Research Article

Biosynthesis of Silver Nanoparticles Using Leaf Extract of *Leea Indica* (Burm.F.) Merr. and their Synergistic Antimicrobial Activity with Antibiotics

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ABSTRACT

The green synthesis of nanoparticles using higher plants is an emerging field of research in nanoscience and technology. In the present investigation, *Leea indica* (Burm.f.) Merr. aqueous leaf extract was used for synthesis of silver nanoparticles. The formation of silver nanoparticles was confirmed by UV-Vis characteristic absorbance peak at 410nm. Influence of leaf extract, metal ion concentrations, temperature and pH of nanoparticles synthesis was evaluated. Synthesized nanoparticles were characterized by FTIR, EDAX, AFM and HR-TEM. The FTIR data revealed that biomolecules involved in the reduction and capping of silver nanoparticles. The morphology of silver nanoparticles was determined by AFM and HR-TEM. The size of nanoparticles ranges between 10-50 nm. The EDX analysis confirmed the purity and elemental nature of silver. Synthesized silver nanoparticles exhibited synergistic antimicrobial activity in combination with antibiotic against *Escherichia coli, Salmonella typhi, Staphylococcus aureus* and *Bacillus subtilis*.

Keywords: Biosynthesis, silver nanoparticles, characterization, *Leea indica* (Burm.f.)Merr. antibacterial activity.

INTRODUCTION

Nanoparticles possess unique features with extensive application in diverse fields.[1] The designing the method of synthesis, in which the size, morphology, stability and properties are controlled has become a major field of interest.[2] The synthesis of nanomaterials is gaining attention in recent years because of their properties which make them useful for catalysis,[3] sensor technology,[4] Biological labeling, optoelectronics recording media and optics.[5]

The size, shape and surface morphology play an important role in controlling the physical, chemical, optical and electronic properties of these nanomaterials. Several physical properties such as Surface Plasmon Resonance (SPR) of silver nanoparticles can be tailored for specific application by controlling the size, shape and morphology of the nanoparticles.[6-8]

This is particularly important for noble metals such as Au and Ag which have strong surface plasmon resonance oscillations.

The metal is poor catalyst in bulk form; nanometer-sized particles can exhibit excellent catalytic activity due to their relative high surface area to volume ratio and their interface-dominated properties, which significantly differ from those of the bulk material.[9]

The nanoparticles are synthesized by different physical and chemical methods, but these methods have certain disadvantages due to involvement of toxic chemicals.

A large amount of toxic chemicals are produced during the synthesis of nanomaterials and these chemicals pose a serious threat to environment. Thus, there is a need for safe, clean, nontoxic and environment-friendly method for the synthesis of nanoparticles. Researchers in the field of nanoparticles have laid emphasis on biological system for synthesis of nanoparticles by biomimetic approach.

The various plant extracts were used for the synthesis of silver and gold nanoparticles such as alfalfa[10,11], *Emblica officinalis* fruit extract[12] and *Aloe vera*.[13] Silver and gold nanoparticles were also synthesized by sundried *Cinnamomum camphora* leaf,[14] *Capsicum annum*,[15] *Dioscorea bulbifera* tube extract,[16] *Citrus sinensis* peel extract,[17] *Cissu quadrangularis*,[18] *Rosa damascence*,[19] *Sesamum laciniatum*[20] and *Santalum album*.[21] Biogenic silver nanoparticles synthesized using *Nicotina tobaccum* leaf extract,[22] *Ocimum tenuiflorum*,[23] lemon leaves,[24] *Dodonaea viscosa*[25] and *Artemisia nilagirica*[26] were evaluated for their antibacterial activity against certain bacteria.

Leea indica (Burm.f.) Merr. is belonging to vitaceae, have been traditionally used as natural remedy in folk medicine by the local. It is a perennial shrub which can be found in tropical and subtropical countries, such as Thailand, Malaysia, India and China.

The leaves and roots of *L. indica* are traditionally used for the treatment of cancer, diabetes, diarrhea, dysentery and skin diseases. The present investigation is undertaken to study phytosynthesis of silver nanoparticles, factors governing the synthesis of nanoparticles and synergistic antibacterial activity of synthesized silver nanoparticles.

MATERIALS AND METHODS

Silver nitrate was obtained from Sigma-Aldrich chemicals. All glassware's were washed with distilled water and dried in oven. Fresh leaves of *L. indica* were collected from botanical garden of Karnatak University, Dharwad.

Preparation of Leaf Extract

Ten gram of leaves were thoroughly washed with de-ionized water and were cut into small pieces and boiled in 100 ml of deionized water for 20 min.

Extract was filtered through whatman 41 filter paper and store at 40°C and used for further experiments.

Synthesis of Silver Nanoparticles

Aqueous solution (1 mM) of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 5 ml of *L. indica* leaf extract was added into 95 ml of aqueous solution 1 mM silver nitrate for reduction of Ag^+ ions to silver nanoparticles.

