

INTRODUCTION

Green synthesis of nanoparticles (NPs) has been a recent development in the field of nanotechnology. This kind of synthesis is a more eco-friendly approach compared to the chemical and physical methods. It involves the use of natural ingredients in the bio-reduction of metallic ions to nanoparticles [1,2]. This simple green eco-friendly method has been utilized to obtain metal nanoparticles using the leaf extract as a precursor [2,3]. Safer and easily metabolized drugs are natural compounds retrieved from plant materials as compared to other synthetic medicinal compounds [1-4]. Metallic nanoparticles are mostly synthesized from precious metals viz., silver (Ag), gold (Au), platinum (Pt), and lead (Pb). From these metals, silver is the most preferred in the area of the biological system due to its wide range of activities over other metals [5-6].

The development of these green nanoparticles has been intensified in recent research not only for their fundamental scientific relevance but also for diverse technological applications [1-6]. Nanoparticles possess catalytic, optical, electronic, antibacterial and magnetic properties and have been found useful in various industries, medical and environmental remediation (Wu et al., 2008; Ejidike et al., 2020).

Carrot (*Daucus carota* L.) are rich in dietary fibre, antioxidants, anthocyanins, carotenoids, vitamins A, B, and C, and minerals. Ethnomedically, the roots of *D. carota* L. have been reported to be useful in the treatment of digestive problems, tonsillitis or constipation, and intestinal parasites. Aqueous hot leaf extract is taken orally as a uterine stimulant during parturition and has abortifacient potentials. The four most commonly kinds of phytochemicals dominant in carrots are polyacetylenes, carotenoids, phenolic, and ascorbic acid [7].

The current study describes the assessment of silver nanoparticles (AgNP05 and AgNP01) content using aqueous solution of *Daucus carota* L. leaf extract (DCLE). Green synthesis was method utilized for the preparation of silver nanoparticles (AgNP05 and AgNP01), and were characterized viz: UV-visible spectroscopy, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), and Transmission Electron Microscope (TEM) analysis. Then *in vitro* cytotoxicity against brain cells and antibacterial properties were investigated.

MATERIALS AND METHODS

Leaves of *Daucus carota* L. was collected during the harvest season from the University of Fort Hare farm, Alice, South Africa with location coordinates of 32°47'09"S 26°60'45"E. About 50 g of the crude powdered sample was weighed and macerated with 500 mL of double-distilled water. The mixture was heated at 90°C for 1 h. The cooled mixture was sieved through Whatman No. 1 filter paper. The extract was then refrigerated at 4°C for further experimental works [1-4]. Silver nitrate (99.9%) as used as source of metal.

Synthesis of Metal Nanoparticles

In a typical synthetic procedure for the preparation of AgNP05, 80 mL of aqueous *D. carota* L. leaf extract (DCLE) was added slowly to 400 mL of 1 mM silver nitrate (AgNO₃) solution. The resultant solution was refluxed for 90 min with continuous stirring at 80 °C. The initial colour when the extract was added to the solution of AgNO₃ was pale cream. The formation of AgNPs was followed by colour change from pale cream to dark brown within 90 min. The appearance of darkish brown precipitation was a clear indication of AgNPs formation a suggestive of surface plasmon resonance. The nanoparticles synthesis success was monitored via UV-vis spectroscopy. The AgNPs precipitates obtained were separated through repeated washing and centrifugation at 5000 rpm for 1 h, followed by oven-drying at 60 °C (4, 8, 10).

Characterization Methods: UV/Vis, FT-IR, XRD, TEM

Antimicrobial activity

The silver nanoparticles (AgNP05 and AgNP01) were tested against the following selected pathogenic microorganisms: *Escherichia coli*, *Staphylococcus aureus*, and α -Hemolytic *streptococcus*. The antimicrobial effects of nanoparticles and DCLE extracts were also screened against the following fungi species *Aspergillus candidus*, *Aspergillus niger*, and *Penicillium cephalosporin* using the disk-diffusion agar method.

MTS end-point toxicity assay

In vitro cytotoxicity assay known as MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium) was utilized to evaluate changes in brain glioblastoma cells (U87MG) viability

xCELLigence real-time toxicity assay

The xCELLigence assay is a real-time, based on cell analysis system that employs impedence to measure cell viability.

