PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.3217

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Synthesis of silver nanoparticles using *Senna sophera* (L.) Roxb. leaf extract and study of antibacterial and anti-cancer properties

Goli Penchala Pratap[1], Mohd Kashif Husain[1*], Nagaraju Vallepu[2] & Sudarsanam Gudivada[3]

[1]Survey of Medicinal Plants Unit, National Research Institute of Unani Medicine for Skin Disorders (NRIUMSD), Hyderabad -500 038, India

[2]Local Health Tradition, National Institute of Indian Medical Heritage (NIIMH), Hyderabad-500 036, India

[3]Department of Botany, Sri Venkateswara University, Tirupati-517 502, India

*Email: kashifptc@gmail.com

OPEN ACCESS

ARTICLE HISTORY
Received: 25 December 2023
Accepted: 30 July 2024
Available online
Version 1.0 : 01 November 2024

Check for updates

Additional information
Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE
Pratap GP, Husain MK, Vallepu N, Gudivada S. Synthesis of silver nanoparticles using *Senna sophera* (L.) Roxb. leaf extract and study of antibacterial and anti-cancer properties. Plant Science Today (Early Access). https:/doi.org/10.14719/pst.3217

Abstract

The present study aimed to synthesise silver nanoparticles using an aqueous leaf extract of *Senna sophera* (L.) Roxb. (Fabaceae) to evaluate their antioxidant, antibacterial and anticancer activity. The silver nanoparticles (AgNPs) produced were characterized by different spectroscopic and microscopic techniques namely; UV-Vis spectroscopy, FTIR, DLS, XRD and TEM. The prominent peak at 424 nm in UV-Vis spectroscopy confirms the synthesis of nanoparticles, FTIR spectra confirms the presence of polyphenols and proteins from the leaf extract which mainly acts as reducing, capping and stabilizing agent. DLS results confirm the negative value of zeta potential and the XRD results revealed that the AgNPs are crystalline in nature with a face-centered cubic nature. TEM micrograph images indicate that the nanoparticles are 5-35 nm without any agglomeration. EDX result revealed that the weight % of nanoparticles is 69.56 %, indicating the purity of the sample. Biosynthesized AgNPs show effective DPPH, H_2O_2 scavenging activity and FRAP assay. AgNPs show strong antibacterial activity against *Escherichia coli, Klebsiella pneumonia, Salmonella typhi, Bacillus subtilis* and *Staphylococcus aureus* with an inhibition zone of 17.6, 16.03, 15.66, 14.13 and 12.33 mm respectively. The antibacterial activity of leaf aqueous extract at different concentrations of AgNPs revealed the efficacy against *Salmonella typhi*. Further AgNPs exhibited good cytotoxic properties against HepG2 cell line with the IC_{50} value of 95.52 µg/mL. The silver nanoparticles were successfully synthesized using *Senna sophera* leaf extract, proving it to be an economical, environmentally benign and sustainable method for its applications in pharmaceutical field.

Keywords

Silver nanoparticles; Phytofabrication; *Senna sophera*; fever; Fourier-transform infrared spectroscopy; HepG2

Introduction

Nanoscience and nanotechnology deal with the study of the application of very minute particles used in various scientific fields, such as chemistry, biology, physics, material science and engineering (1). A high surface area, high optical and magnetic properties and high mechanical and thermal stability are a few of their characteristics (2).

To benefit from enhanced properties like stronger, lighter, more controllable light spectrum and higher chemical reactivity compared to their larger-scale equivalents, modern scientists and engineers are recognising a variety of methods for painstakingly creating materials based on nanoparticles (3). Because of their distinct physical, chemical and biological characteristics, nanoparticles are useful in all industrial sectors (4). Phyto-fabrication of nanoparticles is eco-friendly, non-toxic, cost -effective and more stable when compared to other physical and chemical methods (5).

In recent times, a novel and prominent part of nanotechnology is the plant-mediated green synthesis of silver nanoparticles (AgNPs) (6). Because of its eco-friendly and worthwhileness, with minimum toxicity compared to chemical hazards, it came out and procured importance (7). Green synthesis includes several benefits over physical and chemical nanoparticle creation. The phyto-mediated synthesis process is non-toxic, eco-friendly, cost-effective and requires less energy (8).

Several bioactive compounds like carbohydrates, glycosides, alkaloids, polyphenols, terpenoids, ascorbic acid, amino acids and oxalic acid are present in plant extracts (9). Phytochemicals carry out the reduction of metal ions and the stabilization of nanoparticles. Furthermore, flavonoids with several functional groups have the ability to create nanoparticles (10). Metal nanoparticles (MNPs) have received significant attention due to their applications in different fields of technology (11). Silver, gold, copper, iron, zinc, platinum and other metal-based nanoparticles have got a lot of attention in medicine (12). Due to their remarkable biological activities, AgNPs have acquired a high approach in the present situation (13). AgNPs are widely used in cosmetics, food storage, textiles, health-related products, household, medical device coating, pharmaceutical, drug delivery, anticancer, antimicrobial and optical sensors (14).

In this regard, plant-mediated silver nanoparticles exhibit more biological activities than those nanoparticles synthesized by chemical processes. Previous studies have shown that various parts of plants, such as leaves, roots, stem, bark, fruits and latex may be used to produce AgNPs (8).

According to World Cancer Research Fund International (15), a new case of liver cancer was diagnosed every nine seconds in 2020, making it the sixth most common type of cancer in the world. It is mostly seen in men rather than women and is the second most common reason for death due to cancer. As the age increases, the risk also increases. Most of the cases are diagnosed at 75 years and above. Less developed countries show about 83 % of liver cancer cases, having the highest incidence in Asia and Africa (16). Symptoms like being overweight/obese, drinking alcohol and eating food contaminated by aflatoxins (toxins from certain fungi) are the prime reasons for liver cancer (15). Cirrhosis, chronic viral hepatitis, long use of oral contraceptives having large doses of estrogen and progesterone, and smoking, increase the chances of affecting liver cancer (17). Patients having cirrhosis (scarring of the liver due to earlier damage) develop hepatocellular carcinoma easily. Nearly 90-95 % of people with hepatocellular carcinoma have underlying cirrhosis. So, any reason for cirrhosis, may be viral or chemical, increases the risk of cancer (15, 18). In India, liver cancer is gradually becoming one of the most common cancers and malignancies due to its fast growth. It affects around 3-5 % of every 100000 individuals, resulting in 30000-50000 new cases annually (19).

Senna sophera (L.) Roxb. (Family: Fabaceae) is an annual shrub found in tropical countries. Popularly the plant is used to treat various respiratory diseases (20) and seeds are used for the management of diabetics (21), leaves are used for jaundice, piles, interstitial and bronchial muscle relaxants, all parts are used for skin diseases, bronchitis, asthma (22), roots used for analgesic and anti-inflammatory (23), the plant is used for psoriasis, asthma, pityriasis, acute bronchitis, cough (24). The Yanadis, Chenchus tribals, traditional healers and rural people of the Nellore district of Andhra Pradesh use *S. sophera* roasted leaf powder as a recipe (made by mixing red chilli and allium) for controlling fever, especially mentioned by them as "*sannipata jwaramu*" and in modern terms it is typhoid fever and body ache. It is also claimed to be the best remedy for jaundice. Therefore, present study aims to determine the effect of *S. sophera* leaf aqueous extract mediated synthesized silver nanoparticle (SS-AgNPs) and its antibacterial, antioxidant and anticancer activities.

Materials and Methods

Plant materials

The healthy *Senna sophera* (L.) Roxb. leaves were used for the present study (Fig. 1 a and b). The leaves were collected from the Udayagiri region of Nellore district of Andhra Pradesh, India (14°53'41.1"N; 79°16'11.3"E). The voucher specimen (GP 118) had been deposited in the Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh. All the chemicals and mediums used in this work were procured from Hi-Media Laboratories Pvt. Ltd. Mumbai.

