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PHYTOSYNTHESIS OF SILVER NANOPARTICLES USING FRUIT EXTRACT OF *LEEA INDICA* (BURM. F.) MERR. AND THEIR ANTIMICROBIAL ACTIVITY

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

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ABSTRACT: Green nanotechnology is gaining importance due to the elimination of harmful chemicals and provides a safe, environmental friendly and economic method for synthesis of nanoparticles. In the present investigation fruit extract of *Leea indica* (Burm.f.) Merr. was used for the synthesis of silver nanoparticles. Aqueous fruit extract were added to 1 mm silver nitrate solution by changing the colour of reaction mixture from colourless to brown colour indicates the formation of silver nanoparticles further, confirmed by characteristic UV-Vis absorption peak at 400 nm. Synthesized nanoparticles were characterized by FTIR, EDAX, AFM and HR-TEM. The FTIR data revealed that bio-molecules 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.*

INTRODUCTION: The designing the method of synthesis, in which the size, morphology, stability and properties are controlled has become a major field of interest [1]. Nanoparticles are present at a higher surface-to-volume ratio with decreasing size of nanoparticles. Nanoparticles possess unique optical and electronic properties compared to bulk material. Nanomaterial is used because of their unique physical, mechanical, optical and electromagnetic properties. It has been reported that since ancient times silver is known to have antimicrobial activity [2], antiviral [3], and anti-plasmodial activity [4], anti-inflammatory [5], anti-cancer [6, 7] and anti-diabetic [8].

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The synthesis of nano-materials is gaining attention in recent years because of their properties which make them useful for catalysis, sensor technology, biological labelling, optoelectronics recording media and optics. Nanoparticles can be produced by different source viz. gas, liquid or solid phase. They can be synthesized by physical and chemical methods [9]. But these methods have certain disadvantages due to involvement of toxic chemicals. A large amount of toxic chemicals are produced during the synthesis of nano-materials 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 method for synthesis of nanoparticles by bio mimetic approach. The various plant extracts were used for the synthesis of silver and gold nanoparticles such as alfalfa [10, 11] and *Santalum album* [12]. Biogenic silver nanoparticles synthesized using *Nicotina tobaccum* leaf extract [13], *Ocimum* *tenuiflorum* [14], lemon leaves [15], *Dodonaea viscose* [16], *Sesamum laciniatum* [17] and *Artemisia nilagirica* [18] were evaluated for their antibacterial activity against certain bacteria. Biogenic silver nanoparticles were synthesized by using seed extract *Calendula officinalis* [19], and *Azadirachta indica* [20] were evaluated for their antibacterial activity against certain bacteria. Silver nanoparticles were synthesized by using plant extracts from different origin *Cucurbita maxima* (petals), *Moringa oleifera* (leaves) and *Acorus calamus* (Rhizome) and their anticancer activity against epidermoid A431 carcinoma [21] *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, diarrhoea, dysentery and skin diseases. The present investigation is undertaken to study phytosynthesis of silver 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 fruit of *L. indica* were collected from botanical garden of Karnatak University, Dharwad.

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* fruit extract was added into 95 ml of aqueous solution of 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.

Characterization of silver nanoparticles: The bio-reduction of the Ag+ ions by the fruit extracts of *L. indica* leading to formation of silver nanoparticles were evident by colour change 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 between the ranges from 300 to 800 nm. FTIR characterization of reaction mixture using FTIR (F-7000FL) spectrophotometer for identification of bio-molecules involved in bio-reduction and capping of silver nanoparticles. 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 re-dispersed in 10 mL sterile distilled water. The centrifuging and re-dispersing 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 for 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 biogenic silver nanoparticles was assessed by well diffusion method against gram negative (*Escherichia coli* and *Salmonella typhi)* and gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) microorganisms. Initially, the stock cultures of bacteria were revived by inoculating broth media and grown at 37ºC 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 desired quantity of nanoparticles solution. All the plates were incubated at 37ºC for 24 h and the diameter of inhibition zone was noted.

RESULTS AND DISCUSSION: The fruit extract of *L. indica* was added to 1mM silver nitrate solution for the synthesis of silver nanoparticles. The colour of reaction mixture changes from colourless 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 [22]. The UV-Vis spectra show a sharp intense absorption peak at 400 nm due to the Surface Plasmon Resonance (SPR) band of silver nanoparticles **(Fig. 1)**. The appearance of single prominent peak shows particles are spherical and uniform in size [23].

