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Biogenic Silver Nanoparticles from *Alternanthera philoxeroides* (Mart.) Griseb: Synthesis, Characterization and Bioapplications

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Abstract

Silver nanoparticles have diverse applications, particularly in health and medicine, emphasizing the need for cost-effective, eco-friendly, and scalable production methods. Green synthesis forms nanoparticlesof silver throughbioreduction of silver ions, provides a sustainable alternative that eliminates the requirement for external reducing agents. This study highlights the efficiency of AgNPs synthesized by leaf aqueous extract of *Alternanthera philoxeroides*. The biosynthesized nano-sized particles were characterized using UV-Vis and FTIR spectroscopy. Morphological analysis via Electron Scanning Microscopy and Electron Transmission Microscopy revealed spherically shaped nanoparticles (AP-AgNPs) ofvarious sizes from 13-43 nm. The antimicrobial properties of APAE and AP-AgNPs were assessed using diffusion assay against microbial strains including *S. aureus* MTCC 11949, *B. subtilis* MTCC 10010, *E. coli* MTCC 9721, *P. aeruginosa* MTCC 9800, and *A.niger* MCIN 512, where AP-AgNPs exhibited notable antimicrobial activity. Furthermore, the cytotoxic effects of AP-AgNPs were assessed in contrast to MCF-7 and HCT-116 cell lines, yielding IC_{50} values of 7.33 µg/mL and 13.15 µg/mL, respectively, compared to the positive control cisplatin (IC_{50}: 3.17 µg/mL & 6.24 µg/mL). These observations suggest that the synthesized silver nanoparticles exhibitprominent antimicrobial and anticancer properties, making them valuable for future applications in biomedical research, drug delivery systems and healthcare.

Keywords: Silver nanoparticles, antimicrobial activity, antioxidant properties, cytotoxic effects.

Introduction

The exploration of green technology for metallic nanoparticles synthesis has gained greater emphasis over the past decade because of their advantages, such as cost-effectiveness and environmental aspects. The utilization of plant parts in metal reduction is because of the presence of numerousbioactive compounds. The production of biogenic silver nanoparticles (Ag-NPs) using plant extracts has gained more significance since they are less toxic than chemically synthesized silver nanoparticles (1).

Ag-NPs are a fascinating metal to examine, particularly within the realms of health and medicine. Known for its potent antibacterial properties, silver can also be toxic to cells. Silver possesses the capability to compromise bacterial cell membranes, hinder bacterial growth, and disrupt cellular metabolism due to the interactions between silver ions and cellular macromolecules; proteins and deoxyribonucleic acid. The interaction of silver ions with cellular structures obstructs protein synthesis, reduces membrane permeability, and ultimately results in cell death.

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Ag-NPs have a vast range of benefits, particularly in health and medicine, highlighting the need for anaffordable, eco-friendly synthesis method with scalability potential.Silver ions are converted into nanoparticles through green synthesis via bio-reactions, offering a viable alternative without requiring additional reducing agents.Furthermore, the method's scalable procedures enhance its efficiency compared to chemical and physical synthesis techniques.Numerous studies hadreported the synthesis of Ag-NPs using cellular or plant-derived parts, like extracts from roots, leaves, stems, and calluses of plants as stabilizing agents. Moreover, Ag-NPs produced from different plant sources have reported a variety of biological functions, such as anticoagulant, antimicrobial, anti-inflammatory, anticoagulant, anti-angiogenesis, antioxidant and anticancer activities.However, very few studies are available on the identification of potential plant sources for the production of Ag-NPs(2,3).

Phytochemical analysis of leaves extract shows the details of the possible presence of various biologically active compounds in nature. Apart from water, alkaloids, anthraquinone, carbohydrates, flavonoids, glycosides, phenol, phytosteroids, proteins, saponins, and tannins are major bioactive compounds that are known for their potent antibacterial, enzymatic inhibition, antiviral, anticancer, anticarcinogenic, anti-inflammatory, anti-allergic, and antioxidant activities. Surprisingly, researchers have discovered the mechanism of synthesis of Ag-NPs by reducing metal ions in cytosol in plant cells and production of Ag-NPs by the action of indole moieties from a single protein in the presence of potassium bromide. These results confirm the presence of one or several bioactive compounds in this plant(4-7).

The existence of a biological molecule in the extract is vital in the development of Ag-NPs, demonstrating its important factor to control reduction process and resulting in well-dispersed and stable Ag-NPs. Functional groups like carboxylic, hydroxyl, amine, and thiol groups found in plant compounds, including amino acids, phenols, proteins, enzymes, alkaloids, vitamins, flavonoids, lignin, and pigments, serve as reductive and stabilizing agents, influence the fundamental membrane properties of Gram-ve and Gram+ve bacteria, which allows using a green synthesis strategy for emerging potential antibacterial therapeutic applications (8,9). These functional groups may form covalent and non-covalent bonds with metal ions, thereby resulting in the reduction of Ag+ exchanged in the medium to Ag atoms and the stabilization of Ag-NPs by capping on the surface of the overloaded Ag atoms.The Ag-NPs stabilized by the phytochemicals have a long-term storage due to suppressing the characteristic instability of Ag-NPs and the depletion of the excess reductant.

The current investigation on*A.philoxeroides*leaf aqueous extract was utilized for the formation of Ag-NPs.The biosynthesis of these Ag-NPs offer significant advantages, as the plant extract biomolecules facilitate their formation, capping, and stabilization.Biogenic synthesized nanoparticles are exceptionally stable due to the capping properties of plant biomolecules, and hence they have remarkable antimicrobial effects. *A. philoxeroides* is an invasive weed found in different parts of wetlands in India; however, weed extract-mediated Ag-NPs have not been reported until now. Therefore, this study was carried out to synthesize Ag-NPs utilizing *A. philoxeroides* weed. Furthermore, their enhanced effect against bacterial and fungal pathogens is studied.

Collection of Plant Material

The plant *A. philoxeroides*(Mart.) Grisebwas gathered from the fields around Guntur, Andhra Pradesh. The plant was certified by Botanical Survey of India, Coimbatore. Flourishing leaves were gathered, washed thoroughly, air-driedand then ground into a granular powder.