RESULTS AND DISCUSSION

UV-Vis analysis

UV-visible absorption spectra have been used to prove its significance and sensitivity towards silver colloids formation since silver nanoparticles display an intense absorption peak as a result of the surface plasmon. (Fig. 1). The appearance of darkish-brown colouration evident the formation of silver nanoparticles (AgNP) in the mixture, and the effective bio-reduction of the Ag^+ to Ag^0 , the formed colour solution allowed the measurement of the absorbance against typical wavelength to affirm the preparation of silver nanoparticles. The distinct peak observed at 437 nm for AgNP05 and 429 nm for AgNP01, indicate the surface plasmon resonance (SPR) of the AgNPs [8]. The surface plasmon resonance (SPR) frequency is produced by an electric field of the incident electromagnetic (EM) radiation that displaces the electron of the particles from equilibrium, therefore, a restoring force is produced that results in the electrons oscillatory motion with a distinct frequency.

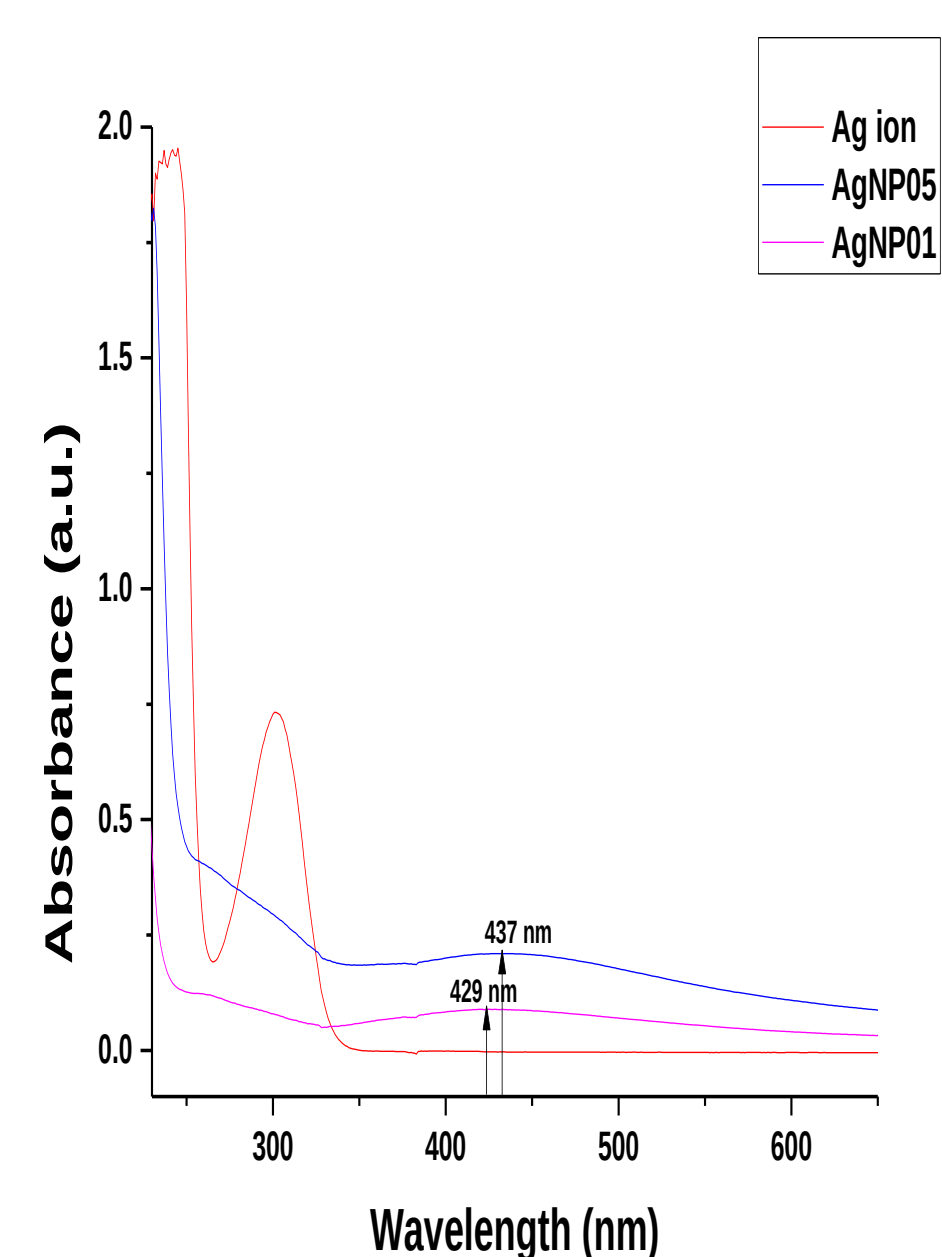


Fig. 1: UV-Vis spectra for the AgNPs

Fourier-transform infrared spectroscopy (FTIR)

The FTIR spectra of AgNP05 and AgNP01 (Figure 2) were recorded along the spectrum of the *D. carota* L. leaf extract (DCLE). An important feature in the spectra of AgNP05 and AgNP01 was the absence of a broad peak at 3291 cm⁻¹ in the spectra of DCLE attributable to ν(O-H) vibrations from water molecule, phenolic and alcoholic compound. The FTIR spectra of DCLE and AgNP05 are displayed in Figure 2. A slight shift was observed in the AgNP05 spectrum and the peaks intensity in the extract at 3291 cm⁻¹, 2920 and 2843 cm⁻¹, 1729 cm⁻¹, 1597 cm⁻¹, 1397 cm⁻¹, and 1015 cm⁻¹ assignable to ν(OH), ν(CH₃/CH₂), ν(C=O), ν(C=C), ν(C-N) and ν(C-O) vibrations, respectively, as this peaks became significantly reduced within the AgNP05 spectrum [4-7]. This is a suggestion that these functional groups are probably participated in the reduction, stabilization, and capping of the AgNP03 leading to the formation of AgNP05. The FT-IR further confirms that some distinctive phytochemicals such as polyphenols were answerable for the reduction and capping of AgNPs as shown from the assorted peaks described.

