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Biogenic synthesis of silver nanoparticles using *Sterculia foetida* seed extract and evaluation of its therapeutic potential

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ABSTRACT

Sterculia foetida, also known as Wild Almond, is a large deciduous tree in the Sterculiaceae family with various medicinal properties. This plant has been used in traditional medicine to treat various ailments like rheumatism, obesity, gonorrhea, emphysema, and skin diseases. Plant extracts have tremendous potential for the biosynthesis of silver nanoparticles (AgNPs) due to their stabilizing, and reducing activity. This study aimed to create silver nanoparticles of *S. foetida* seed (Sf-AgNPs) by utilizing silver nitrate and a water-based extract obtained from *S. foetida* seeds. Subsequently, these particles were characterized and subjected to *in vitro* evaluations to determine their antioxidant, antibacterial, anti-inflammatory, and cytotoxic activities. The production of silver nanoparticles was established by Ultraviolet–Visible (UV – Vis) Spectroscopy, FTIR, and TEM. The UV–Vis spectral analysis revealed a peak of λ_{max} at 440 nm which confirmed the existence of AgNPs. FTIR analysis determined the potential functional groups of the biomolecules present in the *S. foetida* seed extract, which are primarily accountable for the bioreduction of Ag^{+} to Ag^{0}. The TEM image of green synthesized AgNPs confirmed the occurrence of spherical, mono-dispersed nanoparticles with a diameter of < 100 nm. The *in vitro* anti-oxidant studies were conducted using the total antioxidant and reducing power assays. The Sf-AgNPs displayed higher antioxidant capacity than the seed's aqueous extract (Sf-AQSE). Furthermore, unlike its aqueous extract, the Sf-AgNPs showed higher antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. Protein denaturation and anti-proteinase assays were used to carry out *in vitro* anti-inflammatory studies. The potential of the synthesized silver nanoparticles to induce albumin denaturation and proteinase inhibition at all tested concentrations suggested that they could control protein denaturation and inhibit proteinase implicated in the inflammatory process. The MTT assay was used to investigate the cytotoxic action of Sf-AgNPs against SK-MEL-5 cancer cell lines, indicating a dose-dependent decrease in cell viability as the cells were exposed to various dosages of Sf-AgNPs. Thus, the findings of the present study showed that AgNPs synthesized utilizing the water extract of *S. foetida* seed possess potent antioxidant, antibacterial, anti-inflammatory, and cytotoxic properties.

1. Introduction

Nanotechnology primarily deals with developing nanoparticles (NPs) of different forms, sizes, and chemical compositions. Nanoscience has currently exploded in the fields of medicine, sensors, optoelectronics, and catalysis. A lot of biological interest has recently been focused on NPs [1,2]. A small size (1–100 nm) and a high surface area-to-volume ratio are attributed to the exceptional chemical, physical, and optical properties of these NPs [3,4]. Silver nanoparticles (AgNPs), one of the most well-known types of these NPs, have recently drawn a lot of attention because of their high synthesis efficiency and indispensable applications [5,6]. Biosynthesized AgNPs are reported to have many pharmacological effects including cytotoxic [7], anticancer [8], antifungal [9], antibacterial [10], hepatoprotective [11], antioxidant [4], mosquitocidal and antiplasmodial [12].

For the synthesis of AgNPs, two approaches have been employed: "Top-down" and "Bottom-up". The "top-down method" uses size reduction to break up suitable bulk material into tiny particles. Chemical methods are used to synthesize AgNPs through a 'bottom-up process,' in which new nuclei are formed and subsequently grow into nanoscale particles. The preferred technique for producing AgNPs in the bottom-up approach is chemical reduction. 'Ag' ions are reduced using many

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different organic and inorganic reducing agents, which include polyethylene glycol block copolymers, Tollen's reagent, sodium citrate, and others. Additionally, capping agents are utilized to stabilize the NPs' size. [13]. Although these approaches are extensively used in research to create AgNPs, their main disadvantages are their high cost or the use of potentially hazardous compounds as reducing and stabilizing agents, which can result in several environmental problems and biological concerns [1,6]. As an alternative to these traditional methods, a high-performance "bottom-up" approach to synthesizing AgNPs using green and sustainable technology has emerged and is known as the "green synthesis method" [14]. Compared to conventional techniques of synthesis, green synthesis of AgNPs provides several benefits. This method aims to minimize or eliminate the use of harmful chemicals and energy-intensive processes commonly associated with conventional synthesis methods. The biosynthesis of AgNPs also has the key benefit of producing a large number of AgNPs with well-defined sizes and morphologies [15,16]. In the green production of AgNPs, silver ions are reduced to create nanoparticles using plant extracts, bacteria, fungi, yeast, algal extracts, or other biological elements. Among these, the preparation of AgNPs from plant extracts has generated considerable attention and application due to its low cost, high quality, ease of large-scale manufacturing, and environmental friendliness [6,14,17]. AgNPs are made from many plant parts, such as leaves, fruits, flowers, seeds, bark, roots, etc. [16]. Medicinal plants have been widely exploited in the green synthesis of AgNPs due to the benefits of broad sources and various active components. These plants consist of phytochemicals that have a range of pharmacological properties and serve as reducing, capping, or stabilizing agents during the biosynthesis of AgNPs [18].

