

Black Datura Fastuosa Flower Extract Mediated Green Synthesis Of Silver Nanoparticles And Their Antibacterial Activity

N. Kalyanasundaram , N. Sivakumar, Gautham Devendrapandi, P. Selvakumar

Abstract: The fcc crystallized AgNps was synthesized from flower extracts of Black Datura fastuosa plant. The synthesized AgNps was characterized by SEM, FE-SEM, XRD, FT-IR, DLS and UV-vis spectroscopy methods. The formation of the nanoparticles were primarily identified by the UV-vis spectroscopy, it clearly exhibits the absorption band at 420-450nm which is responsible for surface plasmon resonance due to the interaction of the electrons of the silver metal nanoparticles with photons. The surface morphology of the Ag Nps were studied by SEM and FE-SEM, it confirms that the derived nanoparticles have found to be 23-31 nm in size, which is in accordance with the obtained results of DLS data. An employment of various functional groups of bioactive molecules from of B.D. datura flower extract was apparently emphasized by FT-IR technique. Also, the synthesized AgNps were subjected to their antibacterial studies possessing significant activities against the tested microorganism.

Key Word: Black Datura Fastuosa, Silver Nanoparticles (AgNps), Antibacterials study

1. INTRODUCTION

In science and technology era, research on nano material is found to be one of the most impressive and attractive area of interdisciplinary science to the researchers, which have been evidently explored by the several applications in various field including pharmaceutical, biomedical, material science, cosmetics, agriculture, food and etc.¹⁻¹² Particularly, Ag NPs is one such material and its significant uses have been noticeably extended due to their unique physico-chemical properties¹³⁻¹⁸. Remarkably, AgNPs incorporated health care products and diagnostic materials have been intensively increased so for because of its potential interaction with microbes lead to causes cell wall and membrane damage¹⁹⁻²⁸. Generally, a number of methods of synthesize for Ag NPs have been reported in the literature²⁹. However, uses of photochemical, radiational, electrical, chemical and pyrolytic techniques are very common methods which are employing with expensive instruments and tedious conditions³⁰⁻³². Conventionally, the wet chemical approach is considerably being more attentive to synthesize AgNPs in precise shape and morphology³³. Conversely, the chemical processes are often involving in the usage of costly metals and hazardous chemicals like sodium borohydride, hydrazine hydrate, DMF, and ethylene glycol etc³⁴⁻³⁸. Moreover, they may direct to absorb the harsh chemicals on the surfaces of nanoparticles which could possibly induce toxicity effect and harmfulness to the health and environments³⁹. As a result of potential importance of AgNs, in various fields, a cheap, environmentally benign and cost effective protocol for synthesizing Ag Nps is always in demand.

Besides, a green chemistry strategy without using hazardous chemicals, solvents and stabilizer are nowadays very popular in synthesizing AgNPs with desired morphology⁴⁰⁻⁴¹. Alternatively, biochemicals like microbes, fungi, algae, yeast, bacteria, marine organism and plant extract also can be utilised for synthesizing AgNPs since its been immensely admired because of their biocompatibility and environmental benign⁴²⁻⁴³. Interestingly, based on this concept, there are several articles have been published to synthesis of AgNPs using various plants extracts, which are very cheap, easily available and eco-friendly approach⁴⁴⁻⁴⁵. Generally, Datura metal plant is a well known species for traditional medicines, Siddha, Ayurvedha and etc. It has been clearly documented for its pharmacological studies including anti-bacterial anti-fungal, anti-inflammatory, herbicidal, insecticidal, anti-cancer, and anti-rheumatoid activity. Though, the application of Datura plant extract in synthesise of nanoparticles have been well published in several report⁴⁶⁻⁴⁸, but the usefulness of Black Datura Fastuosa flower extract for AgNPs has not been revealed yet. Typically, the biologically active metabolite or scaffolds present in the flower extracts of black Datura Fastuosa is responsible for the reduction of silver ion into Ag Nps in its aqueous solution. Inspired by the above research, we are focussed to derive the AgNps using the flower extract of black datura Fastuosa plant without using stabilizing and capping agents. The flower extracts contains alkaloids like various triglyl esters of tropine, pseudotropine, calystegines, nirotropene and alkaloids of glycosadse linkage, which efficiently involves in the AgNps synthesis. In order to study the concentration effect on the formation of AgNps, various concentration propositions with respect to the metal ion and extract were prepared and used. The obtained AgNps nano particles was characterized by UV-Vis, Particles analyser, SEM, FE-SEM, XRD and EDX. Also, the AgNps were subjected limited against bacterial and fungal strains.

2.1. Experimental Section

2.1 Preparation of Silver Nitrate solutions

- N. Kalyanasundaram M.R. Govt Arts college, Mannargudi, Thiruvarur District, Tamil Nadu, India-614001, E-mail : nks26@gmail.com :9786592871
- N. Sivakumar
- M.R. Govt Arts college, Mannargudi, Thiruvarur District, Tamil Nadu, India-614001, E-mail: suryasiva1981@gmail.com :9840767328
- Gautham Devendrapandi
- University of Madras, Polymer Science, Guindy Campus, Chennai, Tamil Nadu, India- & Indian Traditional Medicine and Herbal medicinal chemistry Research Center, Nagapattinam, Tamil Nadu-India, E-mail : santhanapaneer.d@gmail.com :7418493098
- P. Selvakumar Indian Traditional Medicine and Herbal medicinal chemistry Research Center, Nagapattinam, Tamil Nadu-India, E-mail: selva9899@gmail.com:8973154746

Analytical grade silver nitrate (AgNO_3) a costly chemical, was purchased from local laboratory chemical suppliers. A 0.017g of 1mM stock solution of AgNO_3 was prepared in chloride-free distilled water and the solution was kept dark room.