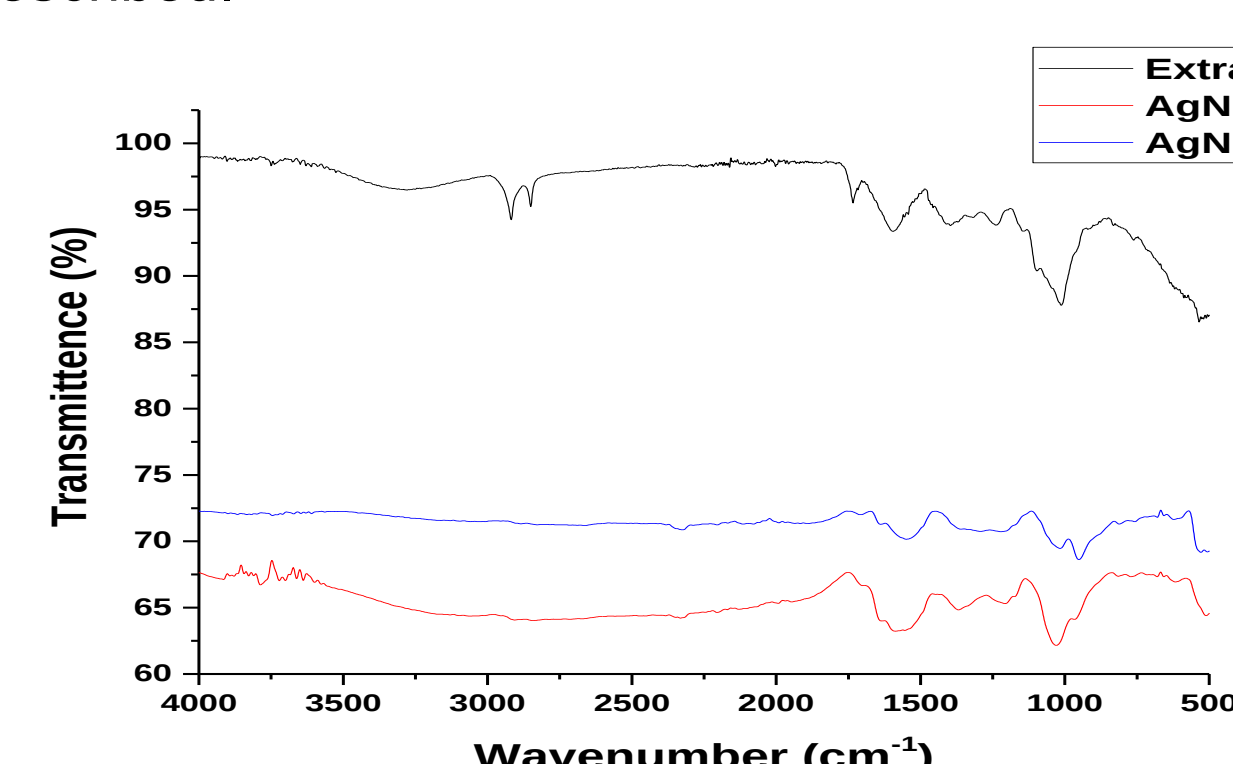


Fig. 2. Comparative FTIR spectra of leaf extract, AgNP05 and AgNP01).