Sterculia foetida, also known as wild almond, bastard poon tree, java olive, and hazel sterculia, is a tall, deciduous tree in the Sterculiaceae family with an array of medicinal properties. This tree can reach heights of 40 m and girths of 3 m, with branches organized in whorls and extending horizontally. This species of plant, which has a lifespan of more than 100 years, is widely distributed in India, Southeast Asia, and along the east coast of Africa. Within 2–3 years, the plant, which grows fast, begins to produce seeds [19]. *S. foetida* has traditionally been a useful ingredient in herbal therapy. Its leaves are natural remedies for conditions like rheumatism, obesity, gonorrhoea, edema, dropsy, and skin diseases. The bark, prepared as a decoction, serves as a remedy for conditions like dropsy and rheumatism, acting as an aperient, diaphoretic, and diuretic. It has also been traditionally employed to address health issues such as emphysema, asthma, and arthritis. The fruit yields a mucilaginous decoction used as an astringent for gonorrhoea and diarrhoea. A root infusion is used for bathing sick children or patients with jaundice. Sterculia gum, another product of the plant, is a mild laxative, that relieves constipation, and it is also used to soothe coughs and sore throats. Furthermore, it stands out for its valuable medicinal properties, which include wound-healing capabilities and anti-inflammatory attributes. The seeds, known for their laxative properties, are also utilized as purgatives. Sterculia oil extracted from the seeds is employed to treat itchy skin conditions. The wood, when combined with seed oil, is used externally to alleviate rheumatism. *S. foetida* seeds are safe to consume either raw or cooked and have no adverse effects on humans or animals. Pharmacological studies on *S. foetida* seed revealed anti-diabetic, anti-dermatophytic, antimicrobial, anticancer, anti-obesity, anti-fertility, antifungal, antiviral, insecticide, and mitogenic effects [20–22]. The aqueous extract of *S. foetida* seed (Sf-AQSE) has been used in this study to synthesize the silver nanoparticles (AgNPs), as plant extracts have considerable potential in the biosynthesis of AgNPs due to their stabilizing and reducing action. The synthesis of AgNPs from an aqueous *S. foetida* seed extract, as well as their characterization and *in vitro* evaluation for antioxidant, antibacterial, anti-inflammatory, and cytotoxic effects, were the primary objectives of this study.

2. Materials and methods

2.1. Chemicals

From Sigma Chemicals Co, USA, we bought silver nitrate ($AgNO_3$), lead acetate, trypsin, and fetal bovine serum. Dimethyl sulfoxide, Chloroform, and Glacial acetic acid were purchased from Merck, India. Ethylene Diamine Tetra Acetic Acid, Ferric Chloride, Sulphuric Acid, Hydrochloric Acid, and Potassium Ferricyanide were procured from Sisco Research Laboratories, India. MTT Assay Kit and Antibiotic Sensitivity Kit were purchased from Himedia, India. The rest of the chemicals/reagents utilized in the experiments were of analytical grade.

2.2. Collection and authentication of S. foetida seeds

The *S. foetida* seeds were authenticated after being collected from the botanical garden of the T.K.M. College of Arts and Science, Kollam, Kerala. A voucher specimen (TKMBC.01) is maintained in the institute. To eliminate surface contaminants, the harvested seeds were rinsed with deionized water and then dried in the shade. The air-dried seeds of *S. foetida* were ground well into a fine powder using a mixer grinder before being stored at room temperature in an airtight container.

2.3. Preliminary phytochemical screening

The aqueous extract of *S. foetida* seed (Sf-AQSE) was made by blending 10 g of seed powder with 50 ml of distilled water and then agitating the mixture for an hour. After stirring the mixture, filtration was conducted using Whatman No. 1 filter paper. The *S. foetida* seed extract was then subjected to preliminary phytochemical screening using standard procedures to detect the phytoconstituents [23].

