Research article

Investigation of Anti-hepatocarcinogenic Effects of *Senna auriculata* Silver Nanoparticle and Evaluation of Their Antioxidant Potential

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Abstract

Plant-based biogenic nanoparticle synthesis has appeared as a feasible alternative to conventional approaches to chemical synthesis. As a result, several environmentally benign methods for the quick production of silver nanoparticles have been published in recent years. The methods employ aqueous extracts of plant components like leaves, bark, and roots. In the present study, silver nanoparticles were synthesized from an aqueous leaf extract of *S. auriculata*. UV-vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and energy dispersive x-ray analysis (EDAX) were employed to validate the synthesized nanoparticles. The UV analysis showed a peak range between 390-420 nm. FTIR showed the functional groups present in the silver nanoparticles (AgNPs). Moreover, the synthesized AgNPs were tested for their antimicrobial activity against both gram-negative and gram-positive bacterial strains. The antioxidant properties were studied with DPPH, hydrogen peroxide, and nitric oxide scavenging activity assays, and a cytotoxic assay was conducted against the Huh-7 cell line by a MTT study. The results showed that the synthesized silver nanoparticles possessed strong antibacterial, antioxidant, and cytotoxic activities against the Huh-7 cell line, indicating that the silver nanoparticles might be used in the pharmaceutical industry and for novel biological applications.

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1. Introduction

Hepatocellular carcinoma (HCC), also known as liver cancer, is among the primary causes of cancer-related death worldwide, particularly in countries with a high prevalence of people with Hepatitis B and Hepatitis C infections, such as India [1]. It is the 5th major cause of mortality in the world. Unfortunately, unlike other cancers, it is only diagnosed in the most advanced stages. HCC is commonly treated with hepatectomy and liver transplantation. Most Indian patients have little chance of receiving highly priced HCC treatments [2]. Many chemotherapeutics are incapable of adequately reaching their target of action in exerting effective pharmacological activity without causing irreversible damage to healthy cells and tissues. Nanotechnology offers diverse tools for treating cancer by directly delivering therapeutic agents across biological barriers [3].

Enzymes, fungi, and plants have been studied for potential roles as biological sources for synthesized nanomaterials that are environmentally safe, non-toxic, economical, and secure options produced by chemical and physical synthesis techniques [4]. In green synthesis, plant extracts function as reductants and system controllers [5]. Plant extracts contain biomolecules that convert metal ions into nanoparticles [6]. Alkaloids, flavonoids, phenols, tannins, and terpenoids are plant secondary metabolites that are fundamentally responsible for reducing ions in bulk metallic nanoparticle production [7]. In the field of nanotechnology, silver nanoparticles have garnered and stimulated research attention due to their numerous medical uses in dentistry [8-10], wound healing [11-13], bone healing [14, 15], catheter modification [16, 17], and drug delivery [18, 19].

Various chemical and physical techniques can be used to synthesize silver nanoparticles, including photochemical [20-22] and chemical reduction [23, 24]. Various hazardous reducing agents are utilized in these processes [25]. Due to the use of noble metal nanoparticles in areas where people come into contact with them, it is critical to developing eco-friendly biogenesis methods that do not rely on hazardous chemicals [26]. Through biological synthesis, often known as "green synthesis", nanoparticles can be synthesized in an alternative and more environmentally friendly manner [27]. There has recently been a strong trend in applying metal nanoparticles as antibacterial agents and as potential medication providers in radiation therapy [28].

In Indian literature, *Senna auriculata*, belonging to the Fabaceae family, is often used to treat acute and common illnesses. Its various components, extracts, and isolated chemicals have various medicinal effects such as antioxidant [29, 30], antibacterial [31, 32], and antihyperglycemic activities in diabetic mice animal models [33], anti-atherosclerotic and cardioprotective actions [34], antifertility activity [35], cytotoxic activity [36-39], immunomodulatory [40, 41], nephroprotective activity [42], antipyretic [43] antimutagenic [44], cardioprotective [45, 46], anxiolytic [47], and diuretic [48, 49] activities.

In this investigation, silver nanoparticles were successfully obtained from the leaf extract of *Senna auriculata.* The characterization study was done using UV analysis, SEM, EDX, and FTIR methodologies. The produced nanoparticles were tested for their anticancer activity against liver cancer cell lines, their antimicrobial activity and antioxidant properties.

2. Materials and Methods

2.1 Chemicals and materials

Silver nitrate was procured from HiMedia, India. Ascorbic acid, DPPH, N-1-Naphthyl ethylenediamine dihydrochloride, Muller-Hilton agar medium, and dimethyl sulfoxide (DMSO) were of analytical grade and purchased from HiMedia, India. Double distilled water was used throughout the experiment. All solutions were made from scratch with double distilled water to

prevent any photochemical reactions and stored in the dark. Before use, all glassware used in the experiments was carefully cleaned with double distilled water and dried in a hot air oven.

