Supplementary information

Analyses of biosynthesized silver nanoparticles produced from strawberry fruit pomace extracts in terms of biocompatibility, cytotoxicity, antioxidant ability, photodegradation, and in-silico studies

1. **Materials and Methods**
	1. *Materials*

Strawberries were purchased at Gyeongju downtown area. Strawberry waste material obtained after juice extraction was selected for AgNPs biosynthesis because of its ease of use and pharmacological properties. The biosynthesis was carried out as previously reported, with a few minor adjustments. Sigma-Aldrich Company supplied silver nitrate (AgNO3) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH). All other reagents were of analytical grade and used exactly as received, and the solutions used for the experiment were freshly made with double distilled water and stored in the dark to avoid any photochemical reaction. Phytochemical analysis of aqueous strawberry waste fruits material was employed to ascertain biochemical active compounds using a standard protocol found in the literature (Farnsworth 1966, Markham 1988, Iqbal et al., 2015).

* 1. *Preparation of aqueous extracts from strawberry waste*

In December-January, 100 grams of freshly purchased strawberries were ringed with distilled water, the calyx was removed, and the strawberries were cut into small pieces before being crushed in a mixer grinder with 100 mL of distilled water. After filtering with filter paper, the solid residues (pomace) as waste material (pomace) were separated and put into a canonical flask containing 125 mL distilled water. The canonical flask containing the solid residue was heated for 25 min at 60°C on a magnetic stirrer. After heating, the solution was cooled and filtered to obtain an aqueous waste strawberry extract that was kept at 4°C for later use.

### *Green synthesis of silver nanoparticles*

A 100 mL aqueous solution of 1 mM silver nitrate was prepared using double distilled water in 250 mL Erlenmeyer flasks. Then add 10 mL of aqueous strawberry extract (waste) to 90 mL of silver nitrate solution, shake gently for 10 min to mix well to initiate the ion reduction process, and leave it at room temperature for 18 h in the dark until the color changes. During this the reduction of Ag+ to Ag0 was confirmed by the colour change of solution from light red to wine red. Its formation was also confirmed by using UV–visible spectroscopy that comes in the range of 431 nm respectively. After centrifuging at 5000 rpm for 8–10 min to remove plant debris and carefully washing with double-distilled sterile water, the biogenic silver nanoparticles were collected. The collected biogenic AgNPs were studied further using spectroscopic methods such as FT-IR, XRD, UV-Vis, SEM, and TEM to determine the shape, size, capping, and stability of the particles.

* 1. *Characterization of biogenic silver nanoparticles*

UV-Vis absorption spectra were obtained with a Shimadzu UV-visible spectrometer (UV-1800, Japan), which is an excellent tool to confirm the formation of silver nanoparticles, in the absorption mode range 300-800 nm using a 1.0 cm lens quartz cell at room temperature. A Perkin Elmer 1750 FTIR spectrophotometer was used to measure the functional groups in the extracts and those that were used to make biogenic nanoparticles in the range of 4000–400 cm-1 using KBr pellet technique. XRD pattern obtained from XPERT-PRO-PANalytical Powder Diffractometer 2θ range from 20 to 80° using monochromatic radiation source Cu K α radiation (λ = 1.5406 Å) at a voltage of 45 kV and a current of 30 mA confirmed the crystallinity of the biogenic nanoparticles. Nanoparticles were subjected to thermal gravimetric analysis (TGA) on a thermal analyser (Perkin Elmer, USA) under N2 at a heating rate of 10 °C min-1. Transmission Electron Microscopy (TEM), scanning electron microscopy (SEM), Dynamic Light Scattering (DLS), and Zeta Potential techniques were utilized to study the particle size, surface shape, and stability of biogenic nanoparticles derived from strawberry extracts in detail.

* 1. *In vitro biocompatibility and cytotoxicity effects on brine shrimps (Artemia salina)*

A biocompatibility assay was performed on fresh human red blood cells (RBCs) to determine the percentage of hemolysis, which was carried out using a previously published approach with some modifications (Malagoli 2007, Li et al., 2012, Alam 2021). In short, 1 mL of human whole blood was added in a tube with 10% EDTA as an anticoagulant and centrifuged at 500 g for 10 minutes to eliminate the serum. The pellet and supernatant were separated by centrifugation, with the supernatant being discarded as serum. Following pellet resuspension in 5 mL of PBS, the RBCs were washed several times by centrifugation (3,000g, 3 min each time). The PBS-erythrocyte suspension is prepared by mixing 200 µL of RBCs with 9.8 mL of PBS. Approximately 800 μl of different concentrations of biogenic AgNPs (200, 100, 50, 25, 12.5, 6.25 and 3.125 μg/mL of phosphate buffered saline) were then added to the erythrocyte suspensions in Eppendorf tubes. Eppendorf tubes were incubated with erythrocyte suspension and biogenic NPs continuously stirring at 150 rpm for one hour at 35°C, followed by centrifugation at 1,377g for 10 min. A 96-well plate was used to measure the absorbance at 570 nm of the supernatant obtained by centrifugation. 5% Triton X-100 served as the positive control, while PBS and sterile water served as the negative controls. Based on the formula below, the percentage of hemolysis in triplicates was calculated.

$$\% of hemolysis=\frac{A-NCA}{\overbar{P}CA-NCA}×100$$

Here, A, NCA, and PCA represent the absorbance of the sample, the negative control, and the positive control, respectively.

