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BIOSYNTHESIS OF SILVER NANOPARTICLES BY USING EDIBLE BAMBOO SHOOTS WITH HIGH ANTIOXIDANT PROPERTIES

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ABSTRACT: Plant mediated synthesis of nanoparticles is a cost effective and environment friendly alternative to the conventional methods. In this study, the syntheses of nanoparticles were carried out from a silver nitrate solution by using extracts of four different species of bamboo shoot as reducing agents. The synthesized silver nanoparticles were characterized by various techniques such as SEM, XRD, EDX, FTIR and UV-Visible spectroscopy. The shoot extracts used in the syntheses have high antioxidant properties and can effectively reduce silver ions. The antioxidant activity was determined by estimating DPPH free radical scavenging activity. The shoot extracts also displayed antimicrobial activities, which were evaluated by agar well diffusion method. The synthesized nanoparticles were quite stable as indicated by the zeta potential values.

KEYWORDS: Silver nanoparticles, antioxidant property, antimicrobial property, bamboo shoot, zeta potential.

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1.INTRODUCTION

The synthesis of noble metal nanoparticles has been able to draw much attention in recent years as it has been pointed out that nanoparticles possess new and interesting characteristics different from those of macroscopic phase. The unique chemical, biological, optical, magnetic and electrical properties of nanoparticles have given rise to widespread applications in various fields such as pharmaceuticals, biotechnology, electronics, optics, catalysis, information storage and energy

conversion. Nanotechnology is the most rapidly growing science of present time which produces new generation materials by manipulating the known properties using nano-sized particles. Thus, nanoparticles are considered as fundamental building blocks of nanotechnology. Among the various metal nanoparticles synthesized and explored, silver nanoparticles are the most widely studied ones because of their "Surface Plasmon Resonance (SPR) which is a strong absorption of the visible light which is observable in UV visible spectra" and also due to their antioxidant and antimicrobial properties [1]. Silver nanoparticles are the particles of silver, with particle size between 1 and 100 nm in size. Like gold, silver nanoparticles have a long history and were initially used to stain glass. In some recent studies silver nanoparticles have shown promising antimicrobial properties [2],[3]. In addition, silver nanoparticles are found to possess anti-inflammatory, anti-angiogenesis, antiviral, anti-platelet activity and activity against cancer cells. "Currently silver nanoparticles are being incorporated in many medical devices" including bone cement, surgical instruments, surgical masks, etc. Moreover, it has also been shown "that right quantities of ionic silver is suitable in treating wounds [4],[5]. In fact, silver nanoparticles are proved to be more effective than silver sulphadiazine in the healing of wounds. Recently many modern techniques of synthesizing silver nanoparticles, such as chemical reduction of Ag^+ with or without stabilizing agents in *aqueous* solutions thermal decomposition in organic solvents, chemical reduction and photo-reduction in reverse micelles and radiation chemical reduction have been reported in the literature [6],[7],[8],[9]. Many of the existing methods for synthesis of nanoparticles involve the use of harmful chemicals, low material conversions and high-energy requirements. As a result, there has been a growing attempt to develop an environment friendly process for synthesis of nanoparticles without using toxic chemicals [10]. An environment friendly process for the synthesis of silver nanoparticles is therefore highly important. Researchers in the last few years have turned to biological systems for nanoparticle synthesis [11]. Synthesis of nanoparticles by biological methods, using microorganisms, enzyme and plant or plant extract, has been suggested as possible eco-friendly alternatives to chemical and physical methods [12]. Plant extracts and micro-organisms have been particularly used for synthesis of silver and gold nanoparticles [13]. Plant cell or plant extract mediated biosynthesis of nanoparticles does not require high energy or temperatures, can easily be scaled up for large-scale synthesis, and is cost effective[3]. Green synthesis of silver nanoparticles using various medicinal plants including, *Acacia leucophloea* [14], *Aegle marmelos* [15], *Alstonia scholaris*[16], Solanum trilobatum, Syzygium cumini, Centella asiatica and Citrus sinensis [10], *Crataegus douglasii*[17], *Ocimum sanctum (Tulsi*) leaf has been reported. Many studies have highlighted the fact that phytochemical constituents such as the flavonoids and polyphenols present in the plant extracts play a major role in the reduction of silver ions into metallic silver and subsequent capping to prevent agglomeration[13]. These phytochemicals are responsible for the antioxidant properties shown by many fruits and vegetables. Bamboo shoots are known to contain assortment of phytochemicals

such as phenols, flavonoids, terpenoids and vitamins possessing antioxidant activity. Different varieties of bamboo species available in North - east India and consumed by local tribes are believed to have potential health benefits such as reducing aging and prevention of cancer and heart diseases. Based on the findings we attempted to biosynthesize silver nanoparticles using aqueous extracts of bamboo shoots. The nanoparticles produced were characterized and their stability was also studied.

2. MATERIALS AND METHODS

Edible bamboo shoots of four different species namely *Bambusa balcooa, Bambusa bambos, Dendrocalamus hamiltonii* and *Bambusa vulgaris,* which are available in north-east India were selected for the synthesis of silver nanoparticles. All chemicals were purchased from Sigma-Aldrich, Himedia chemicals, SRL India, Merck India.