The colour of solution changes from colourless to brown, indicates the formation of silver nanoparticles and further confirmed by characteristic absorption spectra by UV-Vis.

Different Parameters for Investigation

Concentration of Leaf Extract

The varying concentration (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ml) of leaf extract was used for treating 1mM silver nitrate solution. The absorbance of biosynthesized nanoparticles was measured by using UV-vis spectrophotometry.

Concentration of Silver Nitrate Solution

The reaction was monitored by using different concentration of silver nitrate (1, 2, 3, 4 and 5 mM) solution for the synthesis of nanoparticles.

The absorbance of product of reaction mixture was measured spectrophotometry.

Temperature

The reaction temperature was maintained at 0, 10, 37, 40, 50, 60, 70, 80 and 90°C respectively, using water bath. The absorbance of the product of reaction mixture was measured by spectrophotometrically.

Hydrogen ion concentration (pH)

The pH of reaction mixture was maintained at4, 5, 6, 7, 8, 9, 10 and 11. The pH was adjusted using either 0.1 N HCl or 0.1 N NaOH. The absorbance of the end product was measured by using UV-Vis spectrophotometry.

Characterization of Silver Nanoparticles

The bioreduction of the Ag+ ions by the leaf extracts of *L. indica* leading to formation of silver nanoparticles were evident by change of color of reaction mixture from colourless to dark brown and further confirmed by UV–Visible absorption spectra.

The UV–VIS spectrum of the sample was measured on a UV-2450 (Shimadzu) spectrophotometer operated at a resolution of 1 nm. The bio-reduction of silver ions in aqueous solution was monitored by UV–Vis spectrum of the solution which ranges between 300–800 nm. Further characterization was done by FT-IR (F-7000FL) spectrometer. In order to remove any free biomass residue, the residual solution after reaction was centrifuged at 4000 rpm for 20 min and the resulting suspension was redispersed in 10 mL sterile distilled water. The centrifuging and redispersing processes were repeated for three times. Finally, the dried samples were palletized with KBr and analyzed using FT-IR. Morphological characterization of the sample was done by HR-TEM (JEOL JSM6701–F), a pinch of dried sample was coated on a carbon tape.

It was again coated with platinum in an auto fine coater and then the material was subjected to analysis. For EDX analysis, the reduced silver was dried on a carbon tape placed on a copper stub and performed on a HITACHI SU6600.

Antimicrobial Activity

Antimicrobial activity of synthesized silver nanoparticles was analyzed by well diffusion method against gram negative (*Escherichia coli* and *Salmonella typhi)* and gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) bacteria. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37°C for 18 hrs.

The agar plates were poured by muller hinton media and wells were made in the plate. Each plate was inoculated with 18 h old cultures (100 µl, 10^{-4}cfu) and spread evenly on the plate. After 20 min, the wells were filled with different concentration of silver nanoparticles solution, silver nanoparticles and antibiotic, plant extract. All the plates were incubated at 37°C for 24 h and the diameter of inhibition zone were recorded.

RESULTS AND DISCUSSION

Aqueous of 1 mM silver nitrate solution was treated with leaf extract of *L. indica*. The change of color from colouless to brown shows the formation of silver nanoparticles.

The color was arising due to silver nanoparticles absorb radiation from the visible region of electromagnetic spectrum which exhibit surface plasmon vibration.[27] But, control containing 1mM silver nitrate solution remain unchanged during the reaction period. UV-vis spectroscopy is a good analytical technique for characterizing optical properties and electronic structure of silver nanoparticles in solution. The UV-Vis spectra recorded from reaction mixture at 70°C and 9pH (Figure 1). The absorption spectra showed an intense peak at 410 nm due to the Surface Plasmon Resonance (SPR) band of silver nanoparticles and broadening of peak indicated that the particles are polydispersed.

The appearance of single prominent peak shows particles are spherical and uniform in size.[28] Different parameters were optimized including leaf extract concentration, concentration of silver nitrate, temperature and pH were identified as factors affecting the synthesis of silver nanoparticles.

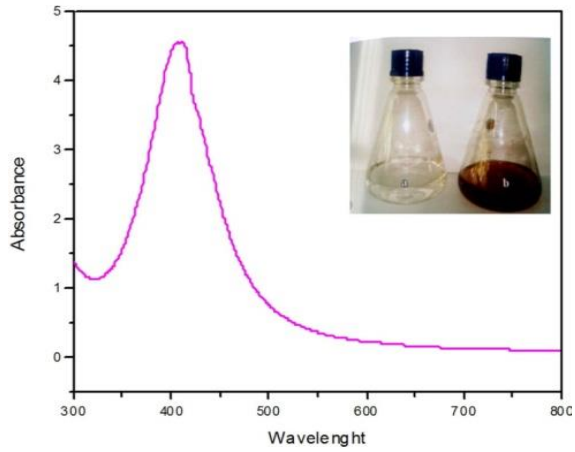

Figure 1: UV-vis spectra of silver nanoparticles, inset picture shows visual observation of silver nanoparticles

Effect of Leaf Extract on Synthesis of Nanoparticles

The quantity of leaf extract plays an important role in the complete conversion of metal salts into metal nanoparticles.10ml of leaf extract is suitable to yield maximum smaller sized silver nanoparticles (Figure 2). The sharpness of the absorption peak is dependent on the concentration of leaf extract, increase in leaf extract quantity decreases the size of particles.[29] The UV-vis spectra of silver nanoparticles show a linear relationship between absorbance and quantity of extract.