2.2 Preparation of extracts from Black Datura Fastuosa flower

Fresh flower of Black Datura Fastuosa (Fig 1) were collected from Nagapattinam district of Tamil Nadu, and washed the flower once with water to remove the dust particles. The 100g of flowers are cut in to small pieces and were placed in a 500 ml Erlenmeyer flask containing 200 ml of sterile DDW. After, the mixture was boiled for 25 min and filtered through filter paper and the extract was stored at 10 ° C at refrigerator.

2.3 Synthesis of silver nanoparticles by adding Black Datura Fastuosa extract to AgNO_3 solutions.

An aqueous solution of flower extracts from Black Datura Fastuosa was used as a reducing solution for AgNO_3 (1mM

in DD water) in aqueous media to produce respective AgNPs. The reaction was carried out at the ambient temperature and the same temperature was maintained throughout the process. Totally, six test tubes were taken and the each test tube was added with 2 mL of extract and 0.5- 3 mL of stock solution (starts from: 0.5,1.0,1.5, 2.0, 2.5 and 3.0 mL respectively), finally make up these respective solutions of each test tube to 10 ml by adding DD water and kept it for 24 h in a dark room. Hence the attained final concentration of each solution is 0.05, 0.10, 0.15, 0.20, 0.25, 0.30mM respectively (Fig.2). The colour of the solution changed gradually to yellow followed by brine and then dark depending on the precursor concentration indicates the formation of AgNPs. The obtained colloidal suspensions of AgNPs were then centrifuged at 15,000 rpm for 20 minutes and washed four times to remove silver ion residue. The precipitated nanoparticles were then dried overnight at 30°C under vacuum to obtain the AgNPs which are dark crystalline solid.



Fig. 1. (a) Black. Datura Fastuosa plants (b) Flower of black Datura Fastuosa

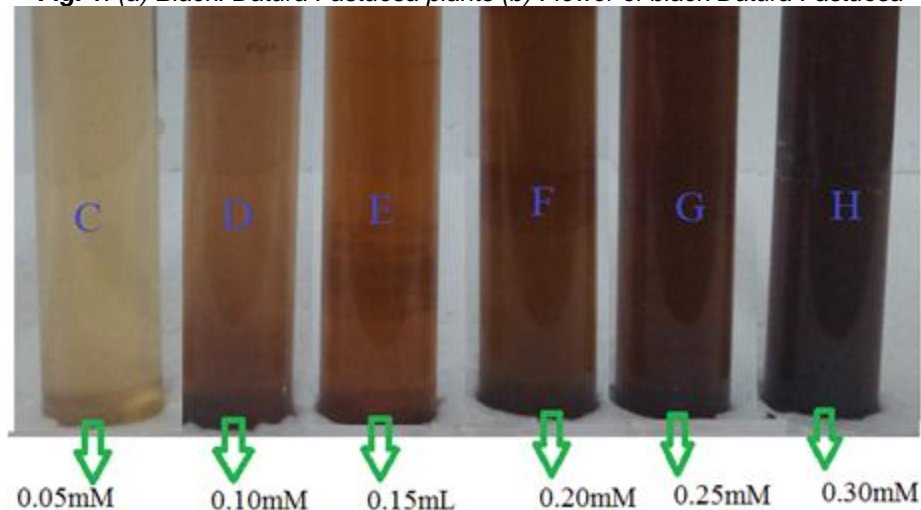


Fig. 2. Colour of AgNPs colloidal solutions after 24 hours

2.4 Characterization of Ag Nps

The flower extract reduced AgNPs was confirmed by absorption UV-Vis spectroscopy (perkin-Elmer LS-55- Luminescence spectrometer) and the average particle size was analysed by DLS particle size

analyzer. The shape, size structure of the synthesized AgNps was clearly Characterised by SEM, FE-SEM, X-ray diffraction (XRD) and FTR-IR analytical techniques.

3. Result and Discussion

3.1 UV visible spectroscopy

Green synthesis of AgNPs was carried out using the flower extract of Black Dutura Fastuosa at room temperature. The reaction of silver ion in the 1mM of aqueous solution with the flower extract of B.D. Fastuosa was monitored by UV spectroscopy. Also, preliminarily, the formation AgNps was confirmed by the colour changes gradually occurs from yellow to wine red and became intensified to black after 24 h. The significant colour changes takes place not only

because of concentration of silver ion but also depends on the quality of the extract, temperature, time and light. In the UV-Vis spectrum, the prominent peak observed at 420-450 nm due to the surface plasmon resonance confirms the characteristics of the silver nano particles (Fig. 4), which are obviously comparable with reported authentic value⁴⁹⁻⁵⁰. Hence, it proves that the phytochemicals of B.D. fastuosa flower successfully facilitate the reduction silver ion into silver nano particles (AgNPs) with controlled shape and size during the reaction.