Preparation of leaf extract

The leaves were brought to the laboratory and washed with tap water, followed by distilled water to remove the dust particles on the surface of the leaves. The rinsed leaves were allowed to shade dry for a week. After completely drying, the leaves were grounded into a fine powder. The aqueous leaf extract of *S. sophera* was prepared by using 5 g of fine powder in 100 mL boiling distilled water for 10 min. The extract was then filtered through cheesecloth followed by Whatman No.1 filter paper. The filtered extract was stored at 4 °C for further use.

Green synthesis of silver nanoparticles

10 mL of aqueous leaf extract of *S. sophera* was added to 90 mL of 1 mM $AgNO_3$ solution and the reaction mixture was allowed to come at room temperature and was kept in


Fig. 1 a. Habitat of *Senna sophera* plant; **b.** A vegetable vendor selling *Senna sophera* leaves to be used as vegetable in the local market of Udayagiri, Nellore district of Andhra Pradesh.

the dark. The reaction mixture's colour changes were often used to identify the bio-reduction of silver ions into nanoparticles.

Characterization of Silver nanoparticles

UV-Vis spectroscopy is used to confirm the formation of silver nanoparticles (AgNPs) in the reaction mixture. UV-Vis spectrum was recorded by using Nanodrop 8000 (Thermo Scientifics, USA) from 200 to 700 nm with a resolution of 1 nm. After complete reduction, the reaction mixture was subjected to centrifugation at 15000 rpm for 15 min. Separated AgNPs were redispersed in distilled water and purified with repeated centrifugation. The obtained AgNPs pellet was allowed to dry in a hot air oven at 40 °C for 24 h. The air-dried AgNPs powder was used for further characterization and applications. FTIR spectroscopy was used to find out the functional groups from the plant aqueous extract which are involved in the reduction reaction. FTIR analysis of dried leaf powder and SS-AgNPs analyzed for the presence of the possible functional groups responsible for reducing nanoparticles using SHIMADZU IR Affinity-1 spectrometer (Bruker Tensor 27, Thermo Scientifics, USA) in the range of 500-4000 cm^{-1} with the resolution of 2 cm^{-1}. Dynamic light scattering was used to determine particle size and zeta potential of synthesized silver nanoparticles (Nano partica SZ 100 Horiba). The morphological structures like the shape and size of SS-AgNPs were analyzed using HR-TEM equipped with EDAX (FEI-TECNAI, G2-20 TWIN). EDAX analyzed the weight % of synthesized SA-AgNPs. XRD analysis revealed the nanoparticles' crystalline nature (Bruker D8 Advance, Panalytical X Pert3).

In vitro Antioxidant activity

DPPH free radical scavenging assay

The SS-AgNPs and plant aqueous extract were tested for *in vitro* antioxidant activity with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay method (25). For the DPPH stock solution preparation, 4 g of DPPH was diluted into 100 mL methanol solution. From stock, 2 mL of 1mM DPPH solution was added to 1 mL of metabolic SS-AgNPs of different concentrations (20, 40, 60, 80 and 100 µg/mL). The reaction mixture was incubated in dark for 45 min. After the incubation period, the absorbance was recorded at 517 nm with a UV-Vis spectrometer (SHIMADZU UV-1800). Methanol was used as the blank, while a 1 mM DPPH solution served as the control. The standard used was ascorbic acid.

H_2O_2 radical scavenging assay

In vitro H_2O_2 radical scavenging assay was followed according to the standard method (26).1 mL of different concentrated leaf extract/ SS-AgNPs (20, 40, 60, 80, 100 µg/mL) was mixed with 2 mL H_2O_2 solution which was prepared in Phosphate buffer (40 mM, pH 7.4). After incubating the reaction mixture for 10 min, UV-visible spectroscopy (SHIMADZU UV-1800) was used to measure the absorbance at 230 nm. The IC_{50} values were calculated through a linear regression coefficient graph.

The scavenging activity of DPPH and H_2O_2 by SS-AgNPs was calculated using the following formula.

$$\text{Radical Scavening activity \%} = \frac{\text{Control Absorbance - Sample Absorbance}}{\text{Control Absorbance}} \text{ X } 100$$

FRAP assay

Ferric reducing antioxidant power (FRAP) assay was followed according to Oyaizu (27) method. Various concentrations of leaf extract/ SS-AgNPs (20, 40, 60, 80, 100 µg/mL) were incubated with phosphate buffer (0.2 M, pH: 6.6, 2.5 mL) and potassium ferricyanide (1 %, 2.5 mL) and the reaction mixture was incubated for 30 min at 50 °C. After the incubation period, trichloroacetic acid (10 %, 2.5 mL) was added to the reaction mixture and centrifuged at 3000 rpm for 10 min. 2.5 mL of supernatant, 2.5 mL of distilled water and $FeCl_3$ (0.01 %, 0.5 mL) were added to the reaction flask and was vortexed for 3 min. The absorbance was recorded at 700 nm.

Antibacterial activity of SS-AgNPs

SS-AgNPs were analyzed for antimicrobial activity against 2 Gram-positive bacterial strains *Staphylococcus aureus* (MTCC-7443) and *Bacillus subtilis* (MTCC-441) and three Gram-negative bacterial strains *Escherichia coli* (MTCC-443), *Klebsiella pneumoniae* (MTCC-8911) and *Salmonella typhi* (MTCC-3224) procured from the Department of Microbiology, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. The disc diffusion assay method was carried

out using a standard protocol (28). 20 µL of plant extract, $AgNO_3$, and 20, 40 µg/mL SS-AgNPs were impregnated in The Whatman No. 1 filter paper, which was then put on the agar medium after being allowed to dry. Ciprofloxacin (5 µg) was used as a standard control. All the samples were kept in the same plate and incubated at 37 °C for 24 h. The experiment was repeated thrice. The diameter of the inhibition zone was measured in millimetres (mm) with the help of a ruler and recorded in a table.

Anticancer activity

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) test, developed by Mosmann, was used to assess the anticancer activity of the SS-AgNPs (29). The National Centre for Cellular Sciences, located in Pune, India, provided the HepG2 cancer cell lines. Penicillin, streptomycin and 10 % FBS were added to Dulbecco's Modified Eagle's medium, which was used to cultivate the cancer cells. In 96-well plates, 5×10^3 cells were kept and the cells were cultured in a CO_2 (5 %) incubator for 12 h. Various SS-AgNP concentrations (12.5, 25, 50, 100 and 200 µg/mL) were added to each well after 12 h and the wells were incubated for another 24 h. Each well received 10 µL of MTT (5 mg/mL in PBS) after 24 h, and was incubated for further 4 h. Following a 4 h incubation period, the medium and MTT were removed and formazan crystals were dissolved in 100 µL of dimethyl sulfoxide (DMSO). An ELISA reader was then used to record the absorbance values at 570 nm. The IC_{50} was calculated by linear regression coefficient ($R^2 = 0.9$).

Results and Discussion

UV-Vis analysis

The SS-AgNPs were synthesized by adding 10 mL of aqueous plant extract as a reducing agent to 90 mL of 1 mM $AgNO_3$. The formation of nanoparticles was initially confirmed by a change in the colour of the solution from yellow to brown. The colour changes of the sample after adding 1 mM $AgNO_3$ was due to the reduction of silver ions by the biomolecules found in the sample. A collective oscillation of electrons occurs in silver nanoparticles. These oscillations affect the light interactions with nanoparticles. A particle's shape and size determine certain oscillations. So, nanoparticles are in various sizes and shapes and have various colors. The concentration of plant extract affects the colour intensity as well (30). The principal mechanism related to AgNP synthesis is the electrostatic interaction between the functional groups of respective active constituents of plant extract and Ag^+ ion (31). UV-Vis spectral pattern confirmed the development of SS-AgNPs in the reaction mixture showing maximum absorbance peak at 424 nm (Fig. 2). The nature and chemistry of the dispersion medium as well as the size and form of the metal nanoparticles, determine the interaction (33). Surface plasma resonance (SPR) produces a large UV-Vis peak between 400 and 450 nm, which suggests that tiny, spherically-shaped AgNPs are forming in solution. The result of the interaction between light photons and the conduction electrons of metal nanoparticles is a resonance phenomenon known as surface plasmon resonance (SPR). Same type of results was found in the tuber-mediated synthesis of AgNPs from *Jatropha heynei* (32).