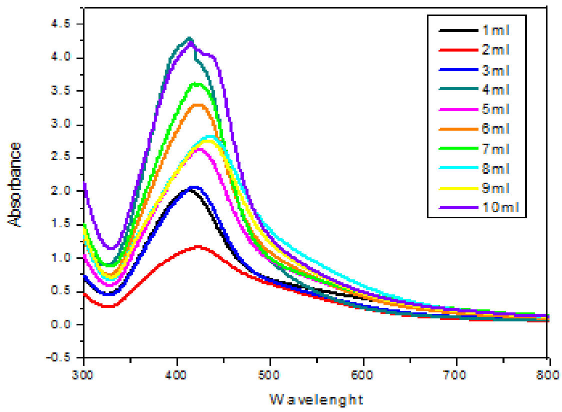

Figure 2: Effect of Leaf Extract on Synthesis of Nanoparticles

Effect of Silver Ion Concentration on Synthesis of Nanoparticles

Variation in silver ion concentration (1-5mM) results in varies the size of nanoparticles. 1 mM silver nitrate solution is suitable to yield maximum of small sized silver nanoparticles. Further, increases in the concentration of silver ions (1, 2, 3, 4 and 5 mM) forms a broader absorption peak which indicates formation of large sized nanoparticles (Figure 3). The concentration of silver ions inversely propositional to the absorbance intensity.

The spectra can exhibit a shift towards the red or the blue end depending upon the particle size, shape, state of aggregation and the surrounding dielectric medium.[30] The spectra shows blue end at 420 nm with small sized nanoparticles later, it shifts to red end at 435 nm indicate formation of larges sized nanoparticles.

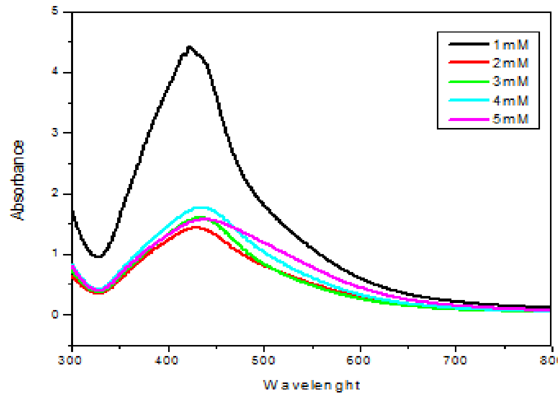

Figure 3: Effect of Silver Nitrate Concentration on Synthesis of Nanoparticles

Effect of Temperature on Synthesis of Nanoparticles

The synthesis of nanoparticles depends on the temperature, increase in the temperature increases rate of formation of silver nanoparticles, to yield maximum small sized nanoparticles at 70°C temperature (Figure 4). The temperature is inversely propositional to the size of nanoparticles.[31]

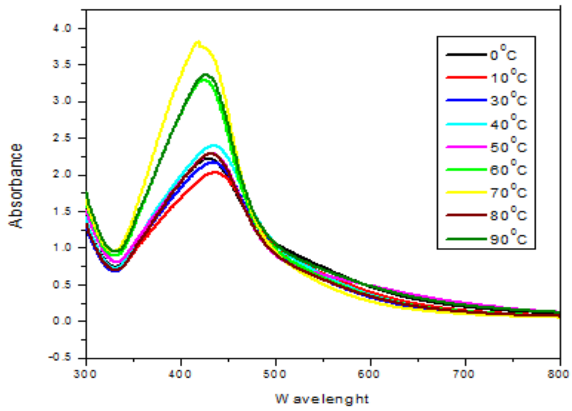

Figure 4: Effect of Temperature on Nanoparticles Synthesis

Effect of pH on Synthesis of Nanoparticles

The pH plays an important role in synthesis of nanoparticles. Acidic condition hinders the formation of nanoparticles and forms a large sized nanoparticles while, small and highly dispersed nanoparticles were formed at basic pH (9) which enhances the formation of nanoparticles (Figure 5). At higher pH large number of functional groups are available for silver binding which results in the formation of large number of silver nanoparticles with smaller dimensions. When NaOH reacts with $AgNO_3$ to form a precipitate of pure silver oxide (Ag_2O) however, the presence of reducing agents in the reaction mixture leads to bioreduction of Ag^+ to Ag(0) using the colloidal system. It is also conceivable that the addition of hydroxide (OH^-) ions deprotonated the active biomolecules with hydroxyl and carboxyl functional groups supplying electrons for the reduction of the Ag^+

ions. The slow rate of formation and aggregation of silver nanoparticles at acidic pH could be related to electrostatic repulsion of anions present in the solution.[32]

