International Journal of Chemistry Studies
www.chemistryjournal.in
ISSN: 2581-348X
Received: 19-07-2022, Accepted: 05-08-2022, Published: 20-08-2022
Volume 6, Issue 2, 2022, Page No. 31-37

Spider silk-based bio fabrication of silver nanoparticles to emphasize anti-bacterial puissance

Sachin B Ghar[1], Yamilee K Das[2], Mulani R M[3*]
[1] Research Scholar, Department of Zoology, School of Life Sciences, SRTMU, Nanded, Maharashtra, India
[2] Assistant Professor, Department of Botany, TACS, (Affiliated to School of Life Sciences, SRTMU), Nanded, Maharashtra, India
[3] Department of Botany, School of Life Sciences, SRTMU, Nanded, Maharashtra, India

Abstract
Silver nanoparticles show potent anti-bacterial dominancy against bacteria. Therefore, its use as an anti-bacterial agent can be helpful in the treatment of bacterial diseases. In the medical field, the silver element has wide applications. To a small extent, it is harmful to living beings as well if the doses are not taken with proper prescription with necessary precautions. The limited quantities of these nanoparticles could be greatly patientstient in treating the disease. The medicinal products can be synthesized from nanomaterials and may also be used to treat fungal infections. The biosynthesized nanoparticles are of interest to study due to their eco-friendliness. The silver nanoparticles are synthesized from the spider silk of Crossopriza lyoni, a house spider. Therefore, it's a novel approach for synthesizing silver nanoparticles from the current inventions in the field of nanotechnology. The current synthesized NPs are 20-30 nm in size with a polydispersity index of -20 mV. The spider silk-based nanoparticles enhance the anti-bacterial potential upto multiple times. Therefore, this study successfully elucidates the anti-bacterial potential against gram-negative bacteria such as Salmonella typhi and Klebsiella pneumoniae at the concentration of 0.6 mg/mL.

Keywords: *Crossopriza lyoni*, Spider silk, Biosynthesis, AgNPs, TEM, AFM, Anti-bacterial activity

Introduction
The era of nanotechnology has arrived to treat, heal and diagnose diseases. In the field of nanotechnology, the particle is defined as a tiny entity that acts as a fundamental element concerning its transportation & properties [1]. The particle fragments are additionally categorized by the bore. Generally, the nanoparticles (NPs) are sized allying 1 & 100 nm in diameter. The granulated particles sweep in the middle of 2,500 to 10,000 nm, air-borne particles are sized between 100 to 2,500 nm, and ultrafine particles are similar in diameter to NPs [2]. Scientists worldwide are interested in NPs research due to its power utilization in electronic, optical & biomedical disciplines. There are multiple procedures for generating NPs; it includes pyrolysis, attrition & hydrothermal synthesis. To determine the properties of NPs, they were coated on metal surfaces. The surface coating can regulate stability, solubility, and targeting [3]. The NPs should be multivalent, bearing multiple targeting groups and able to activate cellular signalling pathways and give strong surface anchoring. Scientists are attracted to the environment-friendly fabrication of NPs because of the lucidity of the task, minimum play with synthetic chemicals and its environment-friendly [4]. Several potent biological substances can be used as capping & stabilization molecules for the fabrication of NPs. The biosynthesis of NPs can be done via biomaterials available in nature. The spider's cobweb is also used in the synthesis of silver nanoparticles (AgNPs) [5]. The spiders are the only dominant animals containing approximately 45,990 species across 114 families worldwide. The spiders belong to the order Araneae. The scorpions are also placed in exact order. Hence both spider and scorpion share the same order. Therefore, the spiders, scorpions, harvestmen, mites and ticks make up the class of Arachnida and are included under the phylum Arthropoda along with insects & crustaceans. Spiders cover a remarkable chunk of the terrene arthropod heterogeneity. Spiders possess great economic value to man because of their role in regulating insect pest populations in agro-ecosystems. Spiders are generalist predators, while several are specialist predators. A spider's body is divided into the front abdomen and the back cephalothorax. The pedicel is a narrow stalk which joins the abdomen and cephalothorax. Spiders are provided with four pairs of legs. They possess six or eight eyes varies species to species. Antennae and jaws are absent, and both the sexes are separate. Spiders have silk glands inside the abdomen, where the silk is produced and then squeezed out by way of spigots that rest on the spinnerets. Spiders are well known for silk production among the arthropods, which is used to make webs or different forms, whichever role as nets or cocoons, to guard their progeny [6]. Spider silk is classified among five fundamental classifieds, *viz.* aciniform, capture spiral, tubiform, major ampullate and minor-ampullate, based on the silk glands in which it is produced. The web's eternal edge & spokes are made by using dragline silk. The dragline web is also a careline, a web filament that the spider

