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Biomedical Applications of *Vitex leucoxylon* Aqueous Leaf Extract and its Bio-fabricated Silver Nanoparticles

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ABSTRACT

Traditional medicine, herbal extracts have historically been utilized to treat a range of ailments, including infections, inflammation, fever, pain, digestive issues and skin diseases. *Vitex leucoxylon* has potential medicinal applications; yet, its use in nanotechnology, particularly for the green synthesis of silver nanoparticles remains limited. The present study examined the potential antibacterial, antioxidant, and cytotoxic activity of *V. leucoxylon* leaf extract and its synthesized silver nanoparticles (Vl-AgNPs). Characterization of Vl-AgNPs were determined by UV-Vis spectroscopy, FTIR, XRD, SEM-EDX, Zeta potential and DLS. Vl-AgNPs showed a comparatively potent antibacterial activity. Additionally, Vl-AgNPs and *V. leucoxylon* extract showed considerable toxicity against HeLa cancer cells, with respective IC_{50} values of 36.40 ± 1.91 and 61.69 ± 1.31 µg/ml, respectively. This study emphasized the antibacterial, antioxidant, and anticancer characteristics of Vl-AgNPs and *V. leucoxylon* crude extract, indicating its potential for medicinal applications.

Key words: *Vitex leucoxylon,* silver nanoparticles, antibacterial, antioxidant, cytotoxicity

INTRODUCTION

Many disciplines, including material sciences, biotechnology, physics, applied microbiology, medicine and chemistry have shown a considerable deal of interest in nanotechnology in the past few decades (Dadhwal *et al*., 2023). In nanotechnology, which typically studies materials smaller than 100 nm, the concepts “creation,” “exploitation” and “synthesis” are associated. Green synthesis, also known as bionanotechnology, is an environmental and economically beneficial approach to create nanoparticles (NPs) utilizing microorganisms and plant extracts derived from fruits, sea algae and plant tissue (Sivagamasundari and Kathirvelu, 2025). When compared to bulk materials, NPs often have superior catalytic and biological capabilities due to their extremely small size (< 100 nm) and larger surface area to volume ratio (Khanal *et al*., 2022).

Because silver nanoparticles (AgNPs) are widely applied in microbiology, pharmacology, chemistry and food technology, they have been thoroughly examined in comparison to other metal NPs (Bhateria *et al*., 2019). AgNPs can generally be fabricated using several techniques, including hydrothermal microwave-assisted combustion, chemical vapor deposition, thermal decomposition, sol-gel, etc (Zhang *et al*., 2019). It was known since early 1900s that AgNPs could be produced sustainably using biomaterials like plant extract as reducing agents. Plant extracts, in various quantities and combinations, affect the properties of nanoparticles. Using various types of biomolecules including alkaloids, proteins, phenolics and terpenoids found in extracts of various plant parts like peels, roots,

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fruits and leaves, AgNPs can in fact be produced by oxidizing Ag+ to Ag^0 (Remya *et al*., 2022). Among the many biomedical uses for these AgNPs are antibacterial, biomolecular detection, antiangiogenetic drugs, antifungal and biosensors (Almashhadani and Qassim, 2025). According to reports, silver is an antibacterial metal that may destroy over 650 harmful bacteria by lysing and damaging their cell membrane (Suresh *et al*., 2021). Furthermore, it has been demonstrated that AgNPs are effective at killing cancer cells by disrupting the mitochondrial respiration chain and breaking down DNA (Barabadi *et al*., 2020). *Vitex leucoxylon* is a common medicinal plant in India, where its active components have demonstrated numerous biological uses (Chinnasamy *et al*., 2024). Several pharmacological actions, including as antibacterial, antioxidant, antihyperlipidemic, antipsychotic, antiparkinsonian, anti-inflammatory and antidepressant properties, have been identified for *Vitex leucoxylon* leaf extract. Particularly in folk medicine, natural plant resources have historically been utilized to treat a diverse array of ailments.