2.2 Collection and preparation of the sample

Fresh *Senna auriculata* leaves were picked from the herbal garden of Thiruvalluvar University in Vellore. The verification of plant material was confirmed by a biologist in the Department of Botany, Ayya Nadar Janaki Ammal College, Sivakasi. The leaves were washed with distilled water. The leaves were dehydrated for 20 days and then ground using an electric mixer. The powdered sample was stored in an airtight container for future use. Five grams of leaf sample was dissolved in 100 mL of distilled water and boiled for 5 min at 80°C to make the leaf extract.

2.3 Synthesis of silver nanoparticles

Senna auriculata leaf extract was prepared, and 1 mL of plant extract was mixed with 19 mL of 1 mM silver nitrate solution dropwise to reduce the silver ions. The mixture was kept in a dark condition. After centrifuging the samples at 6000 rpm for 20 min, the nanoparticles were stored at 4°C.

2.4 Characterization of nanoparticles

The powder sample was dissolved in ionized water and re-suspended to examine the formation of AgNPs. The UV-visible frequency band of the AgNPs was captured between 200 to 600 nm using a Synergy HT Multi-Mode Microplate Reader (BioTek Instruments), and FTIR spectra were recorded on a FTIR-8400S, Shimadzu. The structural and fundamental properties of the produced silver nanoparticles were investigated by a scanning electron microscope (SEM-EDX). The samples were prepared by drop-coating the AgNPs solution onto a carbon-coated copper grid.

2.5 Antioxidant activity of AgNPs

2.5.1 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay

The DPPH radical scavenging process was carried out using the method previously described by Prieto *et al*. [50] with some modifications. A solution of DPPH (0.1 mM) was prepared in methanol and 1 mL of the prepared solution was mixed with various concentrations (250-1000 µg/mL) of synthesized AgNPs. The mixture was kept at room temperature for 30 min. At 517 nm, the absorbance was calculated. Ascorbic acid was used as a standard. Lower absorbance values indicate high activity. In addition, the percentage of DPPH reduction was calculated using the following equation:

$$\% \text{ inhibition} = \left(\frac{A_C - A_E}{A_C}\right) \times 100 \quad (1)$$

Where A_C represents control absorbance and A_E represents tested sample absorbance.

2.5.2 Nitric oxide activity

In phosphate buffer, 10 mM sodium nitroprusside was prepared (pH 7.4) and 0.5 mL of sodium nitroprusside solution was combined with 1 mL of various AgNPs concentrations (250-1000

µg/mL). One hundred and eighty minutes were spent incubating the reaction mixture at 25°C, 180 min later, 0.5 mL of the incubated solution and 0.5 mL of the Griess reagent were combined (1% sulphanilamide, 2% H_2PO_4, 0.1% N-1-naphthyl ethylenediamine dihydrochloride). The sample devoid of AgNPs acted as the control. At 546 nm, the absorbance was measured. Ascorbic acid was utilized as the standard. The % inhibition was calculated using the following equation [51].

$$\% \text{ Scavenging activity} = \left(\frac{A_C - A_t}{A_C}\right) \times 100 \quad (2)$$

Where A_c denotes the control's absorption spectrum, and A_t denotes the test's absorption spectrum.

2.5.3 Hydrogen peroxide scavenging activity

Hydrogen peroxide (20 mM) was prepared in phosphate-buffered saline (pH 7.4). One mL of various extract concentrations (250-1000 µg/mL) was prepared in methanol. The synthesized AgNPs were combined with a 2 mL solution of hydrogen peroxide. For 10 min, at room temperature, the reaction mixture was incubated, and 230 nm were used to measure the absorbance. Ascorbic acid was used as a standard [51]. The hydrogen peroxide activity of the synthesized AgNPs was calculated using the equation:

$$\% \text{ Scavenged } (H_2O_2) = (Ac - At / Ac) \times 100 \quad (3)$$

Where; A_c is the control's absorption spectrum and A_t denotes the test's absorption spectrum.

2.6 Antibacterial activity of silver nanoparticle

The antimicrobial effect of the produced silver nanomaterials was evaluated. In this investigation, we used pathogenic cultures of gram-negative bacteria *Aeromonas hydrophila* (MTCC 1739), *Escherichia coli* (MTCC 1687), and *Vibrio cholera* (MTCC 3906), and gram-positive *Bacillus subtilis* (MTCC 10619), which were kept on agar slants containing nutrients at 4ºC. Agar media with nutrients was made, and 20-25 mL was transmitted into sterilized petri dishes. After the media had solidified, agar wells were punched with a sterile cork borer, and various concentrations of AgNPs (25, 50, 75, and 100 µg/ mL) were filled. The petri dishes were kept for 24 h at 37°C and the appearance of inhibition zones around the agar well on the plates were detected. The activity was compared with the standard streptomycin [52]. The diameters of the clear zones were calculated, and the average values were determined for every pathogen [53].