Extraction of Phytoconstituents

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The dried leaves were pulverized using a mechanical grinder and passed through a 40 µm mesh sieve for uniformity. The resulting powder was extracted with sterile distilled water to obtain the aqueous extract. The mixture was centrifuged at 5,000 rpm, to separate the supernatant, which was then collected. The extract was further purified by vaccumfiltration, and the finalaqueous plant extract (APAE) was preserved at 4ºC for subsequent studies.

Phytochemical Qualitative Analysis

A qualitative analysis of the phytochemicals in APAE was determined through standard experimental techniques to identify common phytoconstituents. The outcomes of these analyses were documented as either positive (+) or negative (−) (10,11).

Phytochemical Quantitative Analysis

Quantification of Phenolic Concentration

The total phenolics was estimated by Folin reagent using gallic acid as standard. The total phenolics in APAE & AP-AgNPs was quantified through standard curve (12,13).

Quantification ofTannin Concentration

Tannin concentration was quantified using an insoluble polymer. The polymer attaches to tannin after incubation and separated by centrifugation. The non-tannin phenolic concentration was estimated in similar way of total phenolics.By deducting non-tannin concentration from total phenolics, tannin concentration was quantifiedin APAE & AP-AgNPs.

Quantification of Flavonoid Concentration

Modified aluminium chloride test is used to quantify the flavonoid concentration in APAE & AP-AgNPs (14,15). Aliquots of samples were diluted with methanol to a final volume of 3 mL. Subsequently, 0.1mL of 10% AlCl3, 0.1 mL $KNaC_4H_4O_6$ and made upto 3 mL with distilled water. Samples weremixed thoroughly and after incubation of 30 min, the optical density was determined. The standard curve was constructed using rutin in concentrations ranging from 2-12 mg/mL.

Synthesis of *Alternanthera philoxeroides* silver nanoparticles (AP-AgNPs)

The AP-AgNPs produced through green synthesis method.Reaction mixture containing 0.5:4.5 ratio of APAE & 1mM $AgNO_3$ was incubated at dark for overnight. Formation of Ag-NPs was identified by color change from green to brown due to bioreduction of silver ions to silver atoms. The developedNPs were separated by centrifugation at 10,000 rpm for 45 minutes (CM-8 plus, REMI, India) and then washed and preserved for further study.

Stock Solutions Preparation

The stock solutions of 10% w/v APAE, 1mM $AgNO_3$ and 10% AP-AgNP were prepared in dimethyl sulphoxide (DMSO). The stock solutions were stored at 4ºC and used to determine antioxidant, cytotoxic, antibacterial and antifungal activities.

Characterization of Ag-NPs

The conversion of silver ions into Ag-NPs was observed using aultraviolet-vis spectrometer (Schimadzu UV-1780). The absorbance values were noted at regular intervals across wavelength range of 200 to 1,000 nm. The surface characteristics, morphology, shape and size of biosynthesized AP-AgNPs were analysed through SEM & TEM.

The spectra of the prepared Ag-NPs was recorded using a UV-Vis spectrophotometer (Schimadzu UV-1780) with a resolution of 1 nm. The FT-IR spectrum was recorded using a JASCO 4400, within the spectral range of 4000–400 cm^{-1}, with the sample prepared as a pellet using potassium iodide (1:100).

Scanning Electron Microscopic Analysis

The shape, morphology, and dispersion of the prepared AP-AgNPs were examined using a SEM. A small quantity of

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Ag-NPs was deposited onto conductive carbon tape bonded to an aluminum stub and then sputter-coated with a thin layer of gold for 3–4 minutes.

Transmission Electron Microscopic Analysis

The fabricated AP-AgNPs shape was examined using TEM. A droplet of the AP-AgNPs solution was deposited onto a carbon-coated copper grid, and pictures were taken at enlargements ranging from 6000 to 8000 with a Hitachi S-3400N apparatus, operated at voltage of 80 kV.

Determination of Antibacterial Efficacy:

The biosynthesized APAgNPs from *A. philoxeroides*leaf extract was checked for its anti-bacterial potency. It was assessed by diffusion assay using Mueller Hinton medium (Himedia)(16,17). The activity was tested against various strains including *S.aureus* MTCC 11949, *B.subtilis*MTCC 10010, *P.aeruginosa*MTCC 9800 and *E.coli* MTCC 9721.

The Mueller Hinton agar media wasseeded with the bacteria cultured for 24 h and the cavities of diameter 6 mm and 2.5 mm depth are prepared equidistantly. Exactly 50 µL of APAE (10% w/v), 1mM $AgNO_3$ and AP-AgNPs (10% w/v) were filled in the wells separately. The antibiotic tetracycline (30 µg per disc) and 1mM AgNO3 were used as +ve and -ve controls. The samples were incubated at 37ºC, 24 h and inhibition zones werecalculated vernier calipers and the results were tabulated. The study was conducted for triplicates and the mean ± SD of inhibition zones were considered for calculating antibacterial potential of extract and silver nanoparticles.

Assessment of Minimum Inhibitory Concentration (MIC):

The MICs of APAE, $AgNO_3$, AP-AgNPs were assessed using the micro-dilution processusing an indicator resazurin. In this procedure, 100µL of each test solution was introduced to the wells in first row while the remaining wells were loaded with 50 µL of sterile nutrient broth. A 50 µL aliquot of the test solutionwas then transferred to the next well to achieve serial dilutions, ensuring all wells contained 50 µL. Subsequently, added 10 µL of 1×10^8 CFU/mL bacterial culture and 30 µL of resazurin of 0.02% to each well and incubated at 37°C for 24 h (18,19).

Determination of Antifungal Activity

The fungicidal property was assessed by the diffusion method against *Aspergillus niger*MCIN 512. The inoculum was spread over sterile PDA (Potato Dextrose Agar – Himedia) medium using sterile cotton swab to get a uniform lawn growth of fungi. The media was punctured to create cups and are added with 100µL of APAE, $AgNO_3$ and AP-AgNPs stock solutions into the labelled cups. The petri dishes were incubated at 25ºC for 48h and inhibition zones were calculated using antibiotic reader. The activity was compared withfluconazole (100µg/disc) and DMSO as positive and negative controls (20,21).