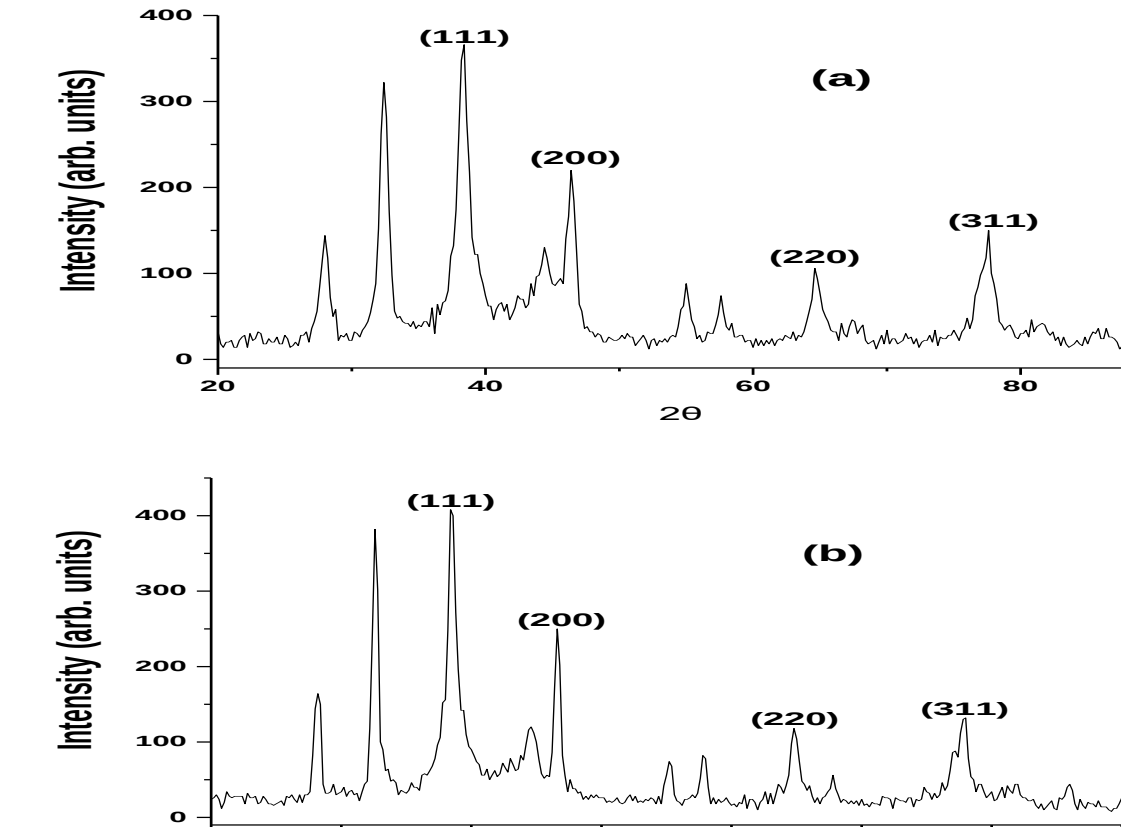


Fig. 3. X-ray diffraction patterns of synthesized (a) AgNP05 and (b) AgNP01 from *D. carota* leaf.

