Research Article

Phytosynthesis of Silver Nanoparticles Using the Leaf Extract of *Diospyros malabarica* (desr.) Kostel and its Antibacterial Activity Against Human Pathogenic Gram Negative *Escherichia coli* and *Pseudomonas aeruginosa*.

T. C. Taranath *, B. R. Hedaginal, P. Rajani, M. Sindhu.
P.G. Dept of Botany, Karnataka University, Dharwad, Karnataka, India.
***Corresponding author's E-mail:** tctaranath@rediffmail.com

Accepted on: 06-12-2014; **Finalized on:** 31-01-2015.

ABSTRACT

Biosynthesis of nanoparticles using higher plants is an emerging area of research in nanoscience and nanotechnology. The present investigation reports a simple eco-friendly method for synthesis of silver nanoparticles using *Diospyros malabarica* (Desr.) Kostel. Leaf extract serve as a source of reducing and capping agents. 1 mM solution of silver nitrate was treated with the aqueous extract of leaf leading to the formation of Ag-NPs was observed visually by change in color from colorless to brown color reaction mixture and confirmed by the surface Plasmon resonance peak at 417 nm in UV-Vis spectroscopy. Further, reduced silver nanoparticles were characterized by Fourier transform infrared spectroscopy (FTIR), atomic force microscopy (AFM) and high resolution transmission electron microscopy. FTIR data reveals the possible functional group of biomolecules involved in the bioreduction and capping for efficient stabilization of Ag-NPs. Atomic force microscopy and High resolution transmission electron microscopy studies showed that the spherical shape silver nanoparticles with size ranges from 10 nm to 50 nm. Finally, biogenic silver nanoparticles tested for antimicrobial activity they showed good zone of inhibition against *E. coil* and *P. aeruginosa*.

Keywords: Phytosynthesis, *Diospyros malabarica* (Desr.) Kostel., HR-TEM, AFM, Antibacterial activity.

INTRODUCTION

Nanoparticles are viewed as the fundamental building blocks of nanotechnology.[1] Nano size particles are quite unique in nature because nano size increase surface to volume ratio and also its physical, chemical and biological properties are different from bulk material. So the main aim to study its minute size is to trigger chemical activity with distinct crystallography that increases the surface area.[2-3]

Silver nanoparticles (Ag-NPs) have become focus of intensive research owing to their wide range of application in areas such as catalyst, optics, antimicrobials and biomaterial production. Ag-NPs exhibit new or improved properties depending upon their size, morphology and distribution.[4] The processes used for nanoparticle synthesis are chemical, physical and recently developed biological method. Chemical methods have various drawbacks including the use of toxic solvents, generation of hazardous by-products and high energy consumption, which pose potential risks to human health and to the environment. Most of the physical methods deal with enormous consumption of energy to maintain the high pressure and temperature employed in the synthesis procedure.

Therefore, the biological method has an advantage over chemical and physical method of nanoparticle synthesis, as it is cost effective and environmentally friendly.[5] The major biological systems involved in this are bacteria; fungi[6] and plant extract[7]. In recent years, the biosynthesis of nanoparticles using plant extracts has gained more importance. The synthesis and application of Ag-NPs from several plants have been studied by many researchers.[8-11]

Diospyros malabarica (Desr.) Kostel. belongs to the family Ebenaceae, is evergreen and it has high medicinal value used in treatment of dyspepsia, leprosia, diarrhoea, dysentry, haemorrhages, skin burning, diabetes, spermatorrhea, vaginal diseases, wounds, flatulence, prolepsis, scabies and intermittent fever.

In this study, for the first time we evaluated the synthesis of silver nanoparticles using the leaf extract of *D. malabarica* and their antibacterial activities against human pathogenic gram negative *Escherichia coli* and *Pseudomonas aeruginosa.*

MATERIALS AND METHODS

Preparation of Leaf Extract

Leaves of *D. malabarica* were collected from the Botanical garden at Karnataka University Dharwad, Karnataka, India. Leaves were washed 2-3 times with tap water followed by double distilled water to remove dust and impurities.

