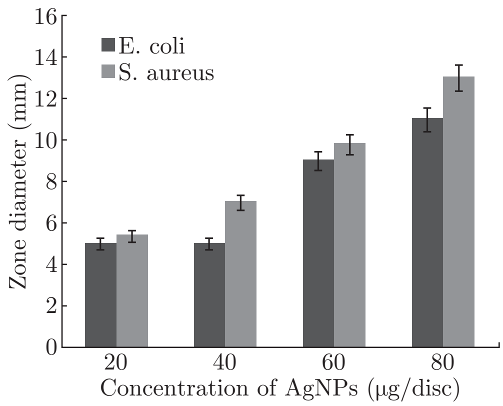
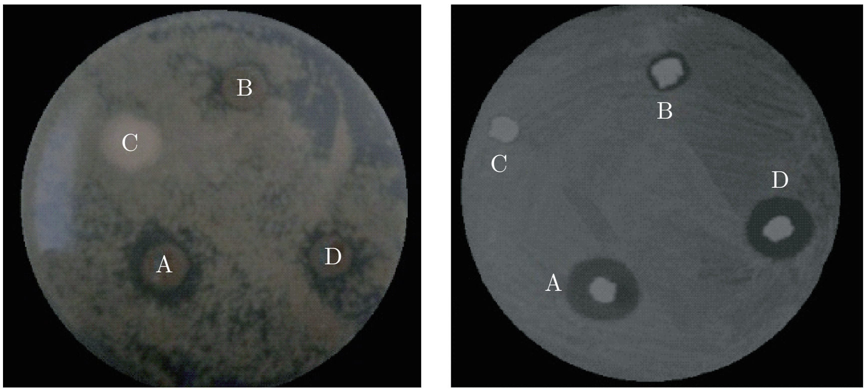



Fig. 6: Bar chart histogram of antibacterial properties against *E. coli* and *S. aureus* using silver nanoparticles




(a) (b)

Fig. 7: Bacterial growth inhibition against bacterial strains (a) *S. aureus* and (b) *E. coli*, by using (A) 80, (D) 60 (B) 20 $\mu$g/mL of silver nanoparticles and (C) taken as control sample

# 4 Conclusion

In this study, a simple approach was attempted to obtain a green eco-friendly way for the synthesis of silver nanoparticles using aqueous bamboo leaves extracts. The silver ions in an aqueous solution was exposed to the bamboo leaves extracts (BLE), the biosynthesis of AgNPs were confirmed by the rapid colour change of plant extracts. The natural benign AgNPs were confirmed further by using UV-Vis spectroscopy. Phenols and flavonoids were present in the leaves, and they serve as an effective reducing agent. AgNPs biosynthesized from bamboo leaves also exhibits great antimicrobial activities against sample bacteria cultures. These biosynthesis silver nanoparticles can potentially be used for different medical applications.

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