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RESEARCH ARTICLE

Free Radical Scavenging and Cytotoxicity of *Dimocarpus Longan* Leaves Mediated Silver Nanoflakes

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Abstract

Objectives: To study the antioxidant and cytotoxicity of biologically synthesised silver nanoflakes using *Dimocarpus longan (D. longan)* leaf extract. **Method:** 1mM Silver nitrate is treated with 20 ml of *D. longan* leaves extract for 1 hour. The phytochemicals like phenols, alkaloids and flavonoids present in the aqueous leaf extract act as a reducing and stabilizing agent which reduces the silver nitrate into silver nanoflakes. **Findings:** The silver nanoflakes show characteristics of surface plasmon resonance at 450 nm in UV-vis spectroscopy. The FT-IR spectrum confirmed the phytoconstituent present in the leaves extracts. The XRD analysis shows the nature of silver nanoflakes. The size and stability were determined using dynamic light scattering (DLS) the values are 87.17 nm and -23 mv. The scanning electron microscope shows a poly-dispersed flake-like structure. It exhibits comparable antioxidant activity with ascorbic acid using DPPH (1, 1-diphenyl-2-picryl hydrazyl) assay and the (IC_{50}) value was approximately 65.5 μgml^{-1}. The remarkable cytotoxicity effect of synthesized silver nanoflakes against MDA-MB-231 and MCF-7 breast cancer cell lines is evidenced by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The Inhibitory concentration (IC-50) values examined were 138.6±2.18 μgml^{-1} and 171.5±2.2 μgml-1. **Novelty:** For the first time, the present study revealed the synthesis of silver nanoflakes of lower size using leaves extract of *D. longan* which was employed for its antioxidant activity and also for *in vitro* cytotoxic effects against breast cancer cells MDA-MB-231 and MCF-7.

Keywords: Silver nanoflakes; D longan leaf; antioxidants; MTT assay and Cytotoxicity

1 Introduction

Among the various noble metal nanoparticles, stabilized silver nanoparticles (AgNPs) have been widely used in pharmaceutical and biomedical potentials such as antimicrobial [1–4], antioxidant, [5], etc. AgNPs are now being utilised to cure cancer as an antitumor molecule, and many attempts have yielded meaningful and positive results [6]. AgNPs were found to have antitumor properties in embryo fibroblast 3T3 cells [7], lung

cancer H1299 cells[8], and breast cancer MCF-7 cells[9]. Biological methods of synthesis of silver nanoparticles utilising various plant extracts give enormous benefits. Plant extracts contain phytochemicals such as terpenoids, flavonoids, phenolic and carboxylic acid which act as reducing and capping agents[10]. Recently various types of plant extracts have been used for the synthesis of silver nanoparticles like *Tamarix articulata* leaves extract[11], lemongrass,[12] *Moringa oleifera* leaves extracts[13], etc.

D. longan Lour. (syn.., Sapindaceae) is a tropical evergreen tree widely distributed in Southeast Asia, which produces edible fruit of high nutritive and economic value. These leaves extracts contain phytochemicals like flavonoids, terpenoids, and phenolic acids[14] which act as reducing and stabilising agents (Figure 1). Different phytochemicals such as flavonoids, tannins, and polyphenols were extracted from the plant's various portions, as well as other substances like vitamins and minerals. This plant's biological activities as a treatment agent included anti-proliferative, antioxidant, anti-cancer, anti-tyrosinase, radical scavenging activity, anti-inflammatory activity, anti-microbial, osteoblast differentiation activation, anti-fungal, immunomodulatory, probiotic, anti-aging, anti-diabetic, obesity, neurological issues, and a suppressive effect on macrophage cells. Different plant sections have shown greater activity in certain disease scenarios. Nonetheless, substances such as gallic acid, ellagic acid, corilagin acid, quercetin, 4-O-methyl gallic acid, and (-)-epicatechin showed more biological activity. Gallic acid, corilagin, and ellagic acid all demonstrated potent anti-cancer action in the cancer cell lines HepG2, A549, and SGC 7901. Additionally, Furthermore, 4-O-methyl gallic acid and (-)-epicatechin have demonstrated exceptional antioxidant and anti-cancer activity[15]. Our interest was piqued by eco-friendly green synthesis using leaves extract to produce excellent AgNPs for antioxidant and anticancer applications.

