Sunlight driven biosynthesis of silver nanoparticles using aqueous stem extract of *Tinospora sinensis* (Lour.) Merr. and evaluation of its catalytic and antibacterial activity

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ABSTRACT

Introduction and Aim: The present study investigated the biosynthesis of silver nanoparticles (AgNPs) using aqueous stem extract of *Tinospora sinensis* under the influence of sunlight irradiation.

Materials and Methods: For the biosynthesis of AgNPs 90 ml of 10^{-3} M $AgNO_3$ was mixed with 10 ml of the aqueous stem extract of *Tinospora sinensis* and the solution was kept under sunlight. The formation of AgNPs were monitored using UV-Vis spectroscopy. Furthermore, the characterization of the synthesized AgNPs were accomplished using X-ray diffraction (XRD), Fourier transforms infr ared spectroscopy (FTIR), Scanning electron microscopy (SEM), Energy Dispersive X-ray (EDX), Dynamic Light Scattering (DLS).

Results: The reaction mixture changes its colour from pale yellow to dark brown within 30 minutes. The UV-Vis spectra of AgNPs showed an absorption peak at 445 nm, which indicated the formation of AgNPs. The SEM and the DLS analysis revealed the spherical shape of the AgNPs with an average particle size of 74.2 nm. The XRD analysis confirmed the crystalline nature of the AgNPs. Furthermore, the synthesized silver nanoparticles act as a catalyst in degradation of 4-nitrophenol by $NaBH_4$. Moreover, the biosynthesized AgNPs showed antibacterial activity against three bacterial strains viz. *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027) and *Staphylococcus aureus* (ATCC 6538P).

Conclusion: In the present study, AgNPs have been successfully synthesized using aqueous stem extract of *Tinospora sinensis* under the influence of sunlight irradiation. The synthesized AgNPs exhibits catalytic as well as antibacterial properties that makes it suitable for environmental remediation.

Keywords: Silver nanoparticles; *Tinospora sinensis*; sunlight irradiation; antibacterial; dye degradation.

INTRODUCTION

In recent years nanoparticle synthesis have gained immense popularity due to their versatile nature in the field of nanotechnology. The word *'nano'* is a Greek word, which stands for dwarf. Silver nanoparticles have gained paramount importance among researchers due to their physiochemical properties (1). Silver nanoparticles have been very popular among the researchers due to their unique properties which enables them to plays a pivotal role in various applications such as catalysis, sensor, biomedicine (2), electronics, optics etc. (3)

Moreover, silver nanoparticles can be synthesized using different methods, which includes chemical, physical and biological methods (4). The physical and chemical methods includes chemical precipitation, sol-gel methods, hydrothermal methods, microwave method (5), ball mining, sonication, flame pyrolysis (6), Laser CVD, emulsion polymerization, physical adsorption etc. However, the chemical methods have several drawbacks such as use of costly, toxic and harmful chemicals and are hazardous for the environment (7).

Due to all these limitations in chemical as well as physical methods, emphasis have been given to the synthesis of AgNPs using biological method. Biological method is considered to be rapid, eco-friendly and cost effective method compare to other two methods (8). Biological methods includes use of fungi, bacteria, enzymes and different parts of the plants (9).

Moreover using plant for the synthesis of silver nanoparticles is convenient, time saving method and can be easily scaled up compare to silver nanoparticles synthesis using microorganisms due to its requirement of highly aseptic condition as well as cell culture maintenance. Literature suggest that silver ions are reduced and stabilized into silver nanoparticles by various phytochemicals which includes terpenoids, flavonoids, phenols, alkaloids etc. present in the plant extract (10, 11).

Studies showed silver nanoparticles have a broad spectrum of antimicrobial activity and exhibits a strong inhibitory effect on the growth of microorganisms. Silver nanoparticles inhibits the growth of bacterial cells by disrupting the outer membrane of the cell, damaging the DNA, inactivating

the metabolic enzymes and generating ROS (Reactive Oxygen Species), increased cell membrane permeability etc (12, 13).

The *Tinospora sinensis* commonly called as "Sudarsana" in Sanskrit, "Giloy" in Hindi and "Hoguni lota" in Assamese which have a wide range of medicinal properties and are used by various native tribes to treat various diseases like fever, urinary infection, Jaundice, diabetes etc. The plant is also known to possess anti-inflammatory and immunomodulatory activity (14, 15). The current study elucidated the process for synthesis of silver nanoparticles using aqueous stem extract of *Tinospora sinensis* (Lour.) Merr. under the influence of sunlight irradiation. Further the synthesized AgNPs were investigated for their catalytic activity in degradation of 4- nitrophenol by $NaBH_4$. Also the AgNPs were tested against pathogenic bacterial strains such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* for their antibacterial activity.

MATERIALS AND METHODS

Chemicals

Nutrient agar was purchased from Himedia (Mumbai). Silver nitrate ($AgNO_3$) and Sodium Borohydride ($NaBH_4$) were procured from Merck (Mumbai), India respectively. Deionized water was used throughout the experiment.