FTIR analysis

The FTIR spectroscopic analysis was performed to identify the functional groups from plant extract responsible for reducing, capping and stabilizing the AgNPs (Fig. 3). The FTIR spectra of SS-AgNPs showing the strong and broad peak in 3228 cm^{-1} represent the O-H stretch vibration of polyphenolic compounds (33). The peak at 2920 and 2850 cm^{-1} corresponds to the C-H stretch vibration of alkenes. The IR band at 1753 cm^{-1} is due to C=O stretch carboxylic acids, band at 1600 cm^{-1} is due to N-H bend 1° amines. The IR band at 1350 cm^{-1} is due to N-O symmetric stretch nitro compounds. The IR band at 1041 cm^{-1} corresponds to C-N stretch aliphatic amines and the band at 547 cm^{-1} corresponds to C-Br stretch alkyl halides. FTIR spectra confirmed the participation of the bioactive compounds present in the SS leaf aqueous extract (hydroxyl groups of polyphenols and amid groups of proteins) involved in the biosynthesis and stabilization of SS-AgNPs. Phytochemical coated/capped SS-AgNPs confer stability for SS-AgNPs by preventing agglomeration (32).

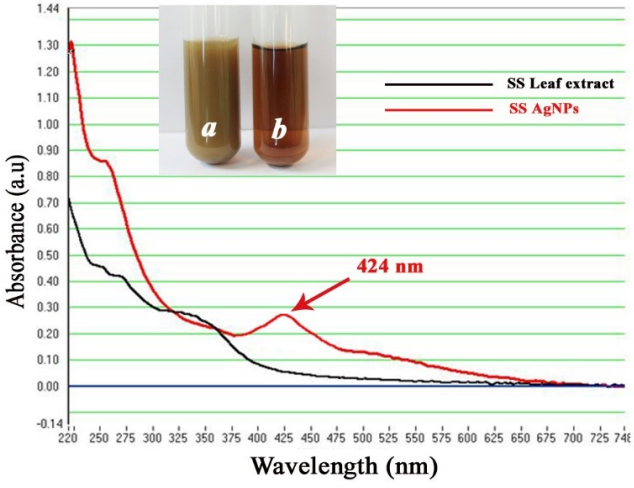


Fig. 2. UV-Vis absorption spectra of SS-AgNPs; **a.** aqueous leaf extract; **b.** SS-AgNPs

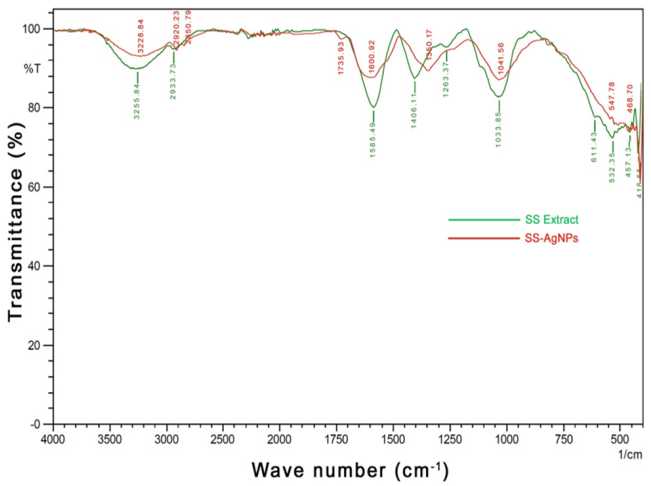


Fig. 3. FTIR analysis of dried powder and SS-AgNPs synthesis of nanoparticles.

DLS analysis

Particle size analysis of SS-AgNPs DLS showed the average hydrodynamic size of NPs to be 20-70 nm in size with an average hydrodynamic radius of 30.6 nm. The polydispersity index was recorded as 0.243 (Fig. 4 a). As a result, it was discovered that the biogenic AgNPs PDI values were less than 0.7, indicating a limited size distribution. As DLS measurement generally depicts the hydrodynamic diameter of a particle with a hydration shell, there is a difference in particle size measurements between TEM and DLS. The hydration cell consists of the stabilizer, which moves with the particle. Due to its poor resolution and lack of robustness, DLS analysis can only determine the qualitative characteristics of AgNPs. Hence, the results of DLS and TEM measurements were compared in this study. The zeta potential value of biosynthesized SS-AgNPs was recorded as -12 mV (Fig. 4 b). The zeta potential value larger than 20 mV and less than -20 mV has stronger electrostatic repulsion and hence is more likely to remain stable in solution. This high negative value indicates that the AgNPs are repelled by one another electrostatically, which keeps the nanoparticles from clumping together in the medium and ensures the formulation's long-term stability (34). Their pH value also influences the stability of any biosynthesized AgNPs; when pH rises, the zeta potential of AgNPs rises (35).

XRD analysis

XRD measurement verified the crystalline form of biogenic SS-AgNPs. The X-ray diffraction patterns of biosynthesized SS-AgNPs powder samples showed distinct diffraction peaks at 2θ angles of 38.13, 44.33, 64.42, 77.38 and 81.51, corresponding to the (111), (200), (220), (311) and (222) Bragg's reflection of the face-centered cubic (FCC) structure of the silver crystal respectively (Fig. 4 c). All these diffraction patterns agree with standard JCPDS data (File No. 89-3722).

TEM analysis and EDX analysis

TEM analysis images reveal that the sizes of SS-AgNPs were in the range of 5-35 nm with almost spherical shapes (Fig. 5 a-b). At 490 kx magnification, biosynthesized nanoparticles clearly showed a 0.231 nm interface between 2 lattice fringes. The lattice fringe spacing value of 0.231 nm corresponds to the (111) crystal plane (Fig. 5 c) and reveals the dominant faces of silver spheres as (111) (32, 36). Six diffraction peaks that corresponded to the (111), (200), (220), (311) and (222) planes were seen in the SAED pattern (Fig. 5 d). The correlation between XRD and SAED data