2.4. Biosynthesis of AgNPs

0.5 g of *S. foetida* seed powder was added to 50 ml of distilled water and agitated for 1 h with a magnetic stirrer. After stirring, the contents were filtered using Whatman No. 1 filter paper. Then, 20 ml of seed extract was mixed with 20 ml of 1 mM $AgNO_3$ solution and the mixture was agitated. Within an hour, the color changes noticeably from colorless to reddish brown, suggesting the formation of silver nanoparticles of *S. foetida* seed (Sf-AgNPs). The reaction mixture was then centrifuged to remove unreacted silver ions.

2.5. Characterization of Sf-AgNPs

Sf-AgNPs are characterized by UV–Vis Spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), and Transmission Electron Microscopy (TEM).

2.5.1. UV–Vis Spectroscopy

UV–Visible spectrum of the biosynthesized Sf-AgNPs was recorded using 'Systronic UV–Vis absorption spectrophotometer'. The sample was scanned between 300 and 800 nm.

2.5.2. FTIR

The FTIR spectroscopic method was employed to find the functional groups responsible for the reduction, stabilization, and capping of Sf-AgNPs. A 'Thermo Fisher Scientific Nicolet Nexus iS50 spectrometer' was used to record the FTIR spectra. The solution containing the synthesized silver nanoparticles was centrifuged at 10000 rpm for 15 min. To remove any enzymes or free proteins that were not capping the AgNPs, the pellet was rinsed three times with 5 ml of deionized water and then it was dried in a vacuum dryer before being used for FTIR analysis. The spectrum was taken in the diffuse reflectance mode with a resolution of 4 cm^{-1} over the wavelength range of 4000–500 cm^{-1} [24].

2.5.3. Transmission Electron Microscopy (TEM)

Sf-AgNPs were characterized by TEM based on particle size and surface morphology. The synthesized silver nanoparticle sample was dipped on the carbon-coated grids and was dried in vacuum for 15 min before measurement. HR-TEM analyses were carried out with FEI-Tecnai G2-30, at an accelerating voltage of 300 kV.

2.6. In vitro antioxidant studies

2.6.1. Determination of total antioxidant capacity

The total antioxidant activity of Sf-AQSE and Sf-AgNPs was evaluated using the phosphomolybdate method with ascorbic acid as a reference. The sample solution (0.1 ml) and the reagent solution (1 ml), which consists of 4 mM ammonium molybdate, 0.6 M sulfuric acid, and 28 mM sodium phosphate, were mixed and incubated at 95 °C in a water bath for 90 min. After the samples attained room temperature, the absorbance of the mixture was recorded against a blank at 695 nm [25]. The total antioxidant capacity was measured in milligrams of ascorbic acid equivalent per gram of extract/Sf-AgNPs.

2.6.2. Reducing power assay

By slightly modifying the method outlined by Linn et al.,[26] the reducing power of the Sf-AQSE and Sf-AgNPs was determined. Sf-AgNPs and Sf-AQSE were prepared at various concentrations and combined with 2.5 ml of 1 % potassium ferricyanide and 2.5 ml of 0.2 M phosphate buffer (pH 6.6). After being kept at 50 °C for 20 min, 2.5 ml of 10 % trichloroacetic acid was added to the mixture. The resultant mixture was centrifuged for ten minutes at 3,000 rpm. 2.5 ml of supernatant was then mixed with an equal volume of distilled water and 0.01 % ferric chloride (0.5 ml). The absorbance at 700 nm was measured using a UV–visible spectrophotometer. In this assay, ascorbic acid served as the standard antioxidant. The % inhibition was calculated using the following formula to determine reducing power.

$$\text{Percentage inhibition} = \frac{(\text{Absorbance of the control} - \text{Absorbance of the sample})}{(\text{Absorbance of the control})} \times 100$$

2.7. Antibacterial studies

The antibacterial efficacy of Sf-AgNPs and Sf-AQSE was tested on clinical isolates of two different bacterial strains, *Staphylococcus aureus* and *Escherichia coli*.

