Biological synthesis and characterization of silver nanoparticles using stem extract of *Langenaria siceraria* and their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT

Introduction and Aim: Nanoparticle synthesis using plants extract has been considered ecologically innocuous. In this study we have reported the synthesis of stable silver nanoparticles (AgNPs) using the stem extract of *Lagenaria siceraria* under two different conditions viz. room temperature and sunlight irradiation.

Materials and Methods: The silver nanoparticles were synthesized using 90 ml of 10^{-3} M $AgNO_3$ was added to 10 ml of the aqueous extract of *L. siceraria*. The solutions were kept under two different condition viz. sunlight irradiation and room temperature. The color change of the solutions was monitored periodically using UV-Vis spectroscopy. The synthesized AgNPs were further characterized using XRD, FTIR, DLS, EDX and SEM.

Results: The UV-Vis spectroscopy result of the synthesized AgNPs under the influence of sunlight irradiation showed highest peak with shorter reaction time compared to AgNPs synthesized at room temperature. The XRD analysis of the AgNPs synthesized using sunlight irradiation were crystalline in nature. In addition, the SEM image revealed the AgNPs were spherical in shape with average particles size of 105 nm. Moreover, the AgNPs showed antibacterial activity against *Escherichia coli* (MTCC 739) and *Staphylococcus aureus* (MTCC 96).

Conclusion: From the above study, we can conclude that the biosynthesis of AgNPs using stem extract of *Lagenaria siceraria* is a cost effective and eco-friendly way to produce AgNPs and can be exploited in the field of biomedicines as well as industries.

Keywords: Silver nanoparticles; *Lagenaria siceraria*; stem extract; antibacterial; biomedicine.

INTRODUCTION

Recently nanotechnology has emerged as the most attractive area of research due to its unique physiochemical properties and wide range of applications (1). Nanoparticles are extensively used in the field of therapeutics, electronics, catalysis, sensor (2), forensic science, biomedicine and waste management (3). Silver nanoparticles have a broad spectrum of antibacterial activity even at a very low concentration (4). Furthermore, silver nanoparticles have been reported to have various properties such as anti-inflammatory, anti-plasmodial activity, antiviral, anti-cancer and antimicrobial (5). The silver nanoparticle is highly toxic towards microorganisms and cause structural changes in the bacterial cell membrane, DNA damage, mitochondrial damage and also produce ROS that interferes with the cellular constituents of microorganisms (6). Different routes are available for the synthesis of silver nanoparticles, which includes physical, chemical, photochemical and biological methods. Though physical and chemical method are most commonly used but many disadvantages are associated with these two methods which includes the use of toxic and expensive chemicals. In addition, are high energy dependent and are not eco-friendly (7). Biological method for the synthesis of silver nanoparticles has proved to be the cost effective and environmentally benign process (8). The biological method for synthesizing silver nanoparticles includes fungi, yeast, bacteria, extract of different parts of plants, bacteria, yeast, Fungi etc. (9).

Synthesis of silver nanoparticles by using micro-organisms are costly and labor intense compare to plant mediated biosynthesis of silver nanoparticles as microbes require maintenance of culture media and also maintenance of sterile environment (10).

In the present study, silver nanoparticles has been synthesized from stem extract of *Lagenaria siceraria* under two different conditions viz. room temperature and sunlight irradiation. The stem extract of *Lagenaria siceraria* acts as a reducing as well as capping agent and reduce the silver ions to stable silver nanoparticles. *Lagenaria siceraria* is a soft pubescent, climbing herb belonging to the family of Cucurbitaceae and is used traditionally for the treatment of various diseases. The plant *Lagenaria siceraria* also known as bottle gourd, is a common fruit vegetable used by Indian people (11).

Many Literatures suggests that the stem extract of *Lagenaria siceraria* is diuretic and have antibacterial activity (12). The synthesized nanoparticles were tested to evaluate the inhibitory effect against the two bacterial strains viz. *Escherichia coli* and *Staphylococcus aureus.*

MATERIALS AND METHODS

Materials

Nutrient agar and Silver Nitrate were purchased from Himedia (Mumbai). All the working stocks were made freshly before experiment using double distilled water. *L. siceraria* stems were collected from Guwahati, Assam, India. *Escherichia coli* (MTCC 739) and *Staphylococcus aureus* (MTCC 96) strains were used to evaluate antibacterial activity of biosynthesized AgNPs along with plant extract and standard antibiotic (gentamycin).

Preparation of stem extract of *L. siceraria*

The *L. siceraria* stems were washed thoroughly under tap water and then finally washed twice with doubled distilled water. 25g of *L. siceraria* stems were crushed in 100 ml of double distilled water and boiled at 60ºC for 10 minutes. The extract was cooled down to room temperature and then filtered using Whatman filter paper No.1 (pore size 25µm). The filtered extract was then stored at 4ºC for further use.