X-ray diffraction analysis

The crystalline nature of the AgNPs formed was confirmed. XRD spectra of silver nanoparticles mediated by aqueous extract of *D. carota* L. (DCLE) are displayed in Figure 3. It was observed that the materials (AgNP05 and AgNP01) are crystalline in nature upon the bio-reduction of AgNO₃. The AgNP05 synthesized from DCLE, (Figure 3a) showed peaks at $2\theta = 38.3, 46.4, 64.5$, and 77.6° which were ascribed to the (111), (200), (220), and (311) face-centered cubic (FCC) structure of the silver in the 2θ range $20\text{--}80^\circ$ [3,7,8]. The XRD peaks for AgNP01 synthesized from DCLE (Figure 3b) at $2\theta = 38.5, 46.6, 64.9$, and 77.8° was also indexed to the (111), (200), (220), and (311) face-centered cubic (FCC) crystallographic planes of metallic silver.

Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) was engaged for the characterization of the sizes, shapes, and morphologies of the designed AgNP05 and AgNP01 from *D. carota* L. leaf extract (DCLE). Figure 4 expresses the TEM micrograph of the silver AgNP05 (Figure a) and AgNP01 (Figure 4b). As evident in the micrographs, it can be detected that spherical-shaped silver NPs were formed with a mean size distribution of 18.26 (± 6.86) nm and 16.81 (± 7.72) nm for AgNP05 and AgNP01 respectively.