2.7.1. Disc diffusion method

The antibacterial properties of Sf-AgNPs and Sf-AQSE were investigated by modifying the Kirby-Bauer disc diffusion assay method. In brief, 100 µl of a bacterium suspension was dispersed over Muller-Hinton agar plates. Sf-AgNPs/ Sf-AQSE stock solutions were prepared in sterile, distilled water (0.5 mg/ml). The Sf-AQSE and Sf-AgNPs were then separately impregnated into the 6 mm diameter sterilized paper discs at two different concentrations (10 µl and 20 µl). After that, the discs were laid on top of Muller-Hinton agar plates. In the *E. coli* and *S. aureus* cultures, positive controls included chloramphenicol and tetracycline (30 µg/disk), respectively, while a negative control was maintained using sterile distilled water. To diffuse the active compounds in the medium, the agar plates were kept at 4 °C for two hours. After that, the plates were incubated for 24 h at 37 °C. The zone of inhibition was determined after the incubation period by measuring the diameter [27].

2.8. In vitro anti-inflammatory studies

2.8.1. Anti proteinase assay

Proteinase inhibitory activity of Sf-AgNPs and Sf-AQSE was tested as detailed by Gunathilake et al., [28] In short, the reaction mixture of 2 ml contained 1 ml of 20 mM Tris-HCI buffer (pH 7.4), 0.06 mg of trypsin, and 1 ml of test samples or a standard drug (diclofenac sodium) at varying concentrations (50, 100, and 200 µg/ml). After 5 min of incubation at 37 °C, 1 ml of 0.8 % (w/v) casein was added, and the mixture was left to incubate for an additional 20 min. Then the reaction ceased by adding 2 ml of 70 % $HClO_4$. After centrifuging the mixture for ten minutes at 3000 rpm, the absorbance of the supernatant was measured at 210 nm against a blank made of buffer. The % inhibition of protein denaturation was calculated after the experiment was repeated three times.

2.8.2. Protein denaturation assay

A modified form of the procedure previously reported by Djuichou Nguemnang et al. [29] was used to conduct this assay. Sf-AgNPs and Sf-AQSE in various concentrations (100, 200, and 500 mg/ml) were added to a 5 % aqueous solution of Bovine Serum Albumin (BSA). Following a 20-minute incubation period at 37 °C for each reaction mixture, protein (BSA) denaturation was induced by immersing the mixture in a 70 °C water bath for 15 min. After allowing the reaction mixture to cool to 23 °C ± 2 °C, absorbance at 660 nm was measured using ultraviolet–visible spectrophotometry to assess turbidity. Diclofenac sodium was used as the positive control, while the BSA solution dissolved in distilled water served as the control. The assay was performed three times, and the inhibition of protein denaturation observed was calculated and expressed as a percentage inhibition.

2.9. Cytotoxicity screening by MTT assay

The Human Skin Malignant Melanoma Cell Line (SK-MEL-5), used in the present study was obtained from the National Centre for Cell Sciences (NCCS), Pune, India. These cells were seeded in 96-well plates (2500 cells/well) and allowed to acclimatize for 24 h in an incubator at 37 °C with 5 % CO_2. Test samples prepared in DMEM medium (100 mg/ml) were sterilized using a 0.2 µm pore size Millipore syringe filter and then further diluted in DMEM to create final concentrations (6.25 to 100 µg/ml). These samples were added to the cell culture wells alongside untreated control wells. After 24 h of treatment, the culture medium was removed, and MTT solution (0.5 mg/ml in PBS) was added (100 µl/well). Following a two-hour incubation to form formazan crystals, the liquid was replaced with pure DMSO (100 µl/well). Absorbance at 570 nm was measured using a microplate reader. Two blank wells without cells were included on each plate. The experiments were conducted in triplicate, and average values were recorded for accuracy, from which the cell viability was calculated [30].

2.10. Statistical analysis

The results are presented as the mean ± standard deviation. To analyze the data, we used IBM SPSS Statistics software and conducted statistical comparisons using the student *t*-test and one-way ANOVA test. We also performed Tukey's post hoc analysis. A *p*-value of ≤ 0.05 indicated statistical significance.

3. Results and discussion

3.1. Phytochemical analysis of the aqueous extract of S. foetida seed

The phytochemical examination of the Sf-AQSE indicated the presence of alkaloids, phenolic compounds, tannins, and terpenoids (Table 1). Phytochemicals are naturally occurring compounds that are present in plants and offer nutrition and medicinal benefits to mankind [31]. The presence of these bioactive secondary metabolites in the *S. foetida* seed may be attributed to its traditional therapeutic uses.

Table 1
Phytochemical analysis of Sf-AQSE.

Sl. No	Phytochemicals	Inference
1.	Alkaloids	+
2.	Phenolic compounds	+
3.	Tannins	+
4.	Terpenoids	+
5.	Saponins	-
6.	Glycosides	-
7.	Flavonoids	-
8.	Steroids	-

(+) Present; (-) Absent.