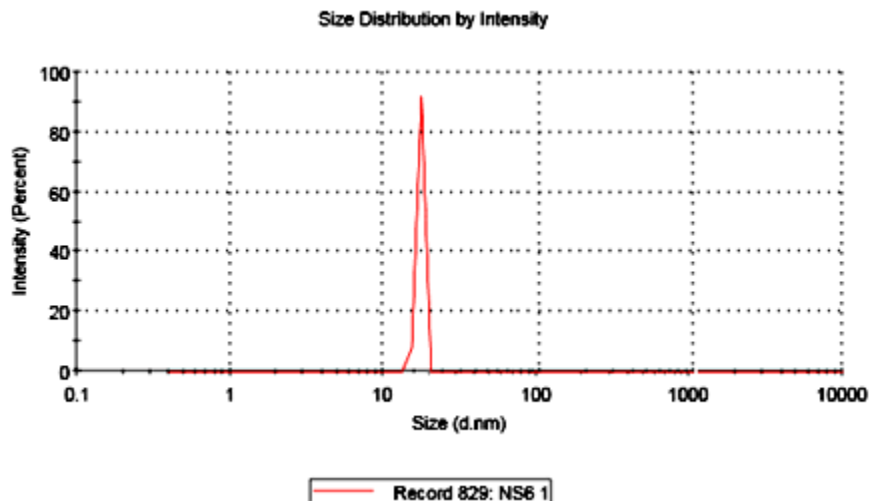


Fig. 3. DLS of B. D. Fastuosa after 24 hrs

3.2 SEM and Fe-SEM analysis

Surface morphology of synthesized AgNps from B.D fastuosa flower extract was characterised by SEM analysis. The results indicate that they are well aggregated and

spherical in size (Fig 5). It was further confirmed by FE-SEM, which manifest the size of the AgNPs from 23 to 31 nm (Fig 6). Additionally, the average particle size is found to be 35 nm

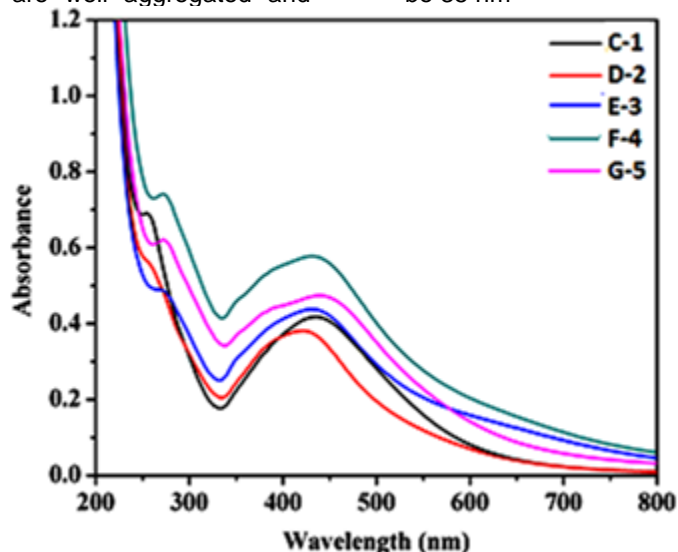


Fig. 4. UV-vis spectra of B. D. Fastuosa after 24 hrs.

which is in good agreement with DLS results (Fig 3). Notably, FE-SEM shows some coating around the formed AgNps, which are responsible for the bioactive molecules that can act as capping and stabilizing agent during the synthesis of AgNps. These functional groups corresponding

to the metabolite are clearly confirmed by FT-IR. In addition, the intense peak of EDAX spectrum offers the clarity on silver element which involved in the process (Fig. 7).

