Green synthesis: silver nanoparticles frommicrobial extract for biogas generation

Pathare Aishwayra, Desai Hindavi, Sarode Sneha, Nibandhe Shalmali and Jamkhedkar Suruchi*
Department of Life Sciences, University of Mumbai, Vidyanagari Campus, Santacruz East, Mumbai-400 098, INDIA
*suruchi.jamkhedkar@gmail.com

Abstract

The primary objective of this study is the green synthesis of silver nanoparticles using the active extract obtained from microorganisms. The microorganism, a new strain of Bacillus hunanensis, was chosen from landfill leachate of Durgadi Fort dumping ground near Kalyan and soil near the decomposer machine of University of Mumbai, Vidyanagari campus. The new strain of Bacillus hunanensis (Accession Number: MK951982) culture was centrifuged and was sonicated for 10 min. The culture was once again centrifuged after being sonicated. The supernatant was decanted and distilled water was used to wash the precipitate.

The process of producing silver nanoparticles involved reacting a standard silver salt solution withan aqueous bio-reducing solution obtained from the microorganism. The reaction progress wasmonitored by analytical techniques. The nanoparticles obtained were characterized by the FTIR, TEM and SEM-EDS. Further the nanoparticles produced were used in the digester for biogas emission.

Keywords: Green synthesis, nanoparticles, biogas.

Introduction

In context of environmental concerns, "green technology" is in high demand and is growingin popularity. Natural processes such as volcanic eruptions, wildfires, weathering or microbial processes can result in the formation of nanoparticles which can be both organic (such as proteins, polysaccharides, and viruses) and inorganic (such as iron oxyhydroxides, aluminosilicates, metals, and others)[13]. Nanoparticles are defined as particulate dispersions of solid particles with at least one dimension at a size range of 10-1000 nm[25]. Nanoparticles (NPs) have wide range of applications in areas such as health care, cancer treatment, therapeutics, cosmetics, food and feed, environmental health, mechanics, optics, biomedical sciences, biosensors, chemical industries, electronics, space industries, drug-gene delivery, energy science, optoelectronics, catalysis, single electron with limitations to be explored[7-9,13,15-17,20,23,26].

A green chemistry technique that links nanotechnology, microbiology and biotechnology is known as microbial synthesis of nanoparticles. There have been reports of bacteria, fungi, yeasts, actinomycetes and viruses biosynthesizing gold, silver, gold–silver alloy, selenium, tellurium, platinum, palladium, silica, titania, zirconia, quantum dots, magnetite and uraninite nanoparticles[18]. Microbes' potential as biological materials for the synthesis of nanoparticles has not yet been extensively investigated, despite their rich biodiversity[16]. It has been noted that intracellular AgNPs are produced by the bacteria *Bacillus licheniformis*[17].

It was discovered that subculturing *Bacillus sp*. into $AgNO_3$ containing media resulted in the creation of intracellular AgNPs[11]. A study was conducted on lactic acid bacteria's ability to synthesise AgNPs. Only four bacterial species: *Lactobacillus sp*., *Pediococcus pentosaceus*, *Enterococcus faecium* and *Lactococcus garvieae* were found to be able to synthesize AgNPs[17,21].

Landfills are the easiest solutions for disposal of waste at minimum cost: Combustion is generally more expensive than landfilling,and air emissions from combustion, such as nitrogen oxides (NOx), sulfur dioxide (SO_2), carbon monoxide (CO), particulate matter (PM), dioxins and furans pose a significant environmental concern[10]. This research mainly focuses on rapid, economical and eco-friendly bio-reduction of silver salts solution for biosynthesis of silver nanoparticles with unique properties including the capacity to generate biogas from a newly discovered strain of *Bacillus hunanensis* from landfill.

Material and Methods

Sample collection: The landfill leachate sample was collected and packed tightly into a zip-lock plastic bag from Durgadi fort dumping ground near Kalyan.

