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Full Length Research Paper

Biogenic synthesis of silver nanoparticles using *Nicotiana tobaccum* leaf extract and study of their antibacterial effect

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A green synthesis of silver nanoparticle was carried out using tobacco leaf extract. Synthesized nanoparticles were characterized using UV-Vis absorption spectroscopy, TEM, EDAX, FT-IR and photoluminescence study, respectively. UV-Vis absorption spectroscopy of prepared silver colloidal solution showed absorption maxima at 418 nm. Excitation maximum and emission maximum obtained from photoluminescence study were found at 414 and 576 nm, respectively. TEM analysis showed average particle size of 8 nm, while SAED pattern confirmed the crystalline nature of synthesized nanoparticles. FT-IR analysis indicated the involvement of carboxyl (-C= O), hydroxyl (-OH) and amine (-NH) functional groups of tobacco leaf extract in preparation of silver nanoparticles. EDAX analysis showed proportion of silver (54.55%) among other elements in nanoparticle. *Pseudomonas aeruginosa* and *Escherichia coli* DH5α showed highest sensitivity towards silver nanoparticles.

Key words: Silver nanoparticles, tobacco, UV-Vis absorption, photoluminescence, FT-IR spectroscopy.

INTRODUCTION

Nanotechnology has achieved status as one of the critical research endeavors of the 21st century as scientists harness the unique properties of atomic and molecular assemblage built at the nanometer scale. These nano-particles have been conventionally produced by physical and chemical means, which have inherent disadvan-tages like increased size, high energy require-ments and capital intensiveness (Joerger et al., 2000). Hence, biogenic synthesis, which involves the action of biological material produce metal nanoparticles, remains pertinent in present context. This method of synthesis is clean, less costly, employs ambient conditions and less energy intensive (Saxena et al., 2010). The diverse application of nanocrystalline silver ranges from catalysis (Jana et al., 1999) to photonics (Velikov et al., 2003), biosensing and diagnostics (Songping and Shuyuan, 2005, Schultz et al., 2000), antimicrobial (Pal et al., 2007) DNA sequencing (Thompson et al., 2008). In one study, antimicrobial activity of silver nanoparticles was shown to promote wound healing (Ghosh et al., 2010). An anti-proliferative activity of silver nanoparticles against human cancer cells have been reported (Rani et al., 2009). The interactions between silver nanoparticle and HIV-1 too have been reported (Elechiguerra et al., 2005). In a recent study, surface-enhanced Raman spectroscopy (SERS) was used to obtain the Raman spectra of the respiratory syn-cytial virus (RSV), using substrates composed of silver nanorods. The study shows that the four virus strains tested were readily detected at very low detection limits (Shanmukh et al., 2008).

A plant species *Gliricidia sepium* used for the synthesis of silver nanoparticles, showed absorption maximum at 440 nm (Raut et al., 2009). Green synthesis of silver nanoparticles using *Argimone maxicana* leaves broth generated particles of 20 nm and found to be effective

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Figure 1. A standing crop of *Nicotiana tobaccum.*

against many bacterial and fungal pathogens (Khandelwal et al., 2010). *Cycas* leaf extract was used to prepare silver nanoparticled of 2 to 6 nm (Jha and Prasad, 2010). A plant species *Solanum torvum* produced silver nanoprticles of 14 nm dimension and showed the absorbance peak at 434 nm. The antimi-crobial activity of synthesized nanoparticles was tested against *Pseudomonas aeruginosa, Staphylococcus aureus, Aspergillus flavus and Aspergillus niger,* showing a zone of inhibition (Govindaraju et al., 2010). Weeds such as *Ipomoea aquatica, Enhydra fluctuans* and *Ludwigia adscendens* were used as a precursor for the synthesis of silver nanoparticles showed absorbance peak between 400 to 480 nm (Roy and Barik, 2010). Silver nanoparticles synthesized from *Boswellia ovalifoliolata* stem bark showed UV-Vis analysis absorption maxima at 430 nm and their size varied from 30 to 40 nm (Ankanna et al., 2010). Dried leaves of *Cinnamomum camphora* have been implicated in synthesis 55 to 80 nm of silver nanoparticles (Huang et al., 2007). *Emblica officinalis* fruit extract was used for fabrication of gold and silver nanoparticles of 10 nm, showed maximum absorption of light at 430 nm (Ankamwar et al., 2005). *Eucalyptus hybrida* (Safeda) leaves have been shown to synthesize silver nanoparticles of 50 nm (Dubey et al., 2009). Dried leaves of *Pongamia pinnata* (L) Pierre were used to synthesize silver nanoparticles of 20 nm size. The study also describes antimicrobial activity of nanoparticles against many Gram negative and Gram positive micro-organisms (Raut et al., 2010). Bio-reduction of silver using various plant extracts such as *Helianthus annus, Basella alba, Oryza sativa, Saccharum officinarum, Sorghum bicolor and Zea mays* have been studied (Leela and Vivekanandan, 2008). Leaf extract of *Parthenium hysterophorus* synthesized silver nanoparticles of average size of 50 nm (Parashar et al., 2009). An aqueous extract of *Azadirachta indica* (Neem) leaves too was studied for the biogenic synthesis of silver nanoparticles, showed maximum absorbance between 440 to 500 nm (Mukherjee et al., 2009). The present study describes the process of synthesis of silver nanoparticles using leaf extract of tobacco plant followed by their characterization.