2 Methodology

2.1 Plant material and Chemicals

D. longan leaves were collected from a local garden. Silver nitrate ($AgNO_3$), Sodium borohydride ($NaBH_4$) were purchased from S.D. Fine chemicals Limited Mumbai. Deionized water was used for all aqueous solutions.

Fig 1. *Dimocarpus longan* leaves

2.2 Preparation of *D. longan* leaves extract

25 g of freshly collected *D. longan* leaves were cleaned completely with running water followed by deionized water. The leaves were kept under shade and dried out for 10 days. Then the leaves were crushed and ground into a fine powder using a mortar and pestle. 10 grams of leaves powder was weighed and boiled in 100 ml double-distilled water in a glass beaker for 30 minutes at 60^{o}C and then filtered using Whatman filter paper and the filtrate was centrifuged to remove any impurities.

2.3 Synthesis of Silver nanoflakes using *D. longan* leaves extract

Biosynthesis of Silver nanoflakes (AgNFs)was carried out by the simple reduction of silver nitrate using aqueous leaves extract of *D. longan* using the standard published literature with slight variations. 20 ml of the prepared leaves extract was added to 80 ml of 1 mM silver nitrate solution and stirred for 1 hour. The colour changed to a brown colour showing the formation of silver nanoparticles. Then confirmed by UV-visible spectroscopy[16].

2.4 Characterisation techniques

The silver nanoflakes were further characterized by Double Beam UV spectrophotometer-2202 in the wavelength range between 200-800 nm. FT-IR spectrum was recorded using a Shimadzu spectrometer to identify the biomolecules responsible for the formation of silver.

X-ray diffraction analysis (XRD) was done by using Bruker, Germany Model: D8 advance Scintillation Detector to identify the proper phase of the silver nanoflakes. The nature of the silver nanoflakes was examined by SEM analysis. The presence of elemental silver nanoflakes was analyzed by EDAX attached with SEM. Malvern and Zeta potential Analyser were used to determine particle size and stability.

2.5 Determination of DPPH scavenging assay

DPPH- radical scavenging activity of the AgNFs was determined according to the technique described by Blois (1958). Different concentrations of silver nanoflakes (20 to 100 μg mL^{-1}) of the sample solution in methanol were blended with 2.5 ml of 0.5 mM methanolic solution of DPPH. The combination was shaken dynamically and incubated for 30 min. The absorbance was measured at 517 nm using a UV spectrophotometer. Ascorbic acid was used as a positive control. DPPH free radical scavenging capacity percentage (%) was determined by using the equation.

% of inhibition = absorbance of control – absorbance of sample/absorbance of control $\times$100.

2.6 Cell Culture and MTT Assay

The Human Breast Cancer cell line and (MDA-MB231) and (MCF-7) were plated distinctly using 96 well plates with the concentration of 1×10^4cells/well in DMEM media with 1X Antibiotic Antimycotic Solution and 10% fetal bovine serum (Himedia, India) in CO2 incubator at 37°C with 5% CO2. The cells were cleaned with 200 μL of 1X PBS, then the cells were treated with various test concentrations (25-500 μg ml^{-1}) of *D. longan* leaves mediated silver nanoflakes in serum-free media and incubated for 24 h. The medium was articulated from cells at the end of the treatment period. 0.5mg/mL MTT prepared in 1X PBS was added and incubated at 37°C for 4 h using a CO2 incubator. After an incubation period, the medium containing MTT was cast off from the cells and washed using 200 μL of PBS. The molded crystals were dissolved with 100 μL of (Dimethyl sulphoxide (DMSO) and thoroughly mixed. The change of colour intensity was estimated at 570nm. The formazan dye goes to purple-blue colour. The absorbance was measured at 570 nm and recorded by a microplate reader. OD value was subjected to sort out the percentage of viability was calculated by using the following formula[17],

% Cell viability = (Mean OD value of experimental sample (AgNFs) / Mean OD value OF experimental control) $\times$ 100

3 Results and Discussion

3.1 UV-Vis spectroscopy Analysis

When aqueous *D. longan* leaves extract was added to Silver Nitrate solution, the colour changed to brown owing to surface plasmon resonance excitation (SPR). Surface plasmon resonance is an interacting electromagnetic field-induced collective oscillation of free-conduction electrons. The location of the SPR band in the UV-visible spectra is affected by particle size and shape. This analysis showed a sharp absorbance at around 450 nm as shown in (Figure 2). The UV-vis Spectroscopy range implies the surface plasmon resonance (SPR) of metal nanoparticles[18].