3.2. Green synthesis of Sf-AgNPs

In this study, AgNPs were synthesized using the aqueous seed extract of *S. foetida* and silver nitrate. As depicted in Fig. 1, the silver nitrate solution changed from its original color to reddish brown at room temperature, indicating the successful reduction of silver ions to silver nanoparticles. The control silver nitrate solution (without seed extract) showed no color change. Reports state that AgNPs display a yellowish-brown hue in water. This is caused by the excitation of surface plasmon vibration in metal nanoparticles [2,27].

3.3. Characterization of Sf-AgNPs

3.3.1. UV–Vis Spectroscopy

To confirm the green synthesis of AgNPs, UV–Visible Spectroscopy is employed to measure the distinctive localized surface plasmon resonance (SPR) absorption peak. Fig. 2 shows the UV–Vis spectrum of green synthesized silver nanoparticles. AgNPs have free electrons, which produce an SPR absorption band due to the mutual vibration of metal nanoparticle electrons in resonance with a light wave. The appearance of the peaks demonstrates the surface plasmon resonance properties of AgNPs [32]. UV–Vis spectrum with λ_{max} at 440 nm has confirmed the production of silver nanoparticles.

3.3.2. FTIR

FTIR spectrum analysis was conducted to determine probable functional groups of the biomolecules in the *S. foetida* seed extract that can reduce Ag^{+} ions to Ag^{0}, capping, and stabilizing the AgNPs. The FTIR spectra of Sf-AgNPs showed distinct peaks across the whole range of observation. For synthesized AgNPs, FTIR analysis revealed clear bands at 3248.44, 2162.34, 1636.22, and 570.80 cm^{-1} (Fig. 3). The band found at 3248.44 cm^{-1} corresponds to O—H stretching H-bonded alcohols and phenols. The peak at 2162.34 cm^{-1} corresponds to C≡C from terminal alkyene. The peak observed at 1636.22 cm^{-1} ascribed to alkenyl C═C stretch, N—H bend primary amines, and amide C═O stretching. Based on the visible peaks, it seems that the Sf-AgNPs contain proteins and phytochemicals like phenolics and terpenoids. These compounds could be responsible for stabilizing and capping the silver nanoparticles. It is possible that the biomolecules connected with the silver ions through their oxygen donor atoms and adhered to the surfaces of the metal ions. Literature reports support the tentative peak assignment [33,34].

3.3.3. TEM

The size, shape, and dispersion of developed silver nanoparticles were assessed by TEM. Fig. 4 shows TEM images of Sf-AgNPs, revealing the presence of spherical mono-dispersed nanoparticles with a size of < 100 nm. The TEM analysis unequivocally confirms the nanoparticle size within the specified range, affirming their existence. Previous reports have also confirmed the spherical shape of green synthesized AgNPs. [35,36]. AgNPs with dark shades indicate the presence of secondary material, which could be biological substances from *S. foetida* seeds. These biocomponents have a key role in facilitating the conversion of Ag + to AgNPs. Additionally, these particles serve as a capping agent that prevents the aggregation of those particles [36].

3.4. In vitro antioxidant activity

The total antioxidant capacities of Sf-AgNPs and Sf-AQSE were 88.57 ± 0.45 and 27.23 ± 0.25 mg equivalent ascorbic acid/g dry extract (Sf-AgNPs), respectively. As shown in Table 2, Sf-AgNPs have a significantly greater ($p \leq 0.05$) antioxidant capacity than Sf-AQSE. Several investigations have documented the potential of natural extracts and microorganisms to generate AgNPs and their superior antioxidant efficacy when compared to substrates. This activity is thought to be caused by the selective sorption of extract constituents onto the nanoparticle surface [37,38].

Reducing power refers to a compound's ability to transfer electrons, which is a key indication of its antioxidant potential. Antioxidants can contribute electrons and neutralize free radicals, protecting cells from oxidative damage. Compounds having higher reducing power are more capable of transferring electrons and are often considered to have stronger antioxidant action. Therefore, evaluating a compound's reducing capacity can reveal important information about its antioxidant potential [39]. Table 3 shows the reductive capacities of Sf-AgNPs and Sf-AQSE at various concentrations. The reducing power of the Sf-AgNPs, which was 62.33 ± 0.44 % at a concentration of 150 µg/ml, was considerably greater than that of the Sf-AQSE, which was 46.23 ± 0.84 % at the same concentration. Ascorbic acid, used as standard, has a