Research on biologically synthesized nanoparticles (Bio-NPs), such as AgNPs, utilizing several plant extracts and their improved biological potential is intriguing. Since different plants have different phytochemical compositions, more medicinally significant plants could be used to enhance or add to the biological potential of these materials (Iftikhar *et al*., 2020). Using this concept, prepared the water-based leaf extract of *V. leucoxylon* for biological fabrication of AgNPs because of its intriguing bioactive chemicals and excellent therapeutic qualities, which may enhance its value for AgNP synthesis (Nahari *et al*., 2022). Additionally, this work sought to evaluate and compare the cytotoxicity, antioxidant and antibacterial properties of Vl-AgNPs and the aqueous leaf extract of *V. leucoxylon*, to inform their biomedical applications.

MATERIALS AND METHODS

Healthy *vitex leucoxylon* leaves were gathered from the Vanjangi Hills Trek forest in Gangaraju Madugula village, Andhra Pradesh, India (18°00’07.63"N 82°29’11.58"E). The leaves were recognized and verified by the Department of Botany, College of Science and Technology, Andhra University; by referring the voucher specimen archived in the Department of Botany at Andhra University. The voucher specimen number 25438 AUV was submitted to the herbarium, Department of Botany, Andhra University.

After cleaning with running tap water, the collected *V. leucoxylon* leaves were kept in the shade for 21 days to dry. Leaf powder was obtained by grinding the dried plant materials in a mechanical grinder and then passing it through a 60-ìm sifter. To prepare 100 ml of the solution, 10 g of the powdered leaves was mixed with demineralized water. After 20 min of boiling at 80°C, the mixture was filtered through a Whatman No. 1 filter to yield an aqueous extract (Fig. 1A). The filtered liquid was stored in a 250 ml conical flask at 4°C for future research. The phytochemical analysis was conducted in accordance with the established methods described.

With minor modifications, the biosynthesis of AgNPs using extracts from *V. leucoxylon* was employed. 10 ml of the extract was mixed with 90 ml of a 1 mM solution of silver nitrate. The mixture was heated at 70°C for 2 h, while continuously stirred at 450 rpm, on a hot plate magnetic stirrer. This was followed by a 24-h incubation period (Shanwaz and Shyam, 2023). The mixture turned greenish-brown, indicating the formation of AgNPs (Fig. 1). The mixture was then centrifuged for 20 min at 18,000 rpm and rinsed three times to separate the Vl-AgNPs. Pellets were air-dried before being used for characterizations, antibacterial, cytotoxicity, and antioxidant properties.

UV-Visible spectroscopy (Shimadzu’s UV-1900i), Fourier-transform infrared spectroscopy (FTIR, Brukers, ALPHA-II). Computer-controlled XRD apparatus (Bruker, D8 Advance), Zeta potential and DLS analyzer (VASCO, Cordouan Technology, France), SEM-EDX (SEM, ZEISS, Germany) and TEM (Hillsboro model, Philips GM-30) analysis were performed for Vl-AgNPs characterization.

The microdilution test was used to determine the antibacterial properties of Vl-AgNPs and *V. leucoxylon* leaf extract against *Bacillus coagulans* (MTCC-13124), *Staphylococcus aureus* (MTCC-3160), *Pseudomonas aeruginosa* (MTCC-6363) and *Escherichia coli* (MTCC-443) bacteria. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