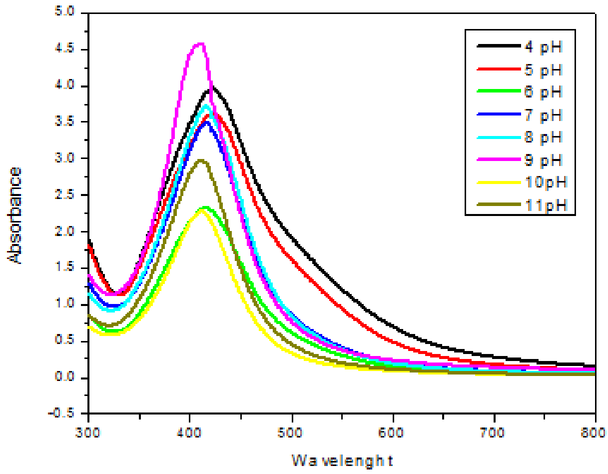

Figure 5: Effect of pH on Nanoparticles Synthesis

FTIR Analysis

The FTIR data of leaf extract and silver nanoparticles reveals the possible biomolecules and chemical change of the functional groups involved in bioreduction and formation of silver nanoparticles (Figure 6 and Table 1). Both the spectra exhibit a broad intense band at 3400 cm^{-1} and 1640 and 1618 cm^{-1} respectively in leaf extract and silver nanoparticles is attributed to N-H and carbonyl stretching (amide band I) frequency arising from the peptide linkage present in the proteins of the extract. The band at 1454 and 1471 cm^{-1} is assigned to methylene scissoring vibrations from the proteins in the solution.[33] The spectra shows bands at 1380, 1379 and 1102 and 1100 cm^{-1} in leaf extract and silver nanoparticles attributed to the –C-O-stretching vibration of water soluble components such as flavones, terpenoids and phenolic compounds. The band at 1022 and 1034 cm^{-1}can be assigned to C-N stretching vibration of primary amines respectively. The band at 1230 cm^{-1} are the bending vibration of C-N (amide III bands) in the proteins. The band observed in extract 688 cm^{-1} which corresponds to Cl^{-}stretching. Both the spectra shows band at 2923 and 2853 cm^{-1} in plant extract and 2925 and 2855 cm^{-1}in silver nanoparticles are arised from methylene antisymmetric and symmetric vibrational mode, these band intensity increased in the silver nanoparticles due to increase in the close packed structure of the biomolecules on the nanoparticles surface.[12] In the leaf extract FTIR spectrum shows band at 764 cm^{-1}, attributed to the bending vibration in the S-H moiety bonded to the CH_2 group. Mukherjee reported that the extract containing cysteine amino acid with S-H bonds which plays a key role in reducing the $AgNO_3$ solution.[34] This represents the release of some protein components into the reaction medium that may bind to the nanoparticles through cysteine residues in the proteins through hydrogen bond or it may cap the silver nanoparticles through electrostatic attraction preventing agglomeration and enhance the stability of the silver nanoparticles. The FTIR results revealed that native proteins which involved in the synthesis of silver nanoparticles and their secondary structure remained unaffected due to its reaction with Ag^+ ions or binding with the silver nanoparticles.[33] The FTIR spectroscopic study confirmed that the carbonyl group of amino acid residue has a stronger ability to bind silver, suggesting that proteins could possibly form a layer covering silver nanoparticles and acting as a capping agent to prevent agglomeration and provide stability to the nanoparticles.

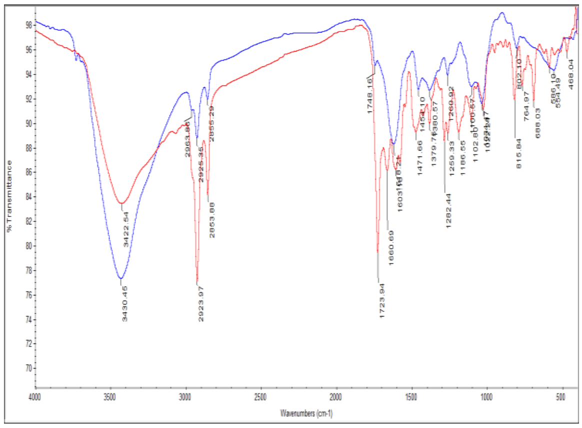

Figure 6: FTIR absorption spectra of aqueous leaf extract before and after complete bioreduction of silver ions

Energy Dispersive X-ray (EDX)

The energy dispersive x-ray spectrum (Figure 7) reveals the elemental nature of the synthesized silver nanoparticles which shows the strong signals of silver correspond to the peaks of the spectrum confirming presence of silver.