2.1 Preparation of extract

The aqueous extract was prepared by using soxhlet extraction method.25 g of fresh bamboo shoot were used for extraction using distilled water and the extraction was done for 3 days at 70°C. Finally, the water was evaporated from the extracts using lyophilizer and kept in refrigerator for further experiments.

2.2 Estimation of phytochemical constituents

Determination of Total Phenolics

Total phenolic content of the extracts was determined by using Folin-Ciocalteu (FC) reagent following the standard procedure. The absorbance was measured at 725 nm after 90 min. A calibration curve was prepared by using gallic acid solution as standard [18].

Determination of Total Flavonoids

The total flavonoid was determined by using aluminium chloride. To the extract 10 % aluminium chloride and 1 M potassium acetate were added. After that 80 % methanol was added to make the volume up to 5 mL. The absorbance was measured at 415 nm. A calibration curve was prepared by using quercetin as standard [19].

2.3 Biosynthesis of silver nano-particles

Aqueous solution (1mM) of silver nitrate ($AgNO_3$) was taken in 250 ml Erlenmeyer flasks and 5 ml of one type of the extract was added to each followed by 5mL of CTAB (0.20 M). The mixture was incubated at 37°C under 200 rpm for 48 hours. The solutions turned dark brown[10] indicating the formation of silver nanoparticles. The spectrophotometric analysis of the solution was carried out using UV–Visible spectrophotometer. The Ag NPs were collected by centrifugation at 18,000 rpm for 20 min. The collected pellet was washed with double distilled water and dried at room temperature. The morphological details of the synthesized silver nanoparticle were studied using scanning electron microscope.

2.4 Antimicrobial activity using agar well diffusion method

The agar well diffusion method was used to test the antimicrobial activity of the nanoparticles. The

micro-organisms such as *Staphylococcus aureus, Klebsiella pneumonia* were maintained at nutrient agar (NA) media for 24 hours. The nutrient agar media was prepared and poured in sterilized petriplates. The pathogenic microorganisms were spread over the plates and allowed to grow. The wells were loaded with 50 µL, 75 µL, 100 µL of the extract (obtained after biosynthesis of AgNPs) respectively. The plates were then kept in incubator for 24 hours at 37°C[10] .The zone of inhibition was measured in mm.

2.5 Antioxidant activity of aqueous extract of bamboo shoots

The antioxidant activity of the bamboo shoot extracts before and after biosynthesis of nanoparticles was measured by using modified DPPH method [20]. The scavenging activity of extracts was measured by spectrophotometer at 517 nm. 2 mL of extract was mixed with 150 µL of DPPH (0.05%) solution and kept the reaction mixtures in dark for 30 minutes. Ascorbic acid (10-100µg/mL) was taken as standard.

The antioxidant activity (%) was calculated using the following equation.

$$\text{Antioxidant activity (\%)} = \frac{\text{Absorbance of the control - Absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

2.6 Methods of Characterization

The initial characterization of synthesized silver nanoparticles was carried out by UV-Visible spectroscopy (Shimadzu UV-1800). The size and morphology of the samples were determined by SEM EDX using (Carl-Zeiss, Σigma -VP) instrument. The binding properties of silver nanoparticles synthesized by different extracts of bamboo shoots were investigated by FTIR analysis. FTIR spectrum (Nicolet 6700 FTIR) was recorded in mid region at 4000-400 cm-1for dry powder of synthesized nanoparticles. The zeta potential of the synthesized nanoparticles was recorded using zeta potential analyzer (Zeta sizer nanoseries. nano-ZS90). The mean particle diameter of silver nanoparticle was measured by X-ray diffractometer (D8 Advance, Bruker, AXS Germany). The dry powder of silver nanoparticle was used for XRD analysis. The diffracted intensities were recorded from 20◦ to 80◦ at 2θ angles.

3. RESULTS AND DISCUSSION

Results

3.1 Estimation of phytochemical constituents

The Total phenolic and total flavonoid contents in the four extracts are shown in Figure 1.

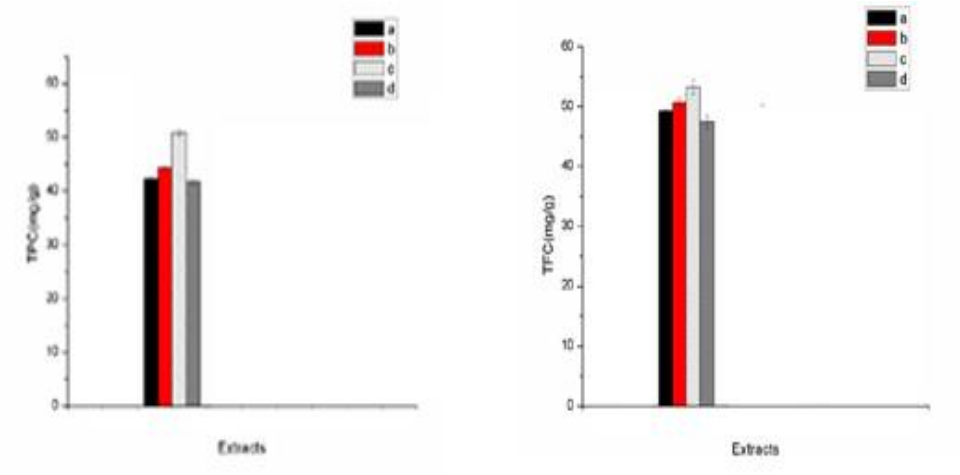

Figure 1: Total Phenolic (TPC) and Total Flavonoid (TFC) contents (mg/g) in aqueous extracts of the four bamboo species a. *B.balcooa*, b. *B.bambos*, c. *D.hamiltonii*, d. *B.vulgaris*.