Preparation of stem extract of *Tinospora sinensis*

The plant *Tinospora sinensis* was collected from the locality of Jalukbari which lies on the western part of the city of Guwahati, Assam, India. The stems were then separated from rest of the plant parts. The stems were cut into smaller pieces and washed thrice with deionized water to remove any contaminants. The stems were then shade dried for 25 days at room temperature. The dried stems were grinded to fine powder using sterile electric grinder. 25g of the dried stem powder of *Tinospora sinensis* was mixed with 100 ml of deionized water and boiled at 60 ºC for 20 minutes. The boiled mixture was cooled and filtered using Whatman filter paper no. 1 (pore size 25µm). The filtrate was stored at 4 ºC for further experiments.

Biosynthesis of silver nanoparticles

For the biosynthesis of the silver nanoparticles 10 ml of the aqueous stem extract of *Tinospora sinensis* was added to 90 ml of 10^{-3} M $AgNO_3$ and the reaction mixture was exposed to bright sunlight for the reduction of Ag^+ to Ag^0. The colour change of the reaction mixture was observed periodically using UV-Vis spectroscopy (Thermo fisher scientific Multiskan go Model no. 1510). The solution was then centrifuged using Eppendorf AG Model No. 5430R at 12,000 rpm for 15 minutes. The pellets were resuspended in deionized water and centrifuged to remove any non-reacting biomolecule in the colloidal matrix, and then dried at 50ºC in hot air oven. The dried pellets were further used for XRD, FTIR and SEM analysis.

Catalytic activity of synthesised AgNPs

The catalytic potential of the synthesised AgNPs was analysed by reducing 4-nitrophenol dye by $NaBH_4$. In the following reaction 2 mM of 4- NP (1.5 ml) was mixed with 15 mM of $NaBH_4$ (1 ml) and 1 ml of distilled water was added. In the reaction mixture, 0.5 ml of the synthesised silver nanoparticles were added. The reaction was measured by using UV-Vis spectrophotometer (Thermo fisher scientific Multiskan go Model no. 1510) at different intervals (16).

Detection and characterization of synthesized silver nanoparticles

The synthesis of the AgNPs using stem extract of *Tinospora sinensis* was monitored using UV-Vis spectroscopy (Thermo fisher scientific Multiskan go Model no. 1510) in the spectrum range of 300 – 800 nm. Further to study the crystallinity of the biosynthesized silver nanoparticles, the dried pellets were subjected to X-ray diffraction analysis (XRD) analysis using Bruker AXS, Germany, D8 Advance, operated at a voltage of 40kV and a current of 40 ma with Cu Kα radiation.

The crude stem extract of *Tinospora sinensis* and the dried pellets of the biosynthesized AgNPs were subjected to FTIR analysis using Thermo Nicolet 6700 FTIR spectrophotometer in the range of 650-4000 cm^{-1} so as to reveal the various biomolecules and the functional groups present in the stem extract of *Tinospora sinensis* that are responsible for the reduction as well as stabilization of the silver ions. To reveal the morphology of the synthesized AgNPs, SEM analysis was done using ZEISS EVO 18 Special Edition to determine the morphology of the biosynthesized AgNPs.

In addition, the EDX spectra of the biosynthesized AgNPs was recorded by using Oxford instrument X act "PentaEFT Precision". The average particle size distribution of the synthesised AgNPs was determined by Dynamic Light Scattering in the range of 0.1-1000 µm at 25 ºC using Nano plus (Micromeritics, USA).

Antibacterial activity of AgNPs

The antibacterial activity of the biosynthesized AgNPs using stem extract of *Tinospora sinensis* was evaluated using three bacterial strains viz. *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* by disc diffusion method. The sterile disc of 6 mm in size were soaked in crude stem extract of *Tinospora sinensis*, synthesized AgNPs and antibiotic (Gentamicin). The discs were then air dried under sterile condition. The bacterial cultures were incubated overnight at 37 ºC in nutrient broth. The bacterial culture having (1×10^5) CFU/ml was then spread uniformly over nutrient agar plates. The petriplates

were loaded with the discs and incubated at 37 ºC for 24 hour. The formation of the zone of inhibition was then measured (17).

RESULTS AND DISCUSSION

Visual observation of biosynthesized AgNPs

In the present research, silver nanoparticles were synthesised using the stem extract of *Tinospora sinensis* (Lour.). The reaction mixture containing 90 ml of 10^{-3} M $AgNO_3$ and 10 ml of aqueous stem extract of *Tinospora sinensis* was exposed to bright sunlight. The colour of the reaction mixture changed after 30 minutes of sunlight exposure from pale yellow to dark brown colour, which visually confirmed the formation of the silver nanoparticles in the reaction mixture (18).

UV-Vis spectroscopy analysis of AgNPs

The biosynthesis of the silver nanoparticles using stem extract of *Tinospora sinensis* was monitored using UV-Vis spectroscopy in the range of 300 nm – 800 nm (Fig: 2). The colour change of the reaction mixture from pale yellow to dark brown is due to the SPR (Surface Plasmon Resonance) that arise due to the collective oscillation of free electron conduction caused by interaction with electromagnetic fields (19). As shown in Fig. 2 there was no remarkable change in the peak initially when $AgNO_3$ was added with stem extract of *Tinospora sinensis* but as the exposure time under sunlight increased the peak between 400 nm to 460 nm started to emerge. After 30 minutes, no further change in the colour was observed and the UV-Vis spectra showed a prominent peak at 445 nm indicating the synthesis of AgNPs.