Radical Quenching Potential

The radical quenching potential of AP-AgNPs and ascorbic acid (10% w/v) was evaluated by measuring their effect on DPPH (2, 2-Diphenyl-1-picrylhydrazyl) (22-24).Graph was prepared between X axis (Sample Concentration) *vs*. Y axis (% inhibition wrt control).

The radical quenching activity of the fabricated nanoparticles was calculated by% DPPH radical scavenged using below equation:

$$\% \text{ scavenging of DPPH radical} = (Ac - As) / Ac \times 100$$

Where Ac & As are the absorbance values of control & sample/ standard.

In-vitro Cytotoxic Evaluation by MTT Assay

The AP-AgNPs cytolytic activity against MCF-7 and HCT-116 cell lines (NCCS Pune) was evaluated by means of MTT assay. Cells (10,000/well) were cultured in a micro titre plate with DMEM F12 medium,

10% FBS, and 1% antibiotics at room temperature with 5% CO_2 for 24 h. The next day, cells were treated with various sample concentrations prepared in DMSO and diluted in FBS-free medium. Untreated cells served as controls, and blanks lacked MTT. After 24 h, 5 mg/mL MTT was mixed and rested for 2 h. Discarded the clarified liquid, and resuspended the cells in 100 µL DMSO. Optical density was recorded at 540 nm using an ELISA plate reader (25-26). IC_{50} was calculated via GraphPad Prism 6, and images were captured using an inverted microscope with an AmScope 10 MP camera. IC_{50} values were reported as Mean ± SEM.

Results

Phytoconstituents Screening

The qualitative phytoconstituents screening of the APAE documented the presence of numerous phytoconstituents as depicted in (Table 1).

Formation of Ag-NPs

The nanoparticles formation commenced upon mixing the crude extract with $AgNO_3$solution. The light green color shifts to dark brown color (Fig. 1) and the subsequent spectral analysis validated the successful synthesis of Ag-NPs.

Quantitative Determination of Tannins & Flavonoids

The total phenolic content in APAE was found to be 12.4 mg of Gallic acid equivalent/g of extract, and the tannin content was 5.6 mg of Gallic acid equivalent/g of extract. These values were determined using the standard calibration curve of gallic acid (Fig. 2).

Flavonoids, a highly diverse and widely distributed group of natural compounds, are among the most significant natural phenols. They possess various chemical and biological activities, including radical scavenging properties. The flavonoid content in APAE was measured at 3.2 mg of rutin per gram of extract, estimated using a standard calibration curve of rutin (Fig. 3). A summary of total phenolics, tannin, and flavonoid contents is provided in (Table 2).

Table 1: Phyto Analysis of APAE

Name of the Test	APAE
Glycosides	+
Alkaloids	+
Carbohydrates	+
Amino Acids	+
Proteins	+
Flavonoids	+
Tannins & Phenolic compounds	+
Steroids	+

Note: '+' indicates positive; '-' indicates negative

UV-VisAnalysis

The reduced stated of fabricated Ag-NPs were evaluated by UV-Vis spectrophotometer (Shimadzu UV 1780).The absorbance peak of AP-AgNPs was recorded at 442 nm (Fig. 4).

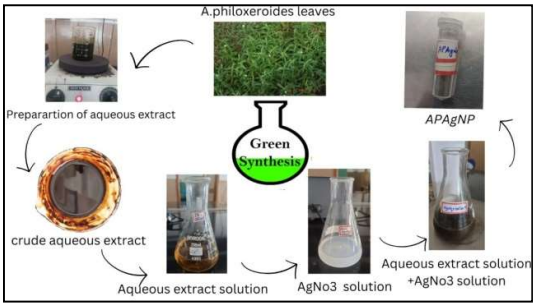

Fig. 1: Graphical representation of synthesis of silver nanoparticles

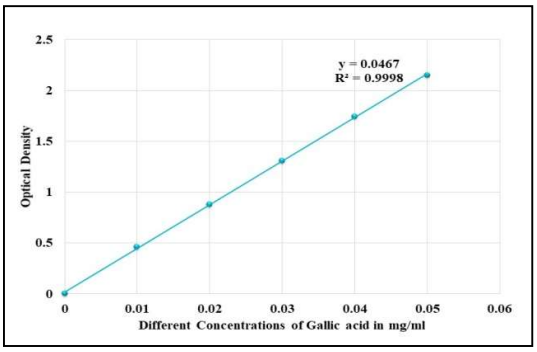

Fig. 2: Gallic acid standard curve and their corresponding optical densities, 725 nm

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FT-IR Analysis

The FT-IR analysis of AP-AgNPs displayed various absorbance bands, specifically at 3399, 3346, 2928, 2347, 2205, 2008, and 1538 cm^{-1}, corresponding to various molecular groups of phytochemicals. These bands indicate the presence of phenolic compounds, flavonoids, tannins, amines, amides, and methyl groups (Fig. 5).

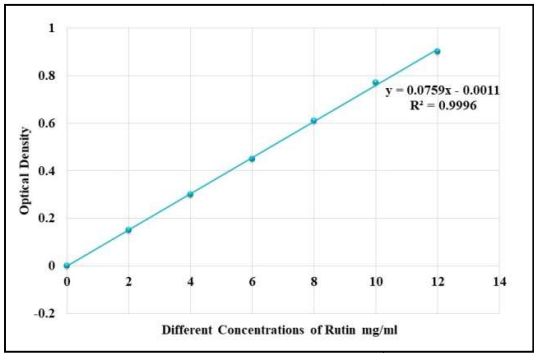

Fig. 3: Rutin standard curve and their corresponding optical densities, 415 nm

SEM & TEM Analysis

The SEM analysis verified the shape of the AP-AgNPs as spherical, withdifferent sizes. TEM results demonstrated the dimensions of the Ag-NPs varied between 13-43 nm (Fig. 6).