MATERIALS AND METHODS

Preparation of tobacco leaf extract

Fresh and young leaves (5 g) of tobacco were washed thoroughly with distilled water and cut in to fine pieces (Figure 1). Leaves were subsequently macerated in 20 ml of Tris.Cl pH 8.0 with the help of mortar and pestle. Thick slurry of leaf thus recovered was subjected to centrifugation at 10,000 rpm for 5 min at 4°C.

The supernatant was transferred in to fresh sterile centrifuge tubes followed by its preservation in refrigerated condition. The extract of tobacco leaf obtained in this manner was used as a precursor for synthesis of silver nanoparticles. All the chemicals

used in the present study were of reagent grade.

Synthesis of silver nanoparticles

In a typical synthesis of silver nanoparticle, requisite amount of $AgNO_3$, 1 mM for 50 ml was added in to 48 ml of deionized miliQ water. Leaf extract (2 ml) was added drop wise in to the solution of $AgNO_3$. The content was later on placed on to a rotatory orbital shaker operating at 200 rpm for 48 h. The incubation of the mixture was carried out at 30°C in dark condition. The reduction of Ag^+ ions was monitored by sampling an aliquot (3 ml) of the mixture at intervals of 24 h, followed by measurement of the UV-Vis spectra using spectrophotometer (OPTIZEN 3220). In order to find the highest peak or λ_{max}, a spectral scanning analysis was carried out by measuring optical density of the content from wavelength, 300 to 700 nm.

Photoluminescence studies

Photoluminescence spectra were recorded in Vrian (Cary Eclipse) spectrofluorimeter using 90° illumination. Intially, prescan was performed to find out the excitation and emission maxima for the silver nanoparticles. Based on the excitation maximum (420 nm), emission scan was carried out in the range of 500 to 600 nm. The excitation and emission slit widths were kept at 5 and 10.0 nm, respectively. The entire scanning was done at the speed of 600 nm/min. The data were analyzed using the WINFLR software.

TEM analysis, EDAX and FTIR analysis

Transmission electron microscopic (TEM) analysis was performed with Techni 20 (Philips, Holland). Thin film of the sample were prepared on a carbon coated copper grid by dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper. Later on, film on the TEM grid was allowed to dry by placing it under a mercury lamp for 5 min. Leaf extract containing silver nanoparticles was freeze dried using a lyophilizer (Labconco, Kanas, USA). The powder obtained in this manner was subjected to EDAX and FTIR analysis. Elemental analysis of synthesized nanoparticles was carried out by using scanning electron microscope (Philips, Netherlands) equipped with energy dispersive x-ray system, EDAX XL-30 operating at 15-25 KV. Disc of 100 mg KBr containing dried 1% freeze dried powder, served as material for recording transmission spectra. Spectra were recorded at 400 to 4000cm^{-1} range using a FT-IR spectrometer (Perkin Elmer, Spectrum GX) with resolution of 0.15 cm^{-1} to evaluate functional groups that might be involved in particle formation.

Anti bacterial assay

Silver nanoperticles synthesized in this study were tested for their antibacterial activity using agar disc diffusion assay, a method adopted from Bauer et al. (1996). Briefly, bacterial strains (obtained from microbial type culture collection MTCC, Chandigarh, India) were inoculated in to Luria Bertani (LB) broth medium followed by their incubation at 37°C for 12 h. Strains of microorganisms used in this study were, *Pseudomonas putida* MTCC 1194, *P. vulgaris* MTCC 1771, *Escherichia coli* DH5α MTCC 1652, *B. subtilis* MTCC 2391, *P. aeruginosa* MTCC 2453, *Salmonella typhi* MTCC 733. Inoculums of 100 µl was drawn from overnight grown culture and spread evenly over the surface of LB agar plate. Sterile discs of six millimeter width were dipped in to leaf extract containing 17 mg/l of $AgNO_3$. Dried disc were placed over culture plates followed by an incubation of 12 h at 37°C. The antibacterial activity was assigned by measuring the inhibition zone formed around the disc.

RESULTS AND DISCUSSION

Aqueous silver ions were reduced to silver nanoparticles after addition of tobacco leaf extract followed by incubation of the mixture for studied period of time. Initially up to 24 h mixture remains whitish yellow (Figure 2b) which ultimately turned in to dark red after 72 h (Figure 2c). Reduction of colloidal silver leading to change in colour has been frequently observed by several authors who successfully synthesized silver nanoparticles using different biomaterial (Saxena et al., 2010; Vigneshwaran et al., 2006; Khandelwal et al., 2010). This colour was primarily due to surface Plasmon resonance of deposited silver nanoparticles. In case of control experiment where leaf extract was not added to silver nitrate solution exhibited no change in colour even left for one week duration.