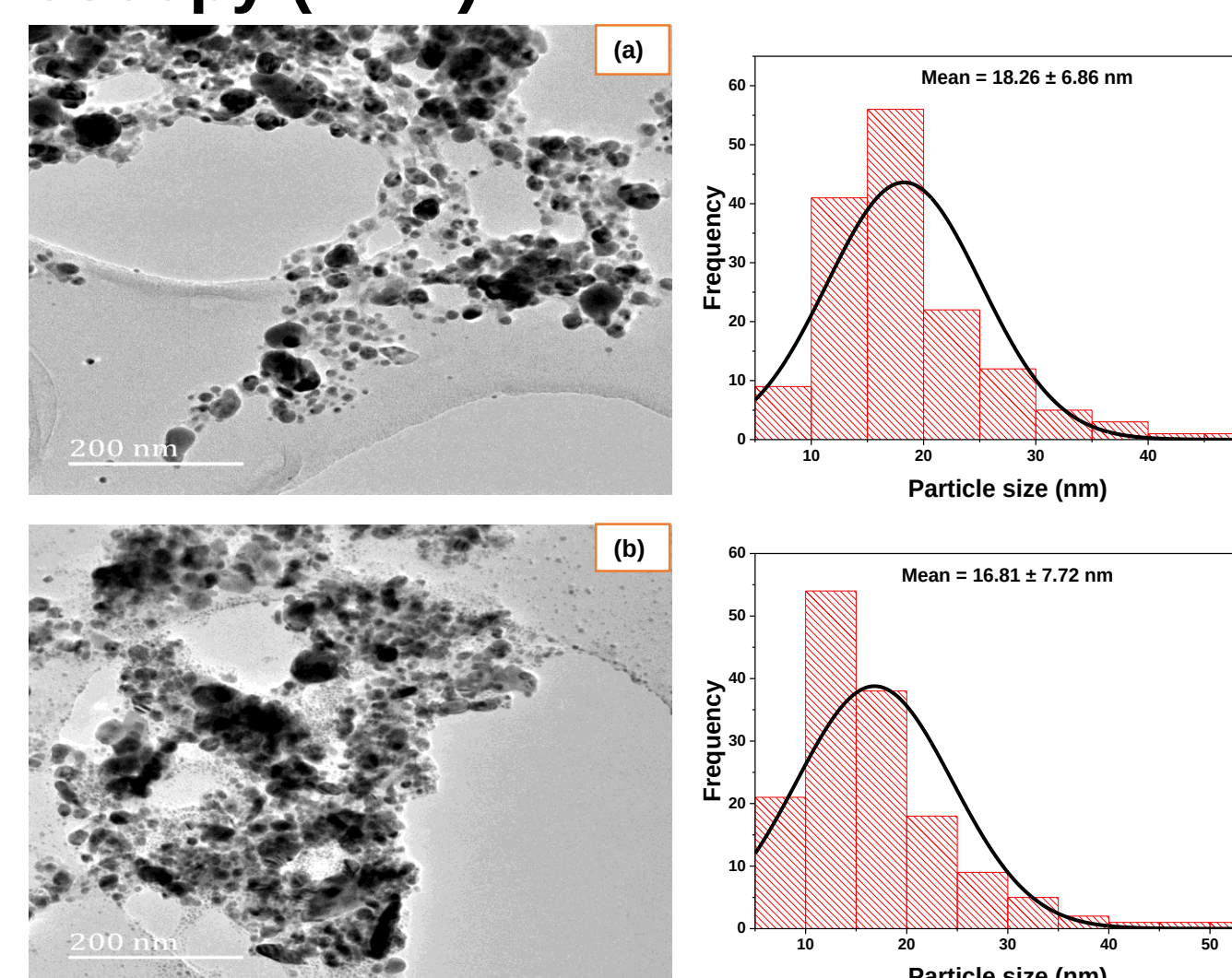


Fig. 4. TEM image of the synthesized (a) AgNP05 and (b) AgNP01

Antimicrobial activity

The nanoparticle synthesis by the green method was found to possess better activities against studied multidrug resistant pathogens than the leaf extracts. AgNP01 from the leaf extract of *D. carota* L. showed a zone of inhibitions: *S. aureus* (20 mm), *α-H. streptococcus* (21 mm), *E. coli* (14 mm), *A. niger* (15 mm), and *P. cephalosporin* (10 mm). Zone of inhibitions of AgNP05 were *S. aureus* (18 mm), *α-H. streptococcus* (17 mm), *E. coli* (14 mm), *A. niger* (15 mm), and *P. cephalosporin* (10 mm). The silver nanoparticles were active against the studies bacterial and fungi strains and but showed no detectable activity was observed on *A. candidus*. Although the standard agents showed activities better than the test agents, the nanoparticles displayed higher bactericidal activity compared to the *D. carota* L. leaf extract (DCLE), and showed favourably comparison with the anti-bacterial activity observed for ciprofloxacin. The synthesized AgNPs showed better antimicrobial properties (Figure 5) towards bacteria pathogens as compared to fungi pathogens under this investigation [3-8].

In vitro cytotoxicity evaluation

The in vitro toxicity of silver nanomaterials (AgNP05 and AgNP01) was investigated against brain glioblastoma cells, U87MG at various concentrations stretching from 0.00 µg/ml to 100 µg/ml by MTS evaluation. The nanomaterials showed a drastic increase in cell proliferation at concentrations 50.0 µg/ml, 25.0 µg/ml, and 12.5 µg/ml. This may be an indication of an attack on the cell nucleus and the cell attempt to rid of themselves of the toxin [6,8]. Also, the xCELLigence system is a kind of technology that supports accurate real-time monitoring of cell behaviour. In this study, xCELLigence real-time toxicity of AgNP05 and AgNP01 on U87MG cells showed growth inhibition and reduction of cell viability (Figure 6), at concentrations (stretching from 0.00 µg/ml to 100 µg/ml) [6,9].