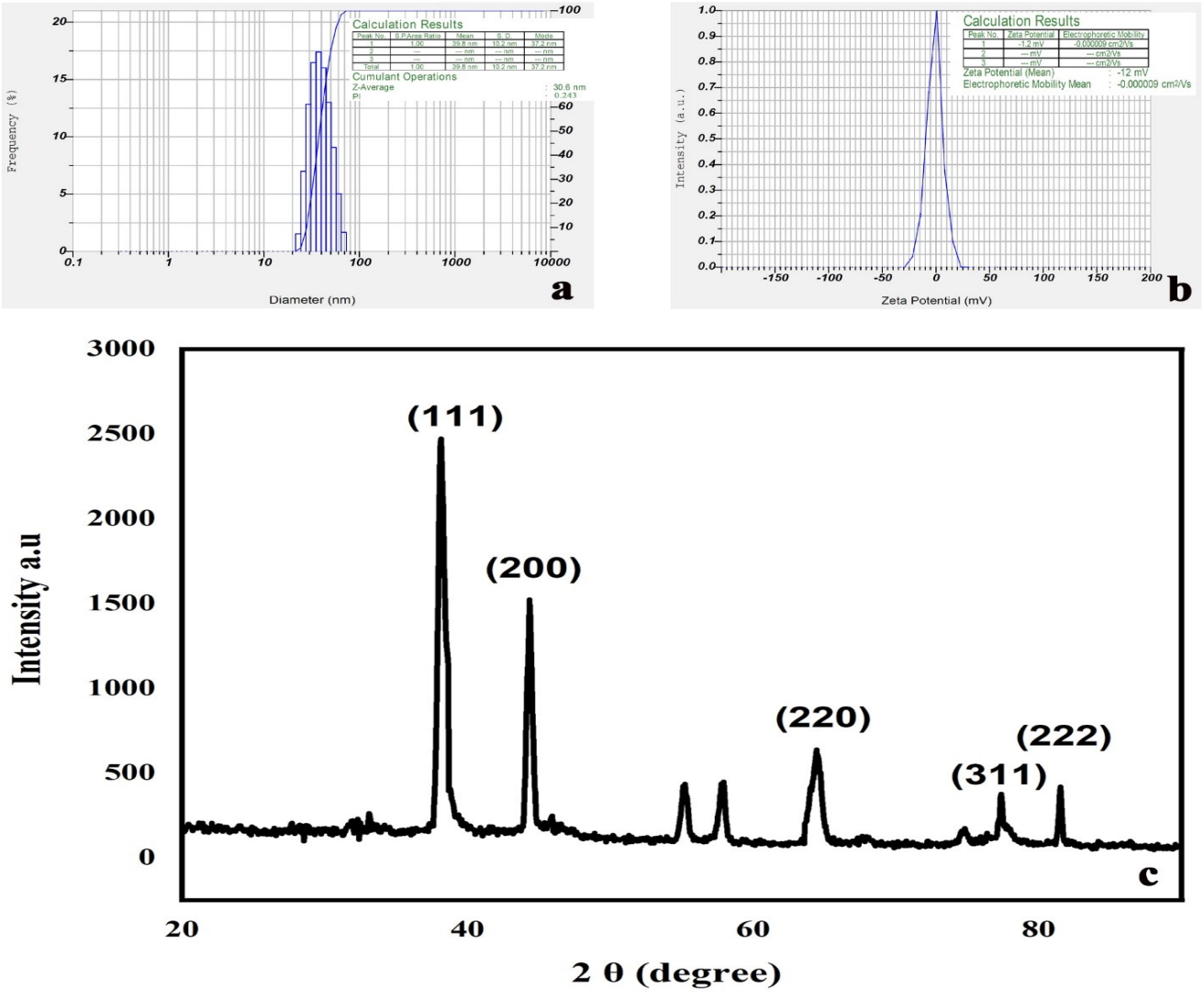


Fig. 4. DLS analysis **a.** Particle size distribution curve and **b.** Zeta potential of SS-AgNPs. **c.** XRD pattern of SS-AgNPs.

verified that the biosynthesized SS-AgNPs are FCC crystals. EDX analysis data revealed that the silver weight percentage in the synthesized sample is 69.56 % which shows strong peaks compared to copper (30.44 %). And some other minor peaks indicate the other elements like carbon and oxygen which are plant sources (Fig. 5 e).

Antioxidant activity

The antioxidant property of biosynthesized SS-AgNPs was estimated through a DPPH scavenging assay. The mean values were represented with the standard deviation (Mean ± SD). In the present experiment, it was determined that the DPPH assay of AgNPs showed 66.45 ± 2.32 % scavenging activity at 100 µg/mL and the value is 92.24 ± 1.73 % for standard ascorbic acid. The 31.6 ± 3.54 % scavenging activity was noticed for aqueous extract at 100 µg/mL (Graph 1a and Supplementary Graph 1). Based on the above results, it was concluded that the SS-AgNPs proved to be the most effective scavenging agent compared to its extract. The IC_{50} values of all the test samples (powder extract, AgNPs and ascorbic acid) calculated were found to be 132.7, 75.53 and 39.63 µg/mL respectively.

H_2O_2 radical scavenging assay SS-AgNPs were carried out and represented in Graph 1b. At 100 µg/mL concentration, AgNPs showed 49.41 ± 2.52 %, 55.38 ± 0.97 % and 67.92 ± 1.35 % inhibition for aqueous extract, AgNPS and ascorbic acid respectively. The IC_{50} values were noticed at 122.93, 87.01 and 65.49 µg/mL (Supplementary Graph 2).

The reducing power was estimated based on the extract/ SS-AgNPs efficiency to reduce the Fe^{+3} to Fe^{+2}. Reducing power assay and absorbance increased when the concentration of leaf extract and SS-AgNPs increased. SS-AgNPs had higher antioxidant activity than the ascorbic acid (standard), with an absorbance of 0.656 ± 0.017, while the standard showed an absorbance of 0.643 ± 0.016 at the concentration of 300 µg/mL. The absorbance of the leaf extract was 0.45 ± 0.02 (Graph 1 c). The H_2O_2 free radical scavenging assay was lower than the DPPH scavenging assay. Previous research reports reveal the relation between antioxidant activity and the total phenolic component of dietary items. The results were in accordance with earlier reports of DPPH (37, 38) and H_2O_2 (39, 40) scavenging assays. The extract's ability to scavenge H_2O_2 is dependent on the phenolic component, which may transfer electrons to H_2O_2 and therefore neutralize it in water (41). Human exposure to H_2O_2 can result in the production of highly reactive hydroxyl radicals (.OH), which can cause cellular damage. Inflammation, aging, intestinal problems, malignancies and Alzheimer's disease have all been linked to the presence of hydroxyl radicals (.OH). As a result, nanoparticles can be used to scavenge H_2O_2 in environmental compartments (42). Nanoparticles's effectiveness as antioxidants depend on