3.2 Formation of Silver nanoparticles

Figure 2 depicted the visual observation of formation of silver nanoparticles using bamboo shoot extracts a. *B.balcooa*, b. *B.bambos*, c. *D.hamiltonii*, d. *B.vulgaris*, e. Control.

3.3 Characterization of silver nanoparticles

The formation of silver nano-particles was confirmed by recording the UV-Visible spectra (Figure 3) after 48 hours of interval measured at wavelength ranging from 200-700 nm. The presence of silver nanoparticles was established from the appearance of absorption peak at 430 nm, which is reported as a characteristic of silver nano-particles [21]

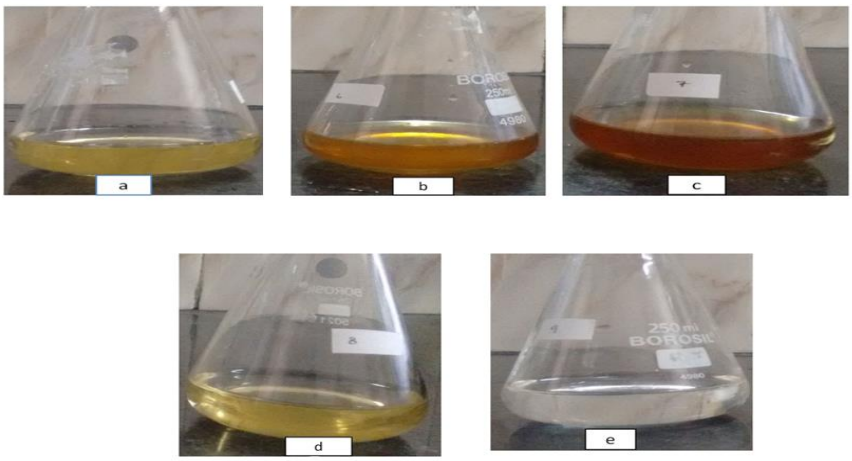

Figure 2: Figure showing colour change of silver nitrate solutions indicating formation of silver nanoparticles on treatment with bamboo shoot extracts a. *B.balcooa*, b. *B.bambos*, c. *D.hamiltonii*, d. *B.vulgaris*, e. Control (no extract).

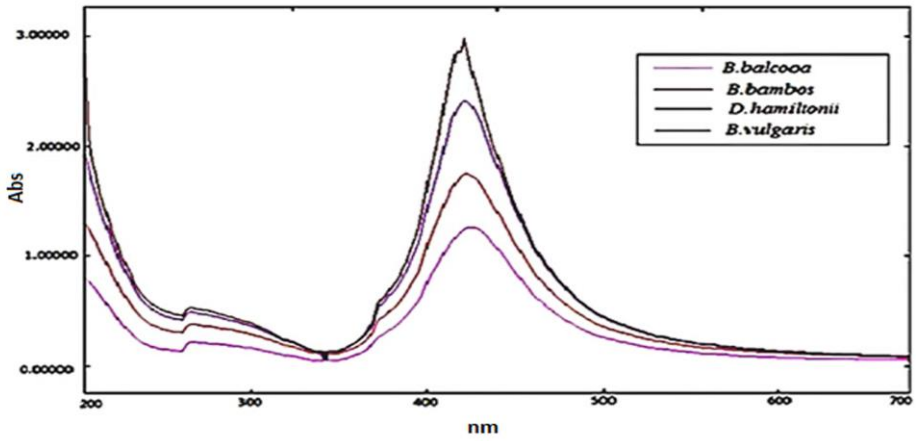

Figure 3: UV-VIS spectra of silver nanoparticles biosynthesized by aqueous extracts of a. *B.balcooa*, b. *B.bambos*, c. *D.hamiltonii*, d. *B.vulgaris*.

3.4 SEM EDX analysis of the synthesized nanoparticles

The SEM analysis was carried out to get the morphological details of silver nanoparticles. It revealed the formation of silver nanoparticles of spherical shape with sizes ranging between 10 nm to 30 nm (Figure 4). The EDX spectrum (Figure 5) of silver nanoparticles showed peaks around 2.12 keV corresponding to the binding energies of AgL.