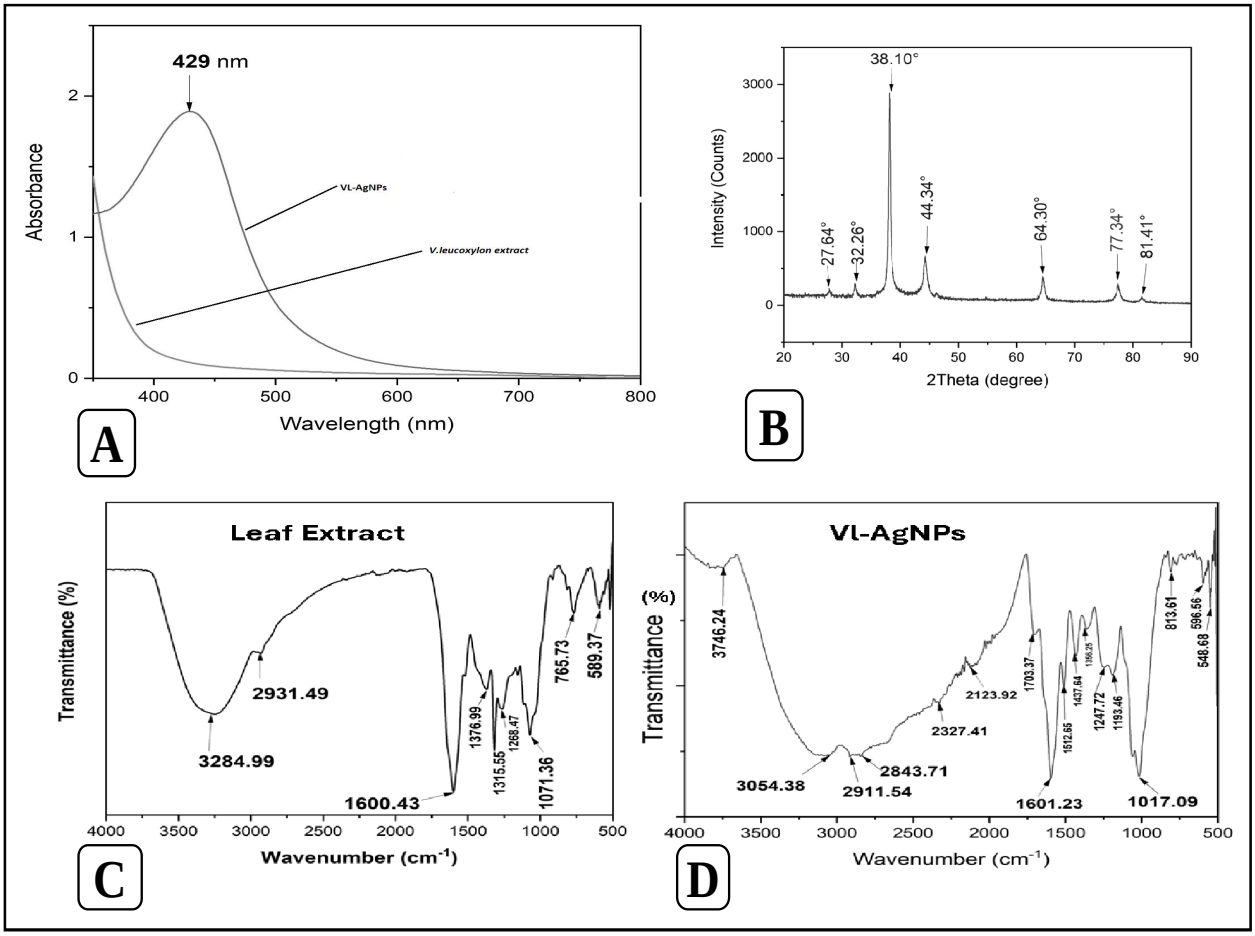

Fig. 1. (A) UV–visible absorption spectra of *V. leucoxylon* leaf extract and Vl-AgNPs; (B) X-ray diffraction (XRD) pattern of Vl-AgNPs; (C) FTIR spectrum of *V. leucoxylon* aqueous leaf extract and (D) FTIR spectrum of the synthesized Vl-AgNPs.

were assessed using 96-well microtiter plates and a double-fold serial microdilution test. Mueller Hinton Broth (MHB, Hi-Media) was used to cultivate the tested bacteria for 18 to 24 h. Subsequently, normal saline was added to the bacterial suspension to achieve a turbidity of 0.5 McFarland (0.5-1 × 10 ^5 CFU/ml). The microplates were incubated at 37°C for 24 hours. The standard antibiotic used was ciprofloxacin (0.01-4 µg/ml). The negative control, which contained only 300 µL of Mueller-Hinton Broth, was placed in the first row of tubes. The test dilution used to estimate MIC on Mueller–Hinton broth plates and MBC was obtained by subculturing with a loop. Following an overnight incubation period at 37°C, the lowest concentration that showed no discernible growth was represented as MBC, signifying 99.5% of the primary inoculum was killed.