Biosynthesis of silver nanoparticles by stem extract of *L. siceraria* under room temperature

For the biosynthesis of the silver nanoparticles, 10 ml of the stem extract of *L. siceraria* was added to 90 ml of 10-3 M AgNO3. The reaction mixture was incubated at room temperature under dark condition and the color change of the reaction mixture was checked periodically. The color change of the reaction mixture from light green to dark brown was observed after 48 hours of incubation at room temperature. The color change was then monitored by using UV-vis spectroscopy.

Biosynthesis of silver nanoparticles using stem extract of *L. siceraria* under sunlight irradiation

10 ml of the of stem extract of *L. siceraria* was added to 90 ml of 10^{-3} M $AgNO_3$. In order to initiate the formation of AgNPs, the reaction mixture was exposed to bright sunlight. The color change of the solution from light green to dark brown started within a few minutes of the exposure and remained unchanged after 32 minutes from the time of exposure. The change in the color confirmed the formation of silver nanoparticles and were monitored by using UV-vis spectroscopy.

Production and recovery of the biosynthesized silver nanoparticles by centrifugation

Among both the methods used for the biosynthesis of silver nanoparticles using stem extract of *L. siceraria*, sunlight irradiation method showed maximum production of silver nanoparticles. Furthermore, it has been selected for bulk production of silver nanoparticles. The biosynthesized AgNPs were then subjected to centrifuge using Eppendorf AG Model No. 5430R at 12,000 rpm for 20 minutes. The pellets were collected and washed 3 times with ethanol to remove any water-soluble biomolecules. The pellets thus obtained was dried at room temperature and were used for XRD, SEM and FTIR analysis.

Characterization of biosynthesized silver nanoparticles

The formation of stable silver nanoparticles under both the conditions viz. room temperature and sunlight were recorded using UV-Vis spectroscopy between 350 to 600 nm. The UV-vis spectral analysis was done using an Analytikjena SPECORD 50 PLUS spectrophotometer. The obtained dried pellets after centrifugation were subjected to XRD analysis by using Bruker AXS, Germany, D8 Advance, operated at a voltage of 40kV and a current of 40mA with Cu Kα radiation. The Scanning Electron Microscope (SEM) analysis has been performed to determine the shape and size of the biosynthesized silver nanoparticles by using ZEISS EVO 18 Special Edition. The crude stem extract of *L. siceraria* (without $AgNO_3$) and the dried pellets of silver nanoparticles were subjected to FTIR (Perkin Elmer FTIR Spectroscopy Spectrum Two) spectroscopy analysis in the range of 500 – 4000 cm^{-1} with KBr pellets. Furthermore, the EDX analysis of the biosynthesized silver nanoparticles were done using Oxford instrument X act "PentaEFT Precision". The average size distribution of the biosynthesized silver nanoparticles was measured using Dynamic Light Scattering (DLS) in the range of 0.1 – 1000 µm at 25 ºC using Nano plus (Micromeritics, USA). The sunlight induced AgNPs were tested for their antibacterial potential against *E. coli* and *S. aureus*. The evaluation of the antibacterial potential of the synthesized silver nanoparticles were done by disc diffusion method on nutrient agar plates. The bacterial culture was grown overnight in nutrient broth having (1 × 105) CFU/ml. The culture was then spread onto the nutrient agar plates. Gentamycin was used as positive control. The crude stem extract of *L. siceraria* were also used to evaluate a comparative analysis of antibacterial activity along with AgNPs against the two bacterial strains. The cultured petri-plates were incubated at 37ºC for 24 hours. After 24 hours of incubation, the zone of inhibition was measured.

RESULTS AND DISCUSSION

UV-VIS Spectroscopy

In this study, the stem extract of *Lagenaria siceraria* (Fig. 1a) were used to synthesize stable silver nanoparticles. 10 ml of the stem extract of *Lagenaria siceraria* was added to 90 ml of 10^{-3} M $AgNO_3$ and were incubated under two conditions viz. room temperature and sunlight irradiation. The color changes from light

green to dark brown was observed in both the conditions as shown in Fig.1 (b) and (c), thus indicating the formation of silver nanoparticles. The colour change takes place due to the excitation of surface Plasmon resonance (SPR) exhibited by the silver nanoparticles (13, 14).

The AgNPs formed under both conditions viz. room temperature and sunlight irradiation were further characterized by using UV-Vis spectroscopy (Fig. 2) in the range between 350-700 nm. The room temperature mediated silver nanoparticles synthesis using stem extract of *Lagenaria siceraria* showed characteristic absorbance peak at 425 nm whereas the sunlight irradiated reaction mixture showed a strong characteristic absorbance peak at 428 nm. Among both the conditions sunlight irradiated nanoparticle synthesis showed highest and sharp peak and were used for further studies.