2.7 Cytotoxic activity

In this investigation, MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] test was employed to measure the impact of AgNPs on the development of human liver cell lines (Huh-7) and normal cell lines. Tumor cells (1×10^4 cells/well) were developed in a 96-well microplate for 48 h to 75% convergence. The cells were maintained for another 48 h in a fresh medium containing sequentially dissolved biosynthesized AgNPs. Following the removal of the culture medium, 100 µL of MTT [3-(4,5-dimethylthiozol-2-yl)-3,5-diphenyl tetrazolium bromide] solution was introduced, and the mixture was stored at 37°C for 4 h. After 4 h, 50 µL of DMSO was added to the medium. Finally, the mixture was maintained for 10 min to dissolve the formazan crystals. The optical density (OD) was evaluated in an ELISA microplate at 620 nm [54]. The following formula

used the OD value to determine the viability percentage. At a rate of 50 µg, the synthesized AgNPs had the lowest inhibitory concentration.

$$\% \text{ of viability} \quad = \frac{X}{Y} \times 100 \tag{4}$$

Where,

X - OD value of experimental sample (AgNPs treated)
Y - OD value of experimental control (untreated cells)
OD is Optical Density

2.8 Statistical analysis

The data were displayed in the form of mean standard deviation. The paired-sample t-test was used to assess statistical significance. ANOVA with repeated measures was used to evaluate comparisons at different times. For the statistical test, SPSS® version 20 was used. The P value of ≤ 0.05 was regarded as relevant.

3. Results and Discussion

3.1 Visual observation

A reaction mixture containing silver nitrate solution and plant extract was kept in the dark for 24 h. After incubation, the colorless solution turned yellowish brown, indicating the presence of silver nanoparticles. Previous studies also observed similar color changes in the reactive media [55]. An aqueous seed extract of *Phoenix dactylifera* was added to a silver nitrate solution, and a color change from pale yellow to dark brown was observed. The primary cause of the brown color was the activation of surface plasmon vibrations, and this color change was a sign that AgNPs had been synthesized [56]. The % yield of AgNPs was as high as 92%. AgNPs were synthesized from *Ocimum tenuiflorum, Solanum tricobatum, Syzygium cumini, Centella asiatica and Citrus sinensis,* and the color of the solution turned from yellow to bright yellow and then to dark brown after 1, 24, and 48 h of the reaction, which indicated the formation of silver nanoparticles [57]. Several researchers also observed the bio-reduction of silver ions to metal nanoparticles utilizing various plant parts, and a color change was noted [58-60].

3.2 UV analysis

The Surface Plasmon Resonance (SPR) absorption band is caused by the combined vibration of the metallic nanoparticles in resonant frequencies with light waves [61]. The powdered material was prepared in distilled water, and the optical density value was evaluated. The UV analysis showed the SPR of peaks corresponding to those of silver nanoparticles (λ, 390 nm to 430 nm) [62]. The UV analysis of the synthesized silver nanoparticles is shown in Figure 1. The SPR band shows that the fluid contained spherical silver nanoparticles. Previous studies showed that the synthesis of silver nanoparticles began from 2 to 10 min on, and the reaction increased rapidly until 24 h [63]. According to several investigations of the synthesis of AgNPs using medicinal herbs, the absorption peak was between 412-470 nm, and the synthesis lasted between 4 and 24 h [64-66]. One medicinal herb contained a range of phytoconstituents, including flavonoids, polyphenols, and terpenoids, which aided in the formation of silver nanomaterials, and may have explained the variation in color change rate [67].

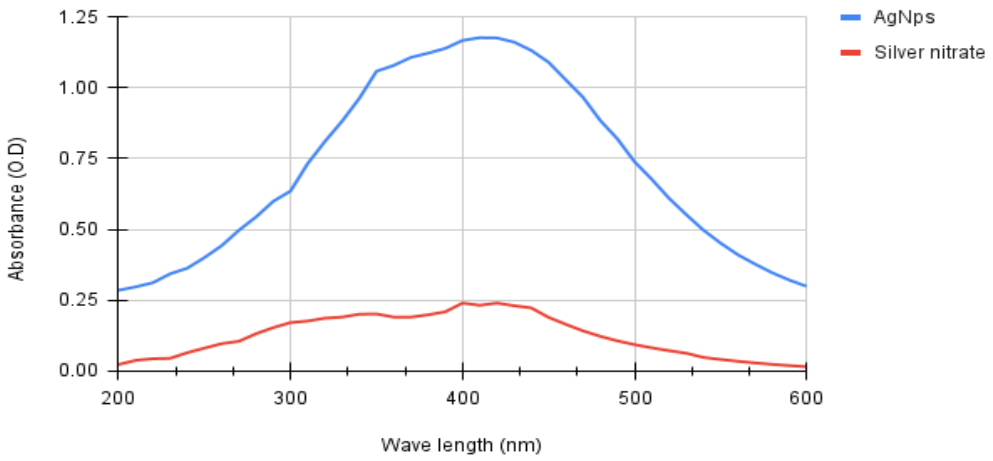

Figure 1. UV analysis of AgNPs synthesized from *S. auriculata* aqueous leaf extract