Leaves were shade dried to remove the residual moisture and weighed about 25g. The weighed leaves were cut into small pieces and boiled in a glass beaker containing 250ml of sterile distilled water for 20 minutes.[12]

The aqueous extract was separated by filtration with Whatman No. 1 filter paper and stored in refrigerator at 4 ˚C for further use.

Preparation of Silver Nitrate Solution

1 mM $AgNO_3$ solution was prepared by dissolving 0.08493g $AgNO_3$ (AR grade) in 500ml double distilled water.

Phytosynthesis of Silver Nanoparticles

For reduction of silver ions, 10 ml of plant extract was added to 250 ml Erleyenmeyer flask containing 90ml of 1mM aqueous $AgNO_3$ solution. Simultaneously, the reaction mixture was adjusted to pH 10. Then the flask containing reaction mixture was incubated at 30-40 ˚C, resulting in the formation of pale yellow to dark brown solution indicating the synthesis of silver nanoparticles.[13]

Characterization

The bioreduction of silver ions in the solution was monitored by using UV-Vis spectroscopy (Jasco V- 670 UV-Vis NIR spectrophotometer) operated at resolution of 1nm. The solution containing bioreduced silver ions was centrifuged at 3000 rpm for 40 min to remove the unwanted biomass residue; the resulting suspension was then dispersed in 10ml of double distilled water and centrifuged again at the same condition. Redispersion and centrifugation process was repeated for 2-3 times to obtain the pellet of silver nanoparticles free from any biomass residue. A sample taken from pellet was dispersed on a slide and dried slide was observed on contact mode of AFM. The pellet thus obtained was redispersed in double distilled water and oven dried at 60 ˚C to obtain the powder. The powder was used for FTIR and HRTEM (TECNAI 20 G2-electron microscope) analysis.

Antibacterial Activities

The silver nanoparticles synthesized using *D. malabarica* leaf extract was tested for antibacterial activity by agar well diffusion method against human pathogenic Gram negative *Escherichia coli (NCIM 2931)* and *Pseudomonas aeruginosa (NCIM 5029).* This method depends on the radial diffusion of an antibiotic from the well through semisolid agar layer in Petri plate, which prevents the growth of bacteria in a circular area or the zone around the well.

The pure cultures of bacteria were sub-cultured on nutrient broth at 35 ˚C. The hot sterile medium was poured into the sterile Petri plates to form 2-3 mm thick uniform layer and allowed to solidify. Each strain was swabbed uniformly on the individual plates using sterile cotton swab. Wells of size 6 mm were made on nutrient agar plates using gel puncture. 15, 30, 45 and 60 µl of nanoparticle solution was poured onto each well on all plates using micropipette. After incubation at 37 ˚C for 24h, the diameter of zone of inhibition was measured in millimeters and tabulated.

RESULTS AND DISCUSSION

The present study was carried out to synthesize Ag-NPs using leaf extract of *D.malabarica* to study their biological properties. Nanoparticles are generally characterized by their size, shape, surface area and dispersity. Homogeneity of these properties is important in many applications.[14] When the leaf extract was mixed with $AgNO_3$ pH was adjusted to 10 and incubated at 30-40 ˚C. The color of the reaction mixture changes from pale yellow to dark brown within few seconds as shown in Fig-1, indicating the formation of AgNPs. It is an efficient and rapid method, which was very well explained by other researchers who worked with different plant system.[15-18] The change in color was due to the excitation of surface Plasmon resonance in the metal nanoparticles[11]. Our results are in conformity with Kiruba who reported the formation of AgNPs with adjustment of pH 10. Acidic condition suppresses the formation of AgNPs (pH 2 and 4); whereas the slight basic condition enhances the formation of the nanoparticles (pH 6-8). Large sized nanoparticles were formed at lower pH which is indicated by the color change and aggregation in the solution, but small and highly dispersed nanoparticles were formed at pH 8-10.[12]