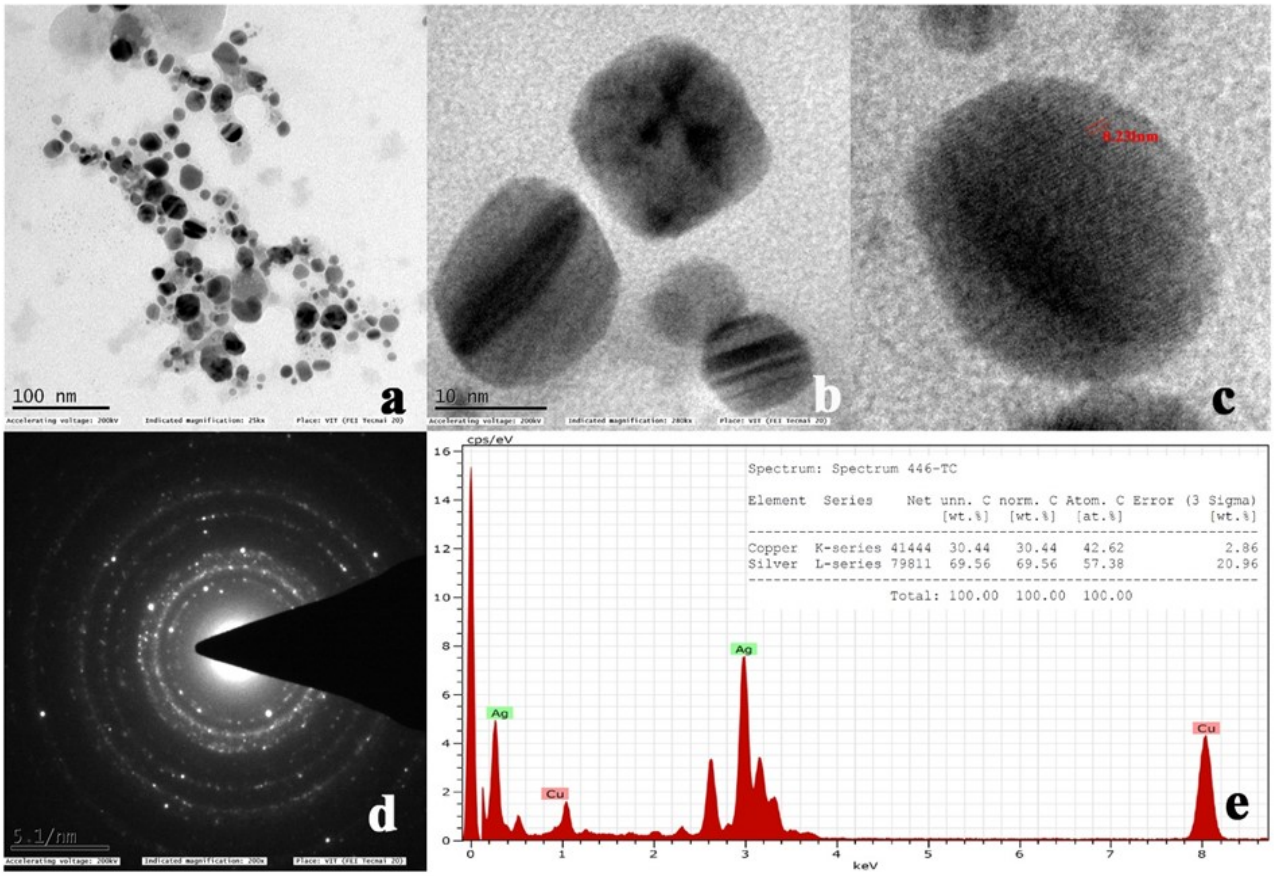
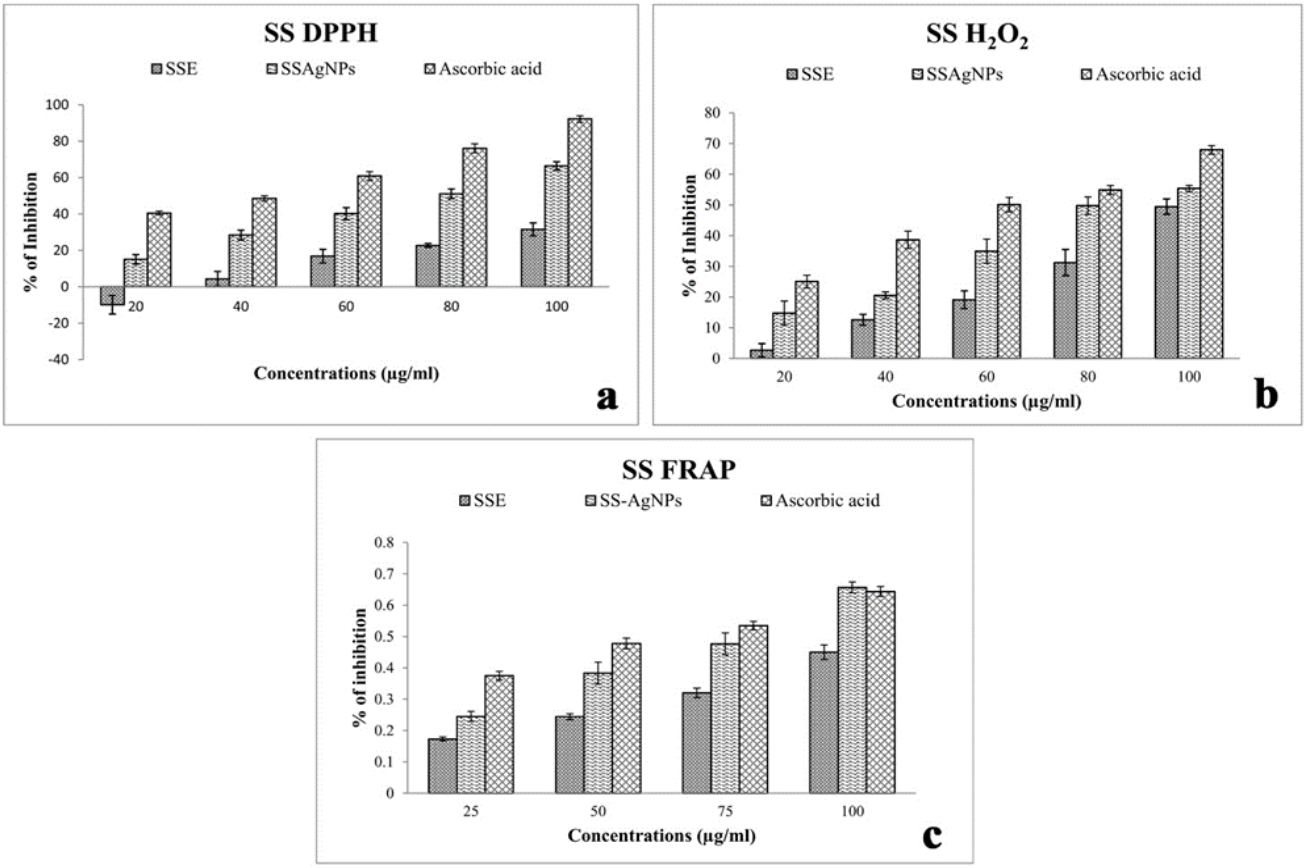


Fig. 5. TEM analysis of SS-AgNPs at magnification of **a.** 25 kx; **b.** 280 kx; **c.** 490 kx; **d.** SAED pattern showed six diffraction rings and **e.** EDX analysis of SS-AgNPs with 69.56 % of Ag weight.

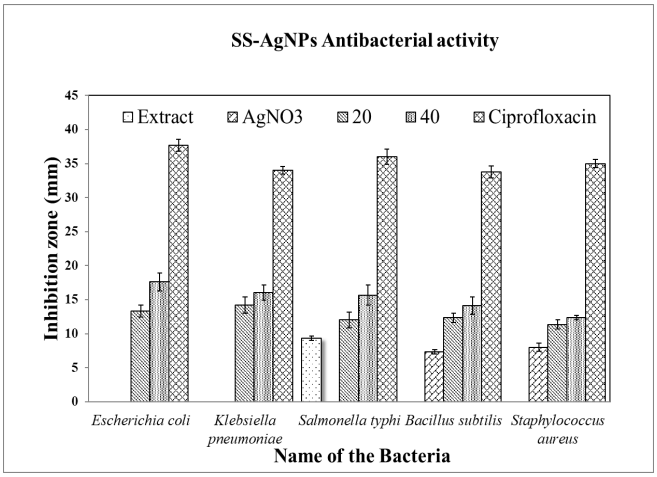


Graph 1. Antioxidant activity of SS-AgNPs **a.** DPPH scavenging assay; **b.** H_2O_2 scavenging assay; and **c.** FRAP assay.

the nanoparticles' size and surface properties. Natural antioxidants that have been nano-formulated improve the impact of antioxidants produced from plants that are unable to produce the same potential in their free form. These antioxidants encapsulated in nanoparticles are now efficiently transported and released at the target location, reducing the amount of antioxidants required (43). Oxidative stress is a result of free radicals like superoxides, peroxides, hydroxyl radicals and singlet oxygen. It is associated with inflammation, obesity, diabetes and a number of neurological diseases. The synthesised AgNPs ability to scavenge free radicals demonstrated their biological significance in combating several oxidative stress-related illnesses. The obtained antioxidant activity result of SS-AgNPs is supported by previous reports using *Ageratum conyzoides* (44) and *Solanum sisymbriifolium* (45).

Antibacterial activity

SS aqueous leaf extract and SS-AgNPs showed antimicrobial activities against various pathogenic Gram-positive and Gram-negative bacteria. The powder aqueous extract and silver nitrate solution served as control. The antibacterial activity of SS-AgNPs was determined by measuring the zone of inhibition around the disc. The SNPs show a higher zone of inhibition against *Escherichia coli* (17.6 ± 1.3 mm) and *Klebsiella pneumoniae* (16.03 ± 1.1 mm). Whereas, for *Salmonella typhi* (15.66 ± 1.4 mm), *Bacillus subtilis* (14.13 ± 1.4 mm) and *Staphylococcus aureus* (12.33 ± 0.33 mm) moderate zone of inhibition was noticed compared to ciprofloxacin (5 µg) (Fig. 6, Graph 2 and Table 1). The antibacterial activity results of SS-AgNPs tested at different concentrations against 5 bacterial strains showed appreciable antibacterial activity compared to the standard. This finding indicates that the larger surface area of the SS-AgNPs can be attached to the bacterial cell membrane and its charge-related aspects (40) and electrostatic attraction, van der waals forces, receptor-ligand and hydrophobic interactions. The silver nanoparticles subsequently penetrate the bacterial membrane and collect along the metabolic route, affecting the cell membrane's structure and function. Following that, damaging the DNA, regulating vital metabolic enzymes and producing reactive oxygen species (ROS) damage the cellular constituents like lysosomes, ribosomes and enzymes in the bacterial cell, electrolyte balance problems, enzyme inhibition, protein deactivation and changes in gene expression (46). The plant kingdom is a variable source of phytochemicals that are



Graph 2. Antimicrobial activity of SS-AgNPs from the powder extract.