UV-Vis spectrophotometry

UV-Vis absorption spectra of silver nanoparticle is shown in Figure 3. The spectra of silver solution containg tobacco leaf exract (72 h of incubation) showed absorption maxima at 418 nm. Several other invstigators have observed absorption maxima of collodal silver solution between 410 to 440 nm which is assigned to surface plasmon of various metal nanoparticles (Sarkar et al., 2010). Scaning of absorption spectra of the mixture was continuely recorded for one month time period, yielded no significant change in the intensity of absorption maxima suggested a stable nanoparticle formation.

TEM analysis of silver nanoparticle

In order to determine the morphology and size detail of silver nanoparticles synthesized by tobacco leaf extract, transmission electron microscopy was carried out. TEM image recorded from synthesized nanoparticles are shown in Figure 4a. Image suggests that synthesized particles are monodisperse with average size of 8.43 ± 1.15 nm. The selected area diffraction pattern (SAED) confirms the crystalline structure of metallic silver (Figure 4b).

Photoluminescence analysis

Photoluminescence spectra of synthesized silver nano-particles were carried out to evaluate its optical property. Figure 5 shows photoluminescence spectra of silver nanoparticles mixed with leaf extract of Tobacco. The excitation peak was found at 414 nm, while emission

Figure 2. A visible observation of change in color during silver nanoparticle formation. Conical flask containing 1 mM $AgNO_3$ solution without addition of leaf extract. (a) A whitish yellow color appeared when silver salt was mixed with extract followed by incubation for 24 h; (b) upon extending the incubation time up to 72 h the colloidal content of privious flask turned to red colour (c).

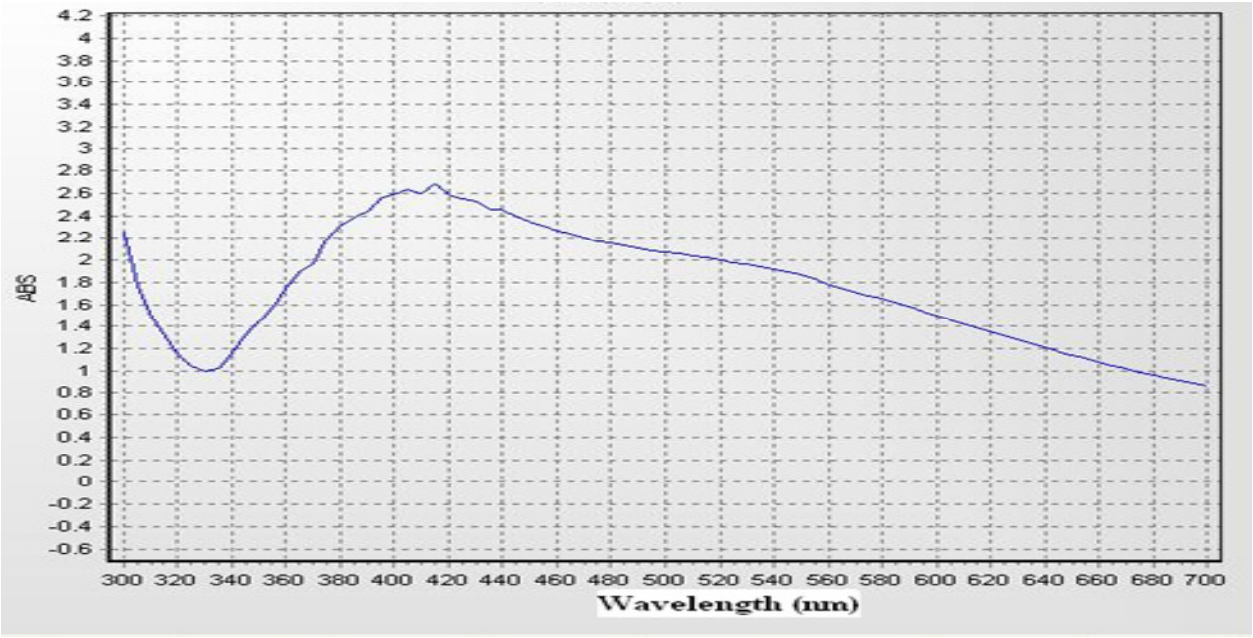

Figure 3. UV-Vis spectra of silver nanoparticles synthesized using tobacco leaf extract.

peak was observed at 576 nm. The excitation peak at 414 nm very well correlates with absorption maxima recorded with UV-Vis spectrophotometer (418 nm). Vigneshwaran et al. (2006) have detected exitation peak at 420 nm where as emission peak was observed at 553 nm.