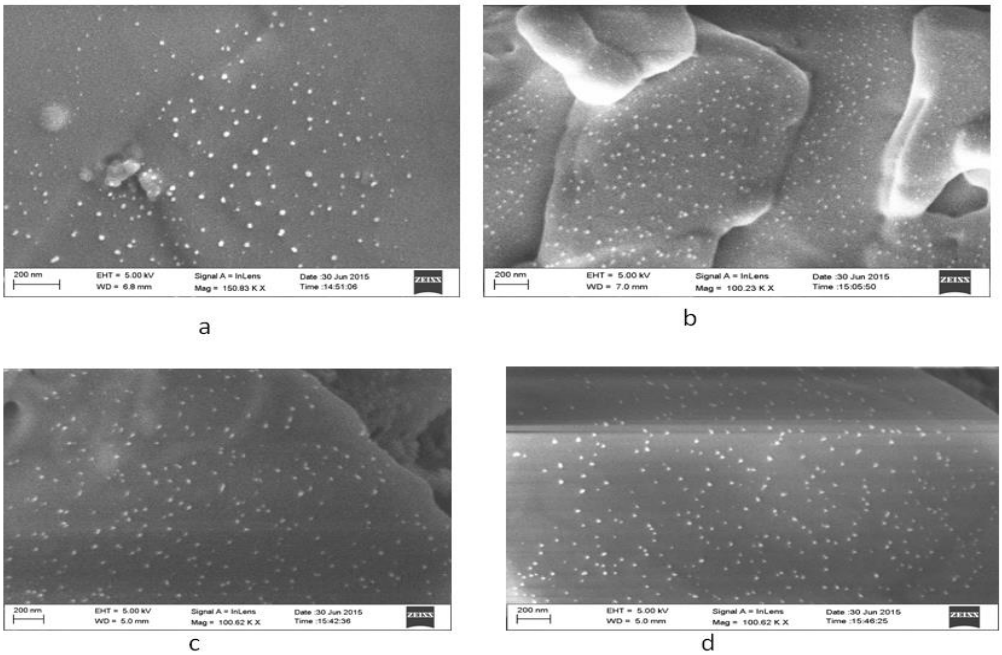

Figure 4: SEM images of silver nanoparticles synthesized using extracts a. *B.balcooa*, b. *B.bambos*, c. *D.hamiltonii*, d. *B.vulgaris*.

3.5 Zeta potential

In the present study, the stability of the colloidal silver nanoparticles was confirmed by analyzing the zeta potential values, which were recorded after 48 hours. The zeta potential values of the silver nanoparticles synthesized by *B. balcooa, B. bambos* and *D.hamiltonii* shoot were found to be 59.1

mV, 51.4 mV and 42.7 mV respectively indicating high stability. However, the silver nanoparticles synthesized by *B.vulgaris* extract had a relatively lower value of 17.7 mV (Figure 6).

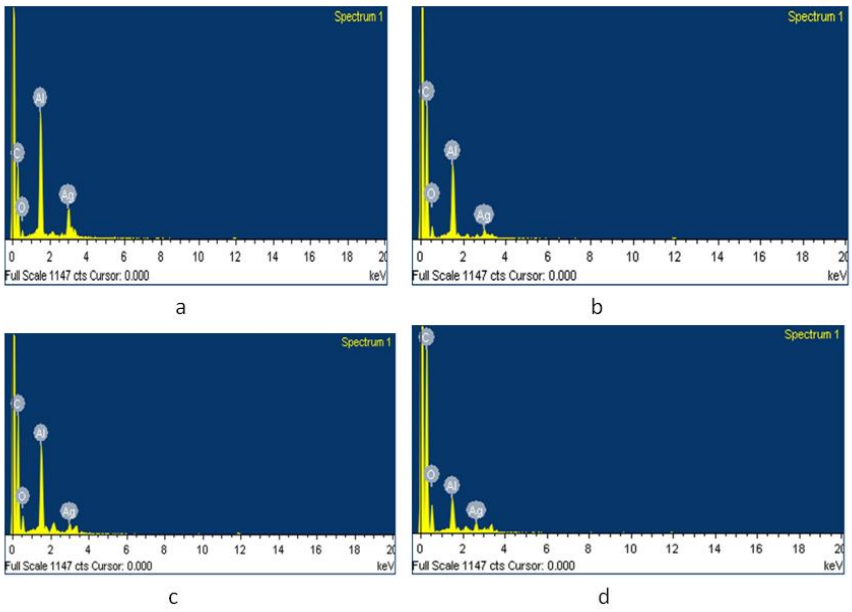

Figure 5: EDX spectra of silver nanoparticles synthesized using edible bamboo shoot extracts a. *B.balcooa*, b. *B.bambos*, c. *D.hamiltonii*, d. *B.vulgaris*.