UV-Vis absorption spectroscopy is one of the main tools to analyze the formation of metal nanopartcles in aqueous solution.[19] The AgNPs formation was confirmed by UV-Vis spectrophotometric analysis. The analysis confirmed surface plasmon resonance peak at 417 nm. (Figure 2). This SPR bands which was specific to the substrate or an organism involved in the biosynthesis of AgNPs. It clear that bioreduction of silver ions occurred in presence of leaf extract. Saifuddin reported SPR band at 410 nm in *Bacillus subtilis.*[20] and 400 nm in *E.coli* by Natarajan.[21] Similarly Baishya reported the strong SPR band at 418 in case of *Bryophyllum pinnatum* (Lam)[22]. Kiruba reported that the characteristic SPR of colloidal nanoparticles ranges between 390 - 420 due to the Mie scattering in case of *Dodonaea viscose* leaf extract.[12-23]

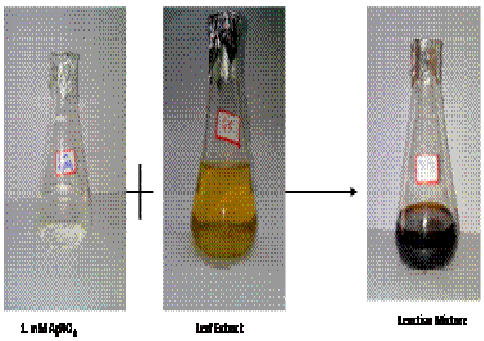


Figure 1: Color change during silver nanoparticle synthesis.

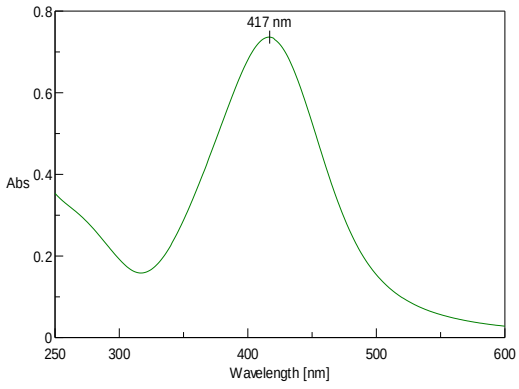


Figure 2: UV-Vis spectrum of AgNPs in an aqueous solution.

Table 1: FTIR absorption peaks value and their functional groups

S. No.	Absorption peak (cm^{-1})	Functional groups
1	3426.10	O-H stretching H-bonded alcohols and phenols
2	2925.22	O-H stretch, carboxylic acid
3	2857.89	Asymmetric stretching of the C-H group
4	1622.56	Amide group from carbonyl stretch in proteins
5	1384.51	C-C , C-N stretching
6	1115.02	Aromatic amines
7	825.20	C-C and C-H phenyl ring substitute
8	673.75	Aromatic compounds

FTIR data reveals the possible functional group of biomolecules involved in the bioreduction and capping for efficient stabilization of AgNPs synthesized using *D. malabarica*. The FTIR spectrum (Figure 3) showed the peaks at 3426.10 cm^{-1}, 2925.22 cm^{-1}, 2857.89 cm^{-1}, 1622.56 cm^{-1}, 1384.51 cm^{-1}, 1115.02 cm^{-1}, 825.20 cm^{-1} and 673.75 cm^{-1}. The broad intense peak at 3426.10 cm^{-1} is associated with inductive O-H stretching bonded to alcohols and phenols arising from carbohydrates and proteins present in the sample.[24] The two small peaks at about 2925.22 cm^{-1} and 2857.89 cm^{-1} are found to be associated with the carboxylic acid and methylene antisymmetric and symmetric vibration of hydrocarbon.[24-25] The band 1622.56 cm^{-1} was characteristics of amide associated with the stretch of carbonyl group coupled to amide linkage.[26] The peak at 1384.51 cm^{-1} arises due to NO^{-}_{3} and associated with C-N stretching vibration of aliphatic and aromatic amines indicating the presence of protein in small concentration.[27] The absorption peak at 825.20 cm^{-1} was associated with C-C and C-H phenyl ring substitute and 673.75 cm^{-1} was with aromatic compounds.[28] These functional groups associated with polyphenolic compounds as flavonoids and also to proteins indicate their involvement in capping of formed nanoparticles leading to stabilization.