Isolation and screening of bacteria (methanogens): The zone of dumping ground from which the leachate sample was collected was rich in cellulose; thus, there was a possibility to obtain cellulose degrading bacteria (methanogens). To confirm the presence of methanogens, 1gm of leachate sample was added into Mineral Salt Medium (MSM) and was enriched by cellulose (2gm/L) and incubated at 42°C for 30 days. After 30 days of incubation, the streaking of the medium was done on a sterile nutrient agar plate. The plate was kept for 24 hrs and growth was observed on the plate after 24 hrs.

The cellulose degrading bacteria were screened by serial dilution (10^{-1} –10^{-7}) of medium enriched by cellulose. The dilution numbers 10^{-5}, 10^{-6} and 10^{-7} were selected for performing spread plate. After 24hrs of incubation at 37˚C, the well isolated colonies were observed and spread plate with dilution 10^{-5} was selected for further studies.

Confirmation of cellulose degrading ability of isolated strain: The ability of the isolated strain to degrade cellulose was verified by inoculating each strain in Congo red cellulose (CRC) medium. The methanogens use cellulose in four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The formation of acid during the acidogenesis phase causes the congo red MSM medium to change colour. Congo red is used in the CRC medium as a pH indicator which gives the medium a red hue when the pH is neutral but turns colourless when the pH is acidic.

Green Synthesis of Silver Nanoparticles: *B. hunanensis* (Strain 5) was inoculated in 100 ml nutrient broth and incubated at 37°C for 48 hrs. The culture was sonicated for 10 mins and then centrifuged at 10000 rpm for 15 mins.The clear supernatant was obtained and filtered through a membrane filter. 0.5 ml of the filtered supernatant was added in 1ml of 1mM $AgNO_3$ stock solution and 10 ml of MilliQ water. Visual observation was conducted periodically to check for the nanoparticle formation[5]. The brown colour was seen after 72 hrs, which indicates the development of nanoparticles.

Characterization of Silver Nanoparticles: The nanoparticles were then collected for further characterization using UV and FTIR Spectral analysis, HR-TEM and FEG-SEM.

a. UV-Vis Spectroscopy studies: The formation of AgNPs was detected through visible changes in the colour of the solution. UV-Vis spectroscopy (Varian Cary 50 UV-Visible Spectrophotometer) with a wavelength range of 200-900 nm was used for the periodic monitoring of the reduction of $AgNO_3$ to AgNPs. AgNPs were measured against Milli Q water in a UV-Vis spectrograph.

b. FTIR (Fourier Transform Infrared spectroscopy): FTIR analysis was performed to identify the potential bio-molecules in the microbial extract responsible for the reduction of the silver ions (Ag) to silver nanoparticles (AgNPs). It was recorded using FTIR (Perkin Elmer) subjected to IR source 400 cm^{-1} to 4000 cm^{-1}.

c. HR-TEM (High Resolution Transmission Electron Microscopy): The surface morphology and size of AgNPs were determined by HR-TEM micrograph which was used to determine the shape and size of the nanoparticles (Tecnai G2, F30 model at 300 kV). The AgNPs sample was loaded on a carbon coated copper grid.

d. FEG-SEM (Field Emission Gun Scanning Electron Microscopy): The surface morphology and size of AgNPs were analysed by FEG-SEM micrograph equipped with Energy-dispersive X-ray Spectroscopy (EDX). The sample was added on double-side coated carbon stubs, dried and observed at different magnifications (JEOL JSM- 7600F).

Biogas Emission: The digester sludge was in 100ml mineral salt medium containing 10g and 25g leachate sample, to this, 2ml of nanoparticles solution was added to both the flasks. The flasks were sealed and kept in incubator at 44°C for 2 weeks. The emission of gas was observed by checking the smell and dipping the tubes in water for the bubbles.

Results and Discussion

Isolation and screening of bacteria (methanogens): In all, 9 strains were isolated from the leachate sample.