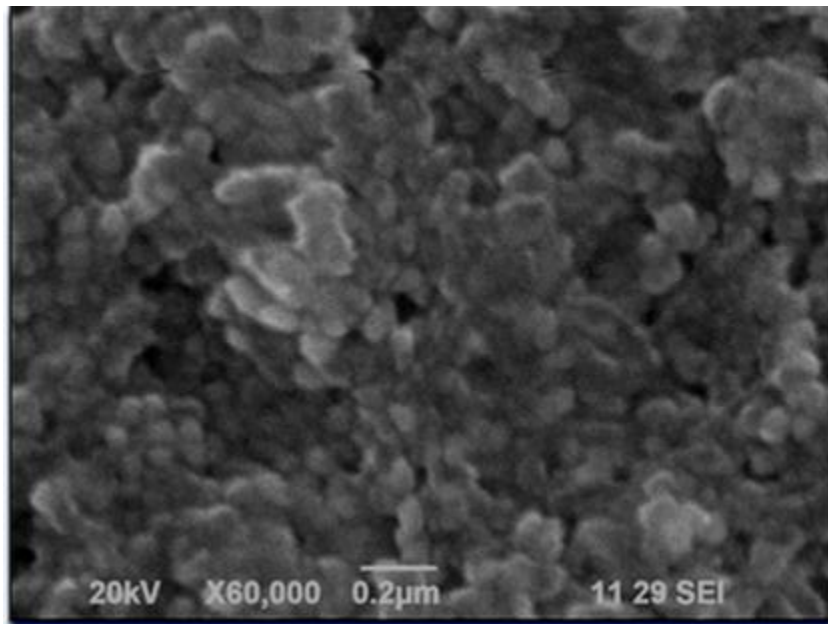


Fig. 5. SEM image showing surface morphology of the silver nanoparticles

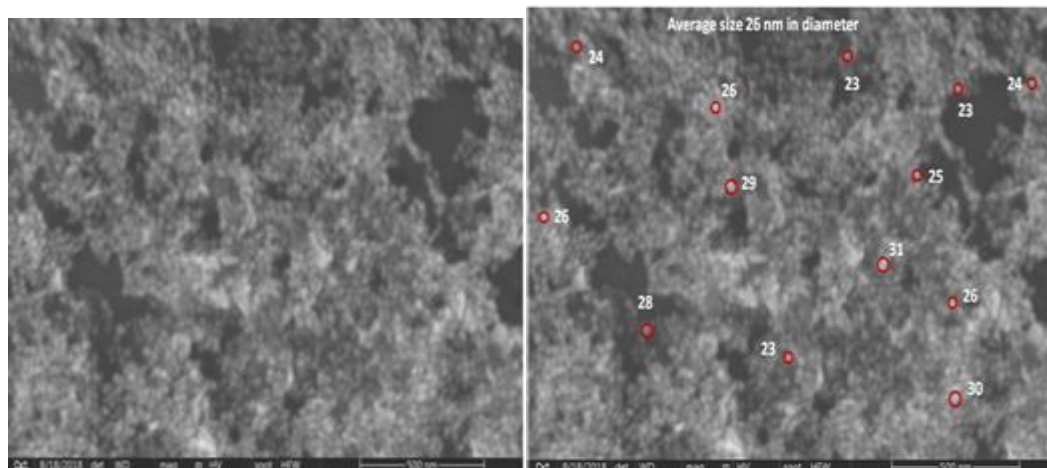


Fig. 6. FE-SEM image showing surface morphology of the silver nanoparticles

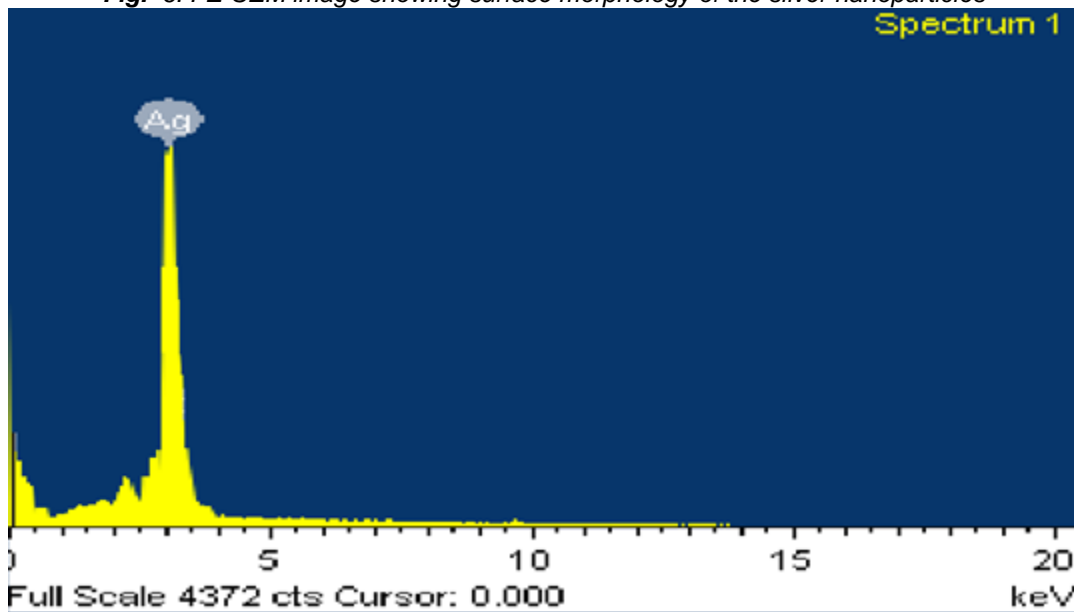


Fig. 7. Energy Dispersive X-ray Spectroscopy for AgNps

3.3 XRD Analysis

The XRD pattern of the synthesized AgNPs clearly poses 2θ value corresponding to the plane (111), (200), (220) and (311) respectively. These planes confirm the crystalline fcc

structure of synthesized Agnes. The other minor peaks or noise observed in the XRD graph could be attributed to the crystal nature of secondary metabolite from the flower extract of B.D.Fastuosa (Fig.8)