Screening of Antimicrobial Potential

The findingsconfirmed the presence of antibacterial activity in APAE, $AgNO_3$, and AP-AgNPs. However, the zone of inhibition indicated that Ag-NPs showed superior antibacterial potential compared to APAE & AgNO3. The zones of inhibition for AP-AgNPs against different microorganisms were 19 mm (*B. subtilis* MTCC 10010), 18 mm (*S. aureus* MTCC 11949), 28 mm (*P. aeruginosa* MTCC 9800) and 20 mm (*E. coli* MTCC 9721), as well as 16 mm against *A. niger* MCIN 512 (Fig. 7).

MIC

The minimal dose needed for bacterial inhibition value of AP-AgNPs towards bacteria was dependent upon the strains. The results were represented in (Fig. 8 and 9), as 6.25

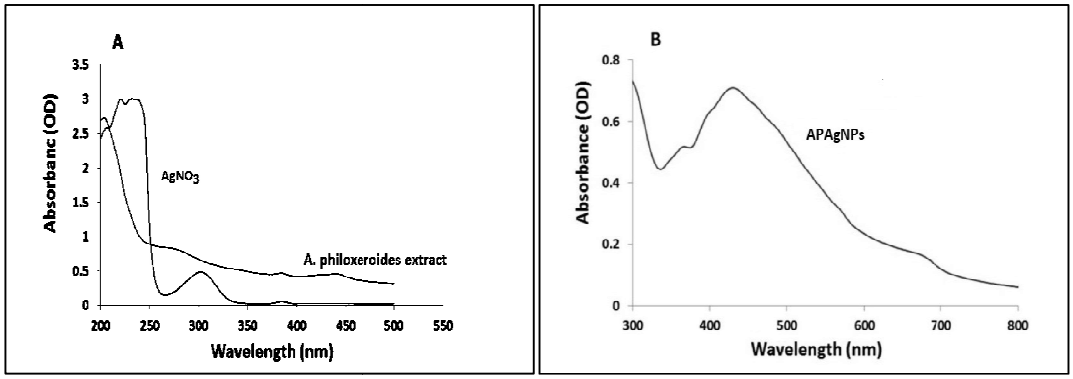

Fig. 4: UV spectra of APAgNPs, AgNO3 and *A.philoxeroides* aqueous extract

Table 2: Quantitative determination of Tannin & Flavonoid contents

Name of the Sample	Total phenolic content (mg of GAE/gm of extract)	Non tannin content (mg of GAE/gm of extract)	Tannin content (mg of GAE/gm of extract)	Flavonoid content (mg of rutin/gm of extract)
APAE	12.4	6.8	5.6	3.2
APAgNPs	21.6	12.3	9.3	4.8

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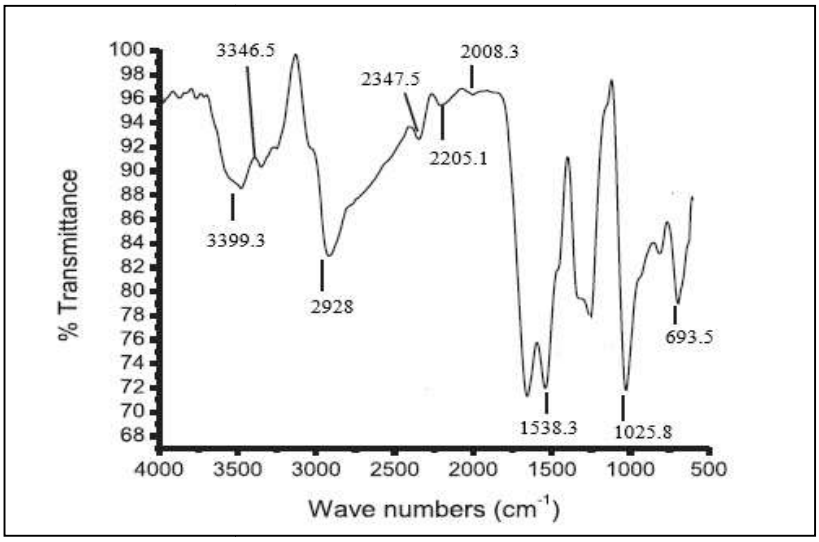

Fig. 5: FTIR spectral analysis of APAgNPs

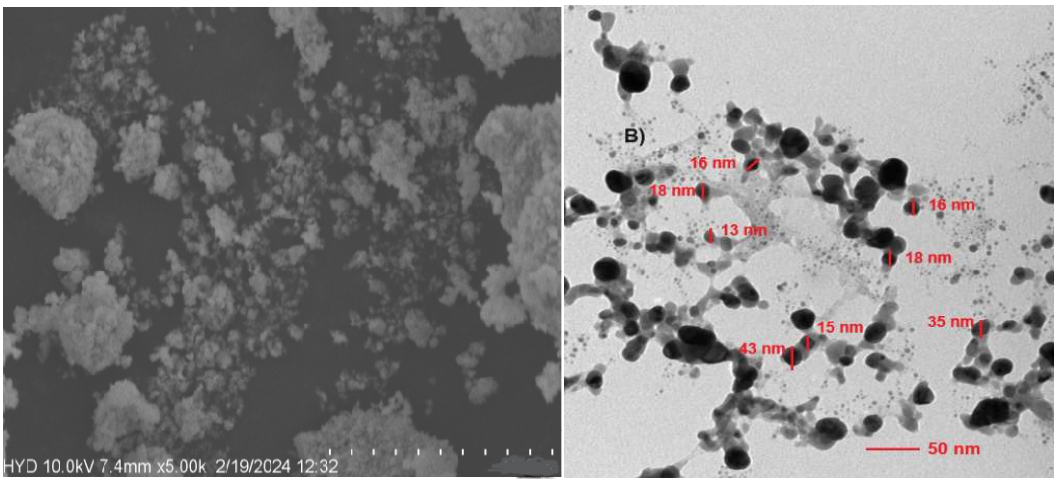

Fig. 6: A) SEM image showing generation of numerous sizes of Ag-NPs, B) TEM image showing generation of spherical Ag-NPs

µg/ml (*S. aureus* MTCC 11949), 3.12 µg/ml (*B. subtilis* MTCC 10010), 6.25 µg/ml (*E. coli* MTCC 9721), 3.12 µg/ml (*P. aeruginosa* MTCC 9800).