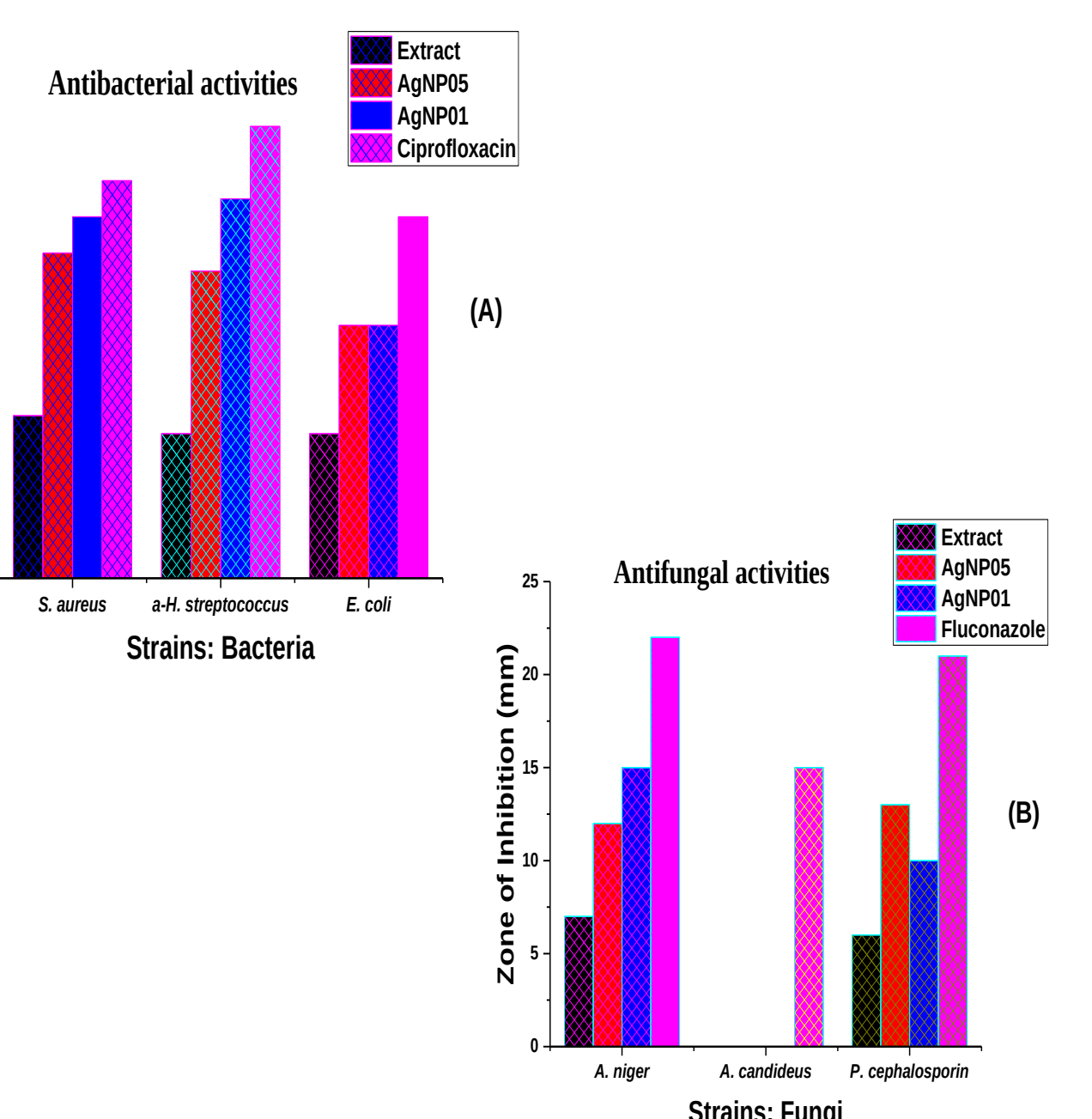


Fig. 5. Antibacterial activities of DCLE) and AgNPs (a) Antifungal activities of DCLE and AgNPs (b).