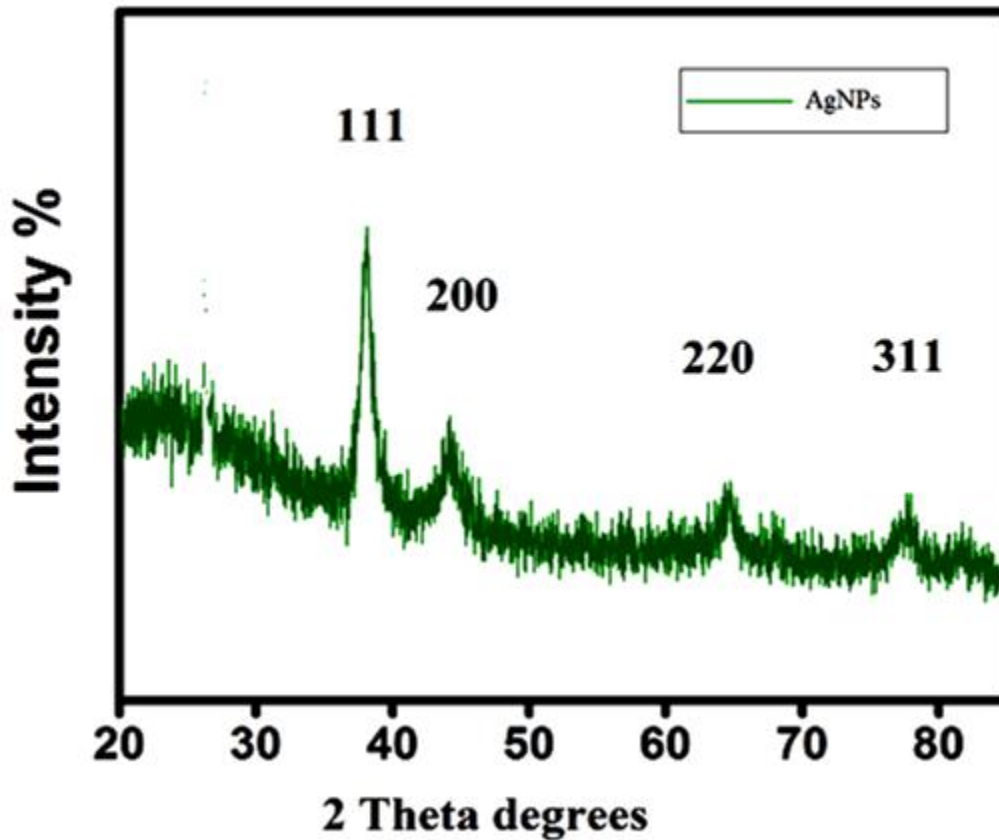


Fig. 8. XRD of Synthesized AgNps

3.4 FT-IR Analysis

Synthesized Agnes was analysed by FT-IR to find out functional groups which are originated from the bioactive motif of B.D. fastuosa flower extract. The spectrum of FT-IR displays the absorption band at 3438, 1636, 2073, 1119, 1231.74 and 1045.74 cm^{-1} respectively (Fig.9). The band corresponding to 3438 cm^{-1} designates the NH stretching

whereas the band at 1636 cm^{-1} responsible for C=O stretching which are the functional units might be participated in the reduction reaction of silver ion into AgNps. The additional band at 2073, 1119, 1231 and 1045 cm^{-1} designates the C-O, alkene and other aliphatic stretching of the metabolite of the flower extracts.

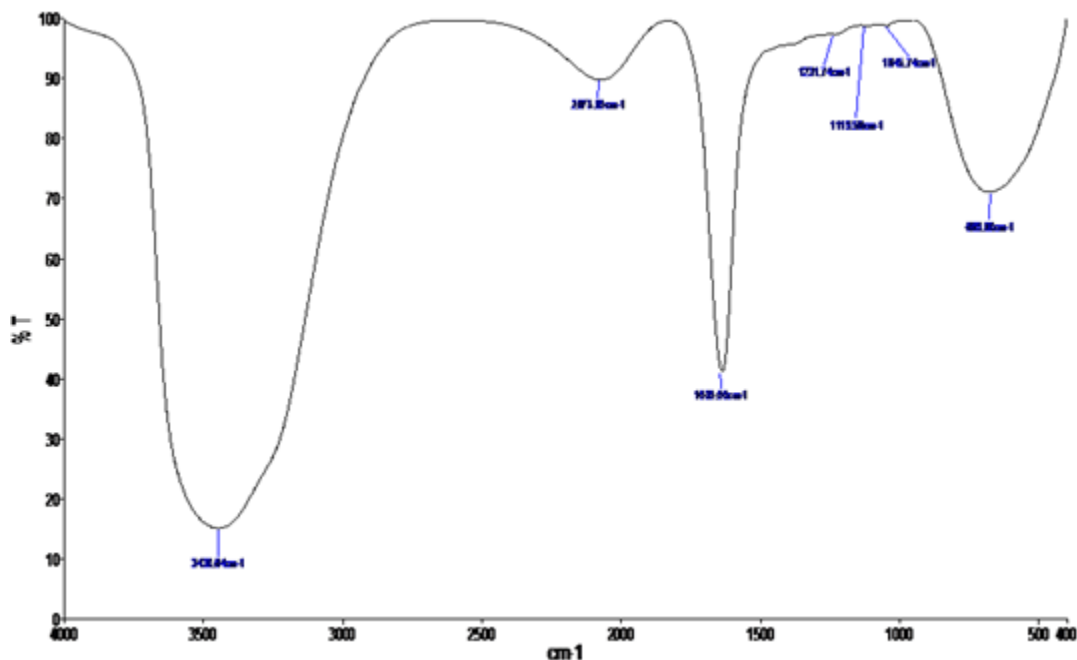
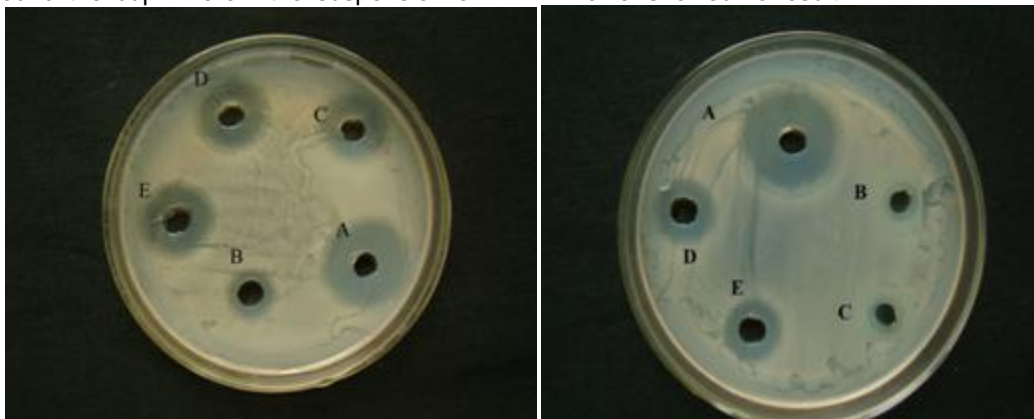