Radical Quenching Potential

Radical quenching activity was done by DPPH method. The findings validated thatAP-AgNPs and ascorbic acid has the radical quenching potential. The IC_{50} values of AP-AgNP was 23.37µg/ml and ascorbic acid 14.29 µg/ml. The results confirmed the antioxidant property of AP-AgNPs.

Cytotoxic Activity of AP-AgNPs

The cell toxicityefficacy of fabricated nanoparticles (AP-AgNPs) on MCF-7 breast cancer cell line and HCT-116 colon cancer

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cell line was studied through MTT assay. The toxic effects of AP-AgNPs against cell lines MCF-7 & HCT-116 was confirmed by microscopic analysis (Fig 12 to 16). The morphological observations clearly showed cell shrinkage, apoptosis and cell death. The cell survival rate of MCF-7 & HCT-116 using varying concentrations of AP-AgNPs and cisplatin were able to inhibit the viability of cell lines in concentration-dependent manner. The cell toxicity effects of variousdoses of AP-AgNPs and cisplatin under controlled experimental conditions on the MCF-7 cell lines presented IC_{50} values 7.33 µg/mL and 3.17 µg/mLin sequence (Fig 10). Similarly, HCT-116 cell lines showed IC_{50} values 13.15 µg/mL and 6.24 µg/mL (Fig. 11).

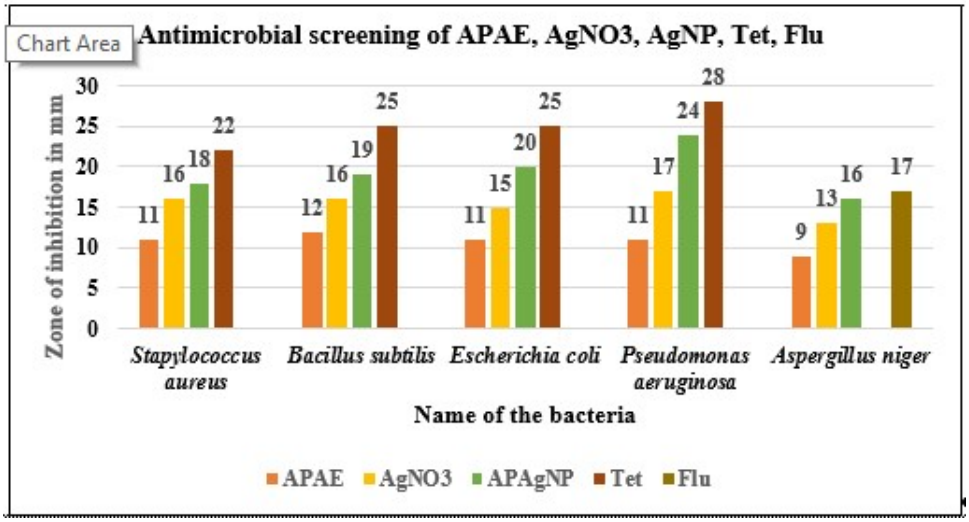

Fig. 7: The graph represents the antimicrobial screening of APAE, $AgNO_3$, AP-AgNP, Tet & Flu against tested bacteria & fungi