Detection of carbon and oxygen suggested the presence of carbohydrate compounds containing sulphur that were either capped on silver nanoparticles or were present in the background.[35] Silver nanoparticles are crystalline in nature and displays an optical absorption peak approximately at 3 keV due to Surface Plasmon Resonance.[36]

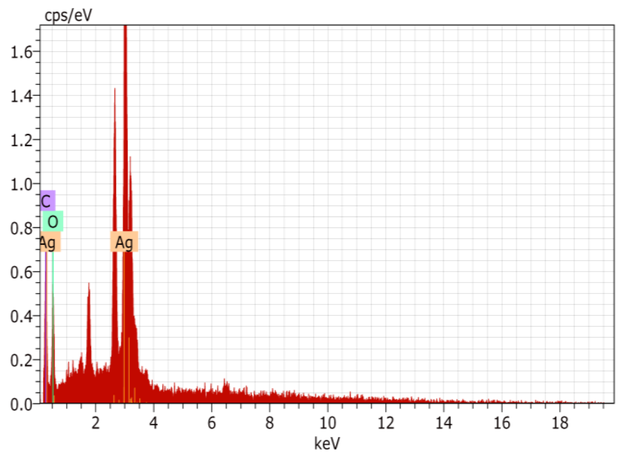

Figure 7: EDX analysis of silver nanoparticles synthesized from leaf extract of *L. indica*

Atomic Force Microscopy and HR-TEM

The atomic force microscopy (AFM) displays surface morphology of silver nanoparticles using *L. indica* leaf extract (Figure 8 a).

The particle size of the silver nanoparticles ranges from 30 to 70 nm were observed. The topographical images of silver nanoparticles are spherical and triangular in shape.

The HR-TEM images of synthesized silver nanoparticles are depicted in Figure 8b which shows the size, shape and crystallinity of nanoparticles.

According to microphograph images particles are spherical in shape and size ranges from 10 to 40nm.

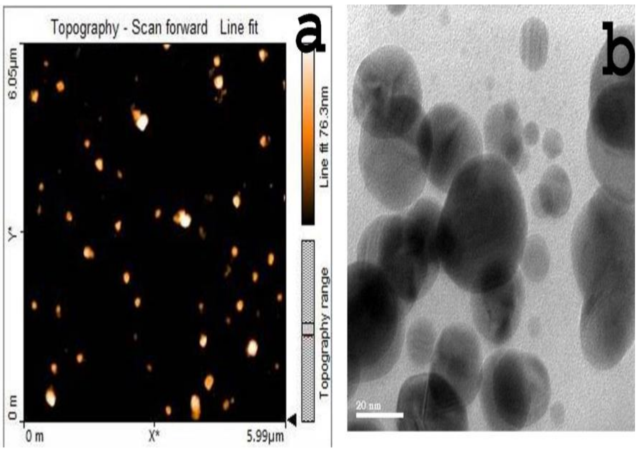

Figure 8: (a) AFM and (b) HR-TEM images of biogenic silver nanoparticles

Antimicrobial Activity

The silver nanoparticles exhibited good antibacterial activity against both gram positive and gram negative bacteria. *E.coli* and *S.typhi.* were more sensitive to silver nanoparticles than the *S.aureus* and *B. subtilis* Silver nanoparticles at different concentration (25µL, 100µL and 250µL) showed different diameter of inhibition zone (DIZ) with respect to different microorganisms (Figure 9). *S. typhi* have shown antimicrobial activity at lower concentration of silver nanoparticles at 100 µL but *S. aureus* and *B. subtilis* shows at 250 µL, no inhibition zone was observed in plant extract and *E.coli*. The researcher reported *S. aureus* were more sensitive to silver nanoparticles than *E.coli* used for assay of microbial activity.[37]

The present study reveals that an increased antimicrobial effect of antibiotics in combination with silver nanoparticles. The increased in activity was due to the synergistic action of both antibiotics and silver nanoparticles. The synergistic effect is a powerful tool against resistant microorganisms.