string or pull afterwards. This is used by spiders to run or break off a boundary; the spider can escalate the careline to comparative welfare. The ampullate minor web is utilized for the interim frame in the course of web assembly. The capture-spiral web silk is produced by the flagelliform gland used to construct capturing lines of the web. Silk produced in the tubuliform gland is used to protect egg sacs. Aciniform silk is 2-3 times more potent than another category of silk & is used to wrap and secure captured prey [7]. Unleash hunt decay more quickly than wrapped hunt; it implies that aciniform silk has an anti-microbial activity which resists the decomposition of prey by microbes. People of the Carpathian mountaintop apply pieces of the tube-shaped silk of *Atypus* spiders as contemporary Band-Aid to cure injury due to their antiseptic properties. Certain pieces of evidence suggest that the silk of *Nephila clavipes* helps in mammalian neuronal regeneration [8]. By considering the variety of essential applications of spider silk, the present study is designed to scrutinize the application of spider silk as a green fabrication material for the environment-friendly biosynthesis of AgNPs and to check its anti-bacterial potential against *Salmonella typhi* and *Klebsiella pneumoniae.*

Materials and Methods

Materials

Spider species, Cobweb, glass rod, petri dish, bacterial culture, Streptomycin, Silver nitrate ($AgNO_3$), Sodium hydroxide (NaOH), Aagar-agar, Cork borer, Canon HD (high definition) camera, Fluorescence microscope, UV-Vis spectrophotometer (Thermo Scientific Multiskan SkyHigh Microplate Spectrophotometer), TEM (Thermo Scientific Talos F200E (Scanning) Transmission Electron Microscope), AFM (The Dimension FastScan® atomic force microscope) etc.

1. Collection of spiders and spider silk

The spider *Crossopriza lyoni* is usually found in human dwelling areas. Therefore, these spider species were collected with the help of a spider catcher early in the morning from our own house in Kolhe Nagar, Tq/Dist. Latur, Maharashtra, India, and identified up to species level based on keys described by Sebastian and Peter. Similarly, the spider silk was collected using a sterile glass rod and moving in a zig-zag position around the spider web so that all the web silk gets rapped over the glass rod. After the collection, a further procedure was carried out.

Fig 1: A) Image of *Crossopriza lyoni*

2. Identification of spiders based on morphological characters

The collected spider was observed under the fluorescence microscope to get better images. The live spider images were captured with the Canon HD camera to get high image resolution; images were stored for the record. Later, the spiders were preserved in a 4 % formalin solution for long-term preservation and the parts of the spider were thoroughly identified under the fluorescence microscope. The images of the head, abdomen and legs were nicely captured and compared with previously published data via a literature survey. These comprehensive observations and records are based on the external morphological characteristics of the spider species [9].