The cellulose degrading ability of isolated strains: The decolorization in strains 1,2,3,4,6,7 and 8 was moderate to minimal (Fig. 1). The medium inoculated with strains 5 and 9 showed significant decolorization after 10 days, demonstrating the breakdown of the cellulose. The methanogens use cellulose in four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis[27]. The acid produced during acidogenesis changes the colour of Congo red in the media. Hence, the strains isolated from leachate were methanogens. Strains 5 and 9 had the greatest cellulose degradation. For the creation of nanoparticles, the strain 5 (*B. hunanensis*) with the greatest capacity to break down cellulose was chosen.

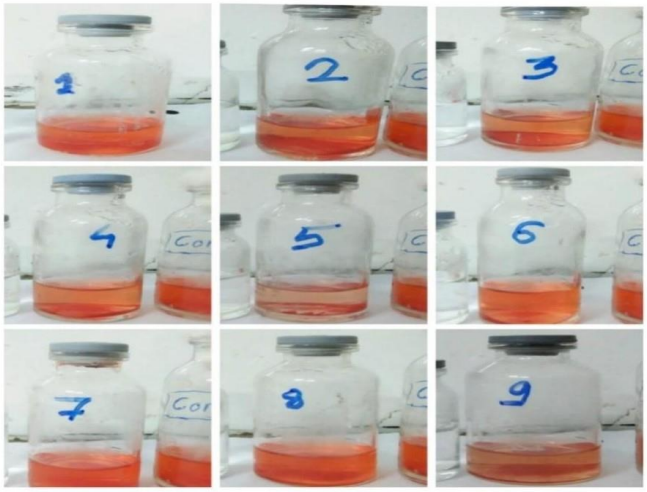

Fig. 1: Cellulose degradation by isolated strains on day 10

Biosynthesis of nanoparticles: The brown colour was seen after 72 hours which indicates the development of nanoparticles using the *B. hunanensis* supernatant (Fig. 2). Brown coloration indicates that the silver nanoparticles are being produced[14].

Characterization of Silver Nanoparticles

a. UV-Vis spectral analysis: The absorption peak was seen in the culture supernatant at 216nm which indicates the formation of silver nanoparticles. The colour changes were seen in the extracellular samples which confirmed the formation of AgNPs. The synthesis, structure and stability of AgNPs are monitored using UV-Visible spectroscopy, which is very effective and dependable technique for AgNPs characterization[2,3]. This colour change confirmed the reduction of silver ions (Ag^+) to a silver atom (Ag^0) and was observed by UV-Visible Spectrophotometer[19,20]. Thus, the colour change and UV absorption data analysis show that the culture supernatant of methanogen (*B. hunanensis*) reduced $AgNO_3$ to silver nanoparticles.

b. FTIR analysis: The peaks were noted at 3308 cm^{-1}, 2104 cm^{-1}, 1634 cm^{-1} and 614 cm^{-1} respectively. Alcohols and phenols with H-bonded bonds that are stretched along the O-H axis make up the peak at 3308 cm^{-1}. Alkynes of the -CC- range are represented by the band at 2104 cm^{-1}. C=C stretch corresponds to the peak in 1634 cm^{-1} (Fig 3). FTIR analysis of AgNPs confirmed the role and presence of certain functional groups of the microbial extract as reducing and capping agent responsible for reduction and capping of silver ions recorded using FTIR (Perkin Elmer) subjected to IR source 400 cm^{-1} to 4000 cm^{-1}.

AgNPs were discovered to havea stretch at about 614 cm^{-1}. In FTIR, nanoparticle production is often seen at a peak between 550 cm^{-1} and 650 cm^{-1}. Similar experiment was done by Begam[4], using marine bacteria *Aeromonas liquefaciens*. The reducing agents were examined using FTIR analysis in order to determine the peak at which the silver nanoparticles are formed. AgNPs were discovered in a span of 618 cm^{-1}, confirming the formation of bacterial supernatant silvernanoparticles.