A strong correlation bonding between antibiotic and silver nanoparticles accelerates antimicrobial activity on the cell membrane at specific point. Silver nanoparticles facilitated the transport of antibiotics to the cell surface acting as a drug carrier.[38]

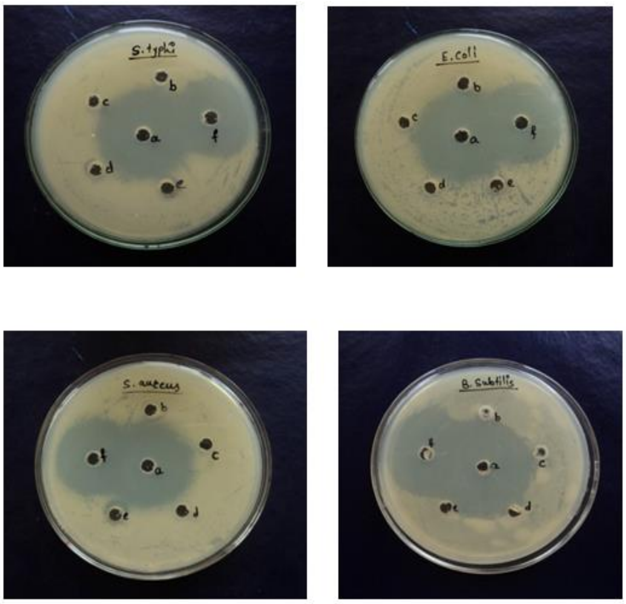

Figure 9: Antibacterial activity of (a) AgNPs+Antibiotics, (b) Plant extract, (c) 25µl (d) 100µl (e) 250µl and (f) Antibiotics

Silver nanoparticles were attached to the cell surface and alters the membrane permeability by immediate accumulation of envelope protein precursors, which results in dissipation of the proton motive force. The sulfur containing proteins in the membrane or inside the cells and phosphorus containing DNA are likely to be the preferential sites forbinding silver nanoparticles[39,40] and affecting the replication machinery, facilitating enhanced infiltration of the antibiotics into the cells. Shrivastava have reported that the silver nanoparticles may modulate the phosphotyrosine profile of putative bacterial peptides. This could affect cellular signaling and inhibit the growth of bacteria.[41]

The oligodynamic effect of silver has antibacterial activity against microorganisms.

The changes in the local electronic structure on the surface of the small sized particles led to the enhancement of their chemical reactivity leading to bactericidal effect. In the present study, a higher inhibition zone was observed for the gram-positive bacteria compared to other gram-negative bacteria.

This observation is in excellent agreement with earlier studies.[42-45] The differential sensitivity of gram negative and gram positive bacteria towards silver nanoparticles is possibly depends on cell surface characteristics.

The negative charge on the cell surface of gram-negative bacterial was higher than the cell surface of gram-positive bacteria.[46] The gram negative bacterial outer membrane consists of proteins, lipids and lipopolysaccharides (LPS) which acts as a barrier and provide effective protection against antibacterial agents.

But gram positive bacteria donot consist of an outer membrane so nanoparticles can easily enters and inhibits the growth.[47]

Table 1: Functional groups involved in the synthesis of silver nanoparticles

S. No.	Leaf Extract (before bioreduction) cm-1	Silver Nanoparticles (after bioreduction) cm-1	Functional Groups
1	3430	3416	N-H stretching
2	2925	2919	methylene antisymmetric and vibrational mode
3	2855	2850	methylene symmetric vibrational mode
4	1748	1748	Carbonyl stretching
5	1640	1618	Amide band I
6	1454	1471	methylene scissoring vibrations
7	1380	1379	–C-O-stretching vibration
8	1260	1223	bending vibration of C-N (amide III bands)
9	1022	1034	C-N stretching vibration of primary amines
10	764	-	bending vibration in the S-H moiety bonded to the CH_2 group
10	688	-	Cl^- stretching

CONCLUSION

The leaf extract of *L. indica* was used for synthesis of silver nanoparticles. The silver nanoparticles are crystalline in nature.

The size of silver nanoparticles synthesized by *L. indica*, ranges between 10 and 50 nm and are spherical and triangular in nature, polydispersed and uniform in size.

The production of silver nanoparticles was very rapid in *L. indica.* Plant mediated biosynthesis offers a rapid, cheap, clean, safe and eco-friendly approach. Synthesized nanoparticles showed good antimicrobial activity in combination of silver nanoparticles with antibiotic had synergistic antibacterial activity against *E.coli, S. typhi, S. aureus*and *B. subtilis*.