3. Biosynthesis of AgNPs from spider silk

The biosynthesis of AgNPs was carried out by following the below procedure. The cobweb collected from the spider web/silk was cleaned neatly with distilled water thrice to detach lint particles and soaked with the help of a hair dryer. In the next step, the protocol described by Tszydel *et al*. was used to electrolyze the spider silk with few modifications to experiment successfully. For the hydrolysis of spider silk, about 100 mg of silk was weighed & added to 10 mL of 0.1 M NaOH and warmed up at 90 °C for 1 h. This step is called the hydrolysis of spider silk. Later, the solution was let to chill and centrifuged at 4000 rpm for 30 minutes. The afloat collected was delegate silk extract utilized to prepare AgNPs as earlier reported by Lateef *et al*. 2015. About 1 mL of silk

extract was adjoined to the 100 mL conical flask carrying 40 mL of 1 mM $AgNO_3$ (0.2 mg/mL) solution to reduce the silver subatomic particles (Ag^{++}). The reaction was fetched out in a steady state at ambient temperature for about half an hour [10]. Finally, the AgNPs were synthesized, and further confirmation was carried out through their characterization.

Fig 2: A) Biosynthesis of silver nanoparticles, a) Effect of increasing pH concentration, b) Colour change with increasing concentrations of AgNPs.

4. Characterization of AgNPs

Characterization is essential in knowing the exact feature, surface, dimensions and shape of the NPs. The optical density of the reaction blend utilizing a UV-visible spectrophotometer and visual observation of the colour change was used to monitor the formation of AgNPs [11]. Based on the UV-vis spectroscopy wavelength, the excitation and emission spectra were measured with the help of a fluorescence spectrophotometer. The TEM images were captured to detect the size of AgNPs [12]. Dynamic Light Scattering (DLS) is performed to check the diameter, size and polydispersity index of AgNPs. The surface properties and roughness were detected using the AFM, and images were captured [13]. Simultaneously, the effects of different parameters such as pH and rising volume of $AgNO_3$ solution on the synthesis of AgNPs were observed by quantifying the penetration at the wavelength where the highest optical density was recorded.

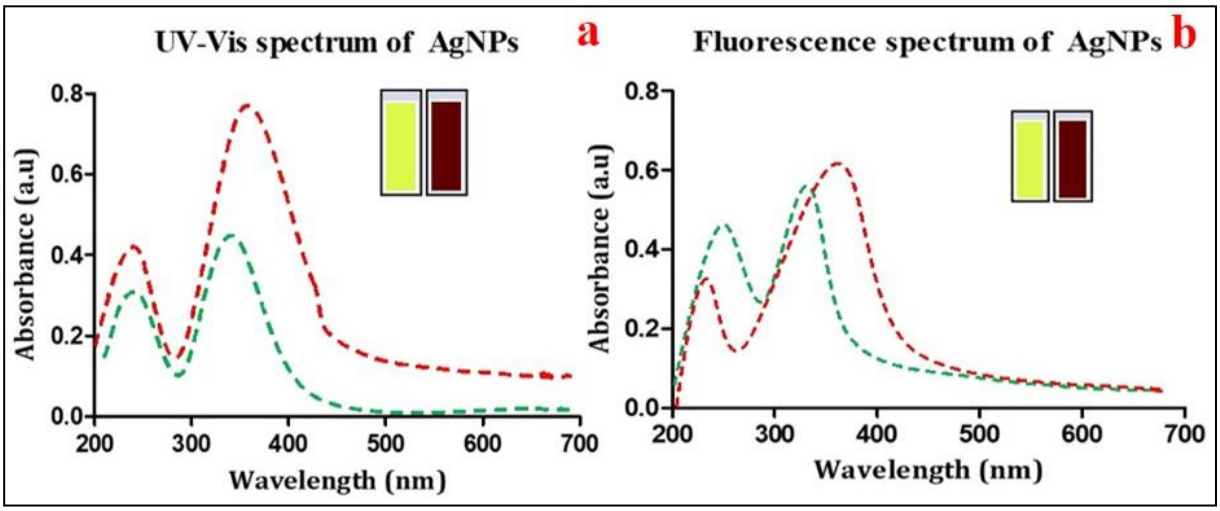

Fig 3: A) The characterization of AgNPs, a) UV-Visible analysis of AgNPs, b) Fluorescence spectra of AgNPs