Fig. 9. FT-IR Spectroscopy for AgNPs

3.5 Antibacterial study and Zone of inhibition

The antibacterial study was carried out against the bacteria *Staphylococcus aureus* and the distinct zone of inhibition was observed around the cup wherein the suspension of

AgNPs was applied as shown below (Fig. 10). The mixtures of AgNO_3 and flower extract with 0.005 to 0.020mM concentration prevented the bacterial growth. The extract of flower showed no result



S.aureus (a)

E.colab (b)

Fig. 10. Antibacterial activity of silver nanoparticles against *S.aureus* (a) and *E.coli* (b)

- A) Antibiotics as control (Chloroemphenicol-100 $\mu\text{g/ml}$).
- B) In hibition zone of Leaf extract - B.D. Fastuosa flower.
- C) In hibition zone of Ag NPs in 0.005 $\mu\text{g/ml}$.
- D) In hibition zone of Ag NPs in 0.01 $\mu\text{g/ml}$.
- E) In hibition zone of Ag NPs in 0.020 $\mu\text{g/ml}$.

CONCLUSION

Green synthesis of silver nanoparticles from B.D. Fastuosa flower leaf provides environmental friendly, simple and efficient route for synthesizing benign nanoparticles. The synthesized AgNPs nanoparticles found to have an average size of 23-31nm scale. The size and morphology and functionality of the capping molecules of synthesized AgNPs were confirmed by SEM, FE-SEM, DLS, FT-IR and XRD techniques. Also, the AgNPS exhibits significant antibacterial activity against the tested microorganism. However, AgNPs from B.D. Fastuosa (rarely cultivated)

would have potential application in various fields including biomedical under validation.

REFERENCES

- [1] S. Linic, U. Aslam, C.Boerigter and M. Morabito, "Photochemical transformations on plasmonic metal nanoparticles", *Nat. Mater* 2015; 14: 567–576.
- [2] K.N.Thakkar, S.S. Mhatre and R.Y.Parikh, "Biological synthesis of metallic nanoparticles. *Nanomед. Nanotechnol*" *Biol. Med* 2010; 6: 257–262.
- [3] W.J .Yoon, K.Y. Jung, J. Liu, T. Duraisamy, R. Revur, F.L .Teixeira, S. Sengupta and P.R. Berger, "Plasmon-enhanced optical absorption and photocurrent in organic bulk heterojunction photovoltaic devices using self-assembled layer of