To examine the antioxidant activity of Vl-AgNPs and *V. leucoxylon* leaf extract, DPPH radical-scavenging assay was conducted according to the established technique and FRAP assay was examined using the technique explained by Manimaran *et al*. (2021). Ascorbic acid was employed as the standard for both the assays.

The conventional MTT assay was employed to evaluate the cytotoxicity effect *V. leucoxylon* aqueous leaf extract and Vl-AgNPs against HeLa cell lines of cancer. The HeLa cancer cell lines were collected from the National Center for Cell Science, India. The experiments were performed in triplicate, and the results were presented as mean values with standard deviation.

RESULTS AND DISCUSSION

The terpenoids, tannins, phenols, flavonoids and saponins were detected by phytochemical screening of water-based leaf extracts of *V.*

Table 1. Phytochemical screening

S. No.	Tests	Aqueous extract
1.	Tannins	+ve
2.	Steroids	–ve
3.	Phenols	+ve
4.	Alkaloids	–ve
5.	Flavonoids	+ve
6.	Phytosterols	+ve
7.	Saponins	+ve
8.	Glycosides	+ve
9.	Terpanoids	+ve
10.	Anthocyanins	+ve

Where, (+ve): present and (–ve): absent.

leucoxylon after biochemical testing (Table 1). The identified phytochemicals acted as reducing and capping agents in the fabrication of Vl-AgNPs and significantly contributed to their therapeutic potential.

The phytochemicals of *V. leucoxylon* leaf extract were used to reduce $AgNO_3$. The colour changed from brown to dark brown over the first 2 h and greenish brown over a 24-h incubation period at room temperature, showing the fabrication of Vl-AgNPs (Dua *et al*., 2023).

The UV-visible spectrum suggested the fabrication of Vl-AgNPs by the noticeable peak seen at 429 nm (Fig. 1A). This was indicative of the absorption of AgNPs' surface plasmonic resonance (SPR) and the colour change of the mixture brought on by the conversion of Ag^+ to Ag^0. In FTIR, the number of functional groups in the Vl-AgNPs and the extract can be estimated from spectral peak intensities. The absence of a peak at 3746.24/cm in the *V. leucoxylon* extract within the 4000-3000/cm region, in contrast to Vl-AgNPs, along with alterations in other peaks, suggested the involvement of functional groups during the reduction process. The presence of alcohol and phenolic groups was confirmed by the band at 3284.99/cm in the FTIR spectrum, corresponding to O–H stretching vibrations (Melkamu and Bitew 2021). Other peaks and their alterations corresponded to functional groups that function as stabilizing and capping agents (Fig. 1C and D). For XRD analysis (Fig. 1B), the prominent peaks at 2è = 27.64°, 32.26°, 38.10°, 44.34°, 64.30°, 77.34° and 81.41° were equivalent to the Bragg's reflections of the face-centered cubic structure of silver at (111), (200), (220), (311), (222), (400), and (331), respectively. The crystalline structure of Vl-AgNPs was confirmed as the peak with the highest intensity, located in the (111) plane, indicating the favoured orientation of the AgNPs' fabrication.

Clearly, the sharp, strong peaks indicated that the silver nanoparticles were highly crystalline as manufactured. The size of particles of Vl-AgNPs ranged from 1 to 10000 nm, in accordance with the size of the particles and zeta potential graphs (Fig. 2). 95% of the particle size diameters were less than 100 nm. The low calculated PDI (0.090), strongly suggested the uniform dispersion of the Vl-AgNPs (Fig. 2a). Some DLS analyzed particle sizes were big because of the condition of the analysis, which led to a tendency to measure the hydration shell of water molecules surrounding the Vl-AgNPs as well as the outer phytochemicals plating the nanoparticles' surface (Zhao *et al*., 2019). Vl-AgNPs' zeta potential was determined to be -79.8 mV (Fig. 2b). These results indicated the high stability of Vl-AgNPs. According to SEM, the surface morphology of most of the Vl-AgNPs was spherical and exhibited irregular agglomerations (Fig. 3a). TEM analysis (Fig. 3b) showed that Vl-AgNPs were uniformly dispersed and sphere-shaped with an average size of 53 nm. The EDX spectra (Fig. 3c) showed a significant Ag signal at about 3 keV, which amply demonstrated that the nanoparticles' metal state was pure silver (Gowda *et al*., 2024).