FTIR analysis

FT-IR analysis was carried out to identify the possible bio-molecules and cell-metal ions interaction responsible for formation and stabilization of silver nanoparticles. The result of FT -IR analysis is presented in Figure 6. The

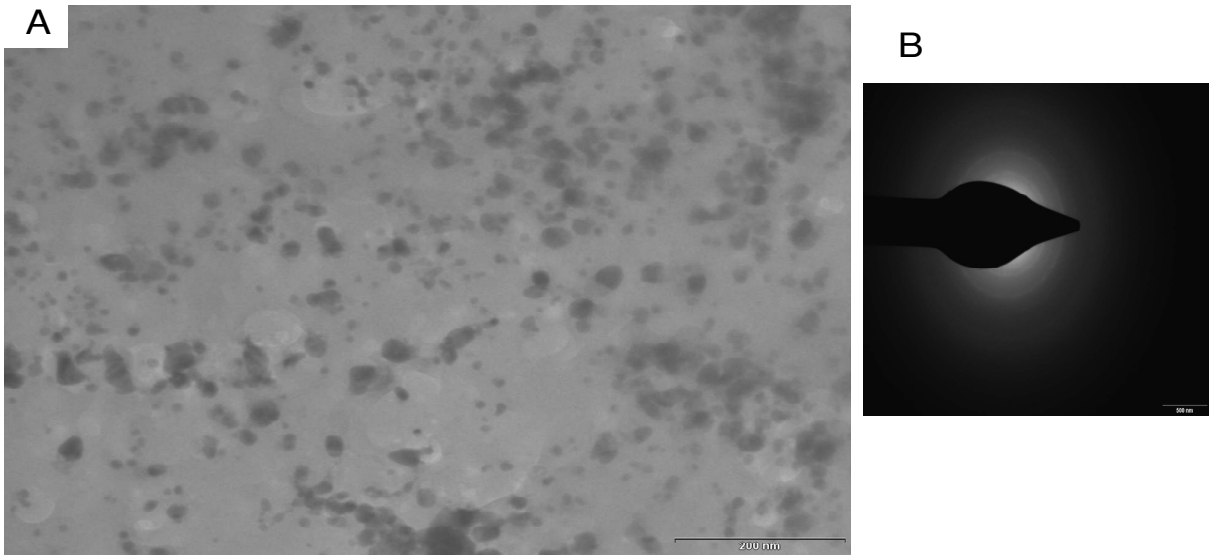

Figure 4. TEM micrographs recorded from drop-cast films of silver nanoparticles solution formed by incubation of $AgNO_3$ solution containing tobacco leaf extract (a). Selected area electron diffraction (SAED) pattern recorded from the silver nanoparticles (b).

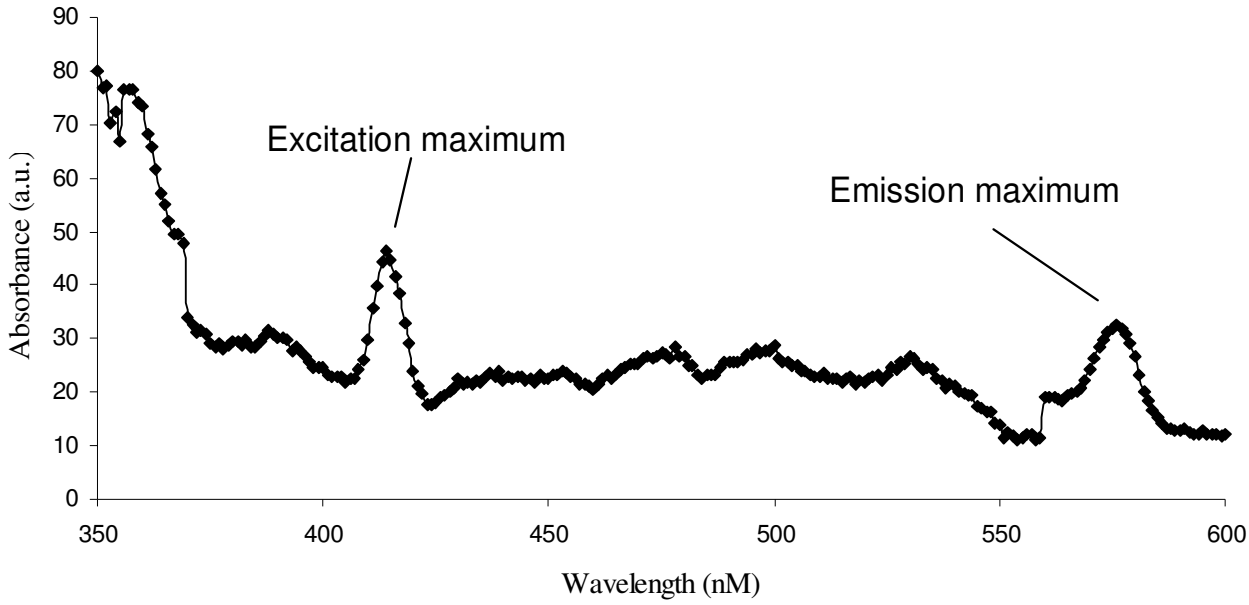

Figure 5. Photoluminescence spectra of silver nanoparticles synthesized by tobacco leaf extract.