3.3 SEM and EDX analysis

SEM and energy-dispersive X-ray spectroscopy (EDX) can be used to examine silver nanoparticle chemical composition and morphology [68]. Morphological traits and atomic configuration were determined by SEM and EDX analysis. Figure 2 shows the surface morphology of the synthesized silver nanoparticles. Large particles of AgNPs were observed due to the aggregation of nanoparticles [69]. The scanning electron microscopic images showed that the morphological sizes of the nanomaterial were in the range of 20-40 nm. EDX spectra showed the integrity and entire chemical properties of the nanoparticles synthesized [70]. The EDX profile revealed a high output of silver, confirming the reduction of silver ions, and is shown in Figure 3 and Table 1.

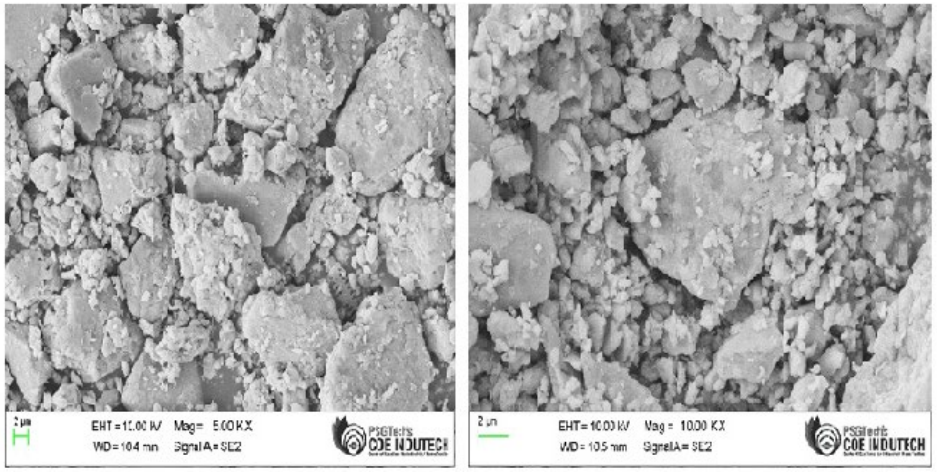

Figure 2. SEM images of AgNPs synthesized from an aqueous extract of *S. auriculata*

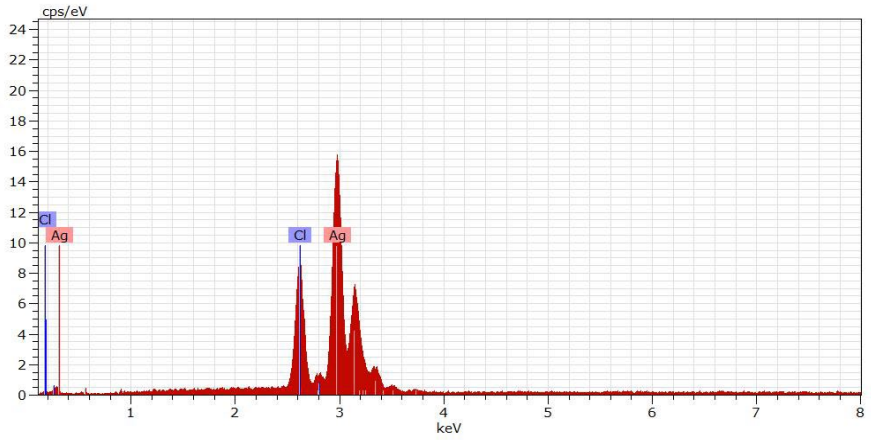

Figure 3. EDX analysis of AgNPs synthesized from *S. auriculata*

Table 1. EDX elemental composition of biosynthesized AgNPs using leaf extract of *S. auriculata*

Elements	Atomic Number (Periodic Table of Elements)	Shells	Weight %	Atomic %
Ag	47	L-series	84.86	64.82
Cl	17	K-series	15.14	35.18
			100	100