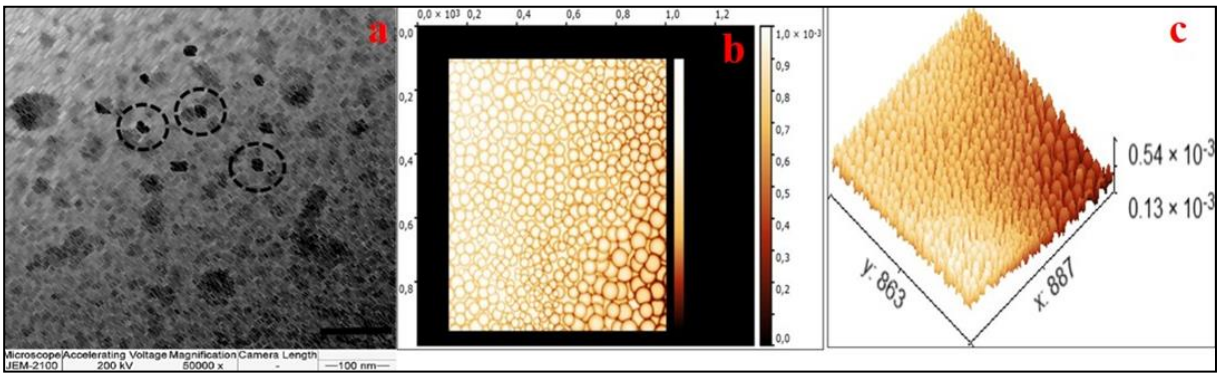

Fig 4: A) The characterization of AgNPs a) TEM image, b) & c) AFM images

5. Anti-bacterial potential of AgNPs

1. Test organisms for anti-bacterial activity

A total of 2-gram negative, harmful bacteria were taken to check the antibacterial potential of prepared AgNPs. These microbes include *Klebsiella pneumoniae* and *Salmonella typhimurium*. Both were gram-negative groups of bacteria. In the next step, these bacteria were inoculated in the nutrient broth for 24 - 48 h with continuous agitating. Simultaneously, the broth with the absence of test bacteria was prepared. These test bacteria acted as diluent. Once the nurture interval was over, the absorbance was measured at 625 nm. The transmission density was calibrated to 0.082 to 0.13 reading by put in sterile broth, and 0.5 McFarland standard of pathogenic bacterial suspension was utilized for the antibacterial assessment [14].

2. *In vitro* anti-bacterial efficacy

The antibacterial puissance of AgNPs was checked by the agar-diffusion method. In brief, the nutrient agar plates were made ready, and 100 µL of the test microbe was pipette out over the centre of the Petri plate & later outspread on all sides employing the glass spreader. The Petri plates were then bored utilizing a cork borer of 7 mm diameter to fabricate wells. The 100 µL of graded concentrations of AgNPs were made in Milli-Q water in increasing order such as 20 %, 40 %, 60 %, 80 %, and 100, % respectively and was loaded in wells. The positive control was streptomycin with a concentration of 50 µg/mL. Then, the Petri plates were put down in an incubator at 37 °C & evacuate after 24 h. The final step recorded the outcome by estimating the inhibition zone calibre [14].

Results and Discussions

1. Collection of spiders and spider silk

In the present study, adult spiders of both sexes were collected with the help of a spider catcher early in the morning from our own house, present in the Kolhe Nagar, Tq/Dist. Latur, Maharashtra, India. The latitude of the Latur district is 18° 24' N & the longitude is 76° 36' E. The spider silk was collected with the help of a glass rod & cleaned minutely with Milli-Q water to liberate the impurity. During the entire collection of the spider cobweb, the thing was kept in mind that only fresh or recently formed cobwebs were collected. The old and new cobwebs were identified with naked eyes. The main difference between the cobwebs was that the old ones looked dirty and blackish in appearance due to the accumulation of dirt particles and the spider's captured prey leftover. In contrast, the fresh cobwebs were white due to the absence of the dirt particle. The spider *Crossopriza lyoni* construct loosely adhered webs called cobweb in neglected areas of the fields at a considerable height from the ground level. This spider species feeds on smaller insects and mosquitoes entangled by the nest [15].