The results showed that Vl-AgNPs exhibited much higher antibacterial activity against the bacterial strains than the *V. leucoxylon* leaf extract. The values obtained for MIC of Vl-AgNPs against *B. coagulans, E. coli, S. aureus* and *P. aeruginosa* strains were 0.5, 0.25, 0.25 and 1 mg/ml, respectively, whereas the values obtained for MIC of *V. leucoxylon* aqueous leaf extract against *B. coagulans, E. coli, S. aureus* and *P. aeruginosa* strains were 2, 1, 0.5 and 4 mg/ml, respectively (Table 2). Additionally, Vl-AgNPs demonstrated greater bactericidal activity against *S. aureus, B. coagulans* and *E. coli* (MBC of 1 mg/ml) than against *P. aeruginosa* (MBC of 2 mg/ml).

Antioxidant activities were investigated by employing the DPPH and FRAP assays. DPPH free radical scavenging activity was investigated at different concentrations (10-50 µg/ml) of Vl-AgNPs, *V. leucoxylon* aqueous leaf extract and ascorbic acid (reference). The assessed DPPH scavenging activity was dose-

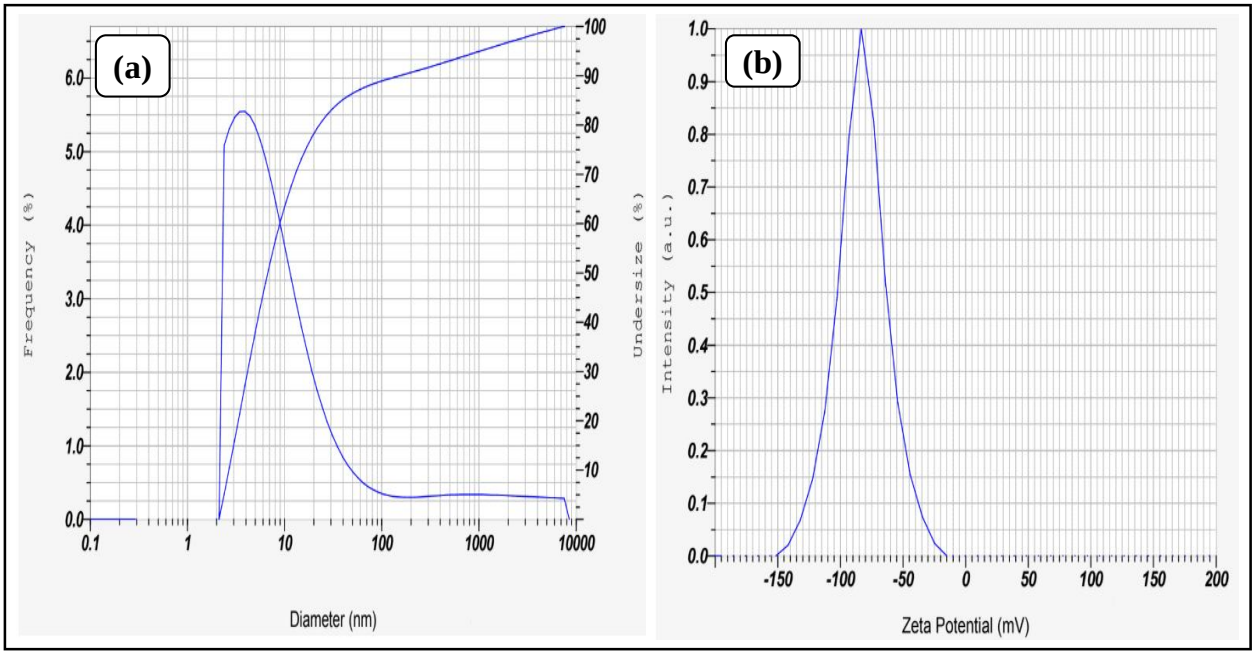

Fig. 2. (a) DLS analysis showing particle size distribution by intensity of synthesized Vl-AgNP and (b) zetapotential of Vl-AgNPs.