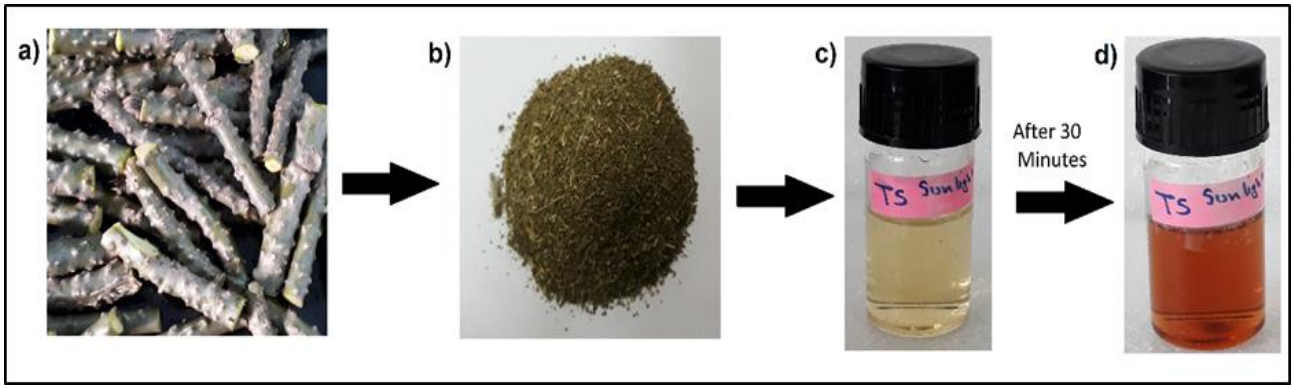

Fig. 1a): Stems of *Tinospora sinensis*. **b)** Dried stem powder of *T. sinensis* **c)** aqueous stem extract of *T. sinensis*. **d)** Synthesized silver nanoparticles after 30 minutes of the sunlight exposure.

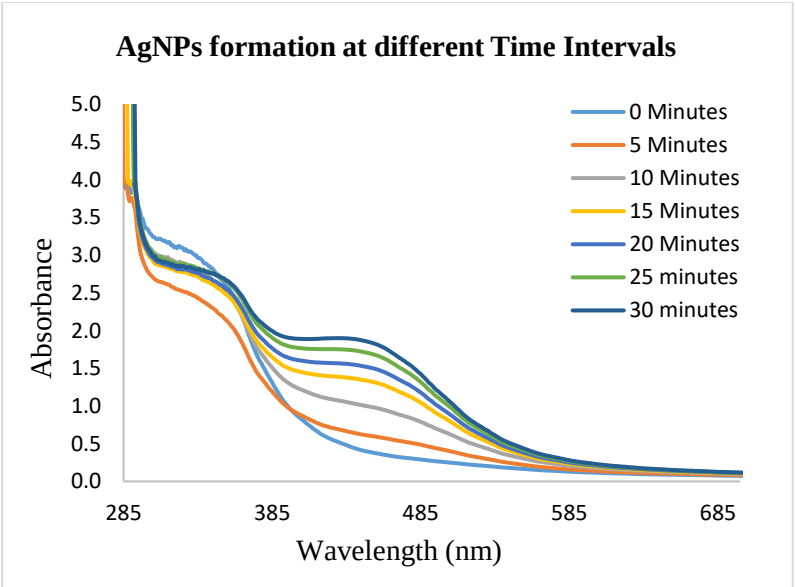

Fig. 2: The UV-Vis spectra of biosynthesized AgNPs at different time intervals

X-ray Diffraction (XRD) analysis

The biosynthesized AgNPs using stem extract of *Tinospora sinensis* were further characterized using XRD analysis. The result as shown in Fig 3. represents four sharp and intense peaks at 2θ = 38.05º, 44.25º, 64.57º and 77.61º, attributed to (111), (200), (220) and (311) planes. From the XRD spectra it is evident that the biosynthesized AgNPs are crystalline in nature (20).

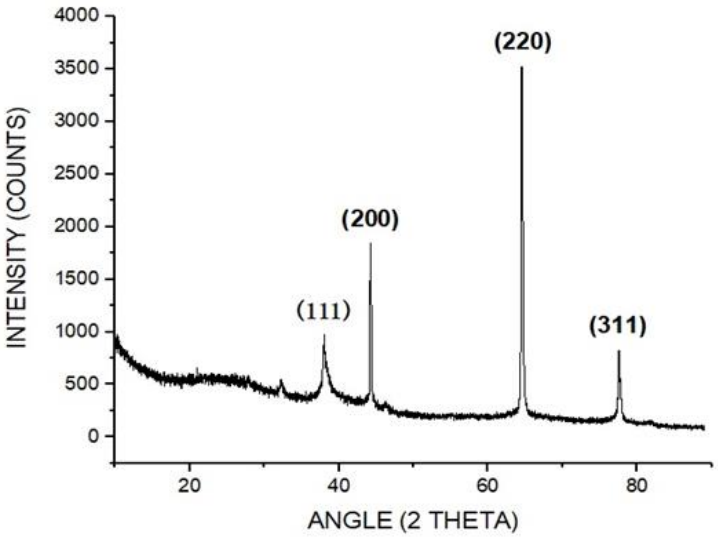

Fig. 3: X-ray diffraction (XRD) spectra of biosynthesized AgNPs

Fourier transform infrared spectroscopy (FTIR) analysis:

The FTIR spectra of both the biosynthesized silver nanoparticles along with the stem extract of *Tinospora sinensis* were recorded as shown in the Fig. 4. The FTIR spectrum of the stem extract of *Tinospora sinensis* showed characteristic peaks at 3336 cm^{-1}, 3231 cm^{-1}, 2946 cm^{-1}, 1745 cm^{-1}, 1635 cm^{-1}, 1557 cm^{-1}, 1019 cm^{-1}, 721 $cm^{-1}$and 694 cm^{-1}. The peak 3336 cm^{-1}, 2946 cm^{-1}, 1745 cm^{-1}, 1635 cm^{-1}, 1557 cm^{-1}, and 694 cm^{-1} corresponds to N-H/O-H vibrational stretch, C-H group, C=O in aldehyde ketone, -C=C group, -C-H- group and -C-C- group.

The FTIR study of the biosynthesized silver nanoparticles showed prominent peak at 3441 cm^{-1}, 2916 cm^{-1}, 2848 cm^{-1}, 1557 cm^{-1}, 1540 cm^{-1}, 1520 cm^{-1} and 1076 cm^{-1}. The peak at 3411 cm^{-1}, 2916 $cm^{-1}$and 2848 cm^{-1}corresponds to –O-H- stretching vibration and –C-H- group stretching due to alkanes (21). 1557 cm^{-1}corresponds to carboxylic functionality. 1540 cm^{-1}could be attributed CONH or NH_2 group. The peak at 1520 cm^{-1}corresponds to N-O asymmetric stretching in nitro compounds and 1076 cm^{-1}corresponds to – C-N- stretching vibration of aliphatic amines (22).

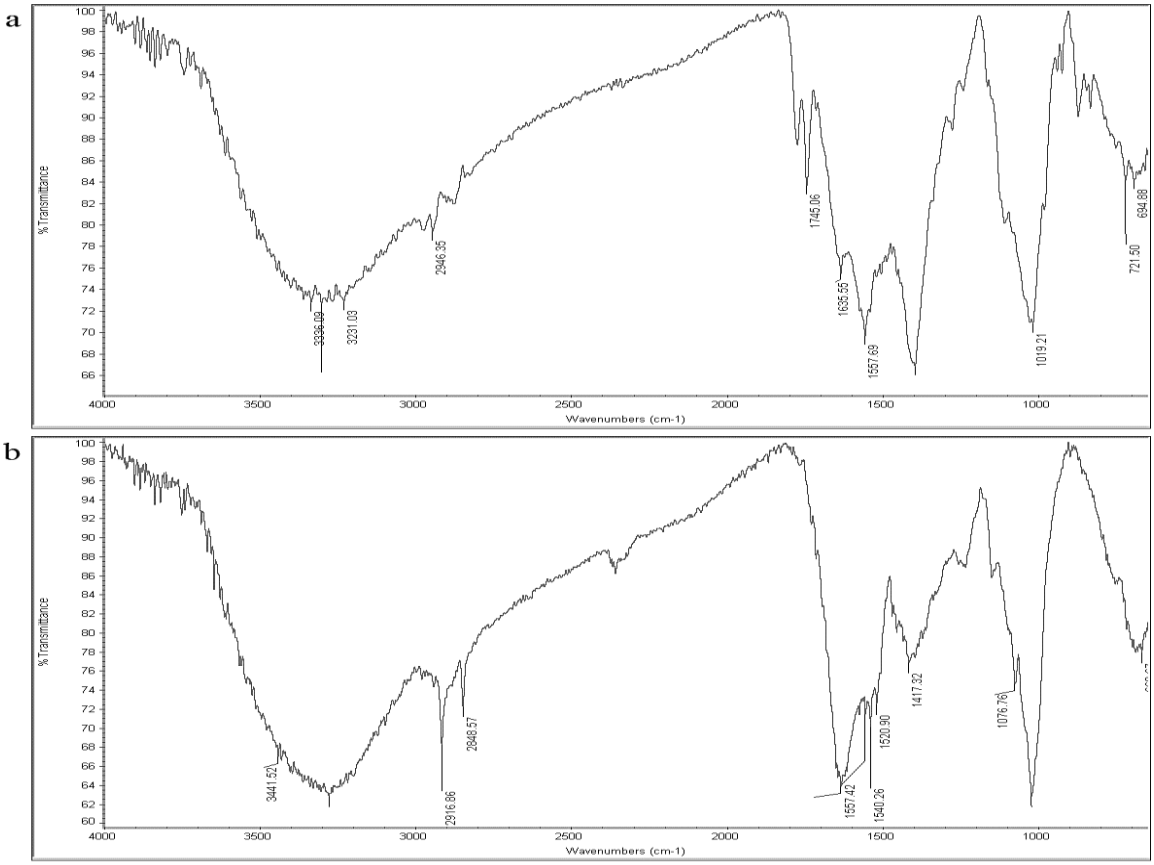

Fig. 4: Fourier transform infrared spectroscopy (FTIR) spectra of (a) Stem extract of *Tinospora sinensis* (b) Synthesized AgNPs.