2. Identification of spiders based on morphological characters

The captured spider species were identified up to the species level based on keys described by Sebastian and Peter (2009). Based on the morphological characteristics, the spider, was classified under the phylum-Arthropoda, Class-Arachnida, Order-Araneae, Family-Pholcidae, Genus-*Crossopriza*, and Species-*lyoni*. The spider species *Crossopriza lyoni* has the following characteristic features: Cephalothoraxrax was greyish-white in colour and broad in size. The long, fragile legs with fine hairs and small black spots jointed to the cephalothorax, which was fifteen times longer than the length of the spider carapace. The front appendages were measured at approximately 6 cm in length, while the hind leg was 4.5 cm in length: early-white eyes, six in number, located on the tip of the cephalothorax. The abdomen observes triangular when viewed from the side, with greyish-white patches on the sides (Table 1., Fig. 1. A). The females were 4-7 mm long, while males were slightly smaller than the females. The female spider carries an egg sac in its chelicerae, which comprises approximately 55-58 eggs held together by only a few silken threads [6].

3. Biofabrication of AgNPs from spider silk

The silk extract was hydrolyzed with the NaOH solution, which facilitated the synthesis of AgNPs at room temperature. The characteristic brown colour indicates the synthesis of AgNPs intensified with time but stabilized after some time. There will be dissimilarity in the shade of AgNPs emulsion suspension because of the fabric of bio-molecules used to synthesize the AgNPs. The synthesized NPs were red-brown. These NPs were further subjected to a UV-visible spectrum to confirm the formation of AgNPs, and the highest pick was observed at 400 nm (Fig-1). It was within the previously revealed fluctuation of 391–440 nm for AgNPs [16]. The final concentration of synthesized AgNPs was 0.18 ≈ 0.2 mg/mL (Fig. 2. A a -b).

4. Characterization of AgNPs

The result of the dilution ratio on the fabrication of AgNPs was determined. It was found that a dilution proportion of 1:10 (silk extract: $AgNO_3$ solution) was optimum for the fabrication of AgNPs. The researcher namely Lateef *et al*. (2016) [17] found that a dilution proportion of 1:40 (cobweb extract: $AgNO_3$ solution) was optimum for the production of NPs.

The effect of pH on the production of NPs was also determined, and it was found that pH 8.0 bring out the elevated amount of AgNPs with maximum absorbance at 400 nm (Fig. 3. A a-b). The excitation & emission spectra were put down for the AgNPs. The NPs were excited at 380 nm & received the emission peak at 400 nm. The AFM images reflect sufficient roughness of the NP's surface for attachment to other adherent surfaces. DLS investigation determined the size of NPs around 20 ±2 nm, and the polydispersity index was -20 mV. The TEM

images showed the dimensions in terms of size and irregular appearance of the NPs (Fig. 4. A a-c). Therefore, the size of the prepared AgNPs was around 20 ±2 in nm diameter [11, 12].