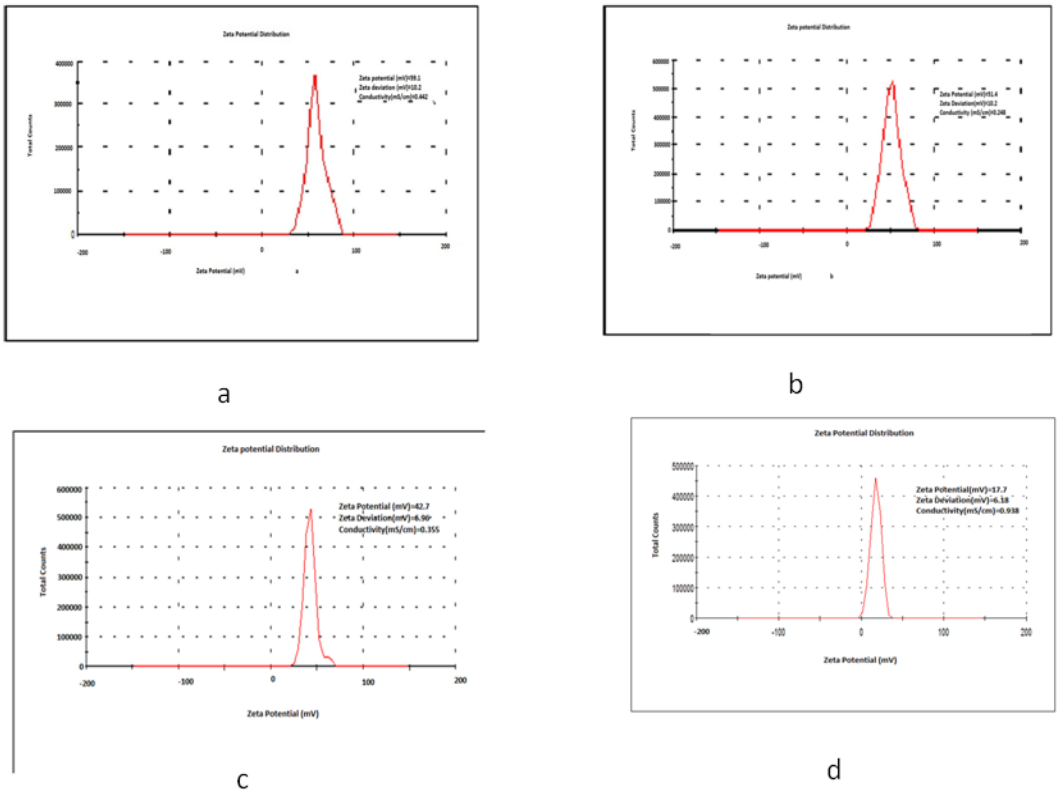

Figure 6: Zeta potential values of silver nanoparticles biosynthesized using extracts of a. *B.balcooa*, b. *B.bambos*, c. *D.hamiltonii*, d. *B.vulgaris*

3.6 XRD analysis

The powder XRD pattern of the prepared nanoparticles is shown in the Figure 7. The diffracted intensities were recorded from 20° to 50° at 2θ angles.

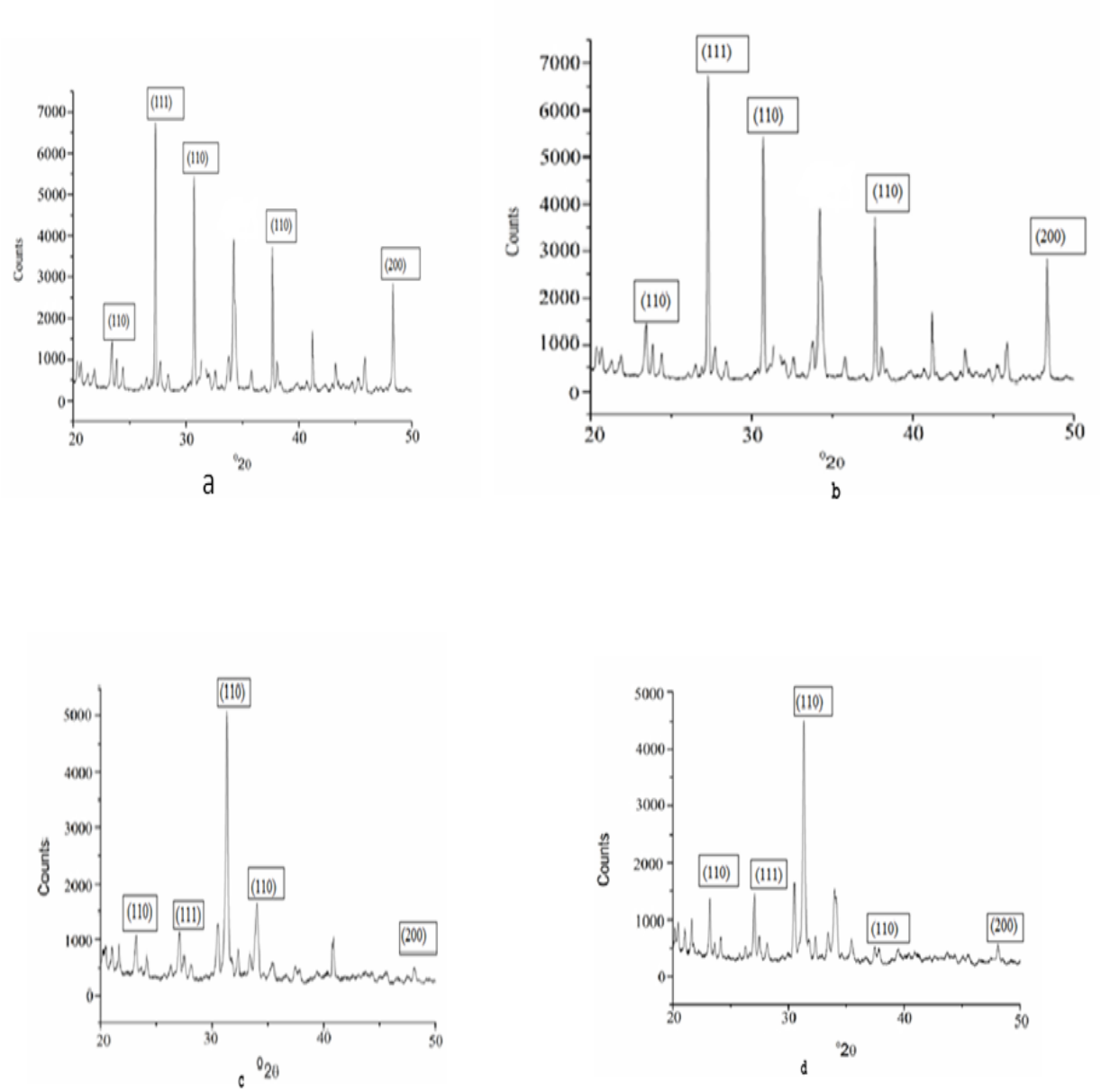

Figure 7: XRD spectra of silver nanoparticles synthesized using extracts a. *B.balcooa*, b. *B.bambos*, c. *D.hamiltonii*, d. *B.vulgaris.*