Fig. 2: Culture supernatant with AgNO3 solution (occurrence of brown colour)

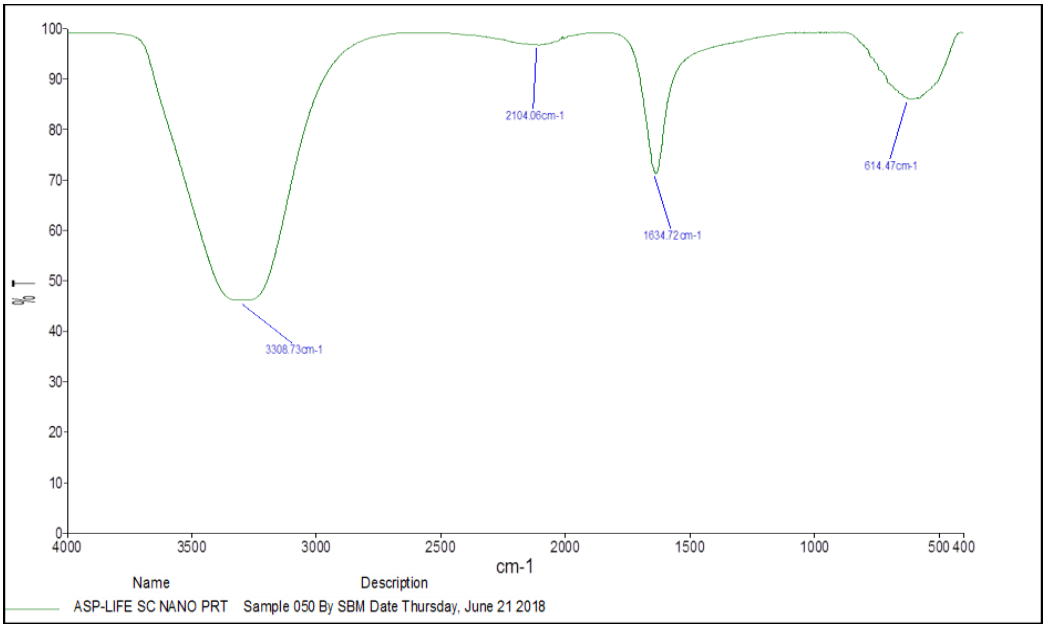

Fig. 3: FTIR Spectrum of silver nanoparticles

c. HR-TEM analysis: The morphology of AgNPs produced by *B. hunanensis* was displayed by HR-TEM micrograph (Fig. 4A to D). The sample under analysis showed differences in particle size; the synthesized AgNPs were mostly spherical and widely scattered. Its dimensions vary from 20 to 50 nm. Concentric rings with bright spots caused by Braggs reflection were visible in fig. 4 (D) of the selected area electron diffraction (SAED) pattern image. The AgNPs' crystallinity was verified by these Bragg reflections from several crystals. AgNPs synthesized from *Elephantopus scaber* leaf extract showed a similar outcome[22].

d. FEG-SEM analysis: The AgNPs' morphology and form were determined using the FEG-SEM micrograph (Fig. 5A). The synthesized AgNPs that were produced, were predominately in spherical shape and ranged in size from 20-50 nm. Chemical characterization or qualitative and quantitative elemental analysis of the sample was measured by EDX[24]. The optical absorption peak at 3 keV caused by surface plasmon resonance in the energy dispersive spectrum verified the existence of nanocrystalline elemental silver[6]. AgNPs synthesized from *Pedalium murex* leaf extract, where particle size ranges from 20-50 nm, showed comparable outcomes[1]. Similar results were obtained using AgNPs synthesized from *Carissa carandas* leaf extract whose particle sizes vary from 20-40 nm[14].

Biogas emission: The synthesized nanoparticles which were utilised in the digester were checked for the emission of biogas. The flask smelled like gas and when the tubes were submerged in water, bubbles were visible, proving that the nanoparticles were responsible for the formation or emission of the biogas (Fig. 6). Kumar et al[12] presented a similar study on nanoparticles and the emission of biogas.