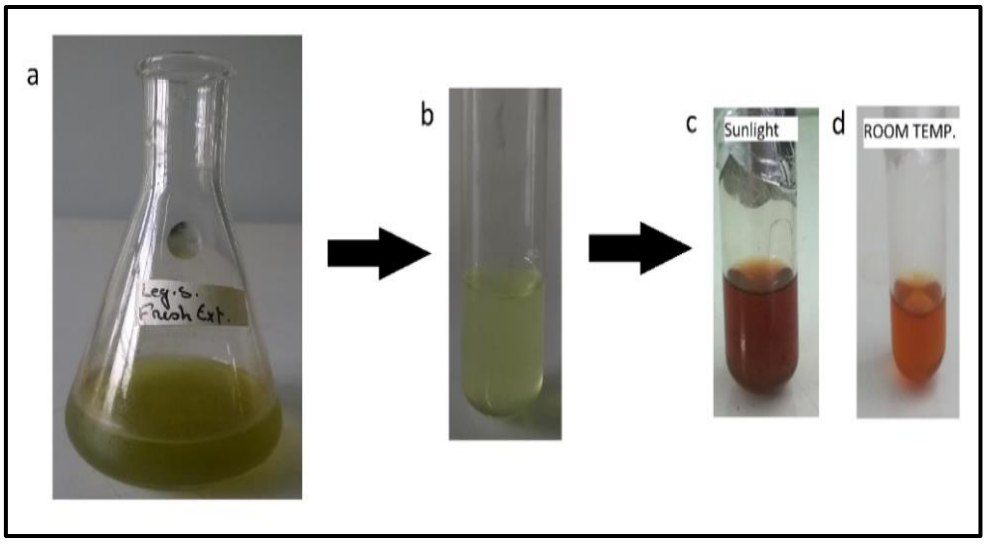

Fig 1: Biosynthesis of AgNPs: (a) Stem extract of *L. siceraria*,(b) Reaction mixture of $AgNO_3$ and plant extract (before reaction), (c) Reaction mixture incubated at room temperature after 48 hours, (d) Reaction mixture exposed to sunlight irradiation after 32 minutes.

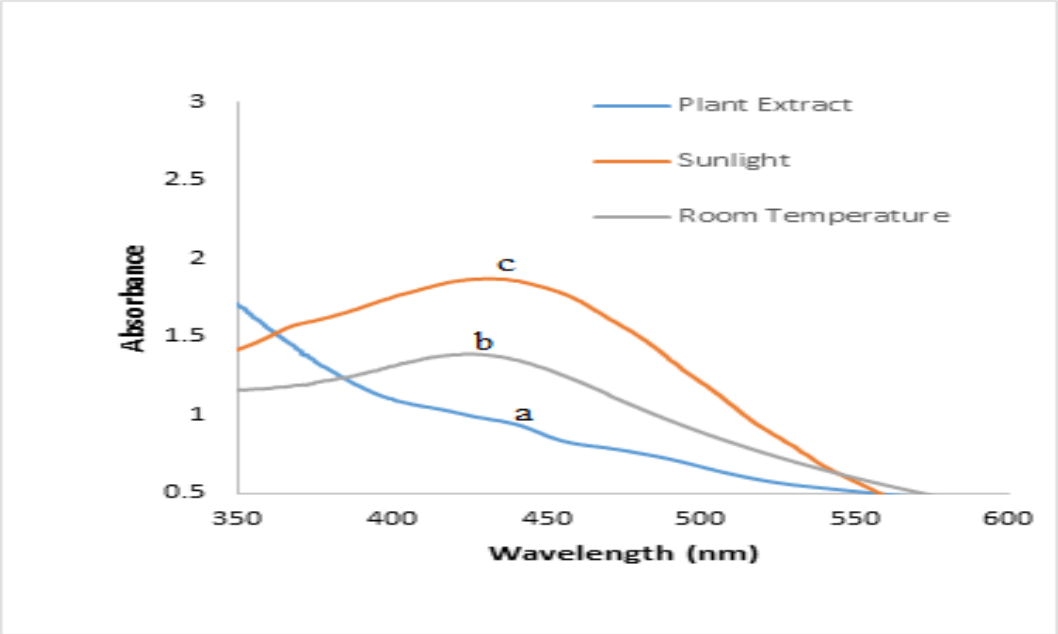

Fig. 2: UV-Vis absorption spectra of: (a) Stem extract *L. siceraria* (control), (b) Biosynthesized AgNPs under room temperature, (c) Biosynthesized AgNPs under sunlight irradiation.

X-Ray diffraction (XRD) analysis

The XRD result (Fig. 3) confirms the synthesis of AgNPs using stem extract of *Lagenaria siceraria*. Silver nanoparticle synthesized showed four intense and sharp peak at $2\theta = 38.10^{o}$, 44.33^{o}, 64.56^{o} and 77.61^{o} and can be assigned to (111), (110), (200) and (311). Thus, from the XRD pattern clearly represents the crystalline nature confirming the formation of silver nanoparticles from the stem extract of *Lagenaria siceraria* (15).