Scanning electron microscopy (SEM) and Energy Dispersive X-ray (EDX) analysis

The biosynthesized silver nanoparticles were subjected to SEM and EDX analysis. The SEM analysis (as shown in Fig. 5) revealed that the biosynthesized silver nanoparticles were spherical in shape. The EDX spectra (as shown in Fig. 6) showed a sharp and intense peak for silver at 3ke V along with other weak signals such as C, O, P, S, Cl which may be due to the surface biomolecules responsible for capping as well as reduction of the synthesized silver nanoparticles originating from the stem extract of *Tinospora sinensis* as reported earlier (23).

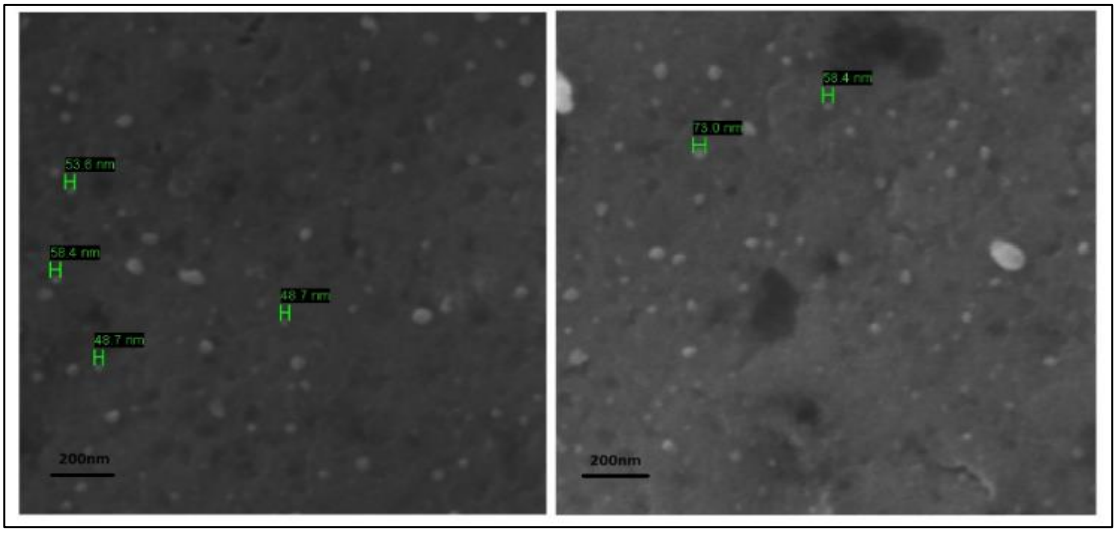

Fig. 5: SEM image of the biosynthesized AgNPs.

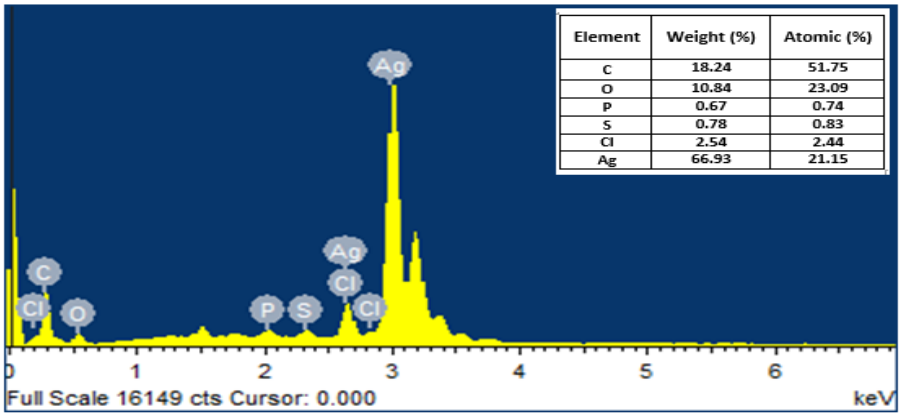

Fig. 6: Energy Dispersive X-ray (EDX) spectra of biosynthesized silver nanoparticles.

Dynamic Light Scattering (DLS)

Dynamic Light Scattering (DLS) analysis of the biosynthesized silver nanoparticles using stem extract of *Tinospora sinensis* is shown in Fig. 7. The average size distribution of the biosynthesized silver nanoparticles was 74.2 nm and the polydispersity index was 0.197.

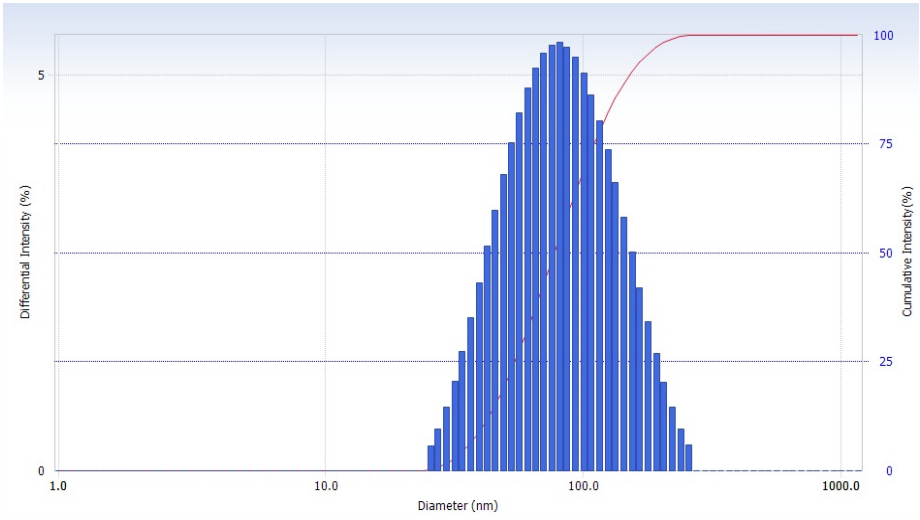

Fig. 7: DLS pattern of the biosynthesized silver nanoparticles.