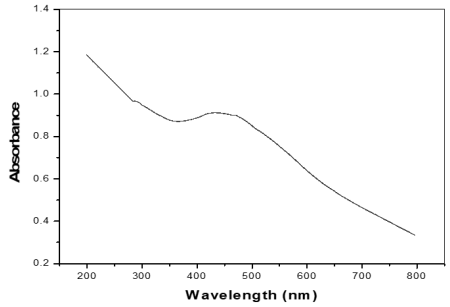

Fig 2. UV-Vis spectrum of silver nanoflakes

3.2 FT-IR spectroscopy analysis

FT-IR spectral analysis confirmed the functional group present in leaves extract is responsible for the synthesis of silver nanoflakes which is shown in (Figures 3 and 4). IR spectrum shows peak of *D. longan* leaves extract appeared at positions 3454, 2924, 2351, 1645, 1384 and 1122 cm.$^{-1}$ shows the spectrum of silver nanoflakes the major peaks appeared at 3446, 2933, 2347 and 1631 cm^{-1}which is due to the presence of stretching vibrations of OH- a group from alcohol, =C-H- a group of vibrations, C-N amide, the N- H stretching vibrations of the carbonyl group. The peaks that appeared at 1382 and 1120 cm^{-1} are allocated to C-H stretching to an alkane and -C-O group of esters. The presence of these peaks confirmed that the several groups of biomolecules exist in the leaves extract might be involved in the reduction process [19].

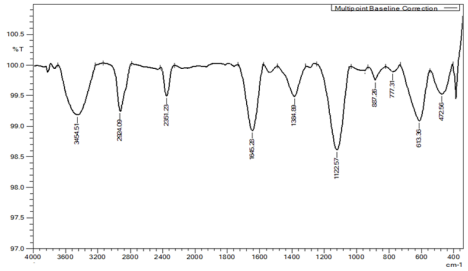

Fig 3. FT-IR spectrum of *D. longan* leaves extract

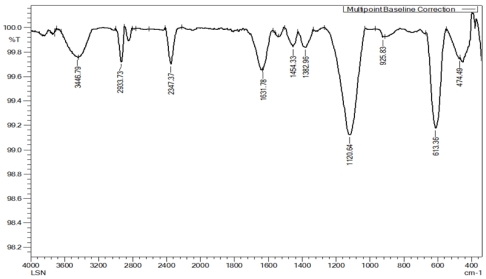

Fig 4. FT-IR spectrum of *D. longan* leaves extract mediated silver nanoflakes

3.3 X-Ray Diffraction Analysis

The XRD analysis was carried out to identify the structure of silver nanoflakes. In (Figure 5). XRD patterns show the diffraction peaks of silver nanoflakes at 2θ [38.43, 44.57, 64.98,] values can be attributed to the miller Indices of the (111) (200) (220) planes of the crystalline structure of silver nanoparticles. XRD diffraction patterns of silver nanoparticles in comparison with

the JCPDS data [04-0783] fairly matched the structure for silver nanoparticles[20]. By using the Debye-Scherrer equation, the average size of silver nanoflakes was determined.

$D = 0.89\ \lambda\ /\ \beta \cos \theta$

Here λ is the X-ray wavelength (Ka = 1.5406 A^0) β is the full width at half the maximum of the peak and θ is the peak position using the above method we obtained and D is the average size of the particle was calculated as 76.45 nm.

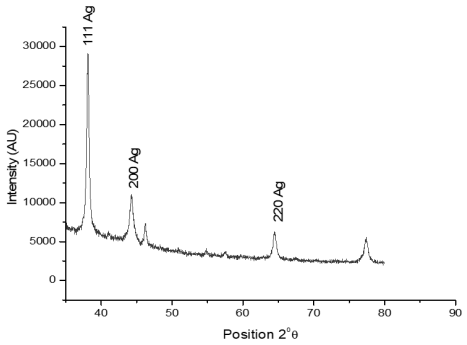

Fig 5. XRD spectrum of leaves-mediated silver nanoflakes

3.4 Scanning electron microscope (SEM) Analysis

SEM analysis was used to identify the surface morphology as shown in (Figure 6) it shows that the particles are flake-like structures. Hence it would be called silver nanoflakes (Ag-NFs)[21].