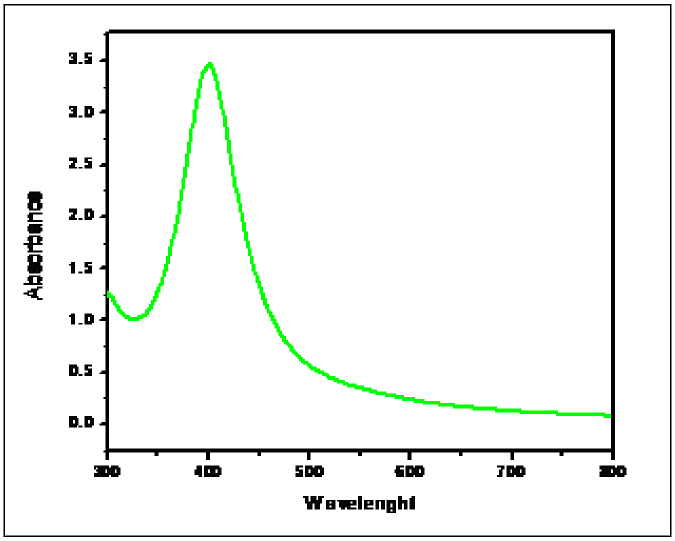

FIG. 1: UV-VIS SPECTRA OF SILVER NANOPARTICLES

FTIR Study: The FTIR data of fruit extract and silver nanoparticles reveals the possible bio-molecules and chemical change of the functional groups involved in bio-reduction and formation of silver nanoparticles **(Fig. 2 and table 1)**. Both the spectra exhibit a broad intense band at 3427 cm^{-1} and 1633 and 1631 cm^{-1} respectively in fruit 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 weak peak appears at 1384 cm^{-1} in the silver nanoparticles may be arised due to C-O stretching of the carboxylate ions present in the amino acid residue. The band at 1034 and 1035 cm^{-1}can be assigned to C-N stretching vibration of primary amines respectively. The band at 1261 and 1265 cm^{-1} are the bending vibration of C-N (amide III bands) in the proteins. The fruit extract shows peak at 1462 cm^{-1} is assigned to methylene scissoring vibrations from the proteins in the solution [24]. Both the spectra shows band at 2854 and 2924 cm^{-1} in plant extract and 2854 and 2924 cm^{-1}in silver nanoparticles are arised from methylene anti-symmetric and symmetric vibrational mode, these band intensity increased in the silver nanoparticles due to increase in the close packed structure of the bio-molecules on the nanoparticles surface [25].

The peaks shift from 1742 to 1745 cm-1 in the spectra of fruit extract and silver nanoparticles could be assigned to C-O (carbonyl) stretching vibration of primary amines. The shift in the band position suggests that functional groups were oxidized during the reaction and were responsible for the formation of 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 [24]. 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.