3.7 FTIR analysis

The silver nanoparticles were concentrated by repeated centrifugation of the reaction mixture at 15,000 rpm in order to remove any free biomass residue or compound which are not the capping ligands of the nanoparticles. The process was repeated for three times and the supernatant was replaced by distilled water each time. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by a Nicolet 6700 FTIR spectrophotometer. The important peaks obtained in the FTIR spectra are shown in Fig.8.

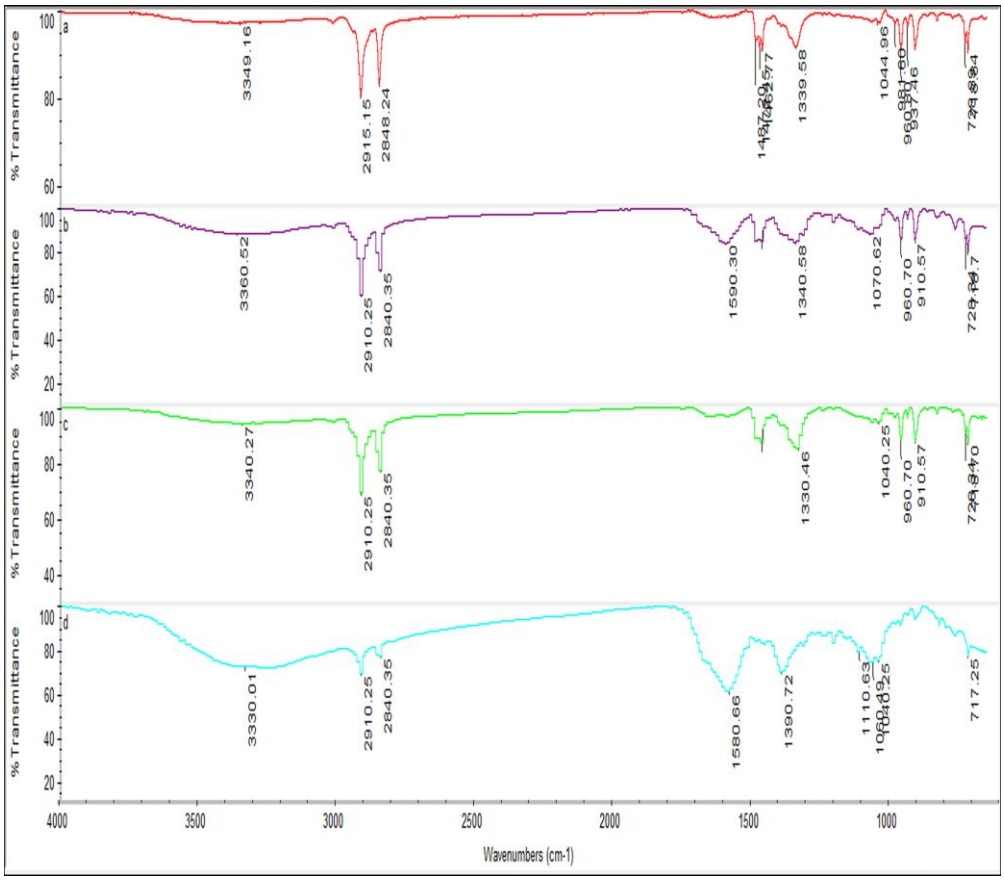

Figure 8: FT-IR spectra of silver nanoparticles synthesized by edible bamboo shoot extracts a. *B.balcooa*, b. *B.bambos*, c. *D.hamiltonii*, d. *B.vulgaris* (from bottom).

3.8 Antioxidant activity

The antioxidant activity of the extract and that of the extract with Ag NPs measured as % DPPH radical scavenging activity are shown in Figure 9.

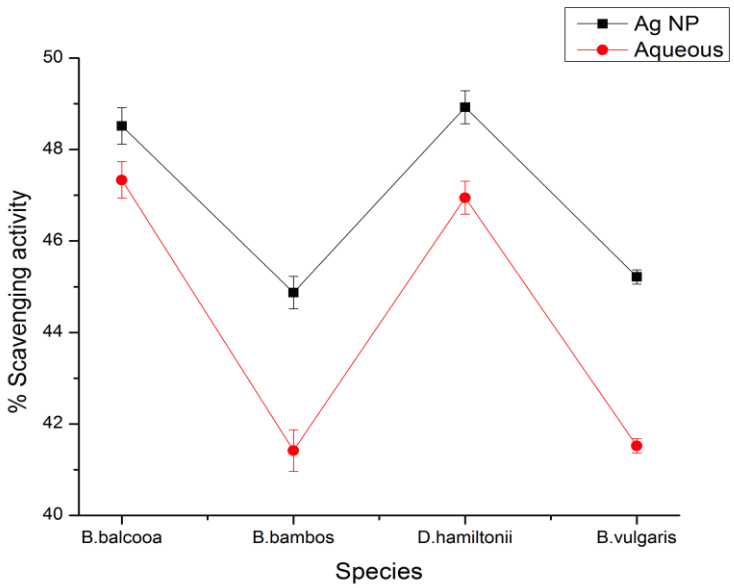

Figure 9: Comparison of DPPH scavenging activity of the aqueous extracts of bamboo shoots