Table 1. Zone of inhibition of SS-AgNPs at different concentrations compared with the extract and standard antibiotic.

	Zone of inhibition (mm)				
	Extract	**$AgNO_3$**	**20 µg/mL SS-AgNPs**	**40 µg/mL SS-AgNPs**	**Ciprofloxacin**
Escherichia coli	0	0	13.3 ± 0.89	17.6 ± 1.30	37.67 ± 0.88
Klebsiella pneumoniae	0	0	14.2 ± 1.17	16.03 ± 1.16	34 ± 0.58
Salmonella typhi	9.33 ± 0.33	9.33 ± 0.67	12 ± 1.15	15.67 ± 1.45	36 ± 1.15
Bacillus subtilis	0	7.33 ± 0.33	12.33 ± 0.68	14.13 ± 1.27	33.76 ± 0.90
Staphylococcus aureus	0	8 ± 0.58	11.33 ± 0.67	12.33 ± 0.33	35 ± 0.58

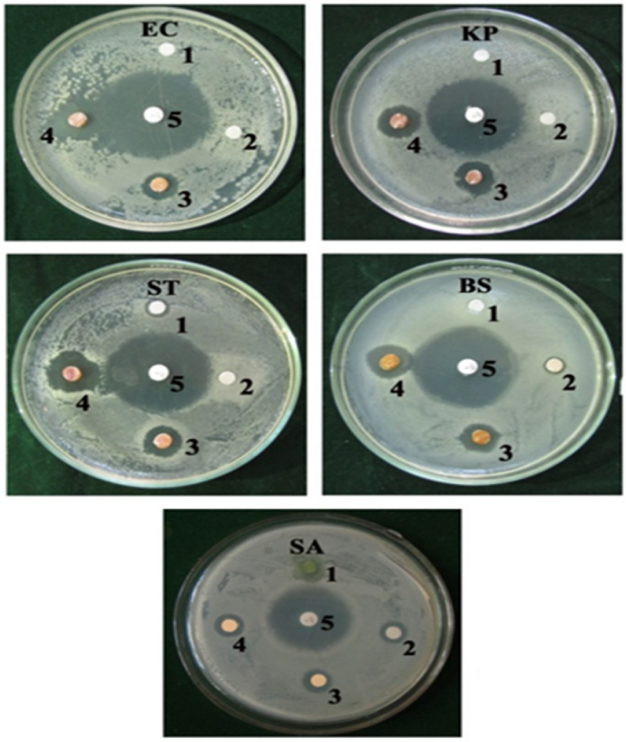


Fig. 6. Antimicrobial activity of SS-AgNPs synthesized from powder extract inhibition zone. **1.** Plant extract; **2.** Silver nitrate solution; **3.** 20 µg AgNPs; **4.** 40 µg SS-AgNPs; **5.** Ciprofloxacin. EC. *Escherichia coli*; KP. *Klebsiella pneumonia*; SA. *Staphylococcus aureus*; BS. *Bacillus subtilis* and ST. *Salmonella typhii*.

biologically active which could be well delivered in coalition with AgNPs to counter the increasing resistance of bacteria. Such a treatment would give a synergistic means from the antimicrobial properties of silver and the biomolecules that are bioactive-capped.

Anticancer activity

Checking the biocompatibility of AgNPs is crucial in biomedical applications. The cell viability percentage of HepG2 cell lines was used in this study for checking SS-AgNPs anticancer properties. The SS-AgNPs did clearly show a dose-dependent increase in cytotoxicity effect. The leaf aqueous extract at 200 µg/mL showed a moderate cytotoxic effect (65.18 %) at high concentrations against HepG2 cell lines. The SS-AgNPs showed 16.28 % and the standard control showed 47.02 % cell viability (Fig. 7, Graph 3). The IC_{50} values of SS-AgNPs were found to be 95.52 µg/mL. From these results, HepG2 cancer cell lines clearly showed more susceptibility to SS-AgNPs than the leaf aqueous extract (Supplementary Graph 3). Due to its lower IC_{50} value on human liver cancer (HepG2) cells, it is considered a potent anticancer agent. The MTT assay results revealed that the SS-AgNPs exhibited a concentration-dependent manner cytotoxic effect against the HepG2 cell line and it is 4-fold higher than the leaf aqueous extract. Higher concentrations of polyphenols, flavonoids and proteins in the leaf of *S.*

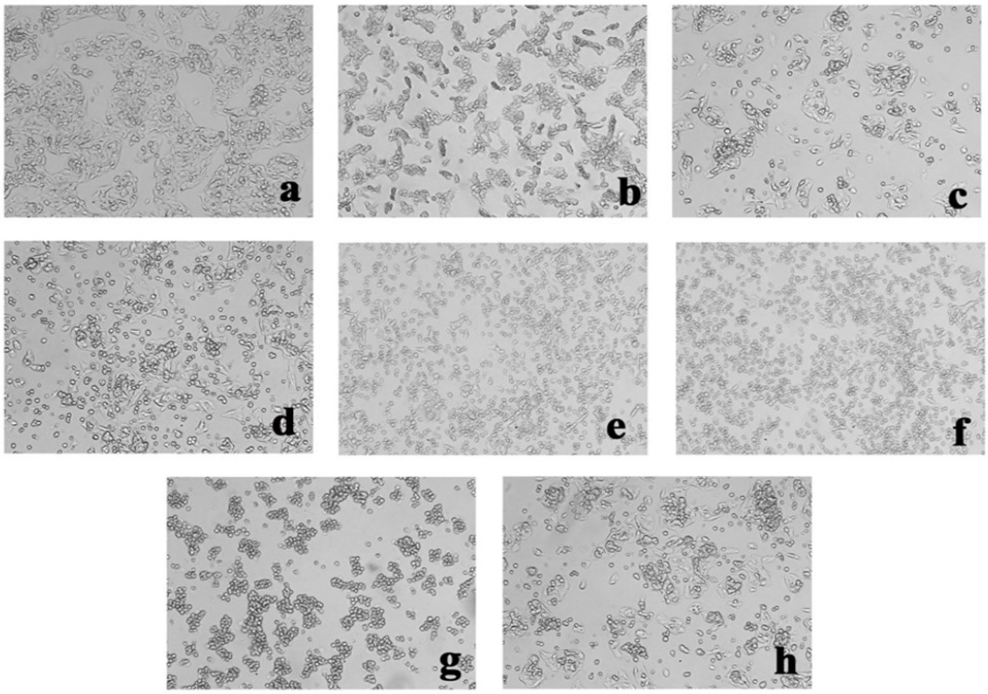
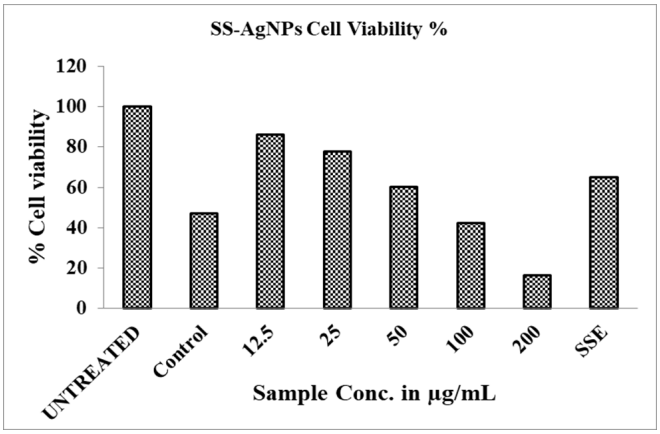


Fig. 7. Anticancer activity of synthesized SS-AgNPs against HepG2; **a.** untreated cell lines (negative control); **b.** Camptothecin (15µM) standard control; SS-AgNPs



Graph 3. Anticancer activity of synthesized SS-AgNPs against HepG2 from the powder extract.

sophera may be the reason for the bestowed anticancer activity. The cytotoxicity could be due to flavonoids, polyphenols, proteins and other phytochemicals present in the aqueous leaf extract of *S. sophera* (47, 48). The antioxidant properties of synthesized AgNPs could damage the viability of cancer cells leading to the release of cytochrome c due to mitochondrial membrane damage. The oxidative stress induces apoptosis-mediated cell death through mitochondrial-dependent pathways (Caspase mediated). Nanoparticles with smaller sizes can penetrate cancer cells more efficiently and alter the proliferation mechanism. The death percentage of cancer cell lines mainly depends on the size of the nanoparticles (49,50).