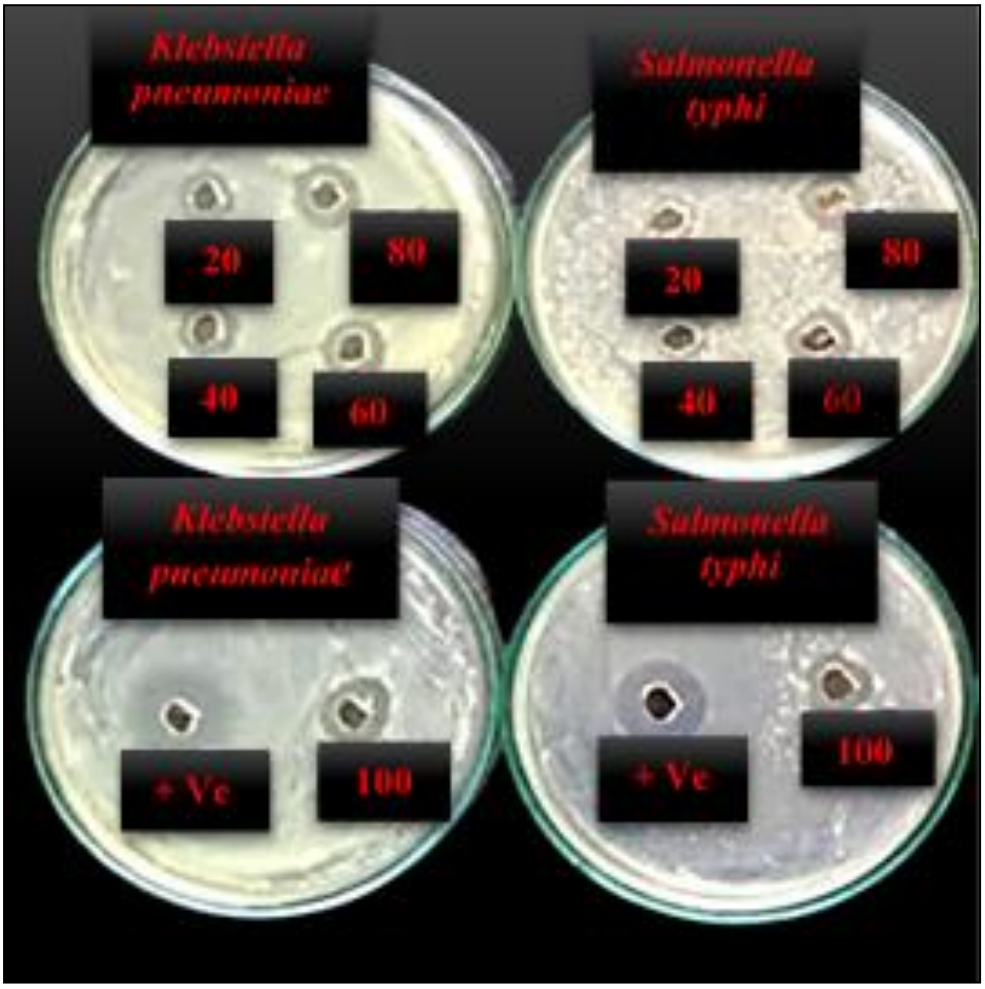

Fig 5: A) Anti-bacterial activity against *Crossopriza lyoni*

Table 1: Classification of *Crossopriza lyoni* spider species.

Classification of *Crossopriza lyoni*	
Phylum	Arthropoda
Class	Arachnida
Order	Araneae
Family	Pholcidae
Genus	*Crossopriza*
Species	*lyoni*

5. Anti-bacterial potential of AgNPs

The synthesized AgNPs were effective against selected microorganisms. The graded concentrations of AgNPs with sterile distilled water were prepared as 20 %, 40 %, 60 %, 80 % and 100 % respectively. The zones of inhibition produced by the AgNPs were recorded (Table 2., Fig 5. A). The highest zone of inhibition was recorded against *Salmonella typhi* and *Klebsiella pneumoniae*, around 15 mm each at 100 % concentration of AgNPs. It was analyzed that with a rise in the concentration, a rise in the zone of inhibition was observed, which indicates that concentration is directly proportional to the inhibition of bacterial growth. Our findings on the anti-bacterial activities of AgNPs resemble with of previously published studies [17]. The positive control streptomycin versus negative control, i.e. 100 % of AgNPs solution, were compared, and it was observed that both the controls resemble in their anti-bacterial potential [11, 14] (Fig. 6. A).

Table 2: Anti-bacterial activity& zone of inhibition by the silver nanoparticles.