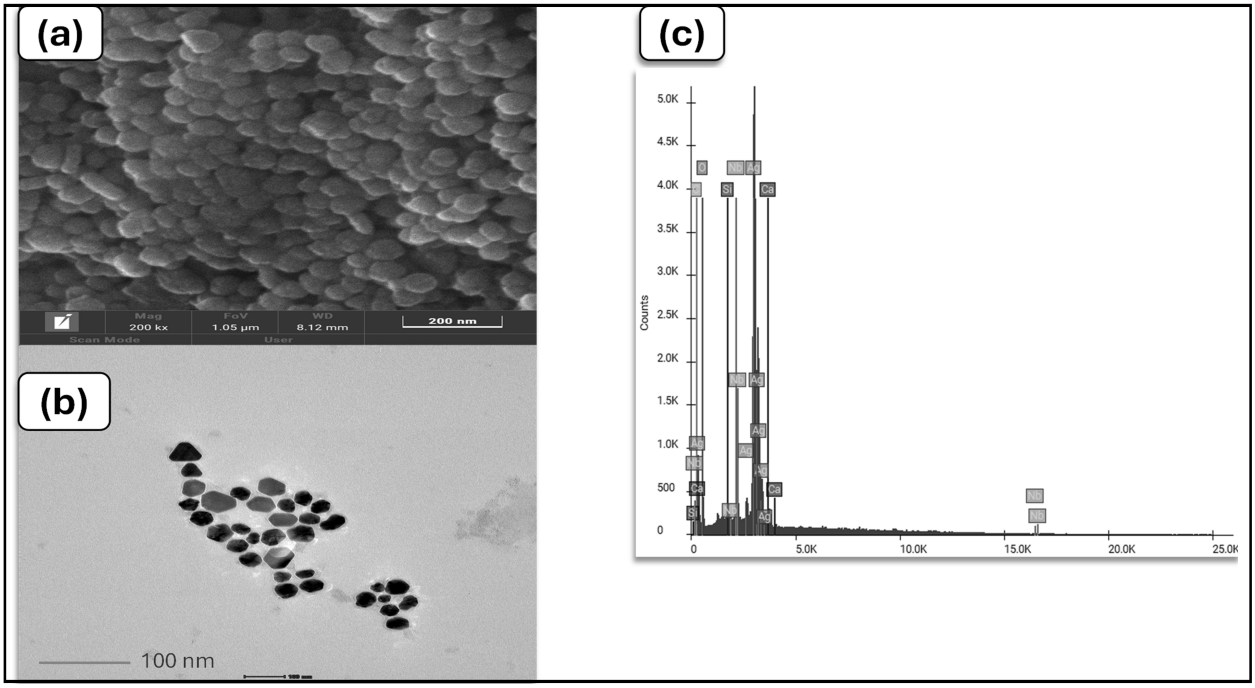

Fig. 3. Characterization of synthesized AgNPs (a) SEM analysis of synthesized AgNPs, (b) TEM analysis of synthesized AgNPs and (c) energy dispersive X-ray analysis (EDX).

dependent. At maximum concentration, the ability to scavenge DPPH shown by Vl-AgNPs, *V. leucoxylon* aqueous leaf extract and ascorbic acid (reference) was 60.89±2.75%, 53.01±2.63% and 66.14±2.38%, respectively. While the Vl-AgNPs demonstrated better inhibition compared to that of aqueous leaf extracts of *V. leucoxylon.* Both demonstrated the antioxidant activity lower than that of the reference ascorbic acid (Fig. 4A). Additionally, the FRAP assay was utilized to investigate the antioxidant capacity of aqueous leaf extracts of *V. leucoxylon* and Vl-AgNPs, with ascorbic acid employed as the standard. The antioxidant

Table 2. Antimicrobial activity of *Vitex leucoxylon* extract and Vl-AgNPs (mg/ml)

Samples	*E. coli*		*S. aureus*		*P. aeruginosa*		*B. coagulans*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
V. leucoxylon extract	1	4	0.5	2	2	4	0.5	4
Vl-AgNPs	0.25	1	0.25	1	0.5	2	0.5	1
Ciprofloxacin	0.02	0.08	0.16	0.64	0.32	1.28	0.16	0.64

Where, MIC and MBC of antibiotics were presented as ìg/ml.