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REFERENCES

1. Matei A, Cernica L, Cadar O, Roman C, Schiopu V. Synthesis and characterization of ZnO polymer nanocomposite, The international journal of material forming, 1, 2008, 767-770.
2. Wiley BJ, Sun Y, Xia Y. Synthesis of silver nanostructure with controlled shapes and properties, Accounts of Chemical Research, 40, 2007, 1067-1076.
3. Narayanan R, El-Sayed MA. Shape-Dependent Catalytic Activity of Platinum Nanoparticles in Colloidal Solution, Nano Letters, 2004, 1343-1348.
4. Gomez-Romero P. Hybrid Organic–Inorganic Materials—In Search of Synergic Activity, Advanced materials, 13, 2001, 163-174.
5. Gracias DH, Tien J, Brcen T. White GMS, Forming electrical networks in three dimensions by self assembly, Science, 289, 2002, 1170-1172.
6. Alivisatos P. Semiconductor clusters, nanocrystals and quantum, Science, 271, 1996, 933-937.
7. Mubarakali D, Thajuddin N, Jeganathan K, Gunasekharan M. Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens, Colloids Surf, 885, 2011, 360-365.
8. Kelly KL, Coronado E, Zhaoand LI, Schatz GC. The optical properties of metal nanoparticles: the influence of size, shape, and dielectric environment, J. Phys. Chem., 107(103), 2003, 668-677.
9. Thangavel S, Ramaraj R. Polymer Membrane Stabilized Gold Nanostructures Modified Electrode and Its Application in Nitric Oxide Detection, J. Phys. Chem., 112, 2008, 19825.
10. Gardea-Torresdey JL, Parsons JG, Gomez E, Peralta-Videa J, Troiani HE, Santiago P, and Jose-Yacaman M. Formation and growth of Au nanoparticles inside live Alfalfa plants, Nano. Lett., 2, 2002, 397-401.
11. Gardea-Torresdey JL, Gomez E, Peralta-Videa J, Parsons JG, Troiani H, Jose-Yacaman M. Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract, Langmuir, 19, 2003 1357-1361.
12. Ankamwar B, Damle C, Ahmad A, Sastry M. Biosynthesis of gold and silver nanoparticles using *Emblica officinalis* fruit extract, their phase transfer and transmetallation in an organic solution, J. Nanosci. Nanotech., 5(10), 2005, 1665-1671.
13. Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M. Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract, Biotechnol. Prog., 22, 2006, 577-583.
14. Huang J, li Q, Sun D, Lu Y, Su Y, Yang X, Wang H, Wang W, Shao Y, He N, Hong J, Chen C. Biosynthesis of silver and gold nanoparticles by novel sun dried *Cinnamomum camphora* leaf, Nanotechnology, 18, 2007, 104-105.
15. Li S, Shen Y, Xie A, Yu X, Qiu L, Zhang L, Zhang Q. Green synthesis of silver nanoparticles using *Capsicum annuum* L. extract, Green Chem., 9, 2007, 852-858.

16. Ghosh S, Patil S, Ahire M, Kitture R, Jabgunde A, Kale S, Pardesi K, Bellare J, Dhavale DD, Cametra S, Chopade B. Synthesis of silver nanoparticles using *Dioscorea bulbifera* tuber extract and evaluation of its synergistic potential in combination with antimicrobial agents, International Journal of nanomedicine, 7, 2012, 483-496.

17. Santhanlakshmi KSJ, Viswanathan B, Muthumary J, Srinivasan K. Biosynthesis of silver nanoparticles using *Citrus sinensis* peel extract and its antibacterial activity, Spectrochimica Acta Part A, 79, 2011, 594-598.

18. Alagumuthu G, Kirirubha R. Green synthesis of silver nanoparticles using *Cissus quadrangularis* plant extract and their antibacterial activity." International Joural of Nanomaterials and Biostructures, 2, 2012, 30-33.

19. Ghoreishi SM, Behpour M, Khayatkashani M. Green synthesis of silver and gold nanoparticles using *Rosa damascene* and its primary application in electrochemistry, Physica E, 44, 2011, 97-104.

20. Rokhade VK, Taranath TC. Synthesis of Biogenic Silver Nanoparticles Using *Sesamum laciniatum* Klein ex Willd, Int. J. Pharm. Sci. Rev. Res., 29(1), 2014, 221-225.

21. Swamy VS, Ram P. Green synthesis of silver nanoparticles from the leaf extract of *Santalum album* and its antimicrobial activity, Materials, 4(3), 2012, 53-59.

22. Prasad KS, Pathak D, Patel A, Dalwadi P, Prasad R, Patel P, and Selvaraj K. Biogenic synthesis of silver nanoparticles using *Nicotiana tobaccum* leaf extract and study of their antibacterial effect, African journal of Biotechnology, 10(41), 2011, 8122-8130.

23. Rupali S, Patil R, Mangesh KS, Sanjay K. Bioinspired synthesis of highly stabilized silver nanoparticles using *Ocimum tenuiflorum* leaf extract and their antibacterial activity, Spectrochimica Acta Part A, 91, 2012, 234–238.

24. Vankar PS, Shukla D. Biosynthesis of silver nanoparticles using lemon leaves extract and its applications for antimicrobial finish on fabric, Applied Nanoscience, 2, 2012, 163-168.

25. Kiruba D, Vinothini GN, Subramanian KN, Sivakumar M. Biosynthesis of Cu, ZVI, and Ag nanoparticles using *Dodonaea viscose* extract for antibacterial activity against human pathogens, J. naopart Res., 15, 2013, 1319.