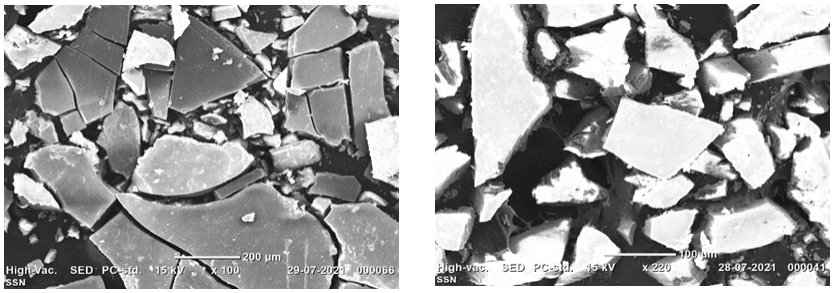

Fig 6. SEM image of leaves mediated silver nanoflakes

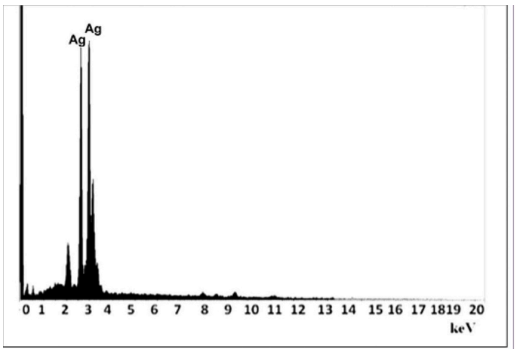

Fig 7. EDAX - The EDAX spectrum shows a clear signal for the silver area and confirms the formation, of silver nanoflakes at 3 Kev

3.5 DLS Measurement and Zeta potential

This study revealed the average size and stability of the nanoparticles in suspension. It was identified that the average size of the nanoparticles was found to be 87.17 nm. The degree of electrostatic charge repulsion or attraction between particles in a liquid suspension was measured by zeta potential. It is important criterion for determining the stability of nanoparticles in an aqueous medium. The particles larger than +30 mV and less than -30 mV are considered stable for colloidal dispersions [22]. As a result, the produced leaves mediated AgNFs with a zeta potential of -23.7 mV are a sufficiently stable colloidal system. Zeta potential with a negative sign indicates the capping of (AgNFs) by negatively charged groups (Figures 8 and 9)