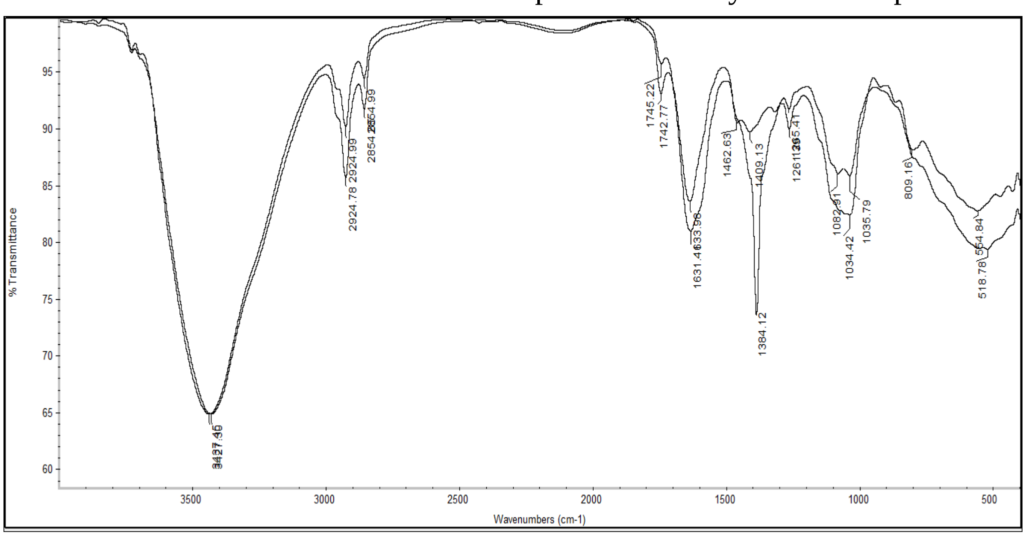

FIG. 2: FTIR ABSORPTION SPECTRA OF AQUEOUS LEAF EXTRACT BEFORE AFTER COMPLETE BIOREDUCTION OF SILVER IONS

TABLE 1: SHOWS FUNCTIONAL GROUPS INVOLVED IN THE SYNTHESIS OF SILVER NANOPARTICLES

S. No	Fruit extract cm-1	Silver nanoparticles cm-1	Functional groups
1	1034	1035	C-N stretching vibration of primary amines
2	1261	1265	Amide band III
3	-	1384	-C-O-stretching of the carboxylate ions
4	1462	-	Methylene scissoring vibrations
5	1633	1631	Amide band I
6	1742	1745	Carbonyl stretching
7	2854	2854	Methylene anti-symmetric vibrational mode
8	2924	2924	Methylene symmetric vibrational mode
9	3427	3427	N-H/O-H stretching

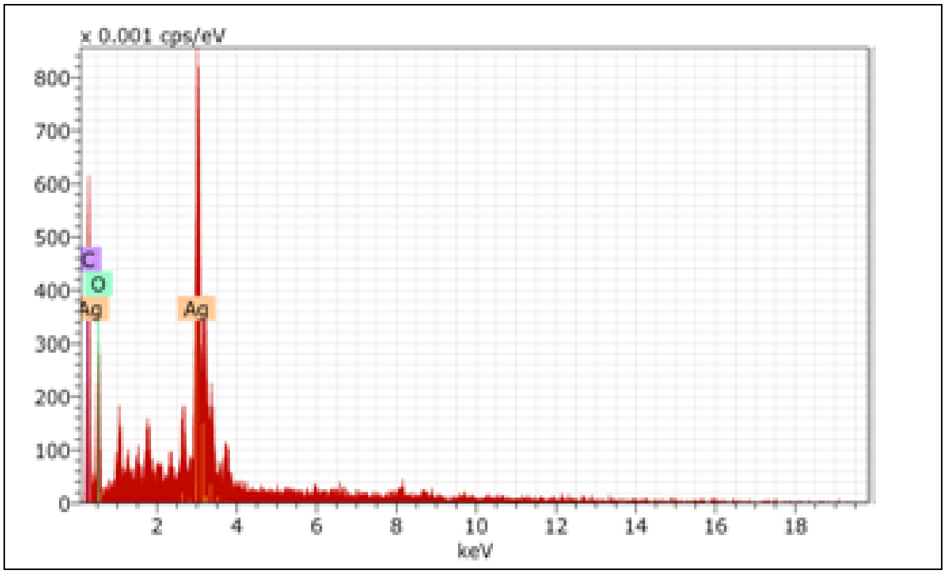

FIG. 3: EDX ANALYSIS OF SILVER NANOPARTICLES SYNTHESIZED FROM FRUIT EXTRACT OF *L. INDICA*

Energy Dispersive X-ray (EDX): The energy dispersive x-ray spectrum (Fig. 3) 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. Silver nanoparticles are crystalline in nature and display an optical absorption peak approximately at 3 keV due to Surface Plasmon Resonance [26].

Atomic force microscopy (AFM): AFM image reveals that surface morphology, shape and size of silver nanoparticles. The silver nanoparticles sizes ranges from 20-40 nm and are uniformly sized and spherical in shape **(Fig.4)**.