Catalysis analysis of synthesised AgNPs

4-Nitrophenol, which is an organic dye used in various industrial purposes, is a harmful compound and cause various health related issues. The synthesised AgNPs showed catalytic activity by increasing the rate of degradation of the 4 nitrophenol by $NaBH_4$ as shown in Fig. 8. Initially the reduction rate of 4-NP (4-Nitrophenol) by $NaBH_4$ was slow but after the addition of the synthesized silver nanoparticles in the reaction, the reduction rate of 4-NP by $NaBH_4$ was increased and the reaction took 55 minutes to completely reduced. In the UV-Vis reading, there is a decrease in the peak at around 400 nm as the time increased due to the reduction of 4-NP and also there is low intensity peak observed at 292 nm due to the formation in 4-AP (4-Aminophenol) that increased progressively. In the reaction, the synthesized AgNPs act as a carrier that absorb the hydrogen and transport it between $NaBH_4$ and 4-NP (24, 25). Hence, the result depicted that the synthesised AgNPs using the stem extract of *Tinospora sinensis* showed the catalytic activity in reduction of 4-NP dye by $NaBH_4$.

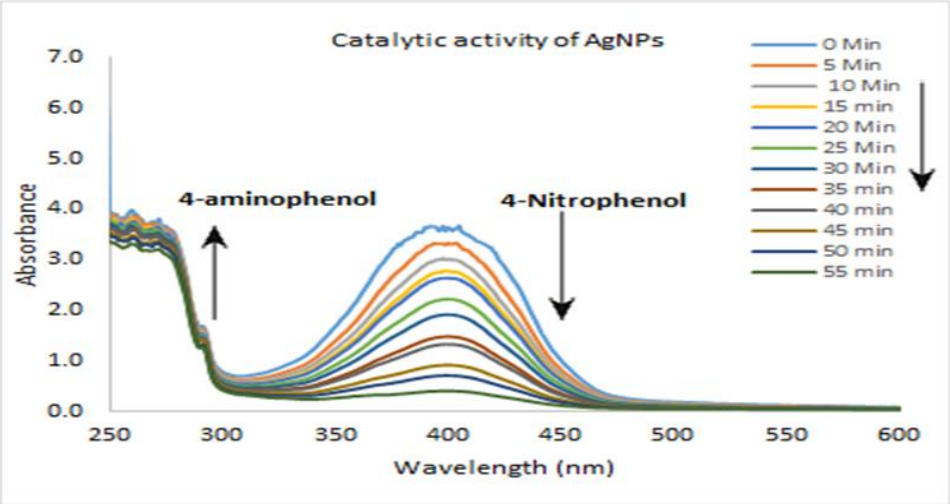

Fig. 8: UV-Vis absorbance spectra of catalytic activity of synthesized AgNPs in reduction of 4-Nitrophenol with $NaBH_4$.

Antibacterial activity of AgNPs

The biosynthesized silver nanoparticles using stem extract of *Tinospora sinensis* was tested for their inhibitory effect against three bacterial strains viz. *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* using disc diffusion methods (Fig. 8) (26). The result as shown in Table 1 suggest that the biosynthesized AgNPs from the stem extract of *Tinospora sinensis* showed high inhibitory effect against three strains of bacteria compare to the aqueous stem extract of *Tinospora sinensis*.

Therefore, the present study revealed that the biosynthesized AgNPs have high inhibitory effect compare to the crude stem extract of *Tinospora sinensis* against *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027) and *Staphylococcus aureus* (ATCC 6538P).