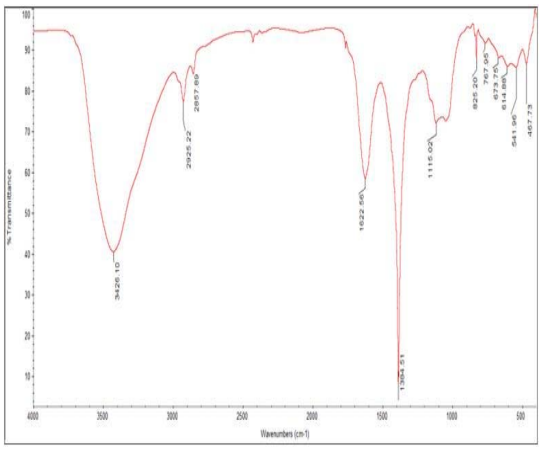


Figure 3: FTIR spectrum of silver nanoparticles.

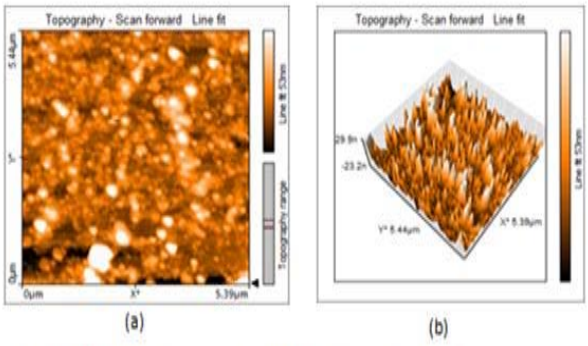


Figure 4: AFM images of silver nanoparticles synthesized from *D. malabarica* (Desc.) Kostel Topography (a) and 3D image (b).

The synthesized silver nanoparticles were characterized by AFM. The topographic image of silver nanoparticles was shown in Figure 4(a). Where the formation of spherical silver nanoparticles and its agglomeration was clearly observed and Figure 4(b) represents three dimensional views of synthesized silver nanoparticles. The size of the silver nanoparticles ranges from 10-50 nm. AFM images were taken with silicon cantilevers with force constant and the particle size was measured using line profile. This could be attributed to the fact that the compounds present in the leaf extract were responsible for the particle morphology and were kinetically controlled.[29]

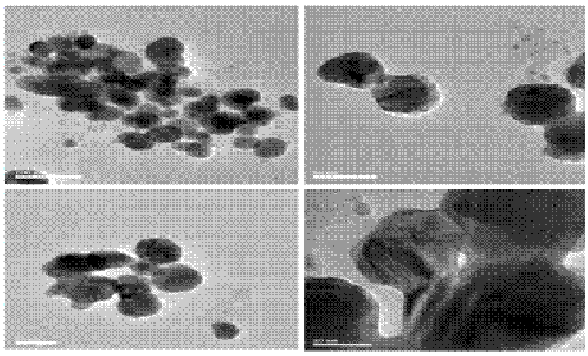


Figure 5: HR-TEM image of silver nano particle synthesized from *D. malabarica*.

The silver nanoparticles were further characterized by HRTEM micrograph, these Silver nanoparticles showed spherical shape with the size range from 10 to 50nm. (Figure 5) also shows that the biomolecules of leaf extract bounded with the nanoparticles acts as capping agents to hinder further oxidation of nanoparticles. Hence from the HRTEM analysis, it was confirmed that all the particles (AgNPs) exist in the nanoscale range and possess spherical shape.