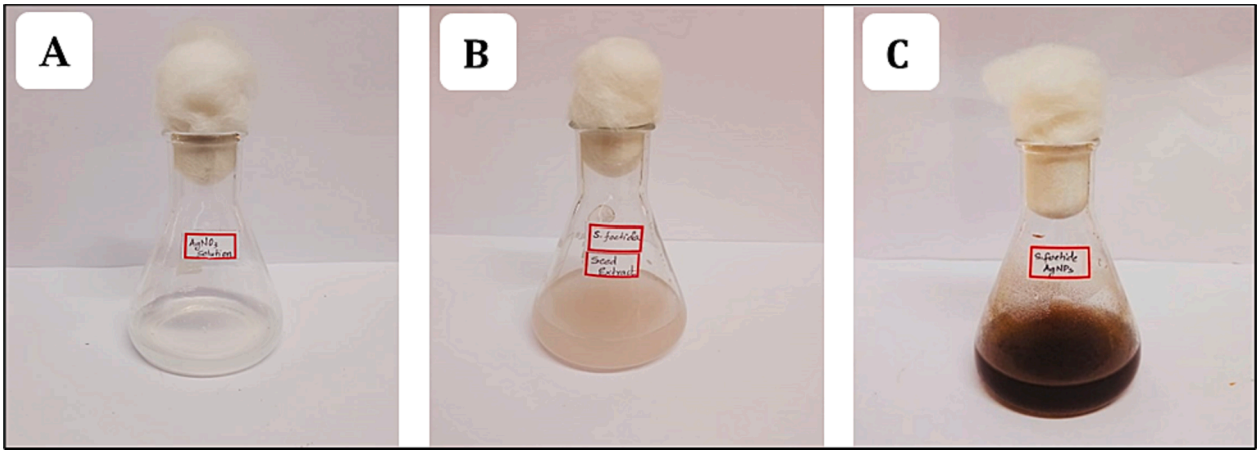

Fig. 1. Photograph showing the green synthesis of AgNPs, using an aqueous extract of *S. foetida* seed. (A) Silver nitrate solution, (B) *S. foetida* seed extract, and (C) Formation of Sf-AgNPs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

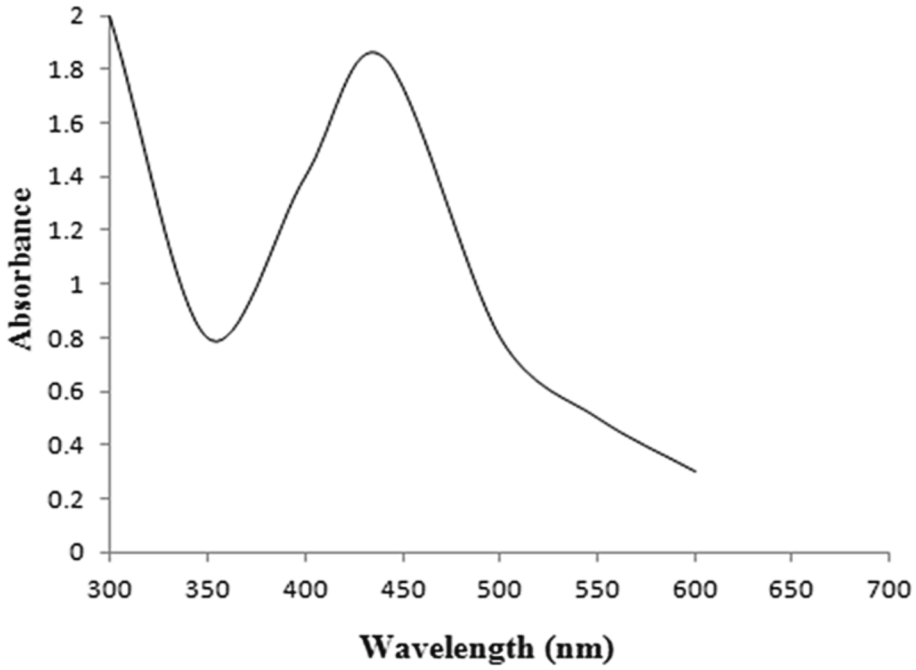

Fig. 2. UV spectrum of the Sf-AgNPs.