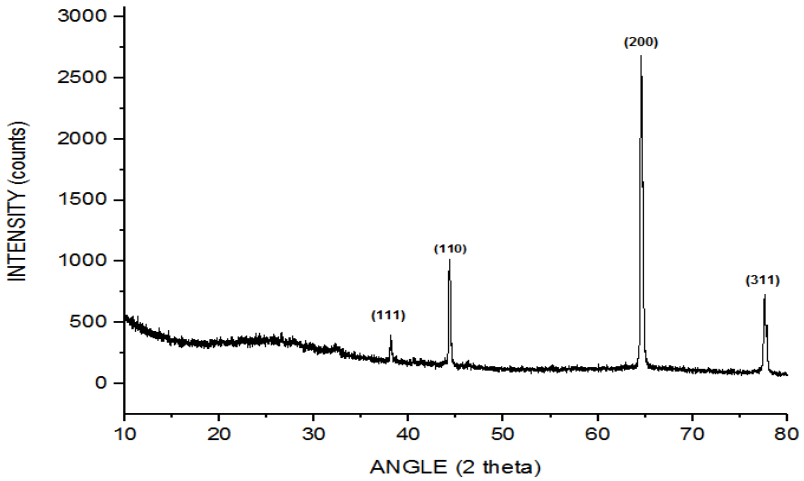

Fig. 3: X-ray diffraction patterns (XRD) of biosynthesized AgNPs

Scanning electron microscopy (SEM) and energy dispersive X-Ray analysis (EDX):

The biosynthesized AgNPs using stem extract of *Lagenaria siceraria* were subjected to SEM analysis for determination of shape of the particles (Fig. 4 a). The spherical shape of the synthesized silver nanoparticles is confirmed through SEM micrograph.

The EDX spectrum (Fig. 4 b) recorded a strong signal of elemental silver, which thus confirms the synthesis of AgNPs using the stem extract of *Lagenaria siceraria*. Other weak signals (C, O, Cl) have also been noted which may be due to the presence of other compounds in the stem extract of *Lagenaria siceraria* (16).

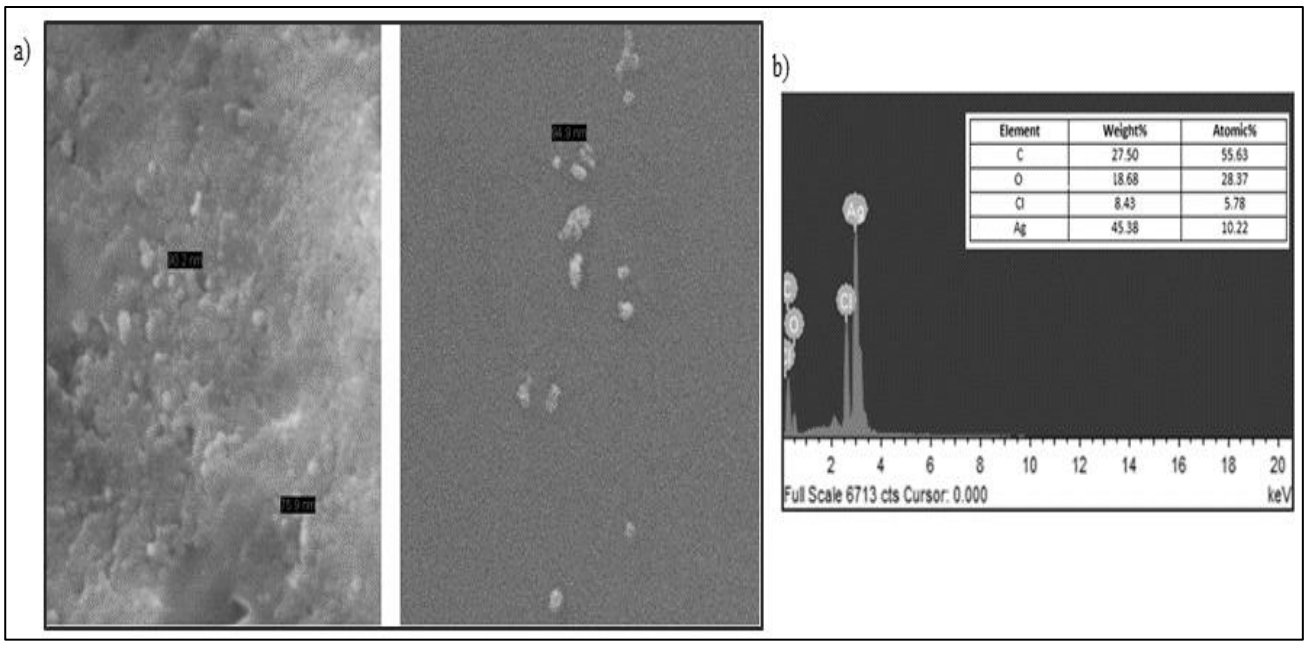

Fig. 4. Scanning Electron Microscopy (SEM) image of biosynthesized AgNPs. a) SEM image of biosynthesized AgNPs and b) EDX Spectrum of biosynthesized AgNPs.