Antibacterial Activity of Silver Nanoparticles

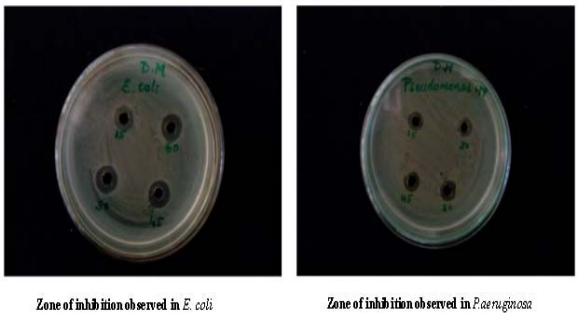
The present investigation reveals that the silver nanoparticles synthesized by leaf extract of *D. malabarica* exhibited antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa*. The inhibitory zone of two replicates of diameter was measured and tabulated

(Table 2). The antibacterial activity of silver nanoparticles tested against gram negative *Escherichia coli* showed an inhibition zone of 13, 14, 14.5 and 15 mm for concentration of 15, 30, 45 and 60 µl respectively and 10, 10.4, 11 and 12mm for concentration of 15, 30, 45, and 60 µl respectively against gram negative *Pseudomonas aeruginosa* (Figure 6). Comparatively maximum zone of inhibition 15 mm was observed against *E. coli* and 12 mm against *Pseudomonas aeruginosa* at 60 µl. It was notice that the zone of inhibition increased with increased concentration of Ag NPs.[30-31] Similarly, Dipankar and Murugan have reported dose-dependent inhibition by Ag NPs synthesized from *Iresine herbstii* leaf aqueous extract.[32] This might be due to the denaturation of bacterial cell wall, blocking bacterial respiration, destabilization of outer membrane, and depletion of intracellular ATP.[33] *E. coli* and *P. aeruginosa* are gram negative bacteria, thus cell wall possesses thinner peptidoglycan layer.[34] The high bactericidal activity is certainly due to the silver cat ions released from Ag nanoparticles that act as reservoirs for the Ag+ bactericidal agent. Changes in the bacterial membrane structure bacteria as a result of the interaction with silver cat ions leads to the increased membrane permeability[35-36] Lin explained that in general, silver ions from silver nanoparticles are believed to become attached to the negatively charged bacterial cell wall and rupture it, which leads in to denaturation of protein and finally cell death.[37] The attachment of either silver ions or nanoparticles to the cell wall causes accumulation of an envelope protein precursor, which results in dissipation of the proton motive force. On the other hand, silver nanoparticles exhibited destabilization of the outer membrane and rupture of the plasma membrane, thereby causing depletion of intracellular ATP.[38] Sarkar reported that for *E. coli* (ATCC 10536) and *Staphylococcus aureus* (ML 422), silver nanoparticles demonstrated greater bactericidal efficiency compared to penicillin.[39] Silver has a greater affinity to react with sulphur or phosphorus-containing biomolecules of the cell. Thus, sulphur-containing proteins the membrane or inside the cells and phosphorus-containing elements like DNA are likely to be the preferential sites for silver nanoparticle binding.[40-41]

Table 2: The biosynthesized silver nanoparticles showing antibacterial activity against *E. coli* and *P. aeruginosa.*

Silver nanoparticle sample	Zone of inhibition(mm)against pathogenic bacteria							
	E. coli				*P. aeruginosa*			
	15µl	30µl	45µl	60µl	15µl	30µl	45µl	60µl
***D. malabarica* (Desr) Kostel.**	13mm	14mm	14.5mm	15mm	10mm	10.4mm	11mm	12mm

Silver nanoparticles are positively charged and it will attach to the negative charged bacteria by the electrostatic attraction in the cell wall[35] and silver nanoparticles associated with thiol groups of cell wall results in the generation of reactive oxygen species and disrupting the cell[42]. Close association of silver nanoparticles with bacterial cell wall and forms the pits in the cell wall affecting the permeability and finally cause cell death.[36] The smaller size of silver nanoparticles facilitates their easy entry into the bacterial cell and affects the intracellular processes such as DNA, RNA and protein synthesis. Silver nanoparticles were binding with bacteria depends on the surface area for the interaction. Smaller particles affect the larger surface area of the bacteria thus it has more antibacterial activity than the larger sized nanoparticles.[34]



Zone of inhibition observed in *E. coli* Zone of inhibition observed in *P.aeruginosa*

Figure 6: Antibacterial assay zore of inhibition seen around phytosynthesized silver nano particles.