3.4 FTIR analysis

The functional groups present in the synthesized AgNPs are determined by FTIR, and the results are shown in Figure 4. The FTIR spectrum of AgNPs showed the band at 3441.74 cm^{-1} corresponding to the OH of carbohydrates and polyphenols, and a band at 2924.85 cm^{-1} corresponding to CH and CH_2 stretching in aliphatic groups. The band at 2853 cm^{-1} corresponded to the N-H amine stretch, 1738.71 cm^{-1} to the C=O ester fatty acid group, 1626.84 cm^{-1} corresponded to C=C bands in alkenes, 1418.55 cm^{-1} to O-H bands in alcohol, and 1388.83 cm^{-1} to the methyl group. Moreover, the band at 1239.18 cm^{-1} corresponded to C-N stretching in amines, 1103.21 cm^{-1} to C-O stretching in secondary alcohols, and 604 cm^{-1} to C-Br stretching in halo compounds. The presence of functional groups C-O, N-H, and C-N in the sample may be accountable for the bio-reduction of Ag+ to AgNPs [71]. The FTIR findings suggested that biological molecules may be involved in the production and stability of AgNPs [72]. Our result showed that the strong bands detected at 3441.74 cm^{-1} and 1388 cm^{-1} corresponded to the presence of -OH stretching. On the other hand, the weak bands detected at 1003 cm^{-1} corresponded to alcohol and alkyl aryl ether stretching. According to Indhumathy *et al.* [73], the major compounds detected in *Cassia fistula* were flavonoids, phenols, saponins, alkanes, aldehydes, nitro compounds, and aliphatic amines. According to Gondwal *et al*. [74], the presence of phenolic content in creating nanostructured materials such as apigenin, emodin, aloe-emodin, rhein, and vitexin was found for *Cassia occidentalis* phytochemical analysis. The FTIR and UV result revealed that these physicochemical properties were the capping and reducing agents associated with AgNP synthesis while the sharp peak at 1652 cm^{-1} corresponded to amide I arising in accordance to carbonyl l stretch in proteins indicating predominant surface capping species mainly responsible for stabilization [75].

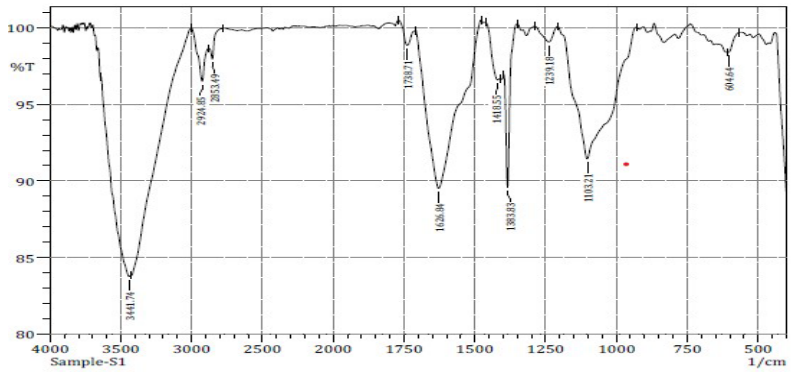

Figure 4. FTIR image of AgNPs synthesized from *S. auriculata*

3.5 Antioxidant activity of AgNPs

3.5.1 DPPH antioxidant activity

One of the most effective ways for determining antioxidant activity is DPPH (1, 1-diphenyl-2-picrylhydazyl) analysis. A stable free radical receives an electron or hydrogen radical from an antioxidant to transform into a stable diamagnetic molecule [76]. According to Akintola *et al*. [77], the stable organic free radical DPPH could be utilized to investigate the free radical activities and consequently the antioxidant activity of diverse natural compounds. The dosages association for the free radical scavenging capability of the produced AgNPs is shown in Figure 5. The synthesized AgNPs had 95.26% scavenging activity at 1000 µg/mL concentration, while the standard ascorbic acid had 97.71% scavenging activity. The antioxidant activity was lower than that of the standard ascorbic acid. Our findings are also related to other studies. The AgNPs synthesized from *C. alata* had 65.72% scavenging activity at a concentration of 1000 µg/mL [78]. Saravanakumar *et al*. [71] reported that AgNPs synthesized from *C. tora* had 51% inhibition at 200 µg/mL, and the activity was lower than that of standard vitamin C.

3.5.2 Nitric oxide scavenging activity

Nitric oxide (NO) is a chemical mediator produced by epithelial cells, macrophages, and neurons. It has crucial signaling functions in physiological and pathological processes such as brain signaling, immunological responses, and blood pressure regulation [79]. Excessive nitric oxide production has been linked to various harmful illnesses, including trauma, dermatitis, and cancer [80]. The nitric oxide scavenging activity of the synthesized AgNPs is given in Figure 6. The biosynthesized AgNPs exhibited a maximum nitric oxide scavenging activity of 97.3% at 1000 µg/mL. Similar results were found with silver nanoparticles synthesized from the plant leaves of *Raphanus sativus*, which had 69.51% nitric oxide scavenging activity at the concentration of 100 µg/mL. The phytochemicals present in *S. auriculata*, tannins and flavonoids, could be attributed to the increase in nitric oxide scavenging activity in a dose-dependent manner [81]. *Cassia auriculata* leaf extract possessed 232.56 µg/mL nitric oxide scavenging activity [82].