figure 6a shows the FT-IR spectrum of tobacco leaf extracts that did not contain $AgNO_3$, where as Figure 6b shows spectrum of extract containing $AgNO_3$. Spectra Figure 6a showed transmission peaks at 3404, 2923, 2856, 1631, 1384, 1320, 1100, 1048, 894, 780, 610 and at 463 cm^{-1}. Similarly, transmission peaks for the leaf extract containing silver nanoparticle were obtained at 3403, 2934, 2396, 1761, 1624, 1384, 1075, 823 and 622 cm^{-1}. Two absorption peaks located around 823 and 1075 cm^{-1} can be assigned as the absorption peaks of –C–O–C. The adsorption at around 1384 cm^{-1} notably showed, NO_3^- existed in residual amount. The peaks at 1624 to 1631 cm^{-1} were attributed to stretching vibration of carboxyl group (-C=O). The peaks observed at 2925 to 2934 cm^{-1} can be assigned to the C-H group. The broad and strong bands at 3403 to 3404 cm^{-1} were due to bounded hydroxyl (-OH) or amine groups (-NH) of leaf extract. The carboxyl peaks at 1631 and 1384.24 cm^{-1}

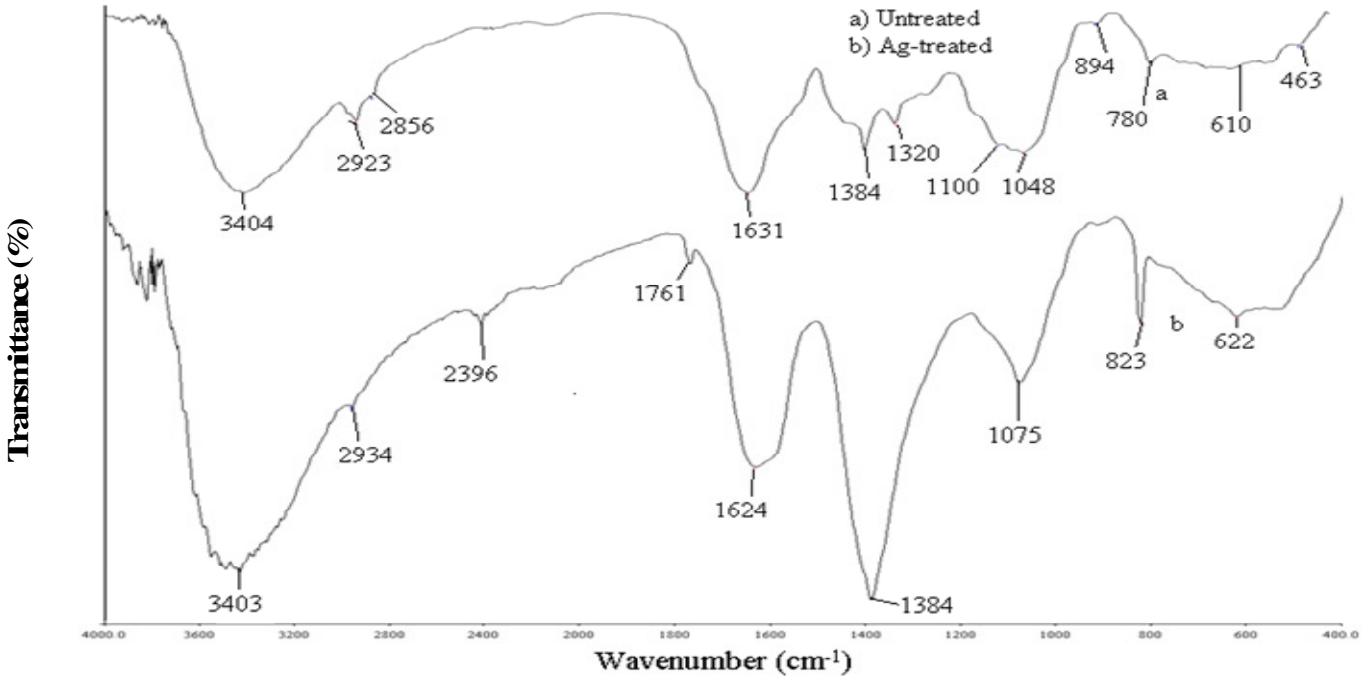

Figure 6. FT-IR spectra of untreated tobacco leaf extract (a); $AgNO_3$ treated tobacco leaf extract (b).