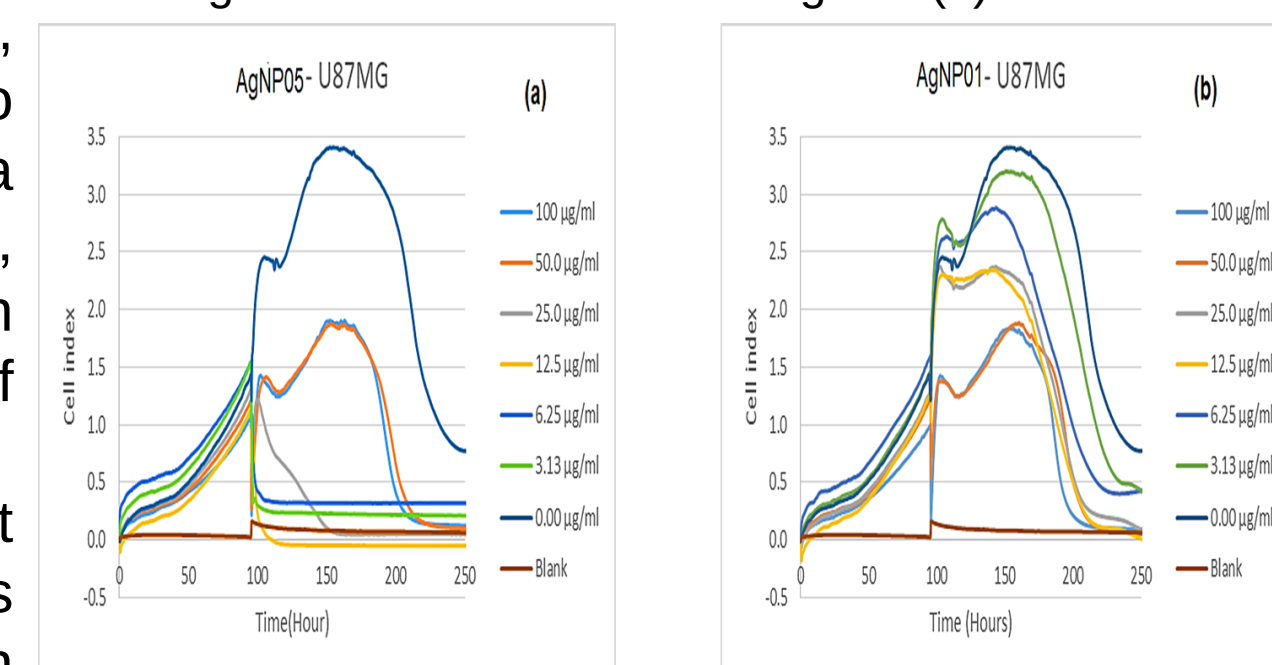


Fig. 6. xCELLigence real-time toxicity for silver nanomaterials AgNP05 (a) and AgNP01 (b) on the U87MG cell line, at concentrations (100, 50.0, 25.0, 12.5, 6.25, 3.125 and 0 $\mu\text{g/ml}$).

CONCLUSION

An economical, efficient and environmentally friendly method has been used for the green synthesis of silver nanoparticles by *D. carota* L. leaf extract (DCLLE). Analytical techniques like UV–Vis spectrophotometer, XRD, FT-IR, and TEM techniques have established the bio-reduction of silver nitrate solution to silver nanoparticles. Phenols and flavonoids were present in the leaves, and they serve as an effective reducing agent. The *in vitro* MTS end-point and xCELLigence real-time toxicity assays of silver nanomaterials showed fewer toxicity activities on brain glioblastoma cells, U87MG. This method for the green AgNPs synthesis may find use in nanomedicine, environmental, and industrial applications.

REFERENCES

- REFERENCES**
- [1] Salleh, A.; Naomi, R.; Utami, N.D.; Mohammad, A.W.; Mahmoudi, E.; Mustafa, N.; Fauzi, M.B. *Nanomaterials (Basel)* **2020**, *10*, 1566.
 - [2] Veijs, H.; Azizi, S.; Mohammadi, P. *J. Clean. Prod.* **2018**, *170*, 1536–1543.
 - [3] Nisar, P.; Ali, N.; Rahman, L.; Ali, M.; Shinwari, Z.K. *J. Clean. Prod.* **2019**, *24*, 929–941.
 - [4] Rotimi, L.; Ojemaye, M.O.; Okoh, O.O.; Okoh, A.I. *J. Mol. Liq.* **2018**, *273*, 615–625.
 - [5] Ejidike, I.P.; Bamigboye, M.O.; Iljimdiya, R.U.; Seyinde, D.O.; Ojo, O.O. *The Proceedings of the Nigerian Academy of Science (PNgAS)*. **2020**, *13*, 136–147.
 - [6] Stefanowicz-Hajduk, J.; Ochocka, J.R. *Toxicology Reports* **2020**, *7*, 335–344.
 - [7] Nouri, A.; Yarak, M.T.; Lajevardi A.; Rezaei, Z.; Ghorbanpour, M.; Tanfizi, F. *Colloids Interface Sci. Commun.* **2020**, *35* 100252.
 - [8] Ogunisile, B.O.; Labulo, A.H.; Fajemilehin, A.M. *Ife Journal of Science*. **2016**, *18*, 245–254.
 - [9] Kikowska, M.A.; Chmielewska, M.; Włodarczyk, A.; Studzińska-Sroka, E.; Zuchowski, J.; Stochmal, A.; Kotwicka, M.; Thiem, B. *Molecules* **2018**, *23*, 3009.