Conclusion

The synthesis of silver NPs by biological organisms containing phytochemicals has become an important area for scientists. Silver NPs are widely used nanoparticles in new biomedical and industrial applications. The present study reports an eco-friendly and cost-effective method for the synthesis of AgNPs from the leaf extract of *Senna sophera*. Due to constraints with chemical and physical methods, the green approach method is the best-opted technique to synthesize AgNPs. During the synthesis of the silver nanoparticles plant extract was involved in the reduction reaction resulting in the formation of metallic silver nanoparticles. The bioactive compounds like polyphenols and amines of proteins from the plant extract are involved in capping, stabilization, and preventing aggregation of AgNPs. FTIR spectra results revealed that the reduction of silver nanoparticles is due to the presence of polyphenols and proteins. Phyto-fabricated silver nanoparticles are Face cantered Cubic crystals, 5-35 nm in size, spherical in shape, non-agglomerated and highly stable with negative surface charge. The synthesized AgNPs show very good radical scavenging activity against DPPH, H_2O_2, FRAP and antibacterial activity against four bacterial species. Both the leaf aqueous extract and AgNPs showed significant effects against *Salmonella typhi* and hence is proving the pharmaceutical and biomedical importance. The MTT assay reveals that the synthesized AgNPs exhibited significant anticancer activity against HepG2 cancer cell lines with the IC_{50} of 95.52 µg/mL. Overall, this study highlights the potential of using plant extracts as a green and sustainable approach for the synthesis of SS-AgNPs with biomedical applications. Further research is needed to fully understand the mechanisms of action of SS-AgNPs and their potential toxicity to healthy cells before they transit into clinical applications.

Acknowledgements

The authors are thankful to the Department of Botany, S.V University, Tirupati and Director General, Central Council for Research in Unani Medicine (CCRUM), New Delhi, for necessary encouragement and support.

Authors' contributions

GPP and MKH: Collected the plant material and ethnobotanical information from the rural and tribes of Nellore, Andhra Pradesh. India. NV: Performed the experiments, data analysis. GPP, MKH, SG and NV: Writing of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical issues: None

References

1. Bayda S, Adeel M, Tuccinardi T, Cordani M, Rizzolio F. The history of nanoscience and nanotechnology: from chemical-physical applications to nanomedicine. Molecules. 2019;25(1):112. https://doi.org/10.3390/molecules25010112
2. Yaqoob AA, Ahmad H, Parveen T, Ahmad A, Oves M, et al. Recent advances in metal decorated nanomaterials and their various biological applications: A review. Front Chem. 2020;8:341. https://doi.org/10.3389/fchem.2020.00341
3. Khan Y, Sadia H, Ali Shah SZ, Khan MN, et al. Classification, synthetic and characterization approaches to nanoparticles and their applications in various fields of nanotechnology: A review. Catalysts. 2022;12(11). https://doi.org/10.3390/catal12111386
4. Mekuye B, Abera B. Nanomaterials: An overview of synthesis, classification, characterization and applications. Nano Select. 2023;4(8):486-01. https://doi.org/10.1002/nano.202300038
5. Viana AD, Nobrega ET, Moraes EP, Neto AOW, et al. Castor oil derivatives in the environmentally friendly one-pot synthesis of silver nanoparticles: application in cysteine sensing. Mater Res Bull. 2020;124. https://doi.org/10.1016/j.materresbull.2019.110755
6. Rahuman HBH, Dhandapani R, Narayanan S, Palanivel V, et al. Medicinal plants mediated the green synthesis of silver nanoparticles and their biomedical applications. IET Nanobiotechnol. 2022;16(4):115-44. https://doi.org/10.1049/nbt2.12078
7. Shah SS, Shaikh MN, Khan MY, Alfasane MA, et al. Present status and future prospects of jute in nanotechnology: A review. Chem Rec. 2021;21(7):1631-65. https://doi.org/10.1002/tcr.202100135
8. Ahmed RH, Mustafa DE. Green synthesis of silver nanoparticles mediated by traditionally used medicinal plants in Sudan. Int Nano Lett. 2020;10:1-14. https://doi.org/10.1007/s40089-019-