It has been a popular model for testing the cytotoxicity of nanoparticles as well as chemical compounds against brine shrimp (Artemia salina). It was highly effective because of its short life cycle, ease of culture, higher offspring production, commercial availability of cysts, and year-round availability for evaluation according to the stated procedure (Arulvasu et al., 2014, Alam et al., 2015, Alam 2021). Additionally, the main advantage of using Artemia as a bioassay organism over other zooplankton species is that it requires no care or storage during testing, requiring only a small amount of the sample to be tested. Brine shrimp with body lengths ranging from 0.8 to 1.0 cm were selected after hatching saline eggs in artificial saltwater prepared by dissolving commercial marine salt (2%) in RO water (mineral water) in a tank well aerated with the aid of an air pump near a light source at 37°C. After that, each well was loaded with test samples (12.5 ‒ 500 μg/mL) and the adjusted volume was 0.5 mL. A control solution was made up of 5% salty water without nanoparticles. During the experiments, the temperature maintained around 27–28°C. Test wells with varied concentrations and 15 brine shrimp were left for 24 h. The number of dead brine shrimp with no visible movement was seen and counted using an optical lens after 24 h of incubation in solutions containing and without NPs. The experiment was repeated three times.

* 1. *Molecular docking*

Molecular docking was performed with silver nanoparticles and the Artemia franciscana serine protease, which is responsible for the hatching of A. salina nauplii, using the PatchDock server (Schneidman-Duhovny et al., 2005). After the removal of organic molecules from the surface of the nanoparticles (NPs), the model structure of the previous study was used (Kyrychenko et al., 2015). The 3D crystal structure of serine protease (UniProt ID: A8D853) (PDB ID: 2HLC) obtained from the RCSB PDB website was prepared by deleting water molecules and supplying missing hydrogen, amino residues, as well as Kollman and Gasteiger charges using AutoDockTools (Morris et al., 2009). After preparation of silver nanoparticle model and receptor, they were supplied to the PatchDock web server for docking results. The PDB format of the silver nanoparticle model and receptor (PDB ID: 2HLC) were provided to the PatchDock web server for docking results after preparation. Docking results were used to determine the optimal orientation of docked silver nanoparticles to the receptor for visualization and analysis of interactions between the receptor and silver nanoparticles using Discover Studio (Biovia 2017).

* 1. *Antioxidant activity*

The antioxidant potential of nanoparticles and chemical compounds has been investigated and documented in literature using 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH solution in methanol shows a luminous purple hue, an intense absorption band at 517 nm and changes color to yellow in the presence of substances with antioxidant activity. The methodology employed (Barros et al., 2007), with some modifications for antioxidant activity was documented elsewhere. In brief, 1 mL of each AgNPs concentration; 12.5, 25, 50, 100, 200, 400, and 500 μg/mL —was added to 1 mL of freshly made solution (1 mM in methanol) and vortexed well. Similarly, the same approach was used to compare results with the reference compound Gallic acid as well as extracts. The biogenic silver nanoparticles and reference compound solutions were then incubated in the dark for 30 min at normal room temperature. The absorbance was recorded at 517 nm using a UV-Vis spectrophotometer for the DPPH solution with and without samples including methanol to express the free radical scavenging activity as percent inhibition, which was determined by the following formula.

% of scavenging = [(Ac -As)/Ac] ×100

Where A*c*represents the absorbance of a negative control (all reagents except the test AgNPs and extracts at varied concentrations) and As represents the absorbance of the antioxidant materials (various concentrations of silver nanoparticles and reference material).

* 1. *Experimental design for photocatalytic activity*

Photocatalytic efficiency of biogenic silver nanoparticles for methyl orange dye degradation was studied. All of the experiments were carried out outside, with the sun serving as the primary source of light for sample solution irradiation (Kumar et al., 2013, Ajmal et al., 2016, Priya et al., 2021). For the photodegradation investigation, triplicate experimental sets of seven beakers (100 mL) were prepared, each with a 50 mL solution of methyl orange (50 mg/mL in deionized water). 20 mg of biosynthesized biogenic nanoparticles were added to each beaker. The dye and NP solution was continually agitated in the dark for one hour to ensure that an equilibrium of silver NPs was achieved in the organic solution. The solution mixture was then exposed to direct sunshine for 9 minutes. At each interval of 0, 15, 30, 45, 60, 75 and 90 minutes, 4 mL of suspension was removed. The remaining dye concentration was then calculated using UV absorption spectra recorded using a UV-Vis spectrophotometer to quantify the degradation of the dye solution at λmax = 464nm after the catalyst was removed by centrifugation. The equation below calculates the percentage of methyl orange degradation rate (MO).

$$\% Degradation=\frac{A\_{0}-At}{A\_{0}}×100$$

According to equation, A0 is the absorbance of MO dye solution at time 0 and At is the absorbance at time t.

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