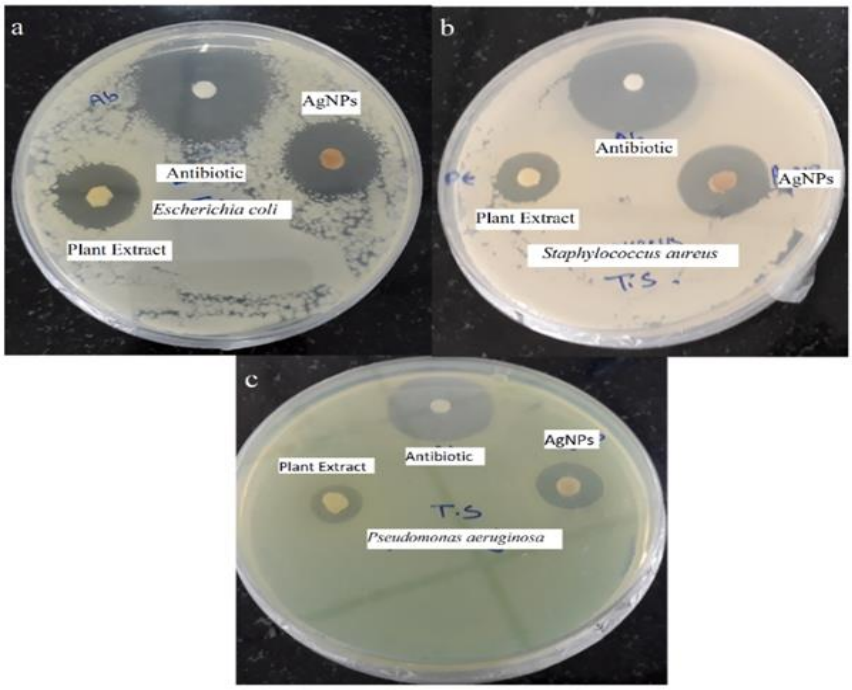

Fig. 9: Antibacterial activity of synthesized AgNPs against (a) *Escherichia coli* (b) *Staphylococcus aureus* (c) *Pseudomonas aeruginosa*

Table 1: Antibacterial effect of biosynthesized AgNPs:

Sl. No.	Species	Antibiotic	AgNPs	Stem extract
1.	*Escherichia coli*	2.9 cm	2.6 cm	1.8 cm
2.	*Staphylococcus aureus*	2.8 cm	2.3 cm	1.5 cm
3.	*Pseudomonas aeruginosa*	2.6 cm	2.5 cm	1 cm

CONCLUSION

According to the present study, the stem extract of *Tinospora sinensis* was found to be an excellent source for the biosynthesis of stable silver nanoparticles under the influence of sunlight irradiation. The biosynthesis of the silver nanoparticles using stem extract of *Tinospora sinensis* under the influence of sunlight was simple, rapid, eco-friendly and energy efficient process. The colour change of the reaction from pale yellow to dark brown have provided a visual confirmation of the AgNPs synthesis in the colloidal solution which has been further characterized using UV-Vis spectroscopy. The UV-Vis have showed an intense peak at 445 nm which corresponds to the synthesis of AgNPs. The XRD analysis also confirmed the crystalline nature of the biosynthesized AgNPs. Further, the SEM analysis revealed the spherical morphology of the AgNPs. In addition, the average particle size of the synthesized AgNPs was found to be 74.2 nm with 0.197 polydispersity index. The FTIR analysis showed the presence of various biomolecules in crude stem extract of *Tinospora sinensis* that are involve in reducing as well as capping the AgNPs. The synthesised AgNPs showed catalytic activity in degradation of 4-NP dye by $NaBH_4$. The biosynthesized AgNPs showed higher inhibitory effect against three bacterial strains viz. *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027) and *Staphylococcus aureus* (ATCC 6538P) as compare to the crude stem extract of *Tinospora sinensis*. The AgNPs thus synthesized could be used as a therapeutic agent in biomedicines as well as to combat various pathogenic bacteria in near future.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Shah, M., Nawaz, S., Jan, H., Uddin, N., Ali, A., Anjum, S., *et al.,* Synthesis of bio-mediated silver nanoparticles from Silybum marianum and their biological and clinical activities. Materials Science and Engineering: C. 2020 Mar 21:110889.
2. Das, S., Gupta, V., Kalyani, M. I., Kalita, M. C., Shukla, S. Biological synthesis and characterization of silver nanoparticles using stem extract of *Langenaria siceraria* and their antibacterial activity against *Echerichia coli* and *Staphylococcus aureus*. Biomedicine. 2019; 39(4): 580-586.
3. Das, S., Kalita, M. C., Shukla, S. Rapid biosynthesis of silver nanoparticles using leaf extract of *Brassica oleracea* Var. Gongylodes and their antimicrobial activity against bacteria. World Journal of Pharmacy and Pharmaceutical sciences. 2018; 7(8): 1135 -1145.
4. Sosnowska, M., Kutwin, M., Adamiak, A., Daniluk, K., Gawin, K., Kutwin, M. P. Green synthesis of silver nanoparticles by using aqueous mint (*Mentha piperita*) and cabbage (*Brassica oleracea* var. capitata) extracts and their antibacterial activity. Annals of Warsaw University of Life Sciences-SGGW. Animal Science. 2017; 56(1): 137-145.
5. Hedaginal, S. R., Taranath, T. C. Characterization and antimicrobial activity of biogenic silver nano-particles using leaf extract of *Thunbergia alata* Bojer Ex Sims. International Journal of Pharmaceutical Sciences and Research. 2017; 8(5): 2070-2081.
6. Shaik, M. R., Khan, M., Kuniyil, M., Al-Warthan, A., Alkhathlan, H. Z., Siddiqui, M. R. H., *et al.,* Plant-extract-assisted green synthesis of silver nanoparticles using *Origanum vulgare* L. extract and their microbicidal activities. Sustainability. 2018; 10(4): 913.
7. Jayaprakash, N., Vijaya, J. J., Kaviyarasu, K., Kombaiah, K., Kennedy, L. J., Ramalingam, R. J., *et al.,* Green synthesis of Ag nanoparticles using Tamarind fruit extract for the antibacterial studies. Journal of Photochemistry and Photobiology B: Biology. 2017; 169: 178-185.
8. Aishwarya, S., Gayathri, R. Synthesis of silver nanoparticles from *Garcinia cambogia* extract and its antimicrobial efficacy. International Journal of Research in Pharmaceutical Sciences. 2018; 9(2): 263-267.
9. Lateef, A., Folarin, B. I., Oladejo, S. M., Akinola, P. O., Beukes , L. S., Gueguim-Kana, E. B. Characterization, antimicrobial, antioxidant, and anticoagulant activities of silver nanoparticles synthesized from *Petiveria alliacea* L. leaf extract. Preparative Biochemistry and Biotechnology. 2018 Aug 9; 48(7): 646-652.
10. Bharali, P., Das, S., Bhandari, N., Das, A. K., Kalita, M. C. Sunlight induced biosynthesis of silver nanoparticles from the bark extract of *Amentotaxus assamica* D. K. Ferguson and its antibacterial activity aganist *Escherichia coli* and Staphylococcus aureus. *IET Nanobiotechnology*. 2018; 13(1): 18-22.
11. Ansari, M. A., Alzohairy, M. A. One-Pot Facile Green Synthesis of Silver Nanoparticles Using Seed Extract of *Phoenix dactylifera* and their Bactericidal Potential against MRSA. Evidence-Based Complementary and Alternative Medicine. 2018; 1-9.
12. Das, S., Sharma, R., Nath, N., Kalita, M. C. Phyto-assisted rapid biosynthesis of silver nanoparticles using leaf extract of *Croton caudatus* Geisel and assessment of their dye degradation and antibacterial activity. International Journal of Scientific Research and Reviews. 2019; 8(2): 2791 -2808.