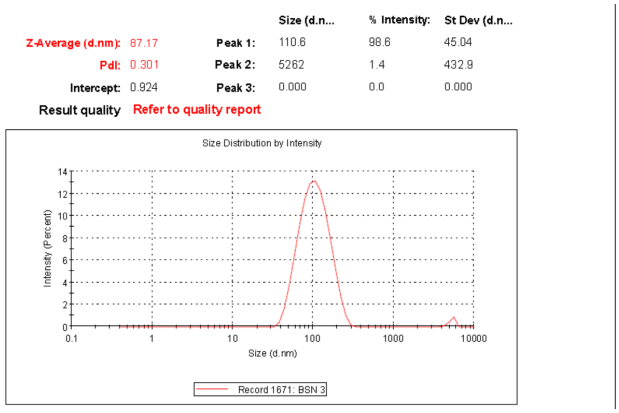

Fig 8. DLS image for particle size measurement

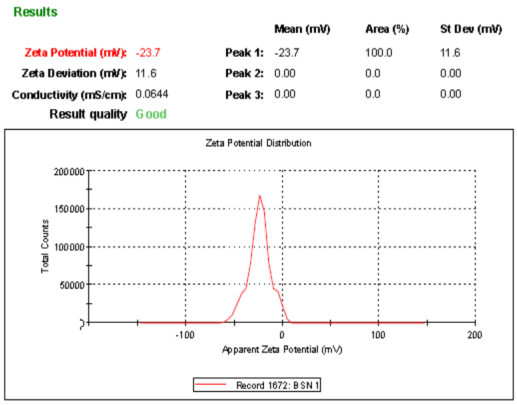

Fig 9. Zeta potential of silver nanoflakes

3.6 Determination of DPPH Scavenging assay

The antioxidant activity of AgNFs was determined using the DPPH radical scavenging test (aliquots of 20,40,60,80,100 μg ml^{-1}) of AgNFs, respectively). Ascorbic acid in concentrations ranging from (20 to 100 μg ml^{-1}) was utilised as a positive control as shown in (Figure 10). Our findings show that produced silver nanoflakes, like aqueous leaves extract, have free radical scavenging activity. The DPPH radical scavenging ability of the samples investigated showed a considerable shift. There are a number of studies undertaken by researchers in the nanotechnology field on various medicinal plants, but no information is known on nanoparticles using *D. longan* leaves. The IC_{50} value of leaves-mediated silver nanoflakes holds at 65.5 $\mu g ml^{-1}$ As a result, an attempt was made to investigate the antioxidant activity of *D. longan*-mediated nanoflakes. The presence of phenolic chemicals is generally responsible for antioxidant activity [23]. AgNPs made from Annona muricata leaves extracts have been shown in earlier investigations to be effective free radical scavengers. According to studies, nanoparticles' antioxidant properties have a comparative advantage over conventional antioxidant delivery systems, including encapsulating shield of the antioxidant agent, enhanced bioavailability, targeted and controlled distribution [24] .

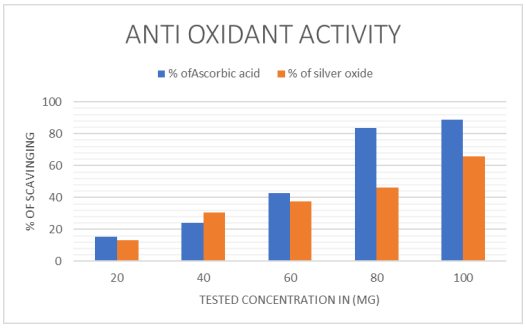

Fig 10. DPPH Scavenging Assay of Ascorbic acid and leaves mediated silver nanoflakes

3.7 Cell culture and MTT assay

Silver nanoparticles are becoming more popular in medical applications. At the same time, the cytotoxic activity of biologically synthesised AgNFs on cancer cell lines has been investigated. The mechanisms involved in the inhibition of cancer cells by silver nanoparticles resulted in DNA damage, adding to the evidence that silver nanoparticles tempted a series of cell death in breast cancer cells[25]. The cytotoxic activity of AgNFs was determined using the MTT test (aliquots of 25, 50, 100, 250, 500 μg ml^{-1}) of AgNF, respectively). The calculated values are shown in (Figures 11 and 12). The MTT assay was done to evaluate the effect of leaves-mediated silver nanoflakes on the growth of MDA-MB-231 and MCF-7 cells. This is the first study to report the cytotoxicity against those breast cancer cell lines. In this study, the cells treated with *D. longan* AgNFs showed dose-dependent cytotoxicity. As concentration increases, cell death also increases. The inhibitory concentration (IC_{50}) value of biosynthesized AgNFs against MDA-MB-231 and MCF-7 cells is determined by 50% of cell death**.** The IC_{50} values are calculated as 138.6±2.18 μgml^{-1} and 171.5±2.2 μgml-1 our findings agree with those of[26] among other studies. Recently carried out a similar investigation on the dose-dependent cytotoxicity of biosynthesized silver nanoparticles utilizing *C. fistula* flower extract on Vero cell line and breast cancer[27]

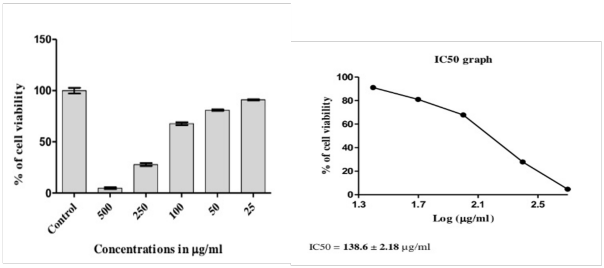

Fig 11. Cytotoxicity of leaves-mediated silver nanoflakes assessed by MTT Assay

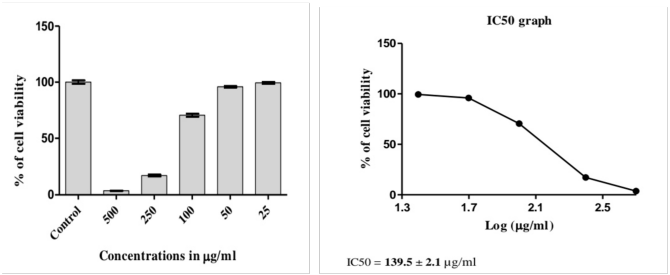

Fig 12. Cytotoxicity of leaves-mediated silver nanoflakes assessed by MTT Assay

3.8 Morphological analysis

When leaves-mediated AgNFs treated with MDA-MB-231 and MCF-7 cells were compared to untreated cells, morphological alterations were detected. The most noticeable morphological alterations in cells are cytoplasmic condensation, cell shrinkage and the development of many cell surface protuberances at the plasma membrane and nuclear chromatin aggregation underneath the nuclear membrane into dense masses as shown in (Figures 13 and 14) [28].