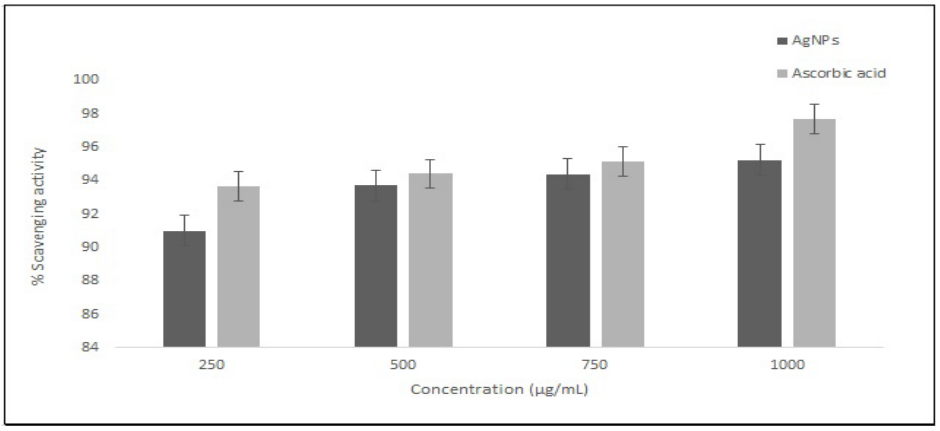

Figure 5. DPPH activity of AgNPs synthesized from *S. auriculata*

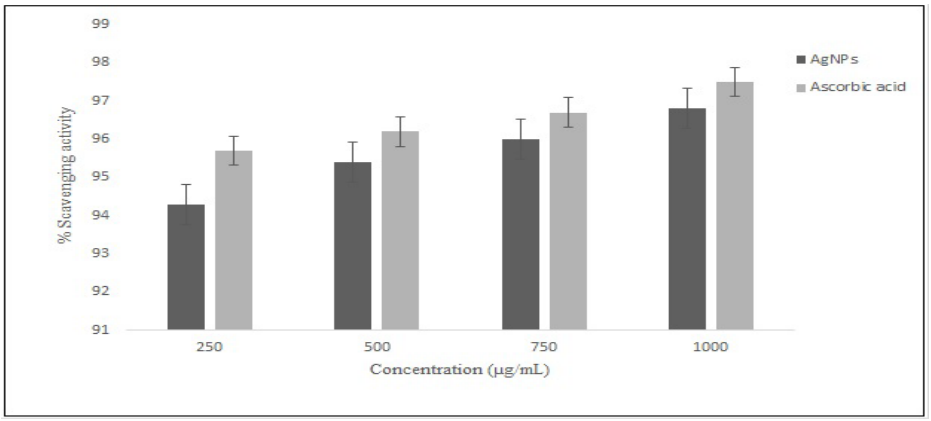

Figure 6. Nitric oxide scavenging activity of AgNPs synthesized from *S. auriculata*

3.5.3 Hydrogen peroxide activity

Every living cell produces hydrogen peroxide as a by-product of respiration. Because it is toxic, H_2O_2 must be eliminated from the cell, and cells produce catalases to eliminate H_2O_2 [66]. The result confirmed that the synthesized AgNPs had 83.74% scavenging activity, whereas the standard ascorbic acid had 98.2% hydrogen peroxide activity. This result proved that standard ascorbic acid has intense hydrogen peroxide scavenging activity compared to AgNPs. The hydrogen peroxide activity of the produced AgNPs is shown in Figure 7. The scavenging H_2O_2 by the extracts may be due to their phenolics, which can donate electrons to H_2O_2 and neutralize it to water [83]. *Cassia auriculata* flower exhibited 79% hydrogen peroxide radical scavenging potential at 1000 µg/mL concentration [84]. Silver nanoparticles were studied extensively due to their potential antioxidant effects in various medicinal plants, e.g., *C. occidentalis* [74], *D. crotonifolia* [85], *A. ampeloprasum* [86].