- silver nanoparticles” ,Sol. Energy Mater. Sol. Cells 2010; 94: 128–132.
- [4] Y. Yang, P. Jin , X. Zhang , N. Ravichandran , H. Ying , C .Yu , H. Ying , Y. Xu , J. Yin and K. Wang , “New epigallocatechin gallate (EGCG) nanocomplexes co-assembled with 3-mercapto-1-hexanol and β -lactoglobulin for improvement of antitumoractivity” ,J. Biomed. Nanotechnol 2017; 13: 805–814.
- [5] M. Sathishkumar , K. Sneha , S.Won ,C.W. Cho , S.Kim and Y.S.Yun, “Cinnamon zeylanicum bark extract and powdermediated green synthesis of nano-crystalline silver particles and its bactericidal activity” ,Colloids Surf 2009;B 73: 332–338.
- [6] L. Gan, S.Zhang, Y.Zhang, S.He and Y. Tian, “Biosynthesis, characterization and antimicrobial activity of silver nanoparticles by a halotolerant *Bacillus endophyticus* SCU-L”, Prep. Biochem. Biotechnol. A, 2018. <https://doi.org/10.1080/10826068.2018.1476880>.
- [7] R.R.Arviso, S.Bhattacharyya, R.A.Kudgus, K. Giri, R.Bhattacharya and P. Mukherjee, “Intrinsic therapeutic applications of noble metal nanoparticles: past, present and future”, Chem.Soc. Rev2012; 41: 2943–2970.
- [8] S. Venkatesh, Manikandan, B. Adhikari and A.Chen, “Nanomaterial based electrochemical sensors for the safety and quality control of food and beverages”, Analyst, 2018; 19.
- [9] T. Banerjee, T. Shelby and S. Santra, “How can nanosensors detect bacterial contamination before it ever reaches the dinner table?”, Future Microbiol 2007; 2: 12.
- [10] E. V. De Francisco and R.M. García-Estapa, “Nanotechnology in the agrofood industry”, Journal of Food Engineering 2018; 238: 1–11.
- [11] N. Milani, M .J. McLaughlin, S. P .Stacey, J. K .Kirby, G. M. Hettiarachchi, D. G. Beak and G. Coprnelis, “ Dissolution kinetics of macronutrient fertilizers coated with manufactured zinc oxide nanoparticles” , J. Agric. Food Chem 2016 ; 60: 3991–3998.
- [12] N. Milani , G. M .Hettiarachchi, J .K. Kirby, D. G .Beak, S. P .Stacey and M. J McLaughlin, “Fate of zinc oxide nanoparticles coated onto macronutrient fertilizers in an alkaline calcareous soil”, PLoS One 2015 ;10 (5). e0126275.
- [13] C.Burda, X. Chen, R. Narayanan and M .A .El-Sayed, “Chemistry and properties of nanocrystals of differenshapes”, Chem. Rev 2005 ; 105: 1025–1102.
- [14] A. N .Grace and K. Pandian , “Antibacterial efficacy of aminoglycosidic antibiotics protected goldnanoparticles—A brief study. Colloids Surf”, A Physicochem. Eng. Asp 2007; 297: 63–70.
- [15] J. S .Lee, “Recent progress in gold nanoparticle-based non-volatile memory devices” Gold Bull 2010; 43:189–199.
- [16] S. J .Van der Molen, J. Liao , T. Kudernac , J. S . Agustsson ,L. Bernard , M. Calame , B. J. Van wees ,B.L. Feringa and C. Schönenberger , “Light-controlled conductance switching of ordered metal molecule metaldevices”, Nano Lett 2008 ; 9: 76–80.
- [17] S. Mackowski “Hybrid nanostructures for efficient light harvesting” J. Phys. Condens. Matter 2010; 22: 193.
- [18] A Khan, R. Rashid, G. Murtaza and A. Zahra, “Gold nanoparticles: Synthesis and applications in drug delivery”, Trop. J. Pharm. Res 2014; 13: 1169–1177.
- [19] R. Bayston, W. Ashraf and L. Fisher, “Prevention of infection in neurosurgery: Role of “antimicrobial” catheters”, J. Hosp. Infect 2007; 65: 39–42.
- [20] K. Galiano , C. Pleifer ,K. Engelhardt ,G. Brössner ,P. Lackner ,C. Huck , C. Lass-Flörl and A. Obwegeser , “Silver segregation and bacterial growth of intraventricular catheters impregnated with silver nanoparticles in cerebrospinal fluid drainages” Neurol. Res 2008 ; 30: 285–287.
- [21] R. E. Weisbarth, M. M. Gabriel, M. George ,J. Rappon , M. Miller , R. Chalmers and L. Winterton , “Creating antimicrobial surfaces and materials for contact lenses and lens cases” Eye Contact Lens 2007 ;33: 426–429.
- [22] V. Alt ,T. Bechert ,P. Steinrücke, M. Wagener ,P. Seidel ,E. Dingeldein ,E. Domann and R. Schnettler , “An in vitro assessment of the antibacterial properties and cytotoxicity of nanoparticulate silver bone cement”, Biomaterials 2004; 25: 4383–4391.
- [23] F. Furno , K.S. Morley,B. Wong , B .L .Sharp, P. L .Arnold, S. M .Howdle,R. Bayston , P. D Brown, Winship and H .J . Reid, “Silver nanoparticles and polymeric medical devices: A new approach to prevention of infection?” J. Antimicrob. Chemother 2004; 54: 1019–1024.
- [24] M. Ip , S. L. Lui, V. K. Poon,I. Lung and A. Burd , “Antimicrobial activities of silver dressings: An in vitro comparison ”, J. Med. Microbiol 2006; 55: 59–63.
- [25] D. J .Leaper, “Silver dressings: Their role in wound management” Int. Wound J 2006; 3: 282–294.
- [26] Y.Li ,P. Leung , L.Yao ,Q. Song and E.Newton , “Antimicrobial effect of surgical masks coated with nanoparticles” J. Hosp. Infect 2006 ; 62: 58–63.
- [27] B. S. Atiyeh, M .Costagliola, S. N. Hayek and S. A. Dibo, “Effect of silver on burn wound infection control andhealing” Review of the literature. Burns 2007; 33: 139–148.
- [28] N .Dur’an, M .Dur’an, M .B .de Jesus, A. B .Seabra, W. J. F’avaro and G. Nakazato, “A New View on Mechanistic Aspects on Antimicrobial Activity”, Nanomedicine 2016 ; 12: 789–799.
- [29] Y. Sun, “Controlled synthesis of colloidal silver nanoparticles in organic solutions: Empirical rules for nucleation engineering”,Chem. Soc. Rev 2013 ; 42: 2497–2511.
- [30] C. B .Farias, A. Ferreira Silva, R. Diniz Rufino, J. Moura Luna, J. E. Gomes Souza and L. A .Sarubbo, “Synthesis of silver nanoparticles using a biosurfactant produced in low-cost medium as