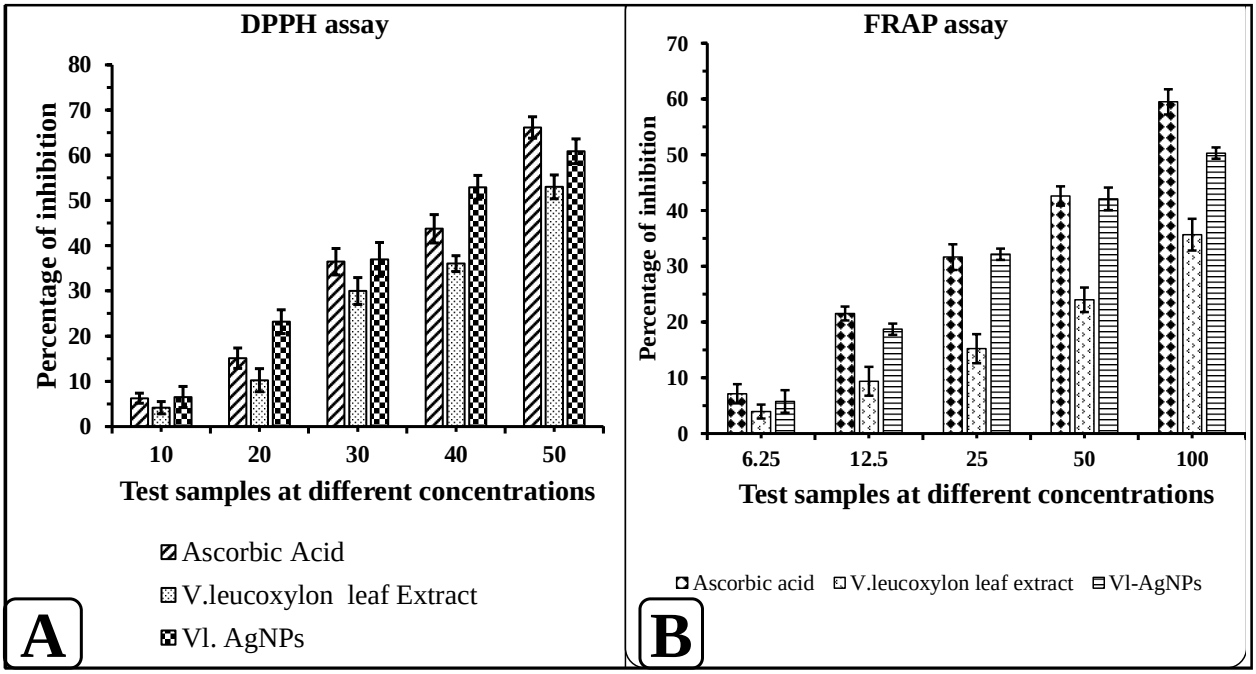

Fig. 4. (A) DPPH assay showing antioxidant activity of Vl-AgNPs and *Vitex leucoxylon* aqueous leaf extract and (B) FRAP assay showing antioxidant activity of Vl-AgNPs and *Vitex leucoxylon* leaf extract.

activity was dose-dependent. Although the standard showed the highest overall antioxidant activity, Vl-AgNPs exhibited higher activity than *V. leucoxylon* leaf extracts (Fig. 4B).

The study evaluated the cytotoxicity of *V. leucoxylon* aqueous leaf extract and Vl-AgNP utilizing a colorimetric MTT assay against the cell line of cervical cancer (HeLa). A dose-dependent decrease in cell viability was noted with increasing concentrations of *V. leucoxylon* extract and Vl-AgNPs (Fig. 5E). The IC_{50} values for the *V. leucoxylon* aqueous leaf extract and Vl-AgNPs against HeLa cell lines were 61.69±1.31 and 36.40±1.91 µg/ml, respectively. These findings suggest that Vl-AgNPs strongly cytotoxically affected the HeLa cancer cell line compared to *V. leucoxylon* leaf extract (Fig. 5). Vl-AgNPs' reduced size and allowed them to pass through the cell wall by phagocytosis, pinocytosis, diffusion, or endocytosis. In cells, AgNPs lower glutathione (GSH) levels and generated reactive oxygen species (ROS), damaged DNA and, ultimately, caused cell death via altered mitochondrial function and the activation of apoptotic genes (Barabadi *et al*., 2020).