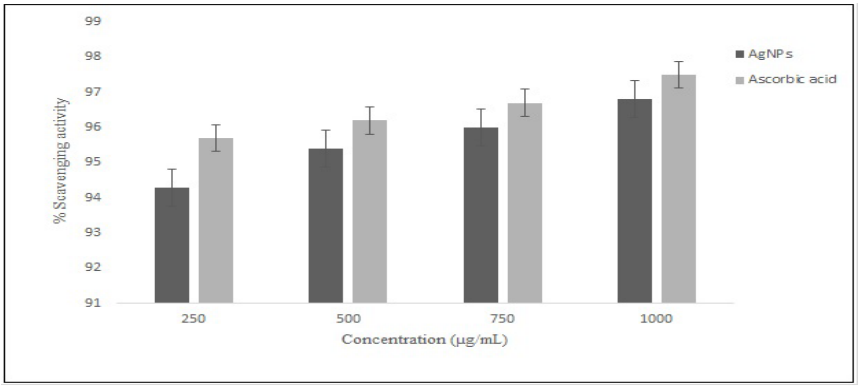

Figure 7. Hydrogen peroxide activity of AgNPs synthesized from *S. auriculata*

3.6 Antibacterial activity

Both gram-positive and gram-negative pathogens were evaluated for antimicrobial activity. The most notable impact of AgNPs was on *A. hydrophila*. The synthesized nanoparticles showed antibacterial activity against all tested bacteria, but maximum activity was recorded against gram-negative *A. hydrophila*, *E. coli*, and *V. cholera,* whereas the least activity was against the gram-positive bacteria *B. subtilis*. The antibacterial property of the produced nanoparticles is given in Figure 8. The bacterial cells are killed when the AgNPs bind to the cytoplasmic membranes. This is due to the electrostatic repulsion between positively charged nanoparticles and the negatively charged cell membranes of the microbes [60]. Slavin *et al.* [87] reported that the higher AgNPs antibacterial activity against gram-negative bacteria is due to their thinner peptidoglycan layer, which allows the nanoparticles to enter into the cell walls and denature or kill the bacteria. According to Actis *et al*. [88], the antimicrobial potential of synthesized AgNPs from *Mangifera indica* leaves increased with increasing concentration of nanoparticles. *Salmonella typhi*, *Enterococcus*, *Escherichia coli*, and *Klebsiella pneumoniae*, which are clinically significant pathogens, were all effectively combated by AgNPs synthesized from *C. auriculata* extract. Shaik *et al*. [75] used the disc diffusion method to examine the AgNPs made from *C. tora* root extract and their antibacterial effectiveness against pathogens such as *Pseudomonas*, *E. coli, S. typhi*, and *Staphylococcus aureus. Pseudomonas* and *Staph. aureus* were the pathogens that responded most negatively to the produced silver nanoparticles. According to some reports, silver nanoparticles prevent bacterial cells from growing and reproducing when they come into contact with the nanoparticles. Silver nanoparticles have thus often been applied in the early detection and management of illnesses, particularly those caused by rapidly developing multidrug-resistant microorganisms. AgNPs are widely used in many different industries and are known to constrain a variety of bacteria [61]. Silver nanoparticle synthesized from *Myrtus communis* had the potency to inhibit the growth of *E. coli* and *S. aureus* compared to vancomycin and gentamycin [89].

3.7 Cytotoxic activity

The cytotoxic effect of the green synthesized silver nanoparticles against the Human hepatic cell line (Huh-7) and the regular cell line was investigated to aid in the advancement of anti-cancer medication. The MTT assay was used to assess cell viability (%) of the Huh-7 cell line after contact

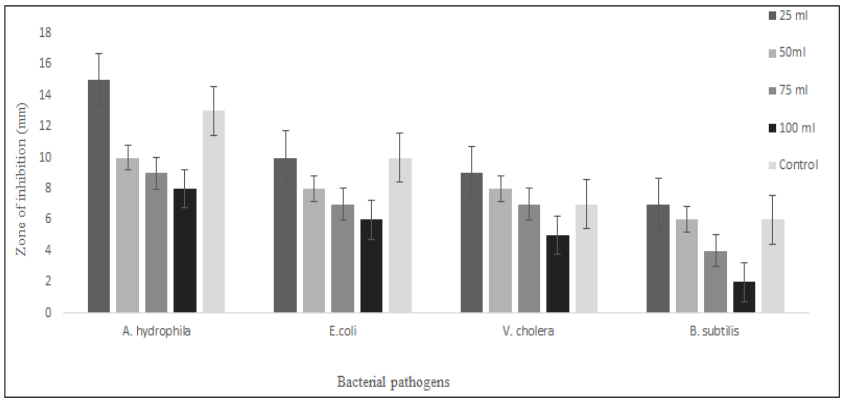

Figure 8. Antibacterial activity of AgNPs synthesized from *S. auriculata*

with biosynthesized silver nanoparticles. Figures 9 and 10 illustrate the viability of cells (%) subjected to different dosages of AgNPs (20, 40, 60, 80, 100 and 120 µg/mL). The IC_{50} value of the synthesized AgNPs was observed at the concentration of 100 µg/mL. This study found that biosynthesized silver nanoparticles had significant cytotoxic effects. This activity, however, could be the outcome of a dynamic relationship between biologically active phytoconstituents adhering to the surfaces of the particles and the nanosized silver. The more significant cytotoxicity of AgNPs might also be related to their size and the capping of biomaterials on the surfaces of nanoparticles, like protein or phenol [90]. The mode of action may involve an increase in neoplastic transformation followed by apoptosis, dose-dependent apoptosis-inducing abilities, necrosis of cancer cell lines, or other mechanisms involving epigenetic and signal transduction pathways [91]. Singh *et al*. [92] evaluated the anticancer activity of nanoparticles embedded in *Madhuca longifolia* extract (ML-AgNPs) with an experimental model of hepatic cancer in rats, and the IC_{50} cell inhibition value was obtained at 41.01 µg mL^{-1}. The cell proliferation activity decreased with increasing concentration of ML-AgNPs. Fine-tuned nanospheres synthesized from *Fagonia cretica*, and the cytotoxicity was checked against the Huh-7 cell line at 200-800 µg/mL, and 60% cytotoxicity was observed at higher concentrations [93].