CONCLUSION

Biosynthesis of stable and spherical shaped nanoparticles using aqueous leaf extract of *Diospyros malabarica* (Desr.) Kostel. has been described in the present investigation. This method offers a viable and an ecofriendly way for fabrication of benign nanoparticles as it is a simple and carried out at room temperature without any huge inputs in terms of energy and waste. It is advantageous over the microbial synthesis as it is carried out using in aqueous solutions at ambient temperature, without any toxic chemicals in lesser time and could be exploited for developing cost effective biosynthesis of Ag nanoparticles at a large scale. Role of phytochemicals such as flavonoids, tannins, saponins and triterpenoids may be significant in reduction and stabilization of the silver nanoparticles. The synthesized silver nanoparticles were evaluated for antibacterial activity against human pathogens viz. *Escherichia coli and Pseudomonas aeruginosa.* The biosynthesized silver nanoparticles showed good antimicrobial activity against both the pathogens.

Acknowledgement: The authors are thankful to the Chairman P. G. Department of Botany, Karnataka University, Dharwad for the facilities and U. G. C. for financial assistance under DSA – I programme. One of the author (B.R.H) thanks to the University for the Award of UGC – UPE Fellowship. Instrumentation facility at USIC (K. U. Dharwad) and DST Unit (HR-TEM) IIT Madras is greatly acknowledged.