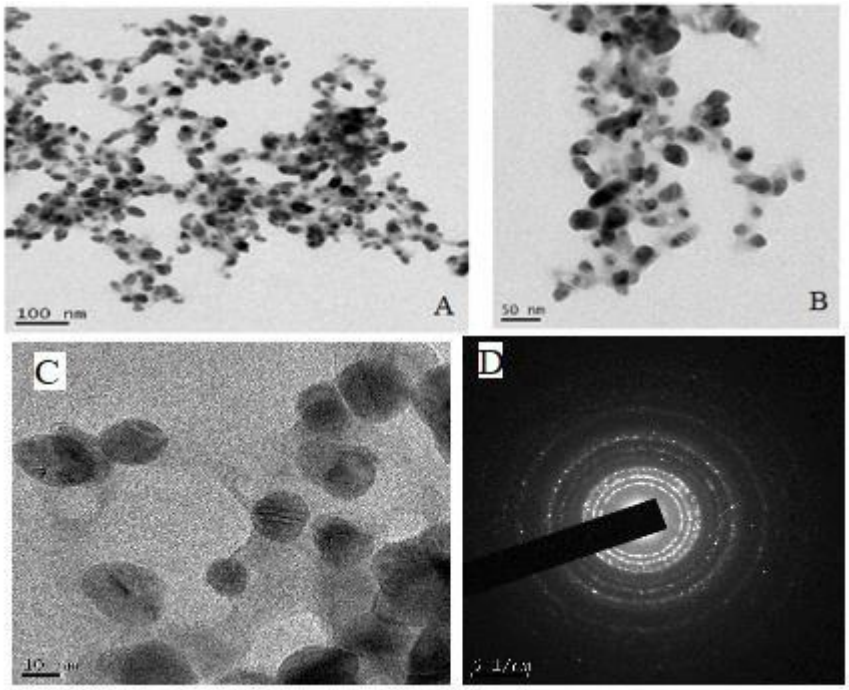

Fig. 4: HR-TEM images of AgNPs at various magnifications (A) 100nm range (B) 50nm range (C) 10nm range (D) SAED patterns of the AgNPs exhibit concentric rings.

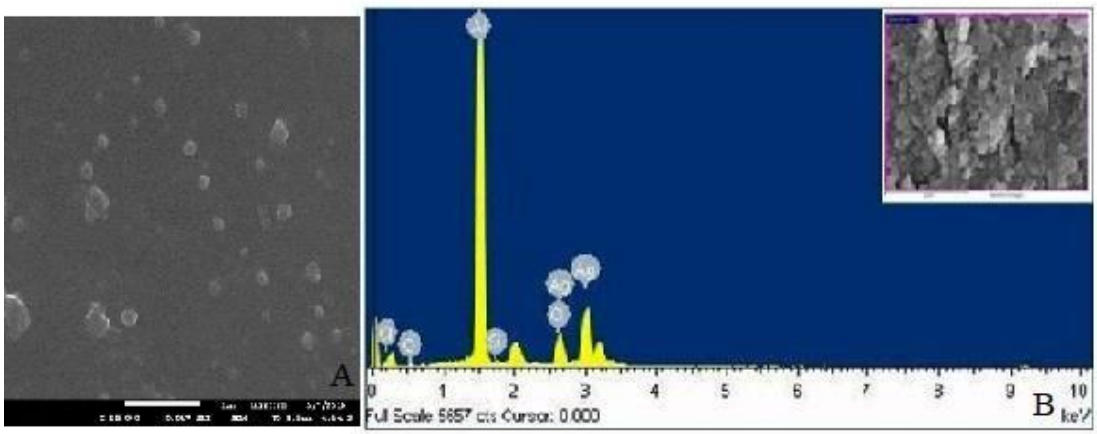

Fig. 5: (A) FEG-SEM image of synthesized AgNPs using microbial extract of *Bacillus hunanensis.* (B) EDX graph