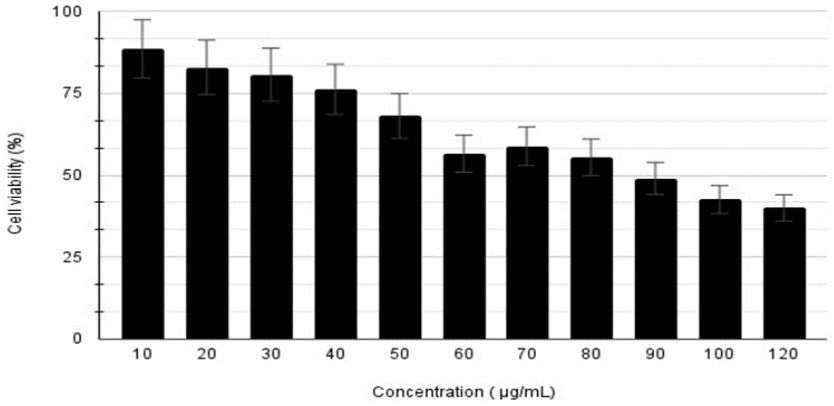

Figure 9. Cytotoxic activity of synthesized AgNps against treated Huh-7 cell line

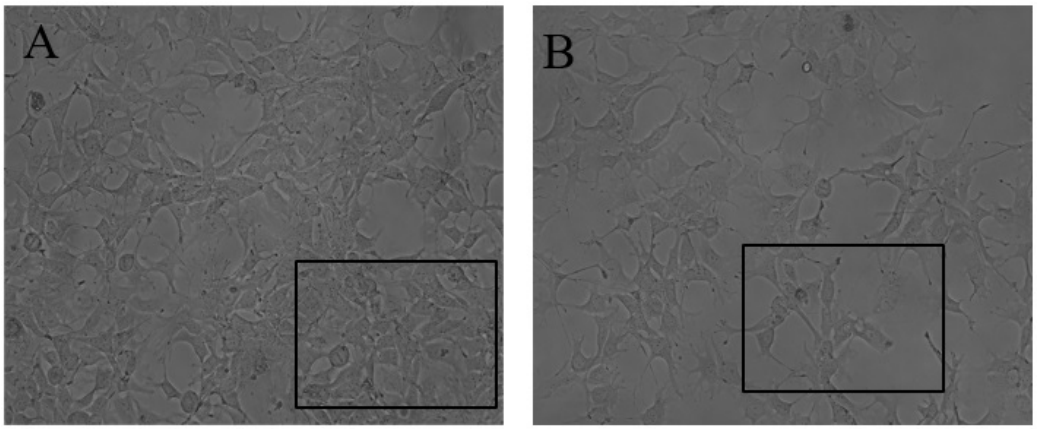

Figure 10. Analysis of cytotoxicity of biogenic AgNPs (*S. auriculata*) against treated human liver cancer cell lines (Huh-7), A-Untreated cell line, B- Treated cell line

4. Conclusions

The creation of a sustainable and environmentally acceptable method for the production of metallic nanoparticles is a crucial requirement in the area of nanotechnology. Due to their appealing physiochemical features, silver nanoparticles significantly impact biology and medicine. In the present study, we successfully synthesized silver nanoparticles from an aqueous extract of *S. auriculata*. The biosynthesized nanoparticle proved to have excellent antibacterial activity against gram-negative and gram-positive bacteria. The activity was dose-dependent. Additionally, the nanoparticles demonstrated intense antioxidant action against DPPH, nitric oxide, and hydrogen peroxide. Overall, the outcomes demonstrated the robust antibacterial and antioxidant capabilities of the AgNPs' efficacy. Furthermore, they exhibited excellent cytotoxic activity against Huh-7 cell line. Green AgNPs appeared to be active against the Huh-7 cell line. The excellent biological results for *Senna auriculata* -AgNPs may be attributed to the synergistic effect of the NP properties and the adsorbed secondary metabolites from the plant leaf extract. As a result of this study, the synthesized SA-AgNPs appear to be a credible candidate for diverse organic and nutraceutical applications. More research is required to establish *in vitro* and *in vivo* dose-dependent biocompatibility. This study will also pave the way for the development of biocompatible nanoparticles obtained from plants that have a range of biological functions.

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