3.9 Antimicrobial activity

The antimicrobial activity of silver nanoparticles synthesized by bamboo shoots extract was investigated against *Staphylococcus aureus* and *Klebsiella pneumonia* bacteria. All the extracts showed maximum zone of inhibition against *Staphylococcus aureus* bacteria. Diameter of the inhibition zones around the well with silver nanoparticles was represented in Table 2. *D.hamiltonii, B.vulgaris* showed highest antimicrobial activity against *S.aureus* than *K.pneumonia* bacteria. The inhibition of growth of the bacterial species is shown in Figure 10.

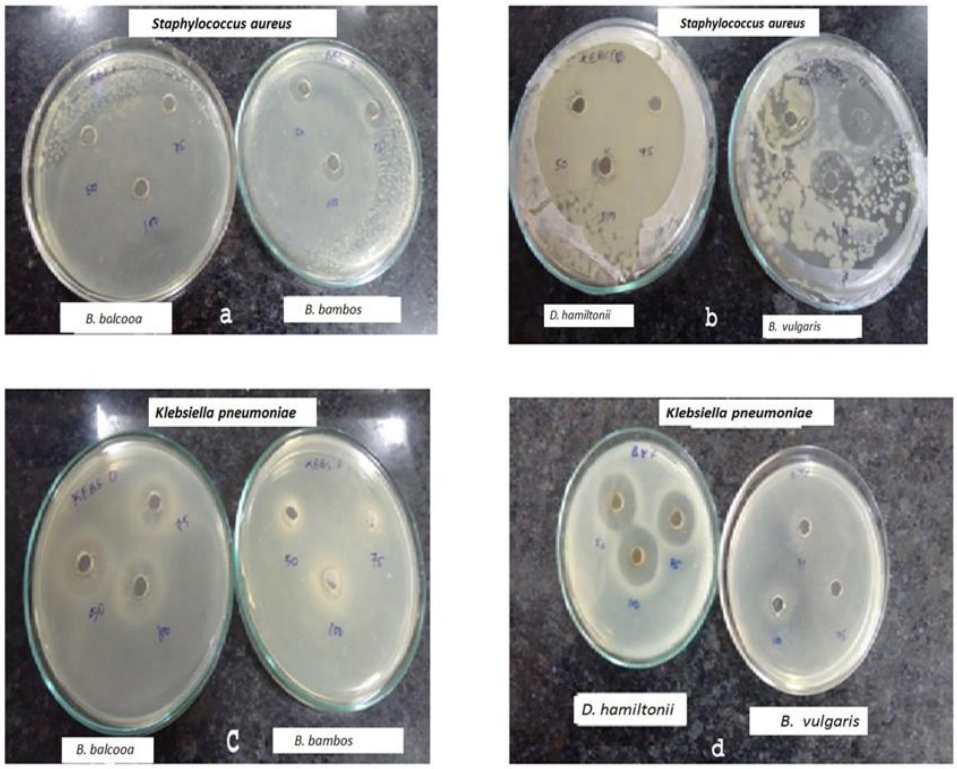

Figure 10: Antibacterial activity of aqueous bamboo shoot extract using *Staphylococcus aureus* and *Klebsiella pneumonia* bacteria.

Table 1: Zone of inhibition (diameter) of bamboo shoot aqueous extract using *Staphylococcus aureus* and *Klebsiella pneumonia* bacteria.

Bamboo shoot	Zone of inhibition (mm) against pathogenic bacteria					
	Staphylococcus aureus			*klebsiella pneumonia*		
	50 µL	75 µL	100µL	50 µL	75 µL	100 µL
B. balcooa	11	14	16	14	18	23
B. bambos	15	18	20	7	10	15
D. hamiltonii	17	20	22	23	27	31
B. vulgaris	22	25	28	21	24	28