CONCLUSION

The use of medicinal plants may enhance the therapeutic capacity of nanoparticles. In the current investigation, capping and green-reducing biomolecules from *V. leucoxylon* leaf extract were used to efficiently fabricate AgNPs. Physiochemical properties of Vl-AgNPs were determined by UV-Vis spectroscopy, FTIR, XRD, SEM-EDX, Zeta potential and DLS. *V. leucoxylon* leaf extracts and Vl-AgNPs demonstrated exceptional antibacterial, antioxidant and cytotoxic activities. These effects were largely attributed to the

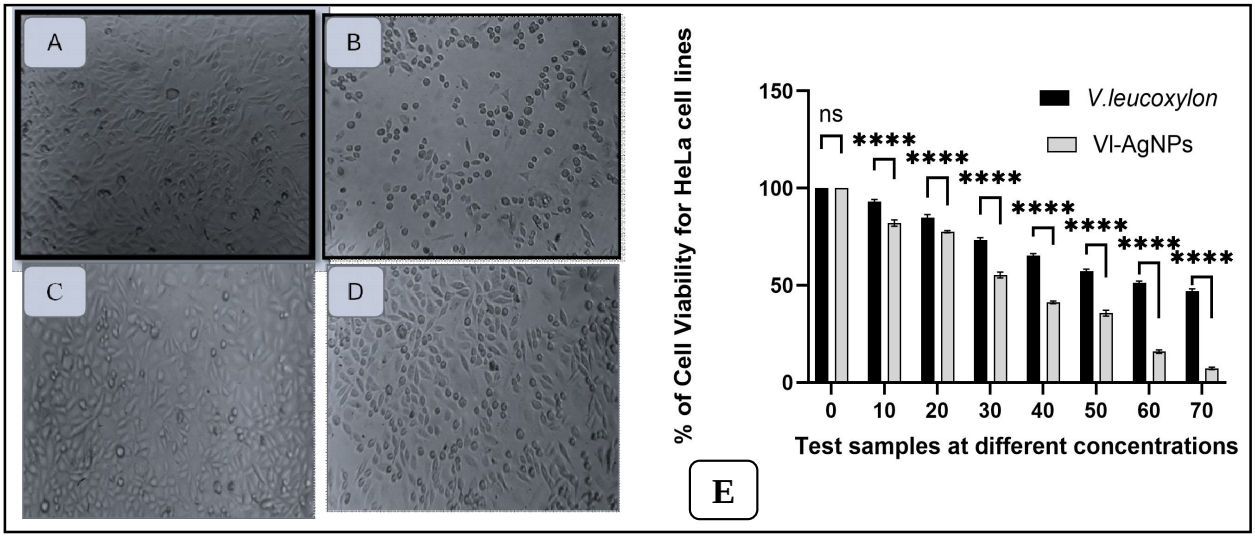

Fig. 5. In vitro cytotoxicity of Vl-AgNPs on HeLa cell lines (A) Control (Without treatment), (B) Treatment with Vl-AgNPs (50 µg/ml). In vitro cytotoxicity of *V. leucoxylon* aqueous leaf extract on HeLa cell lines (C) Control (Without treatment), (D) Treatment with *Vitex leucoxylon* aqueous leaf extract (50 µg/ml) and (E) Graphs showing percentage of cell viability of *Vitex leucoxylon* water-based leaf extract and its phyto-fabricated silver nanoparticles against HeLa cell line.

phytochemicals in the *V. leucoxylon* extract, which were also effective at stabilizing and reducing Vl-AgNPs. Future biological and pharmaceutical applications of phyto-fabricated AgNPs may, therefore, be feasible, with negligible or no adverse effects and without endangering the environment.

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