Fig. 6: Biogas Digester

Conclusion

According to this study, a novel strain of *B. hunanensis* can mediate the manufacture of stable silver nanoparticles quickly, sustainably and affordably. AgNPs range in size from 20- 50nm. The presence of functional groups responsible for easy and effective reduction is indicated by FTIR findings. The synthesized particles are spherical in form and nanoparticles as indicated by the TEM and SEM-EDS data. Two weeks of incubation were enough for the nanoparticles to start producing biogas. The benefits of using the green synthetic method for manufacturing the nanoparticle are energy-efficient, safe for human health and the environment.

Acknowledgement

We express our gratitude to Karamchand Yadav and Pratik Dhavan at the Department of Life Science, University of Mumbai and to the Sophisticated Analytical Instrument Facility (SAIF) at IIT Bombay for granting us the access to use their equipment.

References

1. Anandalakshmi K., Venugobal J. and Ramasamy V., Characterization of silver nanoparticles by green synthesis method using *Pedalium murex* leaf extract and their antibacterial activity, *Applied Nanoscience*, **6(3)**, 399–408 **(2016)**

2. Annamalai J. and Nallamuthu T., Green synthesis of silver nanoparticles: characterization and determination of antibacterial potency, *Applied Nanoscience*, **6(2)**, 259–265 **(2016)**

3. Ajitha B., Reddy Y.A.K., Shameer S., Rajesh K.M., Suneetha Y. and Reddy P.S., Lantana camara leaf extract mediated silver nanoparticles: antibacterial, green catalyst, *Journal of Photochemistry and Photobiology B: Biology*, **149**, 84-92 **(2015)**

4. Begam J.N., Biosynthesis and characterization of silver nanoparticles (AgNPs) using marine bacteria against certain human pathogens, *International Journalof Advances in Scientific Research*, **2(7)**, 152 **(2016)**

5. Das N. and Chandran P., Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview, *Biotechnology Research International*, **2011**, 1–13 **(2011)**

6. Govindappa M., Hemashekhar B., Arthikala M.K., Rai V.R. and Ramachandra Y.L., Characterization, antibacterial, antioxidant, antidiabetic, anti-inflammatory and antityrosinase activity of green synthesized silver nanoparticles using *Calophyllum tomentosum* leaves extract, *Results in Physics*, **9**, 400-408 **(2018)**

7. Horsfall D., Biological Synthesis of Metallic Nanoparticles by Bacteria, Fungi and Plants, *Journal of Nanomedicine and Nanotechnology*, **5**, 233 **(2014)**

8. Husseiny M.I., El-Aziz M.A., Badr Y. and Mahmoud M.A., Biosynthesis of gold nanoparticles using *Pseudomonas aeruginosa*, *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, **67(3–4)**, 1003–1006 **(2007)**

9. Iravani S., Korbekandi H., Mirmohammadi S.V. and Zolfaghari B., Synthesis of silver nanoparticles: Chemical, physical and biological methods, *Research in Pharmaceutical Sciences*, **9(6)**, 385-406 **(2014)**

10. Kim M., The Study of Landfill Microbial Communities Using Landfill Gas and Landfill Gas Condensate, Drexel University **(2003)**

11. Kalimuthu K., Suresh Babu R., Venkataraman D., Bilal M. and Gurunathan S., Biosynthesis of silver nanocrystals by *Bacillus licheniformis*, *Colloids and Surfaces B: Biointerfaces*, **65(1)**, 150–153 **(2008)**

12. Kumar S.S., Ghosh P., Kataria N., Kumar D., Thakur S., Pathania D., Kumar V., Nasrullah M. and Singh L., The role of conductive nanoparticles in anaerobic digestion: Mechanism, current status and future perspectives, *Chemosphere*, **280**, 130601 **(2021)**