Sr.No.	**Name of the bacteria**	**Zone of inhibition (mm)**					
		20 %	**40 %**	**60 %**	**80 %**	**100 % (-Ve control)**	**Streptomycin (+Ve control)**
1.	*Salmonella typhi*	10	12	13	14	15	19
2.	*Klebsiella pneumoniae*	12	13	14	15	15	19

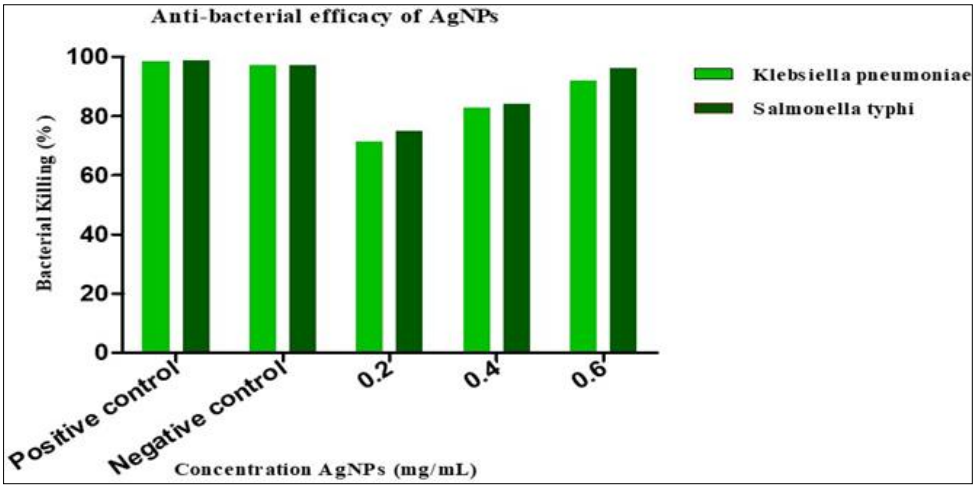

Fig 6: A) Bactericidal effect of AgNPs with increasing concentrations.

Conclusions

Spiders were collected from the region of Kolhe Nagar, Tq/Dist. Latur, Maharashtra, India and identified up to species. The biosynthesis of AgNPs was carried out using hydrolyzed spider silk with NaOH to reduce silver ions to form AgNPs. The characterization technique such as UV-visible spectra, DLS, AFM, and TEM of AgNPs detected the irregular shape of the NPs and their size of around 20 nm on average. Synthesized AgNPs were tested for anti-bacterial potential by the agar diffusion method. The AgNPs effectively impede the growth of *Salmonella typhi* & *Klebsiella pneumoniae* bacterium. Therefore, the final concentration of NPs was 0.2 mg/mL, and the 0.6 mg/mL concentration of AgNPs effectively restricted the bacteria's growth. This research predicts that biosynthesized AgNPs are easy to prepare without using maximum chemicals and are biodegradable due to their eco-friendly nature. This experiment helps the researchers further find ways to improve and prepare the NPs from the spider web, which in turn creates the interest in researchers to use biomaterials and reduce the use of chemicals.

Conflict of interest

All the authors declare there is no conflict to declare.

Acknowledgements

The author S. B. Ghar thanks the DST-INSPIRE (IF 180295) funding agency, New Delhi, Gov. of India, for offering the fellowship.