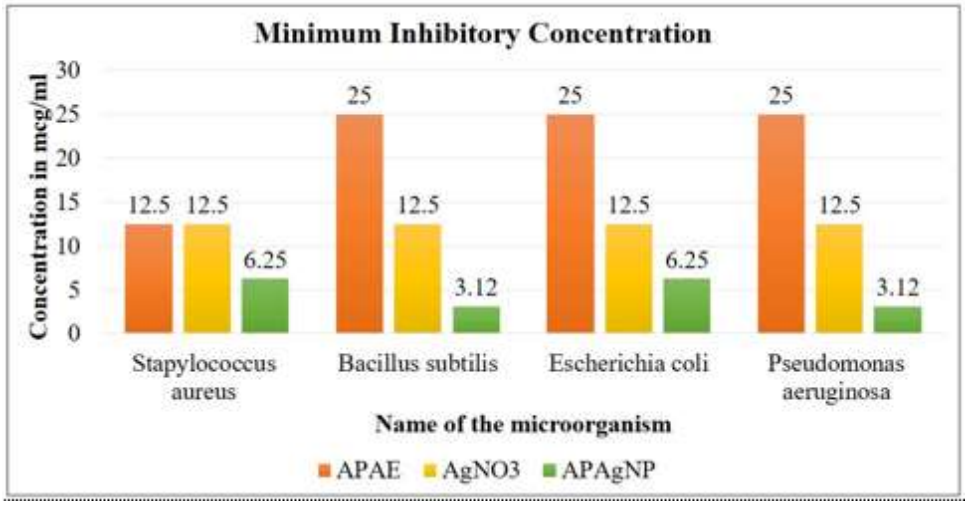

Fig. 8: The graph represents MIC towards assessed microorganisms

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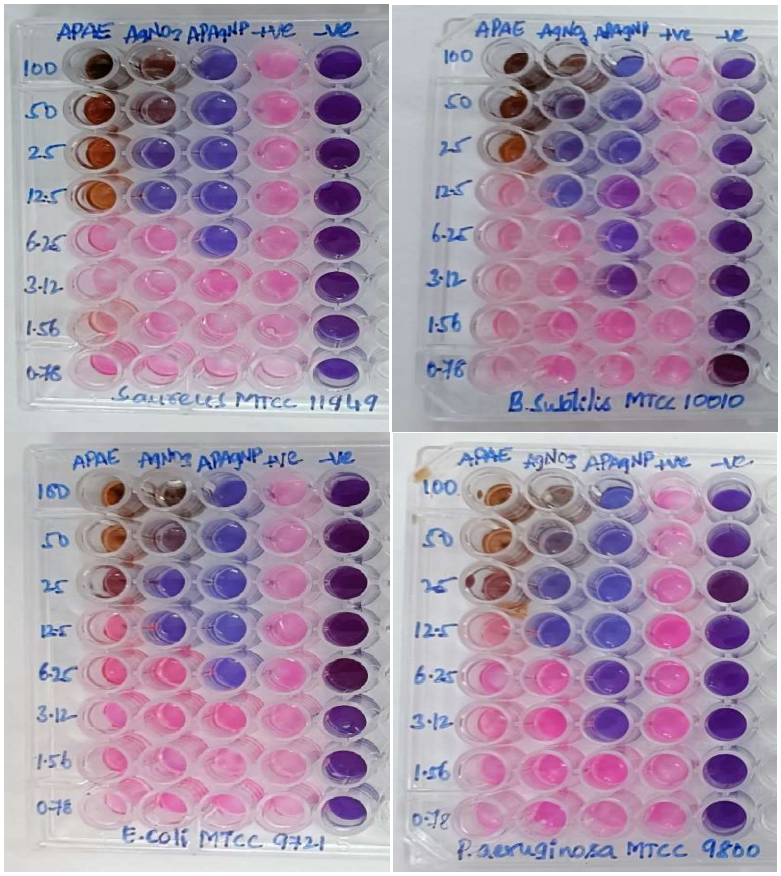

Fig. 9: MIC towards assessed microorganisms

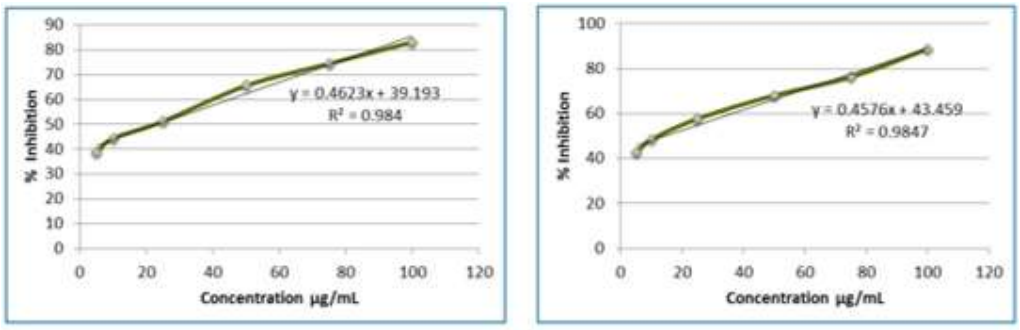

Fig. 10: The graphs represent the Antioxidant property of AP-AgNPs & Ascorbic acid

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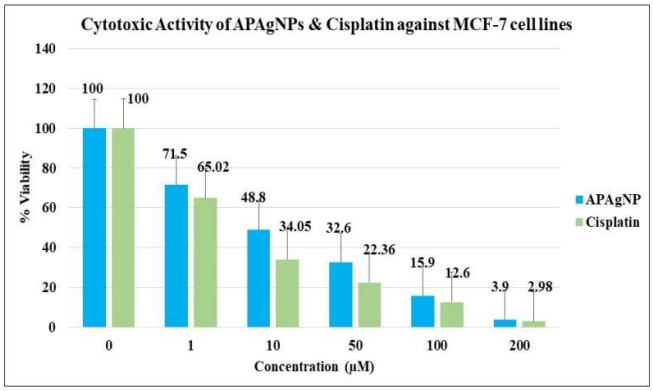

Fig. 11: The graph depicts the MCF-7 cells viability on exposure to varying concentrations of AP-AgNPs & Cisplatin

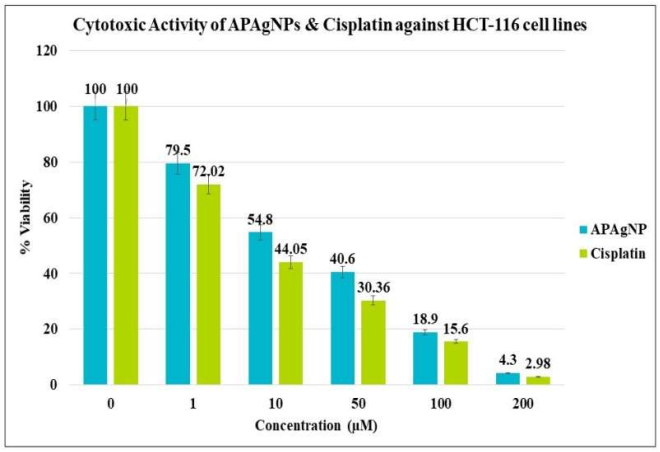

Fig. 12: The graph depicts the HCT-116 cells viability on exposure to varying concentrations of AP-AgNPs & Cisplatin

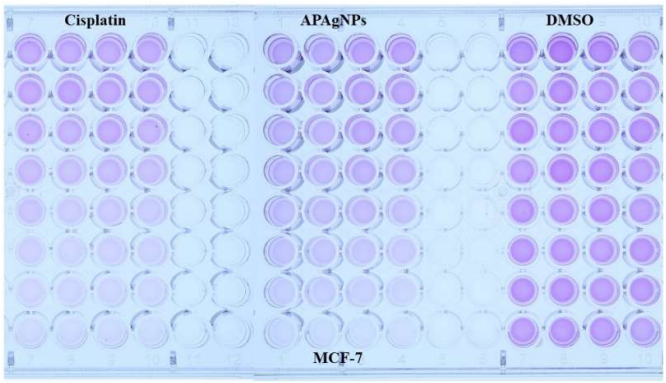

Fig. 13: The image represents the cytotoxic activity of AP-AgNPs & Cisplatin against MCF-7 cells