13 Lead J.R. and Wilkinson K.J., Aquatic colloids and

nanoparticles: Current knowledge and future trends, *Environmental Chemistry*, **3(3)**, 159-171 **(2006)**

14. Madari S.S., Dhavan P.P. and Shah N.J., Biogenic synthesis, characterization of silver nanoparticles from *Carissa carandas* leaf extract and its pharmacological activities, *International Research Journal of Pharmacy*, **11(12)**, 46–52 **(2020)**

15. Mohanraj V.J. and Chen Y., Nanoparticles - A review, *Tropical Journal of Pharmaceutical Research*, **5(1)**, 561-573 **(2007)**

16. Narayanan K.B. and Sakthivel N., Biological synthesis of metal nanoparticles by microbes, *Advances in Colloid and Interface Science*, **15(1-2)**, 1-13 **(2010)**

17. Pantidos N., Biological Synthesis of Metallic Nanoparticles by Bacteria, Fungi and Plants, *Journal of Nanomedicine and Nanotechnology*, **5(5)**, 1 **(2014)**

18. Płaza G.A., Chojnia J. and Banat I.M., Biosurfactant mediated biosynthesis of selected metallic nanoparticles, *International Journal of Molecular Sciences*, **15(8)**, 13720-13737 **(2014)**

19. Sangeetha R., Niranjan P. and Dhanalakshmi N., Characterization of silver nanoparticles synthesized using the extract of the leaves of *Tridax procumbens*, *Research Journal of Medicinal Plants*, **10(2)**, 159-166 **(2016)**

20. Seckler M., Ingle A.P., Gupta I. and Galdiero S., Silver nanoparticles: therapeutical uses, toxicity, and safety issues, *Journal of Pharmaceutical Sciences*, **103(7)**, 1931-1934 **(2014)**

21. Sintubin L., De Windt W., Dick J., Mast J., Ha D.V., Verstraete W. and Boon N., Lacticacid bacteria as reducing and capping agent for the fast and efficient production of silver nanoparticles., *Appl. Microbiol. Biotechnol.*, **84(4)**, 741-749 **(2009)**

22. Sopan N.K. and Vijay D.M., Synthesis, characterization and studies on antioxidant activity of silver nanoparticles using *Elephantopus scaber*, *Materials Science and Engineering C*, **62**, 719–724 **(2016)**

23. Stuchinskaya T., Moreno M., Cook M.J., Edwards D.R. and Russell D.A., Targeted photodynamic therapy of breast cancer cells using antibody-phthalocyanine- gold nanoparticle conjugates, *Photochemical and Photobiological Sciences*, **10(5)**, 822-831 **(2011)**

24. Sudha A., Jeyakanthan J. and Srinivasan P., Green synthesis of silver nanoparticles using *Lippia nodiflora* aerial extract and evaluation of their antioxidant, antibacterial and cytotoxic effects, *Resource-Efficient Technologies*, **3(4)**, 506–515 **(2017)**

25. Thakkar K.N., Mhatre S.S. and Parikh R.Y., Biological synthesis of metallic nanoparticles, *Nanomedicine: Nanotechnology, Biology and Medicine*, **6(2)**, 257-262 **(2010)**

26. Zangeneh M.M., Ghaneialvar H., Akbaribazm M., Ghanimatdan M., Abbasi N., Goorani S., Pirabbasi E. and Zangeneh A., Novel synthesis of *Falcaria vulgaris* leaf extract conjugated copper nanoparticles with potent cytotoxicity, antioxidant, antifungal, antibacterial, and cutaneous wound healing activities under *in vitro* and *in vivo* condition, *Journal of Photochemistry and Photobiology B: Biology*, **197**, 111556 **(2019)**

27. Zinder S.H., Physiological ecology of methanogens, In Methanogenesis: ecology, physiology, biochemistry and genetics, Boston, MA, Springer US, 128-206 **(1993)**.

(Received 07th May 2024, accepted 08th July 2024)