References

1. Murthy SK. Nanoparticles in modern medicine: state of the art and future challenges. Int J Nanomedicine.,2007:2(2):129-41. PMID: 17722542; PMCID: PMC2673971.
2. Jeevanandam J, Barhoum A, Chan YS, Dufresne A, Danquah MK. Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. Beilstein J Nanotechnol.,2018:9:1050-1074. doi: 10.3762/bjnano.9.98. PMID: 29719757; PMCID: PMC5905289.
3. Ali A, Shah T, Ullah R, Zhou P, Guo M, Ovais M, *et al*. Review on Recent Progress in Magnetic Nanoparticles: Synthesis, Characterization, and Diverse Applications. Front Chem.,2021:9:629054. doi: 10.3389/fchem.2021.629054. PMID: 34327190; PMCID: PMC8314212.
4. Mikušová V, Mikuš P. Advances in Chitosan-Based Nanoparticles for Drug Delivery. Int J Mol Sci.,2021:22(17):9652. doi: 10.3390/ijms22179652. PMID: 34502560; PMCID: PMC8431817.
5. Sachin B Ghar, Yamilee K Das. The green & eco-friendly biosynthesized biocompatible metallic silver nanoparticles for anti-bacterial and anti-biofilm efficacy. European Journal of Biotechnology and Bioscience,2022:10(01):43-49. https://doi.org/10.5281/zenodo.6860383
6. Sachin B Ghar, Yamilee K Das, Mulani RM. A study on morphometric and sexual dimorphism characteristics of giant huntsman spider in Kandi, sangareddy region of Telangana state, India. International Journal of Entomology Research,2022:7(8):1-6. https://doi.org/10.5281/zenodo.6957371
7. Römer L, Scheibel T. The elaborate structure of spider silk: structure and function of a natural high performance fiber. Prion.,2008:2(4):154-61. doi: 10.4161/pri.2.4.7490. Epub 2008 Oct 20. PMID: 19221522; PMCID: PMC2658765.
8. Wright S, Goodacre SL. Evidence for antimicrobial activity associated with common house spider silk. BMC Res Notes.,2012:5:326. doi: 10.1186/1756-0500-5-326. PMID: 22731829; PMCID: PMC3443048.
9. North AJ. Seeing is believing? A beginners' guide to practical pitfalls in image acquisition. J Cell Biol.,2006:172(1):9-18. doi: 10.1083/jcb.200507103. PMID: 16390995; PMCID: PMC2063524.
10. Tszydel M, Zabłotni A, Wojciechowska D, Michalak M, Krucińska I, Szustakiewicz K, *et al*. A. Jaruszewska, J. Strzelecki, Research on possible medical use of silk produced by caddisfly larvae of

Hydropsyche angustipennis (Trichoptera, Insecta), Journal of the Mechanical Behavior of Biomedical Materials,2015:45:142-153, ISSN 1751-6161, https://doi.org/10.1016/j.jmbbm.2015.02.003.
11. Yamilee K Das, Sachin B Ghar, RM Mulani. Mohan Karuppayil S. CSH, ADHESION AND ANTI-BIOFILM STUDIES OF TPL AGNPS USING CENTRAL VENOUS CATHETER MODEL. International research journal of modernization in engineering technology and science,2021:03(05):837-849. https://doi.org/10.5281/zenodo.6409671
12. Yamilee K Das, Sachin B Ghar. The implicit remedy to eradicate Culex Pipiens with eco-friendly bio-integrated lemongrass palladium metallic nanoparticles. https://doi.org/10.5281/zenodo.6610664
13. Yamilee K Das, Sachin B Ghar, Ramjan M Mulani, Mohan Karuppayil S. BiTCoN: A Bimodal Therapy Against Candida albicans. International Journal of Scientific Research and Engineering Development,2021:4(3):27-41. https://doi.org/10.5281/zenodo.6409494
14. Ujwala S Deepake, Yamilee Das, Shital Algunde, Gyananath G. Preliminary screening of endophytic fungi from Enicostemma axillare (Lam.) Raynal. for antimicrobial activity. Current Botany,2012:3(5):23-29. https://doi.org/10.5281/zenodo.6860433
15. Strickman D, Sithiprasasna R, Southard D. Bionomics of the Spider, Crossopriza lyoni (Araneae, Pholcidae), a Predator of Dengue Vectors in Thailand. The Journal of Arachnology,1997:25(2):194-201. http://www.jstor.org/stable/3705644
16. Ashraf JM, Ansari MA, Khan HM, Alzohairy MA, Choi I. Green synthesis of silver nanoparticles and characterization of their inhibitory effects on AGEs formation using biophysical techniques. Sci Rep.,2016:6:20414. doi: 10.1038/srep20414. PMID: 26829907; PMCID: PMC4735866.
17. Lateef A, Ojo SA, Azeez MA, *et al.* Cobweb as novel biomaterial for the green and eco-friendly synthesis of silver nanoparticles. Appl Nanosci,2016:6:863-874. https://doi.org/10.1007/s13204-015-0492-9