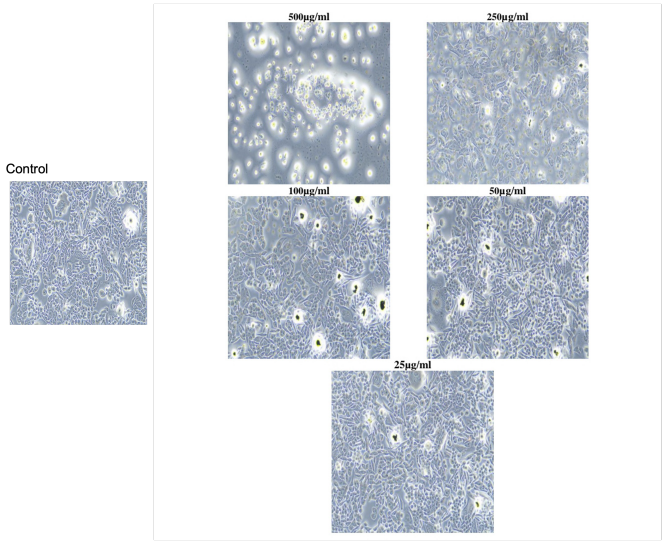

Fig 13. (a) Control and (b) silver nanoflakes treated with MDAMB 231 breast cancer cells

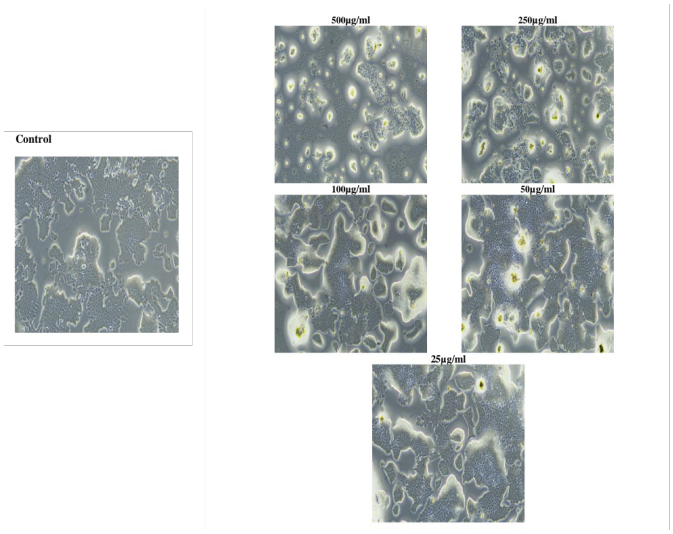

Fig 14. (a) Control and (b) silver nanoflakes treated with MCF-7 breast cancercells

4 Conclusion

In this work, we describe a simple, one-pot, low-cost and eco-friendly method for the synthesis of silver nanoflakes using *Dimocarpus longan (D. longan)* leaves extract. The physiochemical evaluation of the AgNFs in the current studies showed that AgNFs be synthesised in a stable manner and possibly be used in a variety of applications. To the best of our knowledge, we describe the findings of the first investigation of *D. longan* leaves. The silver nanoparticles are shown by the appearance of SPR

at 450 nm. The sample was made up of crystalline face-centered cubic (fcc) lattice structures of elemental silver, according to the XRD pattern. The antioxidant activity was carried out using DPPH assay and found IC_{50} value at 65.5 μgml^{-1}. Using MTT assay evaluates the effect of leaves-mediated silver nanoflakes on the growth of MDA-MB-231 and MCF-7 cells The IC_{50} values are calculated as138.6±2.18 μgml^{-1} and 171.5±2.2 μgml-1. The antioxidant and cytotoxic properties point to their potential use in the medicinal field. The use of *D. longan* leaves extract in the formation of nanoparticles is very favourable since the extract contains a variety of secondary metabolites that impact the generated nanoparticles characteristics and have low toxicity toward healthy cells. It is necessary to explore the mechanism of action of leaves extract in the formation of silver nanoparticles through theoretical simulation approaches.

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