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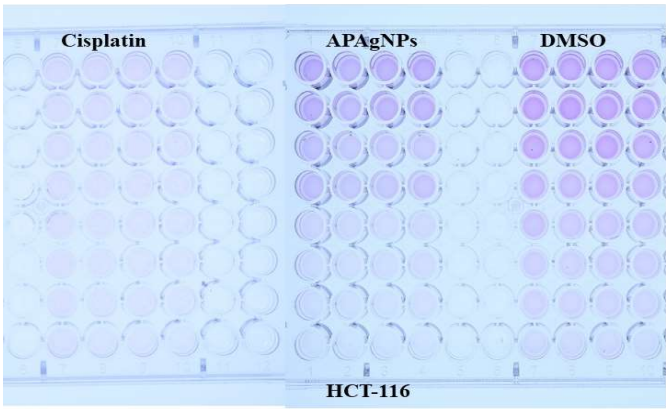

Fig. 14: The image represents the cytotoxic activity of AP-AgNPs & Cisplatin against HCT-116 cells

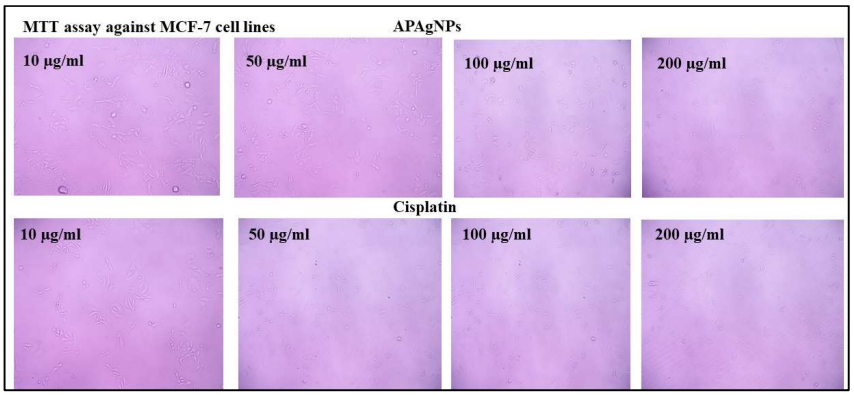

Fig. 15: The image represents the morphological changes of MCF-7 cell lines at various concentrations of AP-AgNPs & Cisplatin

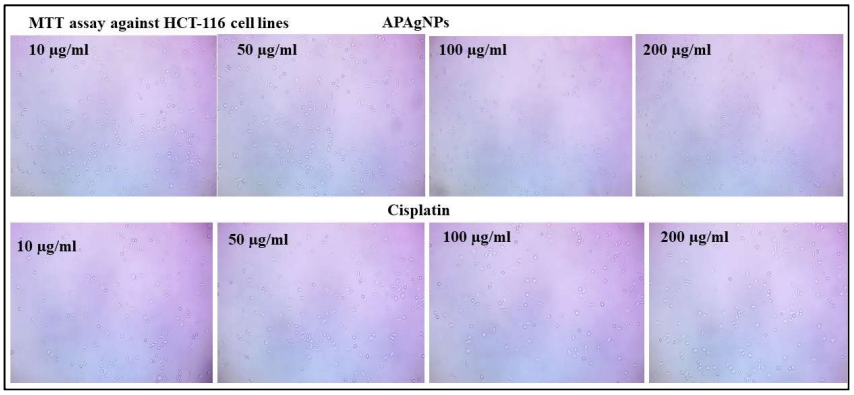

Fig. 16: The image represents the morphological changes of HCT-116 cells at various concentrations of AP-AgNPs & Cisplatin

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Discussion

This study outlines the fabricated Ag-NPs utilizing a raw extract from *A. philoxeroides* leaves. Upon adding the APAE to the $AgNO_3$ solution, the solution's color shifted from light green to dark brown, indicating the reduction of $AgNO_3$ to Ag-NPs, which was confirmed by the appearance of the dark brown color. The efficient reduction Ag+ ions is due to the secondary metabolites that contain active moieties like alkaloids, phenols, flavonoids, tannins, saponins and terpenoids. The outcomes of phyto analysis confirmed the presence of bioactive compoundsthat are known for antibacterial, antiviral, anti-inflammatory, antioxidant and anticancer activities were presented in (Table 1). The quantitative analysis revealed tannin content 5.6 mg of GAE/ gm extract and flavonoid content 3.2 mg of rutin/ gm extract in APAE and 9.3 mg of GAE/ gm extractand flavonoids4.8 mg of rutin/ gm extractin APAgNPsas presented in table 2. The higher levels of tannins and flavonoids in APAgNPs compared to APAE can be attributed to their adsorption onto the nanoparticle surface. Consequently, the nanoparticle formulation shows a relatively greater concentration of these compounds per unit weight than the crude extract. The biomolecules present in APAE play a pivotal role in the synthesis of Ag-NPs, acting as reducing and stabilizing agents, which not only drive the reduction process but also ensure the formation of well-dispersed and stable nanoparticles.

The presence of tannins and flavonoids is high in APAgNPs than APAE because the phytochemicals like tannins and flavonoids get adsorbed on the nanoparticle surface. So, per unit weight, the nanoparticle preparation may show a higher apparent content of these compounds than the crude extract. The existence of a biological molecule in APAE plays a crucial role in the development of Ag-NPs, demonstrating that it is an important factor to control the reduction process and resulting in well-dispersed and stable Ag-NPs.

Further, the development of Ag-NPs was confirmed by UV-Vis spectroscopy with absorbance band at 442 nm (Fig 4). The spectral analysis by FT-IR proved the existence of phenolic compounds, flavonoids, tannins, amines, amides, and methyl groups (Fig 5).

The biogenic synthesis of Ag-NPs has become increasingly widely recognized because it avoids toxic chemicals, is sustainable, affordable, and ideal for medical and drug-relatedapplications (27). The SEM and TEM results confirmed thedevelopment of spherical shape with variable size 13 to 43 nmAg-NPs were synthesized (Fig 6). The significant advantage of the present protocol involves its eco-friendly route. The chemicals employed in this study are easily derived from fresh leaves, are cost-effective, and form metal nanoparticles by a quick, economical method. The synthesis of stable and small-sized nanoparticles is a highly advantageous method to enhance the concentrationpotential of the targeted drug approach. The outcome of current investigation demonstrates that silver nanoparticles with moderate size and monodispersity were synthesized and accumulated in a green route using extract as an effective novel coating agent. The strong adherence of the phytochemicals enhanced the effectiveness of silver nanoparticles with good biocidal properties (28-30).