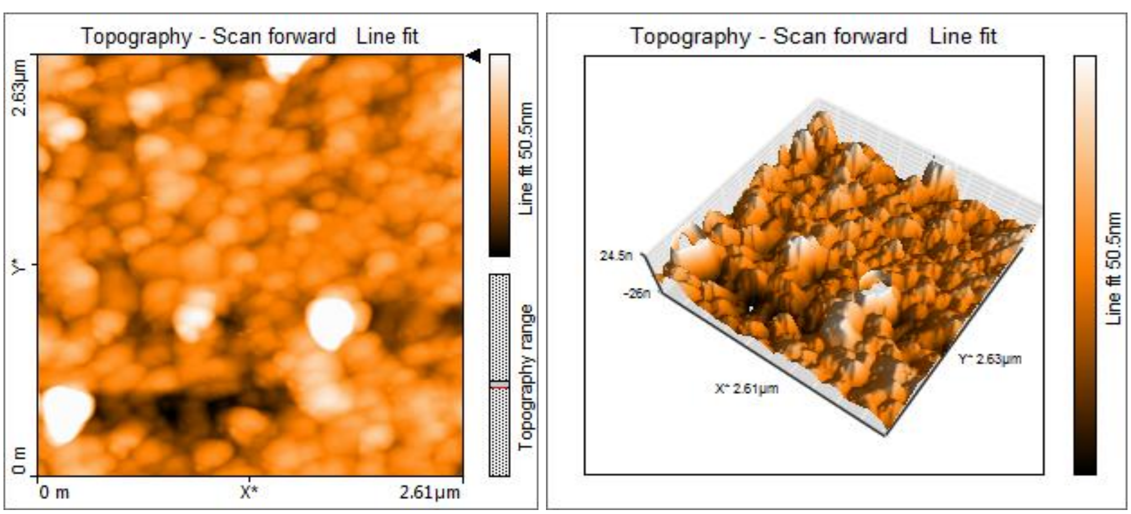

FIG. 4: AFM IMAGES OF BIOGENIC SILVER NANOPARTICLES

High-resolution transmission electron microscopy (HR-TEM): The morphology, size and shape of silver nanoparticles were further asserted by HR-TEM **(Fig. 5)**. It was confirmed that silver nanoparticles possess uniform size 10-40 nm, almost spherical shape and uniformly sized.