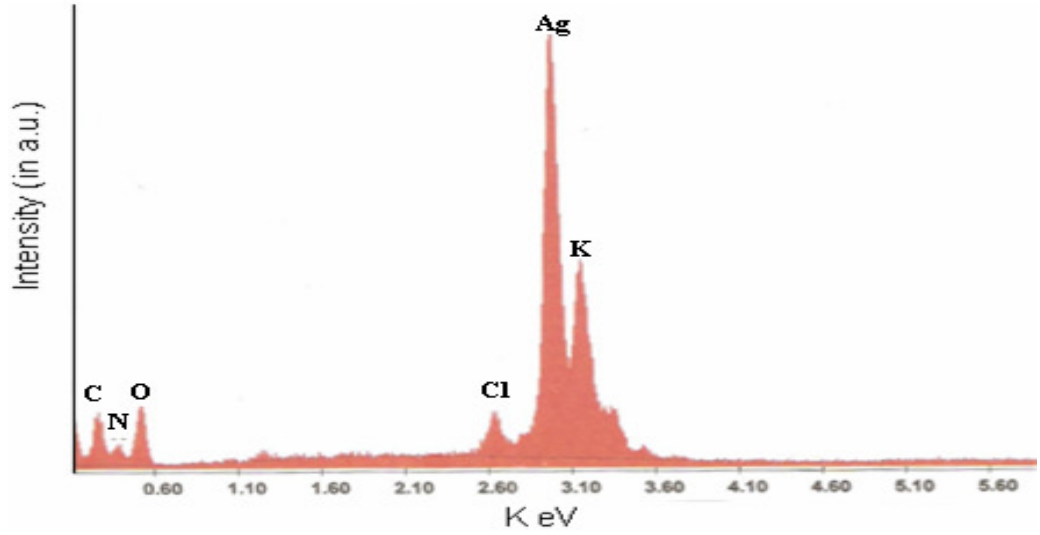

Figure 7. EDAX spectrum of synthesized nanoparticles.

were shifted to 1624 and 1384.22 cm^{-1}. The result indicates that the Carboxyl (-C=O), hydroxyl (-OH) and Amine (-NH) groups of leaf extracts are mainly involved in fabrication of silver nanoparticles.

EDAX analysis

EDAX analysis gives qualitative as well as quantitative status of elements that may be involved in formation of nanoparticles. Figure 7 shows elemental profile of synthesized nanoparticle using tobacco leaf extract. The analysis revealed highest proportion of silver (54.55%) in nanoparticle followed by carbon (14.67%), nitrogen (7.02%), oxygen (15.76%) etc.

Antibacterial assay

Silver nanoparticles were fairly toxic to *P. aeruginosa*

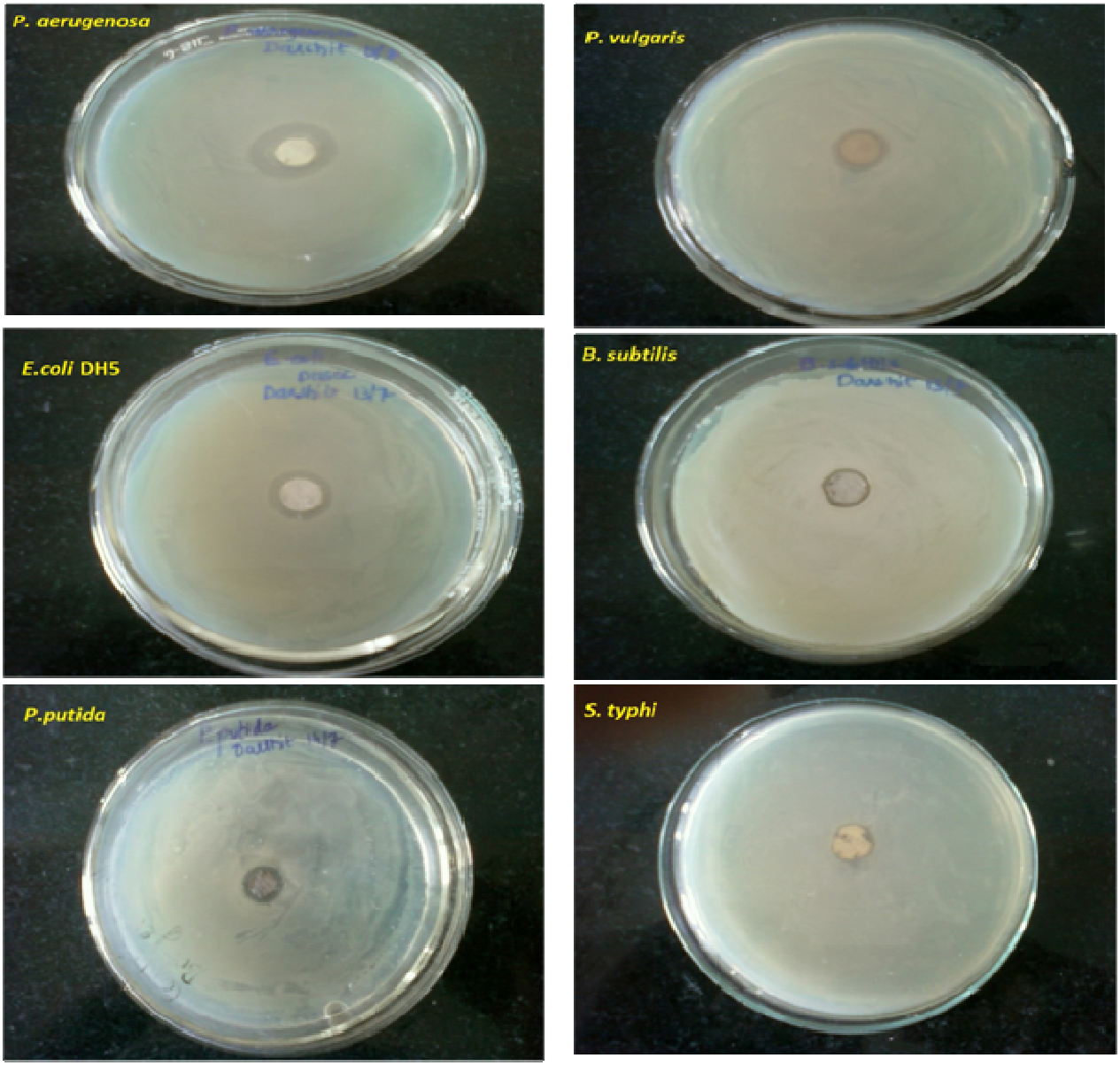

Figure 8. Shows zone of inhibition in response to silver nanoparticles synthesized using tobacco leaf extract.