26. Vijayakumar M, Priya K, Nancy FT, Noorlidah A, Ahmed ABA. Biosynthesis, characterization and anti-bacterial effect of plant-mediated silver nanoparticles using *Artemisia nilagirica*, Industrial crops and products, 41, 2013, 235-240.

27. Mulvaney P. Surface plasmon spectroscopy of nanosized metal particles, Langmuir, 12, 1996, 788-800.

28. Pal S, Tak YK, Song JM. Does the Antibacterial Activity of Silver Nanoparticles depend on the Shape of the Nanoparticle? A Study of the Gram-Negative Bacterium *Escherichia coli*, Appl Environ. Microbiol. 73(6), 2007, 1712-1720.

29. Dubey SP, Lahtinen M, Sarkka H, Sillanpaa M. Bioprospective of *Sorbus aucuparia* leaf extract in development of silver and gold nanocolloids, Colloids and Surfaces B*:* Biointerfaces, 80, 2010, 26-33.

30. Smitha SL, Nissamudeen KM, Philip D, Gopchandran KG. Studies on surface plasmon resonance and photoluminescence of silver nanoparticles, Spectrochim. Acta A*:* Mol. Biomol. Spectroscopy, 71(1), 2008, 186-190.

31. Haung J, Lin L, Li Q, Sun D, Wang Y, Lu Y, He N, Yang K, Yang X, Wang H, Wang W, Lin W. Continuous-flow biosynthesis of silver nanoparticles by lixivium of sundried *Cinnamomum camphora* leaf in tubular microreactors, Ind. Eng. Chem. Res., 47, 2008, 6081-6090.

32. Chou KS, Lu YC, Lee HH. Effect of alkaline ion on the mechanism and kinetics of chemical reduction of silver, Mat. Chem. Phys., 94, 2005, 429-433.

33. Flett M St C 1963 *Characteristic Frequencies of Chemical Groups in the Infrared* (Oxford: Elsevier)

34. Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R, Sastry M. Extracellurlar biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*, Colloids and Surfaces B: Biointerfaces, 28, 2003, 313-318.

35. Mukherjee P, Roy M, Mandal BP, Dey GF, Mukherjee PK, Ghatak J, Tyagi AK, Kale SP. Green synthesis of highly stabilized nanocrystalline silver particles by a non-pathogenic and agriculturally important fungus *T. asperellum*, Nanotechnology, 19, 2008, 075103-109.

36. Sant DG, T. R. Gujarathi, S. R. Harne, S. Ghosh, R. Kitture, S. Kale, B. A. Chopade, & K. R. Pardesi. *Adiantum philippense* L. frond assisted rapid green synthesis of gold and silver nanoparticles, Journal of nanoparticles, 2013, 182320-182329.

37. Ruparelia JP, Chatterjee AK, Duttagupta SP, Mukherjis. Strain specificity in antimicrobial activity of silver and copper nanoparticles, Acta Biomater., 4(3), 2008, 707-716.

38. Magudapatty P, Gangopadhyayrans P, Panigrahi BK, Nair KGM Dhara S. Electrical transport studies of Ag nanoparticles embedded in glass matrix, Physica B, 299, 2001, 142-146.

39. Bragg PD, Rainnie DJ. The effect of silver ions on the respiratory chain of *Esclrerichia coli*, Can. J. Microbiol., 20, 1974, 889.

40. McDonnell, Russell AD. Antiseptics and Disinfectants: Activity, Action, and Resistance, Clin. Microbiol. Rev., 12, 1999, 179.

41. Shrivastava S, Bera T, Roy A, Singh G, Ramachandrarao P, Dash D. Characterization of enhanced antibacterial effects of novel silver nanoparticles, Nanotechnology, 18, 2007, 225103.

42. Cho KH, Park JE, Osaka T Park SG. The study of antimicrobial activity and preservative effects of nano silver ingredient, Electrochimica Acta, 51, 2005, 956-960.

43. Sharma VK, Yngard RA, Lin Y. Silver nanoparticles: Green synthesis and their antimicrobial activities, Advances in colloid and interface science, 145, 2009, 83-96.

44. Xu K, Wang J, Kang X, Chen J. Fabrication of antibacterial monodispersed Ag-SiO2 core-shell nanoparticles with high concentration, Materials Letters, 63, 2009, 31-33.

45. Xu X, Yang Q, Wang Y, Yu H, Chen X, Jing X. Biodegradable electrospun poly(L-lactide) fibers containing antibacterial silver nanoparticles, European polymer journal, 42, 2006, 2081-2087.

46. Chung TC, Su YP, Chen CC, Jia G, Wang HL, Wu JCC. Relationship between antibacterial activity of chitosan and surface characteristics of cell wall, Acta Pharmacologicasinica, 25, 2004, 932-936.

47. Maneerung T, Tokura S, Rujiravanit R. Impregnation of silver nanoparticles into bacterial cellulose for antimicrobial wound dsressing, Carbohydrate polymers, 72, 2008, 43-51.

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