DISCUSSION

The absorption maximum for AgNPs in all the four species was found at ~ 420 nm which is due to the excitation of surface plasmon vibrations of spherical silver nanoparticles[10]. This important feature of absorbance spectra confirms the formation of AgNPs in good yield[7]. The mechanism by which the plant extract could synthesize AgNPs may be explained by the high total phenolics content in the plant. These plant phenolics are strong antioxidants with high reducing capacity [22], which can be used for AgNPs synthesis. The high levels of total phenolics and flavonoid content in the bamboo shoot extracts facilitated the reduction of silver ions to nanoscale-sized silver particles as these phenolic compounds are known for their reducing ability. The total phenolic and total flavonoid contents of *D.hamiltonii* was slightly higher than the other three species which have comparable levels of these two phytochemicals.As a result all the four species were found to be equally efficient in synthesizing AgNPs. The quinoid compounds produced due to the oxidation of the phenol group in phenolics can be adsorbed on the surface of nanoparticles, accounting for their suspension stabilization. The FTIR technique can identify the phytochemicals involved in the reduction and capping of nanoparticle[6]. It can also identify the possible reducing and stabilizing biomolecules. The FTIR spectrum of silver nanoparticles in the extract indicates the presence of various functional groups. The band around 1045.02 cm^{-1} corresponds to C-O stretching vibration of glycosidic linkage[23]. The bands appearing at around 1067.38 cm^{-1} correspond to the C-O stretching vibration whereas the band appearing at 1342.15 show the presence of C-N stretching vibration of the aromatic amines[3]. The band at 1646.46 cm^{-1} appears due to C=C stretching vibration. The band appearing at 1653.94 cm-1 may be assigned to amido group [23] .The band appearing at 1748.40 cm^{-1} corresponds to C=O stretching in ester or ketones while the stretching vibration at 2484.38 cm^{-1} and 3018.32 cm^{-1} correspond to C-H and O-H stretching vibrations. The strong band appearing around at 3348.55 cm^{-1} can be associated to the stretching vibrations of N-H bond in amides and the strong broad band around 3420.99 cm^{-1} may be associated with alcoholic and phenolic O–H[20]. These vibrations indicated the different terpenoids and proteins abundant in aqueous extracts which are responsible for the bio-reduction of Ag ions. As in this study the bio-reduction was done using aqueous extract of bamboo species, it can be inferred that some of the bio-organic molecules such as proteins, flavonoids, phenols and polysaccharids bind to Ag ions and bring about the synthesis and stabilization of Ag nanoparticles[3]. It is also reported that the biological molecules play an important role in the formation and stabilization of silver nanoparticles in aqueous medium and protein can bind to silver nanoparticles through the nitrogen atoms[2]. The SEM image showed relatively spherical shape nanoparticle formed with diameter in the range of 10 nm to 30 nm observed similar phenomenon [24] [25]. Energy dispersive spectrometry (EDS) micro-analysis is performed by measuring the energy and intensity distribution of X-ray signals generated by a focused electron beam on a specimen. EDX spectra were recorded from the silver

nanoparticles[22],[23]. The results pointed out that the reaction product was composed of high quality of Ag nanoparticles. A similar EDX spectrum was found for the AgNPs biosynthesized by extracts of the four bamboo species. From EDX spectra, it is clear that silver nanoparticles produced by the extracts have the atomic percentage of silver between 0.42% and 1.92%. The XRD spectra showed sharp peaks at 24.53°, 28.15°, 31.74°, 38.28° and 44.4° which can be indexed to (110), (111), (110), (110) and (200) crystalline plane of cubic silver respectively. The appearance of these peaks confirmed formation of crystalline silver nanoparticles [26]. In our experiment the pattern of X-ray of synthesized silver nanoparticles matches the FCC structure of the bulk silver. In addition to the Bragg peak representative of FCC silver nanocrystals, additional and yet unassigned peaks were also observed suggesting that the crystallization of bio-organic phase occurs on the surface of the silver nanoparticles. The graph of DPPH scavenging (in figure 8) showed that the percentage scavenging activity for aqueous extracts were lesser (41-47%) compared to aqueous extracts with biosynthesized AgNPs (45-49%). The result clearly indicates that the DPPH scavenging activity of the extract was enhanced due to formation of silver nanoparticle in the aqueous extract. The free radicals may attach to the surface of nanoparticles at the corner or edges. Therefore, the metal nanoparticles have high free radical scavenging activity[27]. The Zeta potential values can be related to the stability of the nanoparticles[2] (Sindhura et al., 2014). Colloidal dispersions with high Zeta potential value (± 30 mV) are electrically stabilized [28],[29] .In the present study, the stability of the colloidal silver nanoparticles was therefore confirmed by analyzing the zeta potential values, which were recorded after 48 hours. The antimicrobial activity of AgNPs was reported in a series of reports[30],[31],[32],[33]. In the current study, plant-AgNPs were effective against *Staphylococcus aureus* and *Klebsiella pneumonia* bacteria. According to some reports the metal nanoparticles show antibacterial activity through the electrostatic attraction between positive charges on the nanoparticles.and negative charged cell membrane of microorganisms. The positive charge on the surface of Ag nanoparticles is extremely important for its antimicrobial activity[34].The nanoparticles of silver showed antimicrobial activity against both gram positive and gram negative bacteria as revealed by formation of zone of inhibition. These biologically synthesized nanoparticles could be used in the medicinal field as it has efficient antimicrobial activity. The findings reported in this paper may contribute to the utilization of bamboo shoots in nanoparticle synthesis.

4. CONCLUSION

Bamboo shoot is reported for the first time to synthesize silver nanoparticles. In the present work silver nanoparticles have been successfully synthesized by the aqueous extracts of young shoots of four different bamboo species with significant antioxidant properties. The method provided an efficient and easy way to synthesize silver nanoparticles. The UV –Visible spectra and XRD confirmed the reduction of Ag^+ ions to Ag nanoparticles. Moreover, the SEM images also confirmed

the formation of relatively spherical shaped nanoparticles with diameters in the range of 10-30 nm. These biologically synthesized nanoparticles show efficient antimicrobial properties and could find application in the pharmaceutical field.

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CONFLICT OF INTERES

The authors declare no conflict of interest.

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