REFERENCES

1. Mansoori G.A., Mohazabi P., Mccormack S.J. Nanotechnology in cancer prevention, detection and treatment: bright future lies ahead. World Review of science, Technology and sustainable development. Vol.4 Nos 2/3, 2007, 226-257.
2. Osaka T., Matsunaga T., Nakanishi T., Arakaki A., Niwa D., Iida H. Synthesis of magnetic nanoparticles and their application to bioassays. Anal Bioanal chem. Vol. 384, 2006, 593-600.
3. Singh L.R., Ningthoujam R.S., Sudarsan V., Srivastava I., Dorendrajit S.S., Dey G.K. & Kulshreshtha S.K. Luminescence study on Eu^{3+} doped Y2O3 nanoparticles: particle size, concentration and core–shell formation effects. Nanotechnology 19, 2008, 055201.
4. Awwad A.M., Nida M.S., Abdeen O.A. "Green synthesis of silver nanoparticles using Carbo leaf extract and its antibacterial activity". International journal of Industrial chemistry. Vol.4, 2013, 29.
5. Nabikha K., Kathiersan A., Raj M., Alikunhi N. Synthesis of antimicrobial silver nanoparticles by callus and leaf extract from salt marsh plants, Sesuvium portulacastrum L. Colloids Surface Biointerfaces 79, 2009, 488–493.
6. Nasreen I.H., Taranath T.C. Biosynthesis of nanoparticles using microbes- a review. Colloids and Surfaces B: Biointerfaces 121, 2014, 474–483.
7. S. Prabhu, E.K. Poulse. Inter. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. Nano letters 2, 2012, 32.
8. 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. Journal of Nanoscience and Nanotechnology. 5, 2005, 1665-1671.
9. Kumar V., Yadav S.C., Yadav S.K. *Syzygium cumini* leaf and seed extract mediated biosynthesis of silver nanoparticles and their characterization. Journal of chemical Technology and Biotechnology, 85, 2010, 1301-1309.
10. Saxena A., Tripathi R.M., Singh R.P. Biological synthesis of silver nanoparticles by using onion (*Allium cepa*) extract and their antibacterial activity. Digest. Journal Nanomaterial and Bioscience. 5(2), 2010, 427-432.
11. Ahmad N., Sharma S., Alam M.K., Singh V.N., Shamsi S.F, Mehta B.R., Fatma A. Rapid synthesis of silver nanoparticles using dried medicinal plant of basil. Colloids Surfaces B: biointerfaces, 81(1), 2010, 81–86.
12. Kiruba Daniel S.C.G., Vinothini G. N., Subramanian, K. Nehru and Sivakumar M. "Biosynthesis of Cu, ZVI, and Ag nanoparticles using *Dodonaea viscosa* extract for antibacterial activity against human pathogens". Journal of Nanoparticle Research, 15, 2013, 1319.
13. Parashar U.K., Saxena & Srivastava A. Bioinspired synthesis of silver nanoparticles. Digest Journal Nanomaterials Biostructures. Digest Journal Nanomaterials Biostructures; 4, 2009, 159–166.
14. Jiang J., Oberdorster G., Biswas P. Characterization of size, surface charge, and agglomeration state of nanoparticle dispersions for toxicological studies. Journal of Nanoparticle Research, 11, 2009, 77-89.
15. Rout Y., Behers, Ojha A.K. and Nayak P.L. Synthesis of silver nanoparticles from Ocimum santum leaf extract and their antimicrobial activity. Journal of Microbiol. Antimicrob. 4, 2012, 103-106.
16. Kumaraswamy M., Sudipta, Jayanta, Balasubramanya S. "The green synthesis' characterization and evaluation of the biological activities of silver nanoparticles synthesized from *Leptadenia reticulata* leaf extract." Applied nanoscience. DOI 10 2014, 1007/s 13 204-014-0293-6.
17. Sahu N., Soni D, Chandrashekhar B., Sarangi B.K., Satpute D., Pandey R.A. Synthesis and characterization of silver nanoparticles using *Cynodon dactylon* leaves and assessment of their antibacterial activity. Bioprocess Biosyst Eng; 36, 2013, 999–1004.
18. Chandran S.P., Chaudhary M., Pasricha R, Ahmad A., Sastry M. Synthesis of gold nanotriangles and silver nanoparticles using Aloevera plant extract. Biotechnol Prog., 22, 2006, 577–583.
19. Wiley BJ, Im SH, Li Z-Y, McLellan J, Siekkinen A, Younan Xia J. Maneuvering the surface plasmon resonance of silver nanostructures through shape-controlled synthesis. Phys Chem B. 110, 2006, 15666–15675.
20. Saifuddin N., Wong C.W., Yasimura A.N. Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation E-journal of chemistry. 6(1), 2009, 61-70.
21. Natarajan N., Selvaraj S., and Murty R.V. Microbial production of Silver nanoparticles. Digest Journal of Nanomaterials and Biostructures. Digest Journal of Nanomaterials and Biostructures; 1, 2010, 135-140.
22. Baishya D., Sharma N. and Bora R. Green Synthesis of Silver Nanoparticle using *Bryophyllum pinnatum* (Lam.) and monitoring their antibacterial activities. Archives of applied science research, 4(5), 2012, 2098-2104.
23. Kleemann W. Random-field induced antiferromagnetic, ferroelectric and structural domain states. Int J Mod Phys B 7, 1993, 2469.
24. Mallikarjuna A., Narasimha B K., Dillip G., Praveen G.R., Sheedhar B., Sree Lakshmi B., Reddy C., Deva B.V.S. Prasad Raju. Digest Journal of Nanomaterials and Biostructers. 6(1), 2011, 181-186.
25. Guidella E.J., Ramos A.P., Elisabete M, Zaniquelli D, Baffa O. Green synthesis of colloidal silver nanoparticles using natural rubber latex extracted from *Hevea brasiliensis.* spectrochimica Acta part A 82, 2011, 140-145.
26. Yallappa S., Manjanna S.J., Pethambar S.K., Rajeshwara A.N., Satayanarayan N.D. "Green synthesis of silvernanoparticles using *Acacia farnesiana* (sweet Acacia) seed extract under microwave irradiation and their biological assessment J. Clust Sci, 24, 2013, 1081-1092.
27. Chidambaram D., Hennebel T., Taghavi S. Mast J., Boon N., Verstraete W., Van Der Lelie D., and Fitts J. Concomitant microbial generation of palladium nanoparticles &

Hydrogen to immobilize chromate. Environ. Sci. Technology, 44, 2010, 7635-7640.