13. 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; 102: 133-142.
14. Narkhede, A. N., Jagtap, S. D., Kasote, D. M, Kulkarni, O. P., Harsulkar, A. M. Comparative immunomodulation potential of *Tinospora cordifolia* (Willd.) Miers ex Hook. F., *Tinospora sinensis* (Lour.) Merrill and *Tinospora cordifolia* growing on *Azadirachta indica* A. Juss. 2014.
15. Lam, S. H., Chen, P. H., Hung, H. Y., Hwang, T. L., Chiang, C. C., Thang, T. D., *et al.*, Chemical constituents from the stems of Tinospora sinensis and their bioactivity. Molecules. 2018 Oct; 23(10): 2541.
16. Mata, R., Nakkala, J. R. Sadras, S. R. Catalytic and biological activities of green silver nanoparticles synthesized from *Plumeria alba* (frangipani) flower extract. Materials science and Engineering: C, 2015; 51: 216-225.
17. Behravan, M., Panahi, A. H., Naghizadeh, A., Ziaee, M., Mahdavi, R., Mirzapour, A. Facile green synthesis of silver nanoparticles using Berberis vulgaris leaf and root aqueous extract and its antibacterial activity. International journal of biological macromolecules. 2019 Mar 1; 124: 148-154.
18. Arya, G., Kumari, R. M., Gupta, N., Kumar, A., Chandra, R., Nimesh, S. Green synthesis of silver nanoparticles using *Prosopis juliflora* bark extract: reaction optimization, antimicrobial and catalytic activities. Artificial cells, nanomedicine, and biotechnology. 2018 Jul 4; 46(5): 985-993.
19. Joshi, N., Jain, N., Pathak, A., Singh, J., Prasad, R., Upadhyaya, C. P. Biosynthesis of silver nanoparticles using Carissa carandas berries and its potential antibacterial activities. Journal of Sol-Gel Science and Technology. 2018 Jun 1; 86(3): 682-689.
20. Rautela, A., Rani, J., Das, M. D. Green synthesis of silver nanoparticles from *Tectona grandis* seeds extract: characterization and mechanism of antimicrobial action on different microorganisms. Journal of Analytical Science and Technology. 2019 Dec; 10(1): 1-10.
21. Supraja, S., Ali, S. M., Chakravarthy, N., Jaya Prakash Priya, A., Sagadevan, E., Kasinathan, M. K., Sindhu, S. Arumugam, P. Green synthesis of silver nanoparticles from *Cynodon dactylon* leaf extract. Int. J. Chem. Tech, 2013; 5(1): 271-277.
22. Sahayaraj, K., Rajesh, S., Rathi, J. M. Silver Nanoparticles biosynthesis using marine alga *Padina pavonica* (Linn.) And its microbicidal activity. Digest Journal of Nanomaterials & Biostructures (DJNB). 2012; 7(4).
23. Jemal, K., Sandeep, B. V., Pola, S. Synthesis, characterization, and evaluation of the antibacterial activity of *Allophylus serratus* leaf and leaf derived callus extracts mediated silver nanoparticles. Journal of Nanomaterials. 2017 Jan 1; 2017.
24. Al-Marhaby, F. A., Seoudi, R. Preparation and characterization of silver nanoparticles and their use in catalytic reduction of 4-Nitrophenol. World Journal of Nano Science and Engineering, 2016; 6(01): 29.
25. Sravanthi, K., Ayodhya, D., Swamy, P. Y. Green synthesis, characterization and catalytic activity of 4-nitrophenol reduction and formation of benzimidazoles using bentonite supported zero valent iron nanoparticles. Materials Science for Energy Technologies. 2019; 2(2): 298-307.
26. 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; 6(2): 223-233.