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR absorption spectra of control dried stem extract of *Lagenaria siceraria* (as shown in Fig. 5a) showed peak at 3368 cm^{-1}, 2932 cm^{-1}, 1598 cm^{-1}, 1386 cm^{-1}, 1121 cm^{-1}, 1084 cm^{-1}, 1047 cm^{-1}, 927 cm^{-1}, 855 cm^{-1}, 824 cm^{-1}, 777 cm^{-1}, 621 cm^{-1}, 666 cm^{-1}. The peak at 3368 cm^{-1} is due to the O-H stretching, the band at 1598 and 2932 indicates C–H group. The peak at 1386 could be assigned to (-COO-) carboxylate ions. The peak at 1121 cm^{-1} is due to the carbonyl group. The band at 1084 cm^{-1} could be due to the presence of phenolic group. The peak at 1047 cm^{-1} is due to the presence of C–OH stretching. The peak in between 400 – 800 cm^{-1} could be due to the presence of aromatic groups.

The FTIR absorption spectra of biosynthesized AgNPs showed prominent peaks at 3432 cm^{-1}, 2925 cm^{-1}, 2100 cm^{-1}, 1636 cm^{-1}, 1547 cm^{-1}, 1402 cm^{-1}, 1241 cm^{-1}, 1081 cm^{-1} and 696 cm-1. The broad band at 3432 cm^{-1} is due to the strong O-H stretching intermolecular bond of alcohol, the band at 2925 cm^{-1} corresponding to C-H stretching bands, the band at 2100 cm^{-1} are due to the N- H stretching band in the free amino groups of AgNPs, the band that appeared at 1636 cm^{-1} represented carbonyl (C=O) group, The band at 1547 cm-1 is due to the amides, 1402 cm^{-1} can be assigned to C=C aromatic.

The band at 1241 cm^{-1} and 1081 cm^{-1} may be due to the C–N stretching.

The above study revealed the interaction between the Ag^{+} ions with the aqueous stem extract of *Lagenaria siceraria*. The following data also explains the multifunction of the stem extract of *Lagenaria siceraria* as both the reducing and stabilizing agent (17).