28. Amarendra D., Dwiveeli and Krishna Gopal. Biosynthesis of silver and gold nanoparticle using *Chenopodium album* leaf extract. Colloids and surfaces A: Phisiochemical and Engineering aspects, 369, 2010, 27-33.
29. Chen D.H., Hsieh C.H. Synthesis of nickel nanoparticles in aqueous cationic surfactant solutions. J. Mater Chem, 12, 2002, 2412–2415.
30. Gokak I. B. and Taranath T.C. Phytosynthesis of silver nanoparticles using leaf Extract of wattakaka volublis (l.f.) stapf. And their Antibacterial activity. International journal of science, Environment and technology, 3, 2014, 93-99.
31. Gokak I. B. and Taranath T.C. Cansjera rheedii j. F. Gmel. A Medicinal Plant-Mediated Synthesis of Silver Nano particles and Their Antibacterial Activity International journal of scientific engineering and technology, 3, 2014, 293-296.
32. Dipankar C., Murugan S. The green synthesis, characterization and evaluation of the biological activities of silver nanoparticles synthesized from Iresine herbstii leaf aqueous extracts. Colloids Surfaces B: Biointerfaces, 98, 2012, 112–119.
33. Maliszewska I., Sadowski Z. Synthesis and antibacterial activity of silver nanoparticles. J Phys Conf Ser, 146(1), 2009, (Article ID 012024).
34. Shrivastava S., Bera T., Roy A., Singh R., Ramachandrarao P. & Dash D. Characterization of enhanced antibacterial effects of novel silver nanoparticles. Nanotechnology, 18, 2007, 225103.
35. Dibrov P., Dzioba J., Gosink K.K., Hase C.C., Dibrov P, Dzioba J, Gosink KK, Hase CC., "Chemiosmotic mechanism of antimicrobial activity of Ag+ in *Vibrio cholerae*,". Antimicrob. Agents Chemother. 46, 2002, 2670.
36. Sondi I., Salopek-Sondi. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. J. Colloids Interface Sci. 275, 177–182.
37. Lin Y.E., Vidic R.D., Stout J.E., McCartney C.A., and Yu V.L. Inactivation of Mycobacterium Avium by Copper Silver Ions. *Water Research,* 32(7), 1998, 1997-2000.
38. Lok CN., Proteomic analysis of the mode of antibacterial action of silver nanoparticles. J. Proteome Res. 5, 2006, 924.
39. Sarkar S., Jana A.D., Samanta S.K., Mostafa G. Facile synthesis of silver nano particles with highly efficient anti-microbial property. Polyhedron, 26, 2007, 4426.
40. Bragg P.D., D.J. Rainnie. The effect of silver ions on the respiratory chain of Escherichia coli. Can. J. Microbiol. 20, 1974, 889.
41. McDonnell G., A.D. Russell. Antiseptics and Disinfectants: Activity, Action, and resistance. Clin. Microbiol. Rev. 12, 1999, 179.
42. Lara G. J. H., F. R. Gomez, G. P. Tamez, C. E. Monreal, G. R. Tamez, and P. C. Rodriguez. Biosynthesis of Silver Nanoparticles by Marine Invertebrate (Polychaete) and Assessment of Its Efficacy against Human Pathogens. British Journal of Medicine and Medical Research, vol. 3, no. 4, 2013, 1308–1316.

Source of Support: Nil, **Conflict of Interest:** None.