00291-9

9. Tran N, Pham B, Le L. Bioactive compounds in anti-diabetic plants: From herbal medicine to modern drug discovery. Biology. 2020;9(9):252. https://doi.org/10.3390/biology9090252
10. Długosz O, Chwastowski J, Banach M. Hawthorn berries extract for the green synthesis of copper and silver nanoparticles. Chem Pap. 2020;74:239-52. https://doi.org/10.1007/s11696-019-00873-z
11. Chandrakala V, Aruna V, Angajala G. Review on metal nanoparticles as nanocarriers: Current challenges and perspectives in drug delivery systems. Emerg Mater. 2022;5 (6):1593-615. https://doi.org/10.1007/s42247-021-00335-x
12. Sánchez-López E, Gomes D, Esteruelas G, Bonilla L, et al. Metal-based nanoparticles as antimicrobial agents: an overview. Nanomater. 2020;10(2):292. https://doi.org/10.3390/nano10020292
13. Crisan CM, Mocan T, Manolea M, Lasca LI, et al. Review on silver nanoparticles as a novel class of antibacterial solutions. Appl Sci. 2021;11(3):1120. https://doi.org/10.3390/app11031120
14. Zhang XF, Liu ZG, Shen W, Gurunathan S. Silver nanoparticles: synthesis, characterization, properties, applications and therapeutic approaches. Int J Mol Sci. 2016;17(3):1-34. https://doi.org/10.3390/ijms17091534
15. World Cancer Research Fund International, WCRF International, London. [cited 2023 December 18]. Available from: https://www.wcrf.org/cancer-trends/liver-cancer-statistics/
16. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer Res. 2015;136 (5):E359-E386. https://doi.org/10.1002/ijc.29210
17. Siddiqui MA, Siddiqui HH, Mishra A, Usmani A. Epidemiology of hepatocellular carcinoma. Int J Pharm Sci Res. 2018;9(12):5050-59. https://doi.org/10.13040/IJPSR.0975-8232.9(12).5050-59
18. Llovet JM, Beaugrand M. Hepatocellular carcinoma: present status and future prospects. J Hepatol. 2003;38:136-49. https://doi.org/10.1016/S0168-8278(02)00432-4
19. Ehealth news, Uttar Pradesh, India [updated 2014 February 04] [cited 2023 December 18]. Available from: https://ehealth.eletsonline.com/2014/02/50000-people-in-india-are-diagnosed-with-liver-cancer-each-year/
20. Deshpande SR, Naik BS. Evaluation of *in vitro* antimicrobial activity of extracts from *Cassia obtusifolia* L. and *Senna sophera* (L.) Roxb. against pathogenic organisms. J Appl Pharm Sci. 2016;6 (1):83-85. https://doi.org/10.7324/JAPS.2016.600114
21. Kharat AR, Kharat K, Jadhav M, Makhija SJ. Antihyperglycemic, antihyperlipidemic and antioxidative evaluation of compounds from *Senna sophera* (L.) Roxb. in streptozotocin-induced diabetic rats. Nat Prod Res. 2019;33(4):602-05. https://doi.org/10.1080/14786419.2017.1399389
22. Khare CP. Indian medicinal plants: an illustrated dictionary. Springer, New York. 2008. https://doi.org/10.1007/978-0-387-70638-2_307
23. Hussain SN, Chaudhry BA, Uzair M, Qaisar MN. Studies on the analgesic and anti-inflammatory effects of *Cassia sophera* roots. Asian Pac J Trop Dis. 2015;5(6):483-88. https://doi.org/10.1016/S2222-1808%2815%2960820-9
24. Rahman MM, Sultana T, Ali MY, Rahman MM, Al-Reza SM, Rahman A. Chemical composition and antibacterial activity of the essential oil and various extracts from *Cassia sophera* L. against *Bacillus* sp. from soil. Arab J Chem. 2017;10(2):S2132-S2137. https://doi.org/10.1016/j.arabjc.2013.07.045
25. Yamaguchi T, Takamura H, Matoba T, Tera, J. HPLC method for evaluation of the free radical-scavenging activity of foods by using 1, 1-diphenyl-2-picrylhydrazyl. Biosci Biotechnol Biochem. 1998;62(6):1201-04. https://doi.org/10.1271/bbb.62.1201
26. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinog. 1989;10 (6):1003-08. https://doi.org/10.1093/carcin/10.6.1003
27. Oyaizu M. Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr Diet. 1986;44(6):307-15. https://doi.org/10.5264/eiyogakuzashi.44.307
28. Cruickshank R. Medical microbiology: a guide to diagnosis and control of infection. E and S. Livingston Ltd., Edinburgh. 1986;888.
29. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65(1-2):55-63. https://doi.org/10.1016/0022-1759(83)90303-4
30. Babu S, Claville MO, Ghebreyessus K. Rapid synthesis of highly stable silver nanoparticles and its application for colourimetric sensing of cysteine. J Exp Nanosci. 2015;10(16):1242-55. https://doi.org/10.1080/17458080.2014.994680
31. Tarannum N, Gautam YK. Facile green synthesis and applications of silver nanoparticles: a state-of-the-art review. RSC Advances. 2019;9(60):34926-48. https://doi.org/10.1039/C9RA04164H
32. Vallepu N, Gaddam SA, Kotakadi VS, Goli PP, et al. Biogenic silver nanoparticles can be an effective and efficient water purification agent of future. Inorg Nano-Met Chem. 2021;51(9):1195-206. https://doi.org/10.1080/24701556.2020.1817940
33. Bethu MS, Netala VR, Domdi L, Tartte V, Janapala VR. Potential anticancer activity of biogenic silver nanoparticles using leaf extract of *Rhynchosia suaveolens*: an insight into the mechanism. Artif Cells Nanomed Biotechnol. 2018;46(sup1):104-14. https://doi.org/10.1080/21691401.2017.1414824
34. Netala VR, Bethu MS, Pushpalatha B, Baki VB, Aishwarya S, Rao JV, et al. Biogenesis of silver nanoparticles using endophytic fungus *Pestalotiopsis microspora* and evaluation of their antioxidant and anticancer activities. Int J Nanomedicine. 2016;11:5683-96. https://doi.org/10.2147/ijn.s112857
35. Velgosová O, Mražíková A, Marcinčáková R. Influence of pH on green synthesis of Ag nanoparticles. Mater Lett. 2016;180:336-39. https://doi.org/10.1016/j.matlet.2016.04.045
36. Annamalai J, Nallamuthu T. Green synthesis of silver nanoparticles: characterization and determination of antibacterial potency. Appl Nanosci. 2016;6:259-65. https://doi.org/10.1007/s13204-015-0426-6
37. Khorrami S, Zarepour A, Zarrabi A. Green synthesis of silver nanoparticles at low temperature in a fast pace with unique DPPH radical scavenging and selective cytotoxicity against MCF-7 and BT-20 tumor cell lines. Biotechnol Rep. 2019;24:e00393. https://doi.org/10.1016/j.btre.2019.e00393
38. Kumar B, Kumari S, Cumbal L, Debut A. *Lantana camara* berry for the synthesis of silver nanoparticles. Asian Pac J Trop Biomed. 2015;5(3):192-95. https://doi.org/10.1016/S2221-1691(15)30005-8
39. Keshari AK, Srivastava R, Singh P, Yadav VB, Nath G. Antioxidant and antibacterial activity of silver nanoparticles synthesized by *Cestrum nocturnum*. J Ayurveda Integr Med. 2020;11(1):37-44. https://doi.org/10.1016/j.jaim.2017.11.003
40. Muniyappan N, Nagarajan NS. Green synthesis of silver nanoparticles with *Dalbergia spinosa* leaves and their applications in biological and catalytic activities. Process Biochem. 2014;49(6):1054-61. https://doi.org/10.1016/j.procbio.2014.03.015
41. Bhatti MZ, Ali A, Ahmad A, Saeed A, Malik SA. Antioxidant and phytochemical analysis of *Ranunculus arvensis* L. extracts. BMC Res Notes. 2015;8:279. https://doi.org/10.1186/s13104-015-1228-3
42. Lateef A, Folarin BI, Oladejo SM, Akinola PO, Beukes LS, Gueguim-Kana EB. Characterization, antimicrobial, antioxidant and anticoagulant activities of silver nanoparticles synthesized from *Petiveria alliacea* L. leaf extract. Prep Biochem Biotech. 2018;48 (7):646-52. https://doi.org/10.1080/10826068.2018.1479864

43. Kumar M, Pratap V, Nigam AK, Sinha BK, et al. Plants as a source of potential antioxidants and their effective nano formulations. J Sci Res. 2021;65(3):57-72. https://doi.org/10.37398/JSR.2021.650308

44. Paramasivam D, Balasubramanian B, Suresh R, Kumaravelu J, et al. One-pot synthesis of silver nanoparticles derived from aqueous leaf extract of *Ageratum conyzoides* and their biological efficacy. Antibiotics. 2023;12(4):688. https://www.mdpi.com/2079-6382/12/4/688#

45. Singh R, Singh R, Parihar P, Mani JV. Green synthesis of silver nanoparticles using *Solanum sisymbriifolium* leaf extract: Characterization and evaluation of antioxidant, antibacterial and photocatalytic degradation activities. Process Biochem. 2024. https://doi.org/10.1016/j.procbio.2024.04.026

46. Wang L, Hu C, Shao L. The antimicrobial activity of nanoparticles: present situation and prospects for the future. Int J Nanomedicine. 2017;12:1227-49. https://doi.org/10.2147/IJN.S121956

47. Essawy E, Abdelfattah, MS, El-Matbouli M, Saleh M. Synergistic effect of biosynthesized silver nanoparticles and natural phenolic compounds against drug-resistant fish pathogens and their cytotoxicity: An *in vitro* study. Mar Drugs. 2021;19(1):22. https://doi.org/10.3390/md19010022

48. Yousefbeyk F, Dabirian S, Ghanbarzadeh S, Eghbali Koohi D, et al. Green synthesis of silver nanoparticles from *Stachys byzantina* K. Koch: characterization, antioxidant, antibacterial and cytotoxic activity. Particul Sci Technol. 2021;42(2):219-32. https://doi.org/10.1080/02726351.2021.1930302

49. Reddy NV, Satyanarayana BM, Sivasankar S, Pragathi D, et al. Eco-friendly synthesis of silver nanoparticles using leaf extract of *Flemingia wightiana*: spectral characterization, antioxidant and anticancer activity studies. SN Appl Sci. 2020;2:884. https://doi.org/10.1007/s42452-020-2702-7

50. Husain MK. Nanotechnology and current advances in plant tissue culture. J Phytonanotech Pharmaceut Sci. 2023;3(1):1-8. https://doi.org/10.54085/jpps.2023.3.1.1