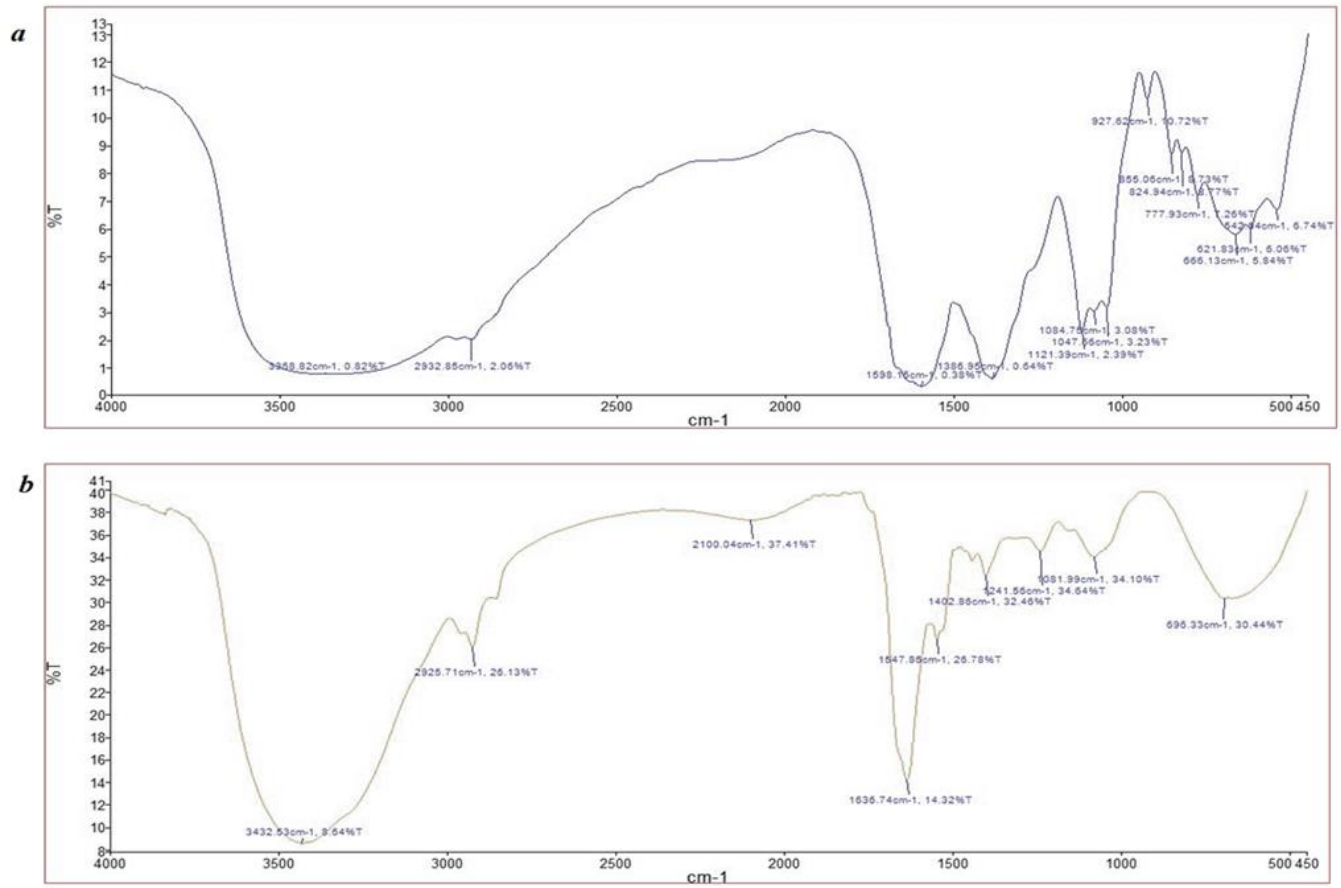

Fig. 5: Fourier transform infrared spectroscopy (FTIR) image of: (a) Control dried stem extract of *L. siceraria* (without $AgNO_3$) *and* (b) Biosynthesized AgNPs (after reaction with $AgNO_3$)

Dynamic light scattering (DLS):

From the dynamic Light Scattering (DLS) analysis, the size distribution of the synthesized silver nanoparticles was obtained by measuring the dynamic variation of the light scattering intensity caused due to the Brownian movement of the synthesized particles. The measurement provide the hydrodynamic diameter that is the particles diameter along with the ion or molecule attached with it (18, 19). The average particle size of the synthesized AgNPs were found to be 105 nm and the polydispersity index was 0.160 as shown in Fig. 6.

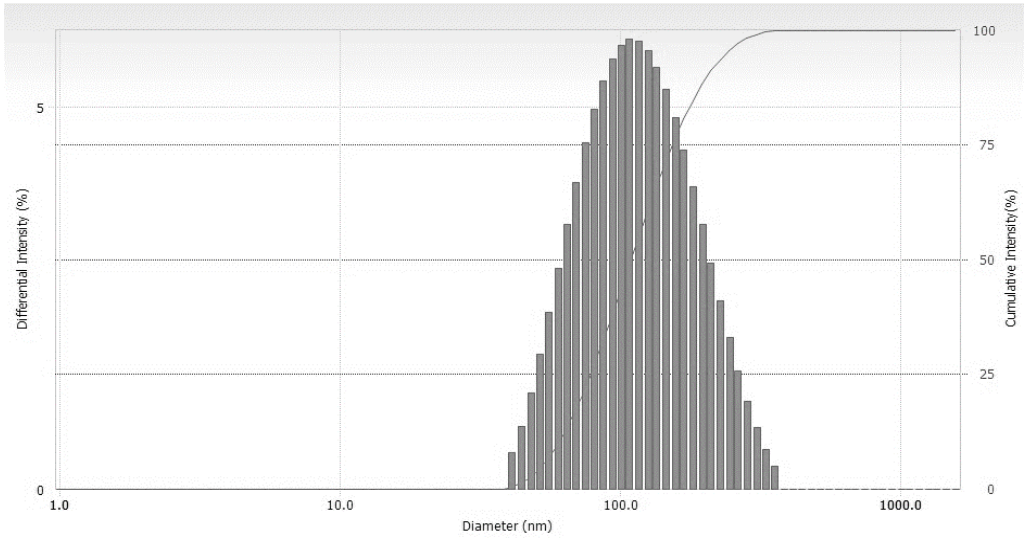

Fig. 6: Particle size distribution of synthesized AgNPs.

Antibacterial activity of AgNPs

The antibacterial activity of the biosynthesized silver nanoparticles was evaluated against gram positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) strains of bacteria using disc diffusion method (20). The antibacterial activity of the synthesized AgNPs was found to be effective against both the strains of bacteria. The result of the study are depicted in [Fig. 8 (a) and (b)] and the zone of inhibition are showed in Table 1. The biosynthesized AgNPs showed satisfactory inhibition activity than the crude stem extract of *Lagenaria siceraria* itself. The exact mechanism behind the antibacterial activity of the silver nanoparticles is still not known. According to literatures silver nanoparticles binds to the thiol group of the cellular enzymes, also report suggests that silver ion interact with the cell membrane and increasing its permeability and the respiration. In addition, AgNPs interact with the DNA by reacting with the sulfur and the phosphorus group (21-23).

In the present study, it has been shown that the biosynthesized silver nanoparticles from stem extract of *L. siceraria* has antibacterial activity against both *S. aureus* and Gram-negative *Escherichia coli* bacterial strains.