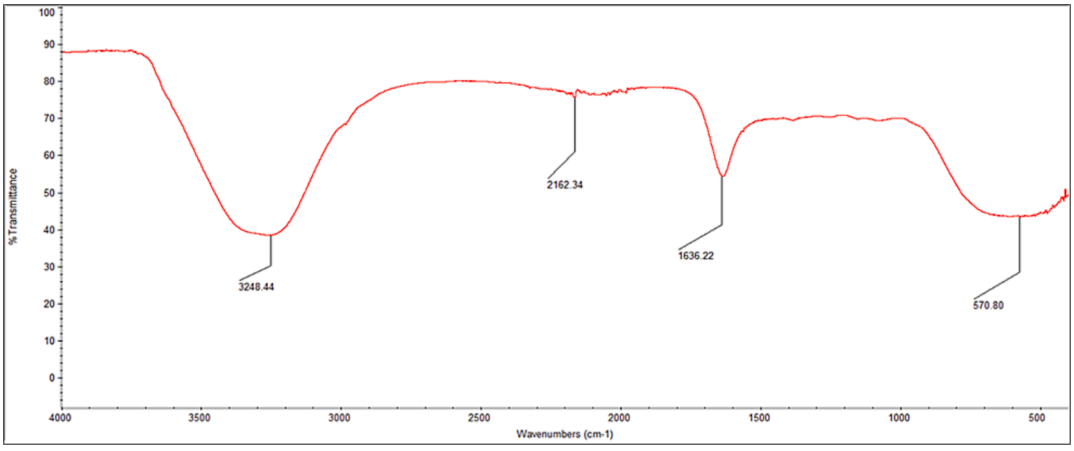

Fig. 3. FTIR spectrum of Sf-AgNP.

maximum reducing power of 78.57 ± 0.31 % at a concentration of 150 µg/ml. In comparison to aqueous seed extracts of *S. foetida*, green synthesized silver nanoparticles had a higher capacity to act as antioxidants.

3.5. Antibacterial activity

The antibacterial action of synthesized Sf-AgNPs was quantified at two different concentrations (10 µl and 20 µl). The average inhibitory zone diameter was measured and calculated for the three replicates. The zone of inhibition was measured (Table 4 and Fig. 5A & B) and compared to the positive control (standard antibiotics) and negative control (sterile distilled water). Chloramphenicol (30 µg/disk) served as a positive control against *E. coli* and demonstrated a zone of inhibition of 17.50 ± 0.33 mm. Tetracycline (30 µg/disk), on the other hand, had a zone of inhibition of 33.67 ± 0.44 mm against *S. aureus* bacteria. 20 µl of Sf-AgNPs demonstrated the highest antibacterial activity against both Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacterial species when compared to 10 µl of Sf-AgNPs. 20 µl of Sf-AgNPs showed a zone of inhibition of 16.33 ± 1.11 mm and 22.67 ± 1.56 mm against *E. coli* and *S. aureus*, respectively. The negative control did not affect any of the organisms. The aqueous seed extracts of *S. foetida* did not show any antibacterial activity against the tested organisms at the given concentrations of 10 µl and 20 µl (0.5 mg/ml) (Table 5 and Fig. 5C & D). The $AgNO_3$ control had a zone of inhibition of 10.00 ± 0.67 mm and 12.17 ± 0.78 mm against *E. coli* and *S. aureus*, respectively. Although antibacterial activity was found in the $AgNO_3$ control, it significantly increased in Sf-AgNPs and was found to have a potent, concentration-dependent antibacterial effect against the tested organisms. It is reported that AgNPs enter bacterial cell membranes and cause a variety of cell malfunctions, including ribosomal, enzyme, protein, and DNA destabilization, ROS formation, and DNA damage, which ultimately leads to cell death [1,16].

3.6. In vitro anti-inflammatory activity

Protein-degrading enzymes, commonly known as proteases (proteinases), are associated with arthritic reactions and can exacerbate