- stabilizing agent. *Electron* J. Biotechnol 2014; 17: 122–125.
- [31] P .Van Dong, C. H .Ha and J Kasbohm, “Chemical synthesis and antibacterial activity of novel-shaped silver nanoparticles” *Int. Nano Let* 2012; 2: 9.
- [32] M. G. Guzmán, J. Dille and S. Godet, “Synthesis of silver nanoparticles by chemical reduction method and their antibacterial activity” *Int. J. Chem. Biomol. Eng* 2009; 2: 104–111.
- [33] M. N .Tahir, F. Natalio, M .A .Cambaz, M. Panthöfer, R .Branscheid, U .Kolb and W.Tremel, “Controlled synthesis of linear and branched Au and ZnO hybrid nanocrystals and their photocatalytic properties”, *Nanoscale* 2013 ; 5 : 9944–9949 .
- [34] L. Polavarapu and L .M .Liz-Marzán, “Growth and galvanic replacement of silver nanocubes in organic media”, *Nanoscale* 2013; 5: 4355–4361.
- [35] S. Mourdikoudis and L .M. Liz-Marzán, “Oleylamine in nanoparticle synthesis”, *Chem. Mater* 2013; 25: 1465–1476.
- [36] V. Montes-García, J. Pérez-Juste, I. Pastoriza-Santos and L.M. Liz-Marzán, “Metal nanoparticles and supramolecular macrocycles: A tale of synergy” *Chem. Eur. J* 2014; 20: 10874–10883.
- [37] N. Erathodiyil and J.Y. Ying, “Functionalization of inorganic nanoparticles for bioimaging applications” *Acc. Chem. Res* 2011; 44: 925–935.
- [38] L. Castro, M.L. Blázquez, F.G. González and A. Ballester, “Mechanism and applications of metal nanoparticles prepared by bio-mediated process” *Rev. Adv. Sci. Eng* 2014; 3: 199–216.
- [39] C A. Rice-evans, N. J. Miller, P .G. Bolwell, P. M. Bramley and J. B. Pridham, “The relative antioxidant activities of plant-derived polyphenolic flavonoids”, *Free Radic. Res* 1995; 22: 375–383.
- [40] K. Riehemann, S.W.Schneider, T.A.Luger, B. Godin , M. Ferrari and H. Fuchs, “Nanomedicine-challenge and perspectives”, *Angewandte Chemie International Edition England* 2009; 48: 872–897.
- [41] T. Mamo, E.A. Moseman, N .Kolishetti, C .Salvador-Morales, J .Shi, D.R .Kuritzkes, R. Langer, U. Von Andrian and O.C. Farokhzad, “Emerging nanotechnology approaches for HIV/AIDS treatment and prevention”, *Nanomedicine* 2010 ; 5: 269–285.
- [42] R. S .Patil, M.R .Kokate and S.S. Kolekar, “Bioinspired synthesis of highly stabilized silver nanoparticles using *Ocimum tenuiflorum* leaf extract and their antibacterial activity, *Spectrochimica Acta*”, Part A: Molecular and Biomolecular Spectroscopy 2012; 91: 234-238.
- [43] K. Vijayaraghavan, S.K. Nalini, N.U. Prakash and D .Madhankumar, “ Biomimetic synthesis of silver nanoparticles by aqueous extract of *Syzygium aromaticum* ” , *Materials Letters* 2012 ; 75: 33-35.
- [44] F. K. Alsammarraie, W .Wang, P. Zhou, A. Mustapha and M. Lin, “Green synthesis of silver nanoparticles using turmeric extracts and investigation of their antibacterial activities” , *Colloids and Surfaces B: Biointerfaces* 2018 ; 171: 398-405.
- [45] S.Das, J.Das, A. Samadder , S.S .Bhattacharyya and A R. Khuda-Bukhsh, “Biosynthesized silver nanoparticles by ethanolic extracts of *Phytolacca decandra*, *Gelsemium sempervirens*, *Hydrastis canadensis* and *Thuja occidentalis* induce differential cytotoxicity through G2/M arrest in A375 cells”, *Colloids and Surfaces B: Biointerfaces* 2013 ; 101: 325-336.
- [46] B. Gajendran, P. Durai, K.M. Varier, W. Liu, Y. Li, S . Rajendran , R. Nagarathnam and A . Chinnasamy, “Green Synthesis of Silver Nanoparticle from *Datura innoxia* Flower Extract and Its Cytotoxic Activity”, *Bionano science*. <https://doi.org/10.1007/s12668-019-00645-9>.
- [47] M. Gomathi, P.V .Rajkumar, A. Prakasam and K. Ravichandran, “Green synthesis of silver nanoparticles using *Datura stramonium* leaf extract and assessment of their antibacterial activity”. <https://doi.org/10.1016/j.reffit.2016.12.005>.
- [48] J. Kasthuri, S. Veerapandian and N. Rajendiran, “Biological synthesis of silver and gold nanoparticles using apiin as reducing agent”, *Colloids and Surfaces B: Biointerfaces* 2009; 68: 55–60. <https://doi.org/10.1016/j.colsurfb.2008.09.021>.
- [49] D. Philip , “Biosynthesis of Au, Ag and Au-Ag nanoparticles using edible mushroom extract”, *Spectrochimica Acta Part A* 2009 ; 73: 374–381. <https://doi.org/10.1016/j.saa.2009.02.037>.
- [50] N.S. Shaligram, M. Bule, R. Bhambure, R.S. Singhal, S.K. Singh and G. Szakacs, “Biosynthesis of silver nanoparticles using aqueous extract from the compactin producing fungal strain *Process*” , *Biochemistry* 2009 ; 44: 939–943. <https://doi.org/10.1016/j.procbio.2009.04.009>.