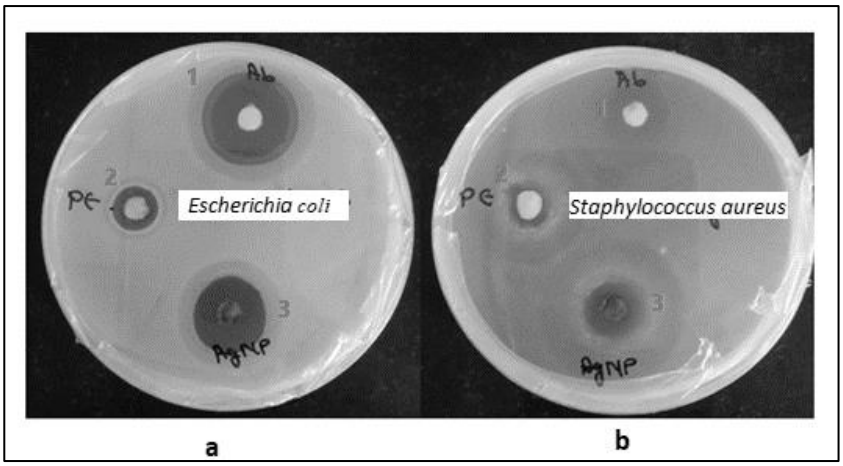

Fig. 7: Anti-bacterial activity of synthesized AgNPs

Table 1: Anti-bacterial activity of biosynthesized $AgNO_3$

Sl. No.	Species	Antibiotics	Stem Extract	AgNPs
1.	*S. aureus*	1 cm	0.7 cm	1.2 cm
2.	*E. coli*	2.4 cm	1.1 cm	1.5 cm

CONCLUSION

In the present study, silver nanoparticles were biosynthesized by using the stem extract of *L. siceraria* under two different conditions viz. room temperature and sunlight irradiation. The colour change of the reaction mixtures containing ($AgNO_3$ + stem extract of *L. siceraria*), visually confirmed the formation of silver nanoparticles. In addition, UV-Vis spectroscopy further confirmed the formation of AgNPs using stem extract of *L. siceraria* between 350 -700 nm. UV-Vis analysis results thus obtained showed characteristic absorbance peak at 425 nm and 428 nm for both the conditions. The synthesis of silver nanoparticles by sunlight irradiation was found to be faster and effective method in terms of reaction time as compared to silver nanoparticle synthesized at room temperature. The synthesized AgNPs via sunlight were characterized further using X-ray diffraction (XRD) analysis (FTIR), FTIR spectroscopy, Scanning Electron Microscopy (SEM), Dynamic Light Scattering (DLS) and Energy Dispersive X-ray analysis (EDX). SEM and DLS analysis revealed that the AgNPs were spherical in shape with the average particle size of 105 nm and the polydispersity index was 0.160 respectively. The XRD pattern obtained thus clearly proves that the biosynthesized AgNPs are crystalline face centered cubic (fcc) in nature. Additional to this, The EDX analysis showed strong signal of Ag (Silver) that confirmed the AgNPs formation and FTIR analysis revealed various bands shifting which is due to the biomolecules present in the crude extract involved in reducing and capping of the Ag ions. Furthermore, the biosynthesized AgNPs showed antibacterial activity against both the Gram positive (*S. aureus*) and Gram-negative *(E. coli*) bacterial strains.

From the above results, it can be concluded that biosynthesis of silver nanoparticles using stem extract of *L. siceraria* is an energy efficient process for producing silver nanoparticles and can be exploited in production of biomedicine in near future.