Bactericidal activities of the Ag-NPs towards both Gram+ve and Gram-ve bacteria were assessed using agar well diffusion assay. The inhibition zones were observed along with the increasing concentration of the Ag-NPs, denoting the dose-dependent antimicrobial activity. The maximum zone formed with respect toGram+ve bacteria due to the heavier peptidoglycan layer in cell boundarywhen compared to that of Gram-ve bacteria. Thus, the Ag-NPs showed a powerful effect against *K. pneumoniae* compared to *C. violaceum*. The synergistic effect of Ag-NPs and antibiotics revealed enhanced bactericidal and fungicidal activities with the reduced dosage of antibiotics. The fabricated silver nanoparticles present an

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excellent reduction potential and high antimicrobial properties useful in the preparation of noble antimicrobial coatings for different surfaces(31-33).

The antimicrobial activity of AP-AgNPs was evaluated against selected bacteria & fungi. The inhibition zones of AP-AgNPs against bacteria were 28 mm (*P. aeruginosa* MTCC 9800), 20mm (*E. coli* MTCC 9721), 19mm (*B. subtilis* MTCC 10010), 18 mm (*S. aureus* MTCC 11949) and 16mm against *A. niger* MCIN 512 in increasing zone diameters (Fig. 7). The MIC of AP-AgNPs varied from 3.12 to 6.25 µg/ml, the results were reported in Fig 8 & 9.The outcomes of antimicrobial susceptibility validated the Ag-NPs and $AgNO_3$ have bacteriostatic potential.Antimicrobial mechanism of AP-AgNPs likely involves interaction with the microbial membrane, leading to significant membrane damage and resulting bacterial cell destruction.The antimicrobial activity can additionally result from the interaction between small bacterial cells and silver nanoparticles, which induces a disruption in microbial cell metabolism. Following the absorption of AP-AgNPs, this imbalance results in formation of intracellular oxidative species, ultimately leadmortality of bacteria (34-36).

Another report revealed that methanolic extracts of *A. philoxeroides* displayed 94.02%, 84.30% ± 0.42, and 67.40% of antioxidant activities in the DPPH test at 1000, 750, and 500 µg/mL extract doses, respectively. The FRAP value of methanolic extracts of *A. philoxeroides* was also found to be 691.21 ± 3.48 µM trolox/g. In comparison, the values of acetone:ethyl acetate (7:3), ethyl acetate, n-hexane, and ethyl ether extracts of *A. philoxeroides* were found to be 254.80 ± 4.21, 148.57 ± 2.94, 105.51 ± 3.20, and 156.24 ± 1.82 µM trolox/g respectively. *A. philoxeroides* possesses excellent radical scavenging properties with respect to inhibition proportion of free radicals formation and total antioxidant capacity value compared to a similar class of plant extracts. Hence, it can be used to protect living organisms against several oxidative stress-mediated ailments (37).

Radical quenching activity by DPPH method confirmed that the AP-AgNPs has antioxidant property. The activity was performed comparing with standard ascorbic acid. The IC_{50} values of AP-AgNP was 23.37µg/ml and ascorbic acid 14.29 µg/ml shows the effective radical scavenging process (Fig 10).

Numerous plant-derived NPs have demonstrated capability in combating cancer cells. Specifically, ZnONPs synthesized from leaf extract of*Cassia auriculata*demonstrated cancer-killing activity against MCF-7 cells while remaining non-toxic to normal human breast cell lines, MCF-12A (38). The cell toxicity effect of AP-AgNPs on cell lines of MCF-7 and HCT-116 was studied through MTT assay. The IC_{50} values of AP-AgNPs and cisplatin against MCF-7 cells were7.33 µg/mL and 3.17 µg/mLin the same order (Fig 10). Similarly, HCT-116 cells showed IC_{50} values 13.15 µg/mL and 6.24 µg/mL (Fig.11). Toxic property of AP-AgNPs and cisplatin were confirmed by microscopic analysis.

In summary, the green synthesis of AP-AgNPs offers an efficient approach to targeting cancer cells and microbial pathogens.

Conclusion

The increasing need for sustainable and nano-science has fuelled the advancement of eco-friendly processes in producing nano-materials utilizingnatural resources like flora, microorganisms etc. Researchers have focused on green synthesis techniques for nanoparticles, prioritizing environmental sustainability. Given their cost-effectiveness, non-toxic properties, easy accessibility, and eco-friendliness, plant extract-mediated nanoparticle synthesis has become a major area of research, with potential applications spanning various industries.

The cuurent study showcases the fabrication of Ag-NPs with*A. philoxeroides* extract. Bioactive compounds present in *A. philoxeroides* leaves are essential for reducing and capping silver nitrates leading to

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development of stable Ag-NPs. The synthesized Ag-NPs exhibit antimicrobial, antioxidant, and cytotoxic activities, primarily due to the molecular groups present on their surface.

Furthermore, Ag-NPs demonstrate strong antibacterial properties against selected bacterial strains, attributed to their small size and the presence of capping agents. Given their non-toxic, cost-effective, eco-friendly, and highly effective nature, these silver nanoparticles hold potential for future use as antibiotics.Integrating traditional medicine with nanotechnology presents a valuable opportunity to create innovative antimicrobial agents.

Cell culture studies have shown that AP-AgNPs can induce cytotoxic effects in human cell lines, including MCF-7 and HCT-116. The cytotoxicity of AP-AgNPs has been observed to be dose-dependent. *A. philoxeroides* leaf extract contains valuable biomolecules for Ag-NPs synthesis, making these nanoparticles beneficial for various applications. Therefore, further research on AgNPs should be pursued to explore their full potential.

Statistical Analysis

Data was analyzed and IC_{50} values were determined using GraphPad Prism 6, and results of antibacterial and antifungal were expressed as mean ± SD.

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