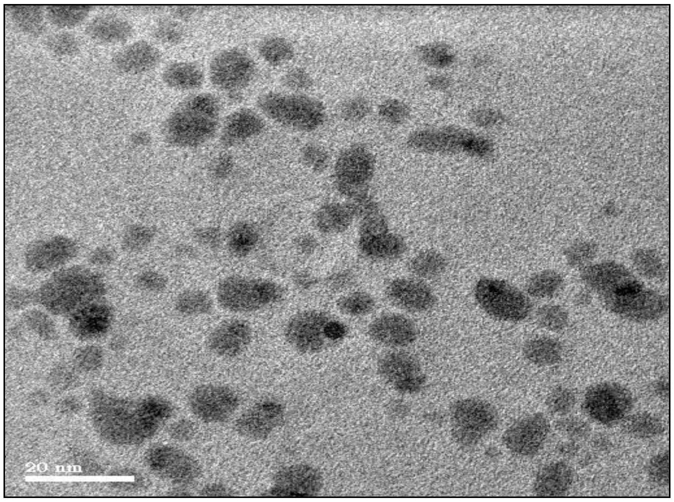

FIG. 5: HR-TEM IMAGES OF BIOGENIC SILVER NANOPARTICLES

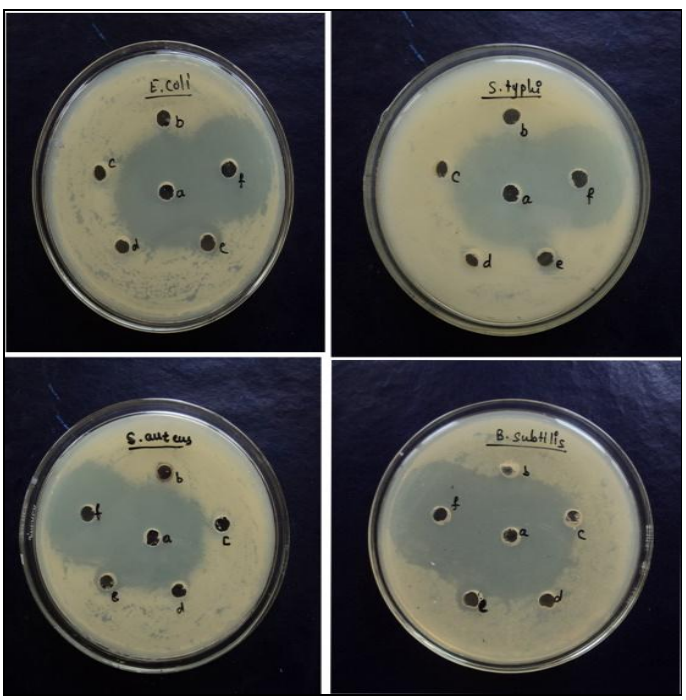

FIG. 6: ANTIBACTERIAL ACTIVITY OF (A) AgNPs +ANTIBIOTICS, (B) PLANT EXTRACT, (C) 25µL (D) 100µL (E) 250µL AND (F) ANTIBIOTICS

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 6). The researcher reported that *S. aureus* were more sensitive to silver nanoparticles than *E. coli* used for assay of microbial activity [27]. 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 [28]. 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 sulphur containing proteins in the membrane or inside the cells and phosphorus containing DNA are likely to be the preferential sites for binding silver nanoparticles [29, 30] and affecting the replication machinery, facilitating enhanced infiltration of the antibiotics into the cells. The differential sensitivity of gram negative and gram positive bacteria towards silver nanoparticles are 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 [31]. The gram negative bacterial outer membrane consists of proteins, lipids and lipo-polysaccharides (LPS) which acts as a barrier and provide effective protection against antibacterial agents.

CONCLUSION: The fruit extract of *L. indica* was used for synthesis of silver nanoparticles. The silver nanoparticles are crystalline in nature. The sizes 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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CONFLICT OF INTEREST: The author has none to declare.

REFERENCES:

1. Wiley BJ, Sun Y and Xia Y: Synthesis of silver nanostructure with controlled shapes and properties. Accounts of Chemical Research Review 2007; 40:1067-1076.
2. Kaviya S, Santhanalakshmi K, Viswanathan B, Muthumary J and Srinivasan K: Biosynthesis of silver nanoparticles using *Citrus sinensis* peel extract and its antibacterial activity. Spectrochim. Acta A 2011; 79:594-598.
3. Suriyakalaa U, Antony JJ, Suganya S, Siva D, Sukirtha R, Kamalakkannan S, Pichiash PBT and Archiraman S: Hepatocurative activity of biosynthesized silver nanoparticles fabricated using *Andrographis paniculata*. Colloid Surf. B 2013; 102:189-194.
4. Jayaseelan C, Rahuman AA, Rajakumar G, Kirthi AV, Santhoshkumar T and Marimuthu S: Synthesis of pediculocidal and larvicidal silver nanoparticles by leaf extract from heart leaf moon seed plant *Tinospora cordifolia* Miers. Parasitol. Res. 2011; 109:185-194.
5. Jacob SJP, Finub JS and Narayanan A: Synthesis of silver nanoparticles using *Piper longum* leaf extracts and its cytotoxic activity against Hep-2 cell line. Colloid Surf. B 2012; 91:212-214.
6. Akhtar MS, Panwar J and Yun YS. Biogenic synthesis of metallic nanoparticles by plant extracts. ACS Sustain. Chem. Eng. 2013; 1:591-602.
7. Das S, Das J, Samadder A. Bhattacharyya SS, Das D and Khuda-Bukhsh AR: Biosynthesized silver nanoparticles by ethanolic extracts of *Phytolocca decandra*, *Gelsemium sempervirens*, *Hydrastis canadensis* and *Thuja occidentalis* induce differential cytotoxicity through G2/M arrest in A375 cells. Colloid Surf. B 2013; 101:325-336.
8. Swarnalatha C, Rachela S, Ranjan P and Baradwaj P: Evaluation of invitro antidiabetic activity of *Sphaeranthus amaranthoides* silver nanoparticles. Int. J. Nanomat. Biostr. 2012; 2:25-29.
9. Iravani S, Korbekandi H, Mirmohammadi SV and Zolfaghari B:Synthesis of silver nanoparticles: chemical, physical and biological. Res. Pharm. Sci. 2014; 9(6):385-406.
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. 2002; 2:397-401.
11. Gardea-Torresdey JL, Gomez E, Peralta-Videa J, Parsons JG, Troiani H and Jose-Yacaman M: Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract. Langmuir 2003; 19:1357-1361.
12. Swamy VS, Ram P: Green synthesis of silver nanoparticles from the leaf extract of *Santalum album* and its antimicrobial activity. Journal of Optoelectronic and Biomedical Materials 2012; 4 (3):53–59.
13. 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 2011; 10(41):8122-8130.
14. Rupali S, Patil R, Mangesh KS and Sanjay K: Bio-inspired synthesis of highly stabilized silver nanoparticles using *Ocimum tenuiflorum* leaf extract and their antibacterial activity. Spectrochimica Acta Part A 2012; 91: 234– 238.
15. Vankar PS and Shukla D: Biosynthesis of silver nanoparticles using lemon leaves extract and its applications for antimicrobial finish on fabric. Applied Nano-science 2012; 2:163-168.
16. Kiruba D, Vinothini GN, Subramanian KN and Sivakumar M: Biosynthesis of Cu, ZVI, and Ag nanoparticles using *Dodonaea viscose* extract for antibacterial activity against human pathogens. J. naopart Res. 2013; 15:1319.
17. Rokhade VK and Taranath TC: Synthesis of Biogenic Silver Nanoparticles Using *Sesamum laciniatum* Klein ex Willd . Int. J. Pharm. Sci. Rev. Res., 2014; 29(1): 221-225.
18. Vijayakumar M, Priya K, Nancy FT, Noorlidah A and Ahmed ABA: Biosynthesis, characterization and anti-bacterial effect of plant-mediated silver nanoparticles using *Artemisia nilagirica*. Industrial crops and products 2013; 41: 235-240.
19. Baghizadeh A, Ranjbar S, Gupta VK, Asif M, Pourseyedi S, Karimi MJ and Mohammadinejad R: Green synthesis of silver nanoparticles using seed extract of *Calendula officinalis* in liquid phase. Journal of molecular liquids 2015; 207:159-163.
20. Verma A and Mehata MS: Controlled synthesis of silver nanoparticles using Neem leaves and their antimicrobial activity. Journal of radiation Research and applied sciences 2016; 9:109-115.
21. Nayak D, Pradhan S, Ashe S, Rauta PR and Nayak B: Biologically synthesized silver nanoparticles from three diverse family of plant extracts and their anticancer activity against epidermoid A431 carcinoma. Journal of colloid and Interface science 2015; 457:329-338.
22. Mulvaney P: Surface plasmon spectroscopy of nano-sized metal particles. Langmuir 1996; 12:788-800.
23. Pal S, Tak YK and 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 2007; 73(6):1712-1720.
24. Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R and Sastry M: Extracellurlar biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Colloids and Surfaces B: Biointerfaces 2003; 28**:** 313-318.
25. Ankamwar B, Damle C, Ahmad A and 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. 2005; 5(10):1665-1671.

26. Sant DG, Gujarathi TR, Harne SR, Ghosh S, Kitture R, Kale S, Chopade BA and Pardesi KR:Adiantum philippense L. frond assisted rapid green synthesis of gold and silver nanoparticles. Journal of nanoparticles 2013; 182320-182329.
27. Ruparelia JP, Chatterjee AK, Duttagupta SP, and Mukherjis: Strain specificity in antimicrobial activity of silver and copper nanoparticles. Acta Biomater., 2008; 4(3): 707-716.
28. Magudapatty P, Gangopadhyayrans P, Panigrahi BK, Nair KGM and Dhara S:Electrical transport studies of Ag nanoparticles embedded in glass matrix. Physica B, 2001; 299: 142-146.
29. Bragg PD, and Rainnie DJ:The effect of silver ions on the respiratory chain of *Esclrerichia coli*. Can. J. Microbiol. 1974; 20:889.
30. McDonnell, AND Russell AD: Antiseptics and Disinfectants: Activity, Action, and Resistance. Clin. Microbiol. Rev. 1999; 12: 179.
31. Chung TC, Su YP, Chen CC, Jia G, Wang HL and Wu JCC: Relationship between antibacterial activity of chitosan and surface characteristics of cell wall. Acta Pharmacologicasinica, 2004; 25:932-936.

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