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REFERENCES

1. Singh, P., Kim, Y. J., Wang, C., Mathiyalagan, R., El-Agamy Farh, M., Yang, D. C. Biogenic silver and gold nanoparticles synthesized using red ginseng root extract, and their applications. Artificial cells, nanomedicine, and biotechnology. 2016 Apr 2; 44(3): 811-816.
2. Dhand, V., Soumya, L., Bharadwaj, S., Chakra, S., Bhatt, D., Sreedhar, B. Green synthesis of silver nanoparticles using Coffea arabica seed extract and its antibacterial activity. Materials Science and Engineering: C. 2016 Jan 1; 58: 36-43.
3. Basu, S., Maji, P., Ganguly, J. Rapid green synthesis of silver nanoparticles by aqueous extract of seeds of Nyctanthes arbor-tristis. Applied Nanoscience. 2016 Jan 1; 6(1): 1-5.
4. Yugandhar, P., Savithramma, N. Biosynthesis, characterization and antimicrobial studies of green synthesized silver nanoparticles from fruit extract of *Syzygium alternifolium* (Wt.) Walp. an endemic, endangered medicinal tree taxon. Applied Nanoscience. 2016 Feb 1; 6(2): 223-233.
5. Rokhade, V. K., Taranath, T. C. Phytosynthesis of silver nanoparticles using fruit extract of *Leea indica* (Burm. f.) Merr. And their antimicrobial activity. Int J Pharm Sci Res. 2017 Mar 1; 8: 1319-1325.
6. Ahmad, A., Wei, Y., Syed, F., Tahir, K., Rehman, A. U., Khan, A., *et al*. The effects of bacteria-nanoparticles interface on the antibacterial activity of green synthesized silver nanoparticles. Microbial pathogenesis. 2017 Jan 1; 102: 133-142.
7. Selim, H. M., Mohamed, D. S., Eskander, H. M. Silver nanoparticles: synthesis, medical application, and toxicity effects. International Journal of Nanotechnology and Allied Sciences. 2017 Dec 13; 1(1): 45-53.
8. Sengottaiyan, A., Mythili, R., Selvankumar, T., Aravinthan, A., Kamala-Kannan, S., Manoharan, K., *et al*. Green synthesis of silver nanoparticles using Solanum indicum L. and their antibacterial, splenocyte cytotoxic potentials. Research on Chemical Intermediates. 2016 Apr 1; 42(4): 3095-3103.
9. Haghighi Pak, Z., Abbaspour, H., Karimi, N., Fattahi, A. Eco-friendly synthesis and antimicrobial activity of silver nanoparticles using *Dracocephalum moldavica* seed extract. Applied sciences. 2016 Mar 2; 6(3): 69.
10. Ahmed, S., Ikram, S. Silver nanoparticles: one pot green synthesis using Terminalia arjuna extract for biological application. J Nanomed Nanotechnol. 2015 Jul 1; 6(4): 309.
11. Roopan, S. M., Rajeswari, V. D., Kalpana, V. N., Elango, G. Biotechnology and pharmacological evaluation of Indian vegetable crop *Lagenaria siceraria*: an overview. Applied microbiology and biotechnology. 2016 Feb 1; 100(3): 1153-1162.
12. Kumar, D., Sharma, C., Singh, B., Singh, D. Pharmacognostical, phytochemical and pharmacological profile of natural remedy *Lagenaria siceraria* (Mol.) Standly: A review. Brit. J. Pharmaceut. Res. 2015 Jan 1; 7(5): 340-352.
13. Abdullah, N. I., Ahmad, M. B., Shameli, K. Biosynthesis of silver nanoparticles using Artocarpus elasticus stem bark extract. Chemistry Central Journal. 2015 Dec; 9(1): 61.
14. Moodley, J. S., Krishna, S. B., Pillay, K., Govender, P. Green synthesis of silver nanoparticles from Moringa oleifera leaf extracts and its antimicrobial potential. Advances in Natural Sciences: Nanoscience and Nanotechnology. 2018 Mar 9; 9(1): 015011.
15. Mary, R. C., Panneerselvam, A., Dhanasekaram, D. Biofabrication of silver nanoparticles by Marine fungus *Aspergillus fumigatu:* Synthesis, characterization and antibacterial activity' International Journal of Scientific Research and Reviews, 2018; 7(2): 59-71.
16. Khan, F. A., Zahoor, M., Jalal, A., Rahman, A. U. Green synthesis of silver nanoparticles by using Ziziphus nummularia leaves aqueous extract and their biological activities. Journal of Nanomaterials. 2016 Mar 1; 2016: 21.
17. Pirtarighat, S., Ghannadnia, M., Baghshahi, S. Green synthesis of silver nanoparticles using the plant extract of Salvia spinosa grown in vitro and their antibacterial activity assessment. Journal of Nanostructure in Chemistry. 2019 Mar 1; 9(1): 1-9.
18. Elamawi, R. M., Al-Harbi, R. E., Hendi, A. A. Biosynthesis and characterization of silver nanoparticles using *Trichoderma longibrachiatum* and their effect on phytopathogenic fungi. Egyptian journal of biological pest control. 2018 Dec 1; 28(1): 28.
19. Erjaee, H., Rajaian, H., Nazifi, S. Synthesis and characterization of novel silver nanoparticles using Chamaemelum nobile extract for antibacterial application. Advances in Natural Sciences: Nanoscience and Nanotechnology. 2017 May 5; 8(2): 025004.
20. Abbasifar, A., Ghani, S., Irvani, M. A., Rafiee, B., Kaji, B. V., Akbari, A. Antibacterial activity of silver nanoparticles synthesized by using extracts of Hedera helix. Zahedan Journal of Research in Medical Sciences. 2017; 19(1).
21. Prasad, R., Swamy, V. S. Antibacterial activity of silver nanoparticles synthesized by bark extract of Syzygium cumini. Journal of Nanoparticles. 2013 Apr 30; 2013.
22. Rao, B., Tang, R. C. Green synthesis of silver nanoparticles with antibacterial activities using aqueous Eriobotrya japonica leaf extract. Advances in Natural Sciences: Nanoscience and Nanotechnology. 2017 Mar 2; 8(1): 015014.
23. Salomoni, R., Léo, P., Montemor, A. F., Rinaldi, B. G., Rodrigues, M. F. A. Antibacterial effect of silver nanoparticles in Pseudomonas aeruginosa. Nanotechnology, science and applications. 2017; 10: 115.