while they showed a moderate toxicity against *P. vulgaris, E. coli, B. subtilis* and *P. putida.* However, nanoparticles exhibited low toxicity against *S. typhi.* Zone of inhibition around silver nanoparticle impregnated disc for individual bacterial culture is shown in Figure 8, while numerical value of inhibition zone is presented in Table 1. Dilute solution of silver nanoparticles have been used to treat various infections and burns (Raut et al., 2010). A number of theory for antimicrobial actions of colloidal silver solution have been proposed for example, alteration of permeability of cell membrane (Sondi and Sondi, 2004), release of lipopolysaccharides and membrane proteins (Amro et al., 2000), generation of free radicals responsible for the damage of membrane (Kim et al., 2007), dissipation of the proton motive force resulting in the collapse of the membrane potential (Chun-Nam et al., 2006), however, exact mechanism has not been fully deciphered. Tripathi et al. (2010) studied effect of silver nano balls on *E. coli*, *S. typhimurium*, *B. subtilis* and *P. aeruginosa* by colony forming unit (CFU) and growth curve at 40 µg/ml concentration. The study showed significant reduction of bacterial population and their growth pattern at studied concentration.

Conclusions

The present study describes synthesis, characterization

Table 1. Zone of inhibition (mm) of nanoparticles against different bacterial strains.

Culture	Zone of inhibition (mm)
P. vulgaris	3
E. coli DH5α	4
B. subtilis	2
P. putida	2
P. aeruginosa	8
S. typhi	1

and antimicrobial activity of silver nanoparticles using tobacco leaf extract. Characteristic red colour appeared after incubation of the mixture for 72 h. UV-Vis spectroscopy showed absorption maxima at 418 nm. TEM study showed the average size of silver nanoparticles was 8.43 ± 1.15 nm, while SAED pattern indicated about crystalline nature of nanoparticles. Photoluminescence study shows that nanoparticles possessed fluorescent property. EDAX analysis shows that silver was the main constituent of synthesized nanoparticles. FTIR analysis indicated the possible role of carboxyl (-C=O), hydroxyl (-OH) and amine (-NH) groups of leaf extract in fabrication of silver nanoparticle. An antimicrobial activity assay of synthesized nanoparticle showed maximum zone of inhibition when tested against *P. aeruginosa* followed by *E. coli* DH5α, *P. vulgaris*, *B. subtilis* and *S. typhi,* respectively. Use of tobacco leaf extract offers an affordable, environment friendly technique for synthesis of large scale silver nanoparticles.

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REFERENCES

Amro NA, Kotra LP, Wadu-Mesthrige K, Bulchevy A, Mobashery S, Liu GY (2000). High resolution atomic force microscopy studies of the *Escherichia coli* outer membrane: The structural basis for permeability. Langmuir, 16: 2789- 2796.

Ankamwar B, Damle C, Ahmad A, Sastry M (2005). Biosynthesis of gold and silver nanoparticles using *Emblica officinalis* fruit extract, their phase transfer, Transmetallation in an organic solution. J. Nanosci. Nanotechnol. 5: 1665-1671.

Ankanna S, Prasad TNVKV, Elumalai EK, Savithramma N (2010). Production of biogenic silver nanoparticles using *Bosvellia ovalifoliolata* stem bark. Digest J. Nanomater. Biostructures, 5: 369-372.

Bauer AW, Kirby WMM, Sherries JC, Truck M (1996). Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol. 45: 493-496.

Chun-Nam L, Ho CM, Chen R, He QY, Yu WY, Sun HP, Tam KH, Chiu JF, Che CM (2006). Proteomic analysis of the mode of antibacterial action of silver nanoparticles. J. Proteome Res. 5: 916-924.

Dubey M, Bhadauria S, Kushwah BS (2009). Green synthesis of nanosilver particles from the extract of *Eucalyptus hybrid* (Safeda leaves). Digest J. Nanomater. Biostructures, 5: 537-543.

Elechiguerra JL, Burt JL, Morones JR, Camacho-Bragado A, Gao X, Lara HH, Yacaman MJ (2005). Interaction of silver nanoparticles with HIV-I. J. NanoBiotechnol. 3: p. 29.

Ghosh S, Upadhay A, Singh AK, Kumar A (2010). Investigation of antimicrobial activity of silver nanoparticles loaded cotton fabric which may promote wound healing. Int. J. Pharm. Biol.sci. 1: 1-10.

Govindaraju K, Tamilselvan S, Kiruthogs V, Simgaravelu G (2010). Biogenic silver nanoparicles by *Solanum torvum* and their promising antimicrobial activity. J. Biopesticide, 3: 394-399.

Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, Wang H, Wang Y, Shao W, He N, Hong J, Chen C (2007). Biosynthesis of silver and gold nanoparticles by using novel sun-dried *Cinnamomum camphora* leaves. Nanotechnol. 18: 105104.

Jana NR, Sau TK, Pal T (1999). Growing small silver particle as redox catalyst. J. Phys. Chem. B. 103: 115-121.

Jha AK, Prasad K (2010). Green synthesis of silver nanoparticles using *Cycas* leaf. Int. J. Green Nanotech: Phy. Chem. 1: 110-117.

Joerger R, Klaus T, Granqvist CG (2000). Biologically produced silver–carbon composite materials for optically functional thin film coatings. Adv. Mater. 12: 407-409.

Khandelwal N, Singh A, Jain D, Upadhyay MK, Verma HN (2010). Green synthesis of silver nanoparticles using *Argimone maxicana* leaf extract and evaluation of their activity. Digest J. Nanomater. Biostructures, 5: 483-489.

Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Huwang CY, Kim YK, Lee YS, Jeong DH, Cho MH (2007). Antimicrobial effects of silver nanoparticles. Nanomed. Nanotechnol. Biol. Med. 3: 95-101.

Leela A, Vivekanandan M (2008). Tapping the unexploited plant resources for the synthesis of silver nanoparticles. Afr. J. Biotechnol. 7: 3162-3165.

Mukherjee A, Chandrashekharan N, Raichur AM, Tripathi A (2009). Antibacterial activity of silver nanoparticles synthesized by aqueous extract of *Azardirachta indica* (Neem) leaves. J. Biomed. Nanotechnol. 5: 93-98.

Pal S, Tak YK, Song JM (2007). Does antimicrobial activity of silver nanoparticles depends on the shape of nanoparticle? A study on the Gram-Negative bacterium *E. coli.* Appl. Environ. Microbiol. 73: 1712-1720.

Parashar V, Parashar R, Sharma B, Pandey AC (2009). Parthenium leaf extract mediated synthesis of silver nanoparticle: A novel approach for weed utilization. Digest J. Nanomater. Biostructures, 4: 45-50.

Raut RW, Kolekar NS, Lakkakula JR, Mendhulkar VD, Kashid SB (2009). Photosynthesis of silver nanoparticles using *Gliricidia sepium* (Jecq.). Curr. Nanosci. 5: 117-122.

Rani APV, Hande M P, Valiyaveettil S (2009). Anti-proliferative activity of silver nanoparticles. BMC Cell Biol. 10: 65.

Raut RW, Kolekar NS, Lakkakula JR, Mendhulkar VD, Kashid SB (2010). Extracellular synthesis of silver nanoparticles using dried leaves of *Pongamia pinnata* (L) Pierre, Nano-Micro Lett. 2: 106-113.

Roy N, Barik A (2010). Green synthesis of silver nanoparticles using unexploited weed resources. Int. J. Nanotech. Appl. 4: 95-101.

Sarkar R, Kumbhakar P, Mitra AK (2010). Green synthesis of silver nanoparticles and its optical properties. Digest J. Nanomater. Biostructures, 5: 491-496.

Saxena A, Tripathi RM, Singh RP (2010). Biological synthesis of silver nanoparticles by using onion (*Allium cepa*) extract and their antibacterial activity. Digest J. Nanomater. Biostructures, 5: 427-432.

Schultz S, Smith DR, Mock JJ, Schultz DA (2000). Single-target molecule detection with nonbleaching multicolor optical immunolabels. PNAS, 97: 996-1001.

Shanmukh S, Jones L, Zhao YP, Driskell JD, Tripp RA, Dluhy RA (2008). Identification and classification of respiratory syncytial virus

(RSV) strains by surface-enhanced Raman spectroscopy and multivariate statistical techniques. Anal. Bioanal. Chem. 390: 1551-1555.
Sondi I, Sondi BS (2004). Silver nanoparticles as antimicrobial agents a case study on *E.coli* as a model for Gram-negative bacteria. J. Colloid Interface Sci. 275: 117-182.
Songping W, Shuyuan M (2005). Preparation of ultra fine silver powder using ascorbic acid. Mater. Chem. Phy. 89: 423-427.
Thompson DG, Enright A, Faulds K, Smith WE, Graham D (2008). Ultra sensitive DNA detection using oligonucleotide-silver nanoparticle conjugates. Anal. Chem. 80: 2805-2810.
Tripathi RM, Saxena A, Gupta N, Kapoor H, Singh RP (2010). High antibacterial activity of silver nanoballs against *E. Coli* MTCC 1302, *S. Typhimurium* MTCC 1254, *B. Subtilis* MTCC 1133 and *P. Aeruginosa* MTCC 2295. Digest J. Nanomater. Biostructures, 5: 323-330.
Velikov KP, Zegers GE, Blaaderen A van (2003). Synthesis and characterization of large colloidal silver particles. Langmuir, 19: 1384-1389.
Vigneshwaran N, Kathe A, Varadrajan PV, Nachane RP, Balasubramanya RH (2006). Biomimetics of silver nanoparticles by white rot fungi *Phaenerochaete chrysosporium*. Colloids Surf. B. 53: 55-59.