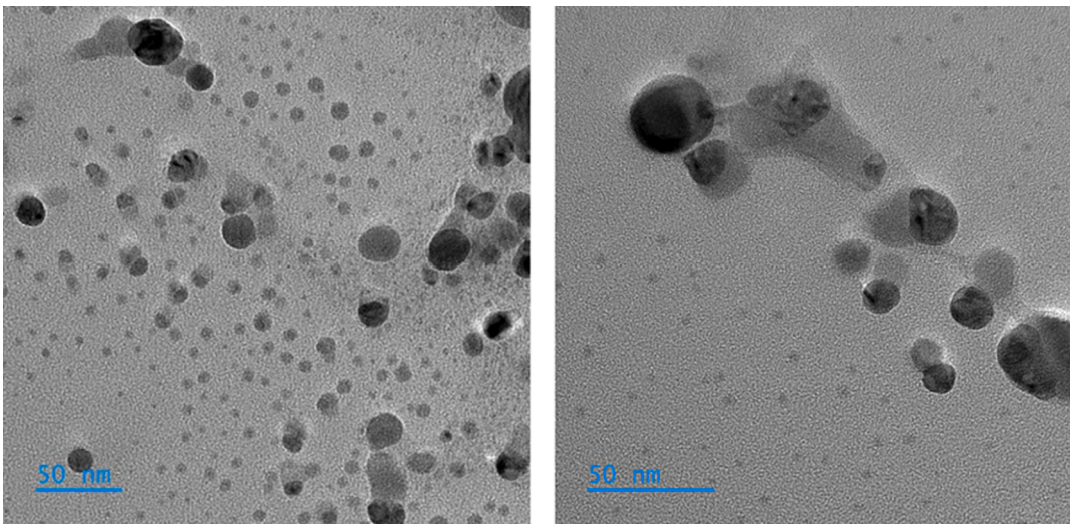

Fig. 4. TEM image of Sf-AgNPs.

Table 2
Total antioxidant capacity of Sf-AQSE and Sf-AgNPs.

Treatment	Total antioxidant capacity (mg equivalent ascorbic acid/g dry extract [Sf-AgNPs])
Sf-AQSE	27.23 ± 0.25
Sf-AgNPs	88.57 ± 0.45[a]

*Values are mean ± SD (n = 3); [a] $p \leq 0.05$ versus Sf-AQSE.

Table 3
Reducing power of Sf-AQSE and Sf-AgNPs.

Treatment	% of reducing power		
	50 µg/ml	**100 µg/ml**	**150 µg/ml**
Sf-AQSE	23.53 ± 0.38[a]	34.13 ± 0.44[a]	46.23 ± 0.84[a]
Sf-AgNPs	45.47 ± 0.62[b]	53.27 ± 0.36[b]	62.33 ± 0.44[b]
Ascorbic acid	61.27 ± 0.49[c]	68.30 ± 0.47[c]	78.57 ± 0.31[c]

*Values are mean ± SD (n = 3); Superscripts with different letters in the same column indicate significant statistical differences with a significance level of $p \leq 0.05$.

Table 4
Antibacterial activity of Sf-AgNPs.

Sl. No.	Name of bacterial species	Zone of inhibition (mm)			
		Positive control (PC)	**Negative control (NC)**	**Sf-AgNPs (10 µl)**	**Sf–AgNPs (20 µl)**
1	*Escherichia coli*	17.50 ± 0.33	Nil	14.33 ± 0.89[a]	16.33 ± 1.11
2	*Staphylococcus aureus*	33.67 ± 0.44[a]	Nil	17.00 ± 0.67[b]	22.67 ± 1.56[c]

*Values are mean ± SD (n = 3); In the row *of E. coli*, [a]$p \leq 0.05$ versus positive control. Superscripts labelled with different letters in the same row for *S. aureus* indicate significant statistical differences ($p \leq 0.05$).

tissue damage in inflammatory responses. It has already been proven that leukocyte proteinase is a crucial component in the process of tissue damage during inflammatory responses. Inhibition of proteinases can be a potential therapeutic approach to mitigate tissue damage and inflammation in arthritic conditions [28]. Table 6 presents the exhibited proteinase inhibitory activity of Sf-AQSE and Sf-AgNPs at different concentrations. The proteinase inhibition level of the Sf-AgNPs, which was 63.43 ± 0.60 % at a concentration of 200 µg/ml, was significantly greater than that of the Sf-AQSE, which was 53.50 ± 0.87 % at the corresponding concentration. However, diclofenac sodium, the standard drug, displayed maximal inhibition of 72.60 ± 0.40 % at the same concentration. The effect of Sf-AQSE, Sf-AgNPs, and diclofenac sodium on proteinase inhibitory activity was concentration-dependent. Sf-AgNPs exhibit more proteinase inhibitory activity than their corresponding aqueous extract, indicating greater potential as an anti-proteinase inhibitor.

Tissue protein denaturation is one of the most well-known causes of inflammation in conditions such as rheumatism and arthritis. The prevention of protein denaturation is one of the key mechanisms of action of nonsteroidal anti-inflammatory drugs (NSAIDs), which may have a considerable impact on their antirheumatic effect [28,40]. In this study, the anti-inflammatory activity of Sf-AgNPs and Sf-AQSE were tested *in vitro* against the denaturation of bovine serum albumin. The anti-inflammatory activity of Sf-AgNPs and Sf-AQSE was found to be concentration-dependent within the concentration ranges studied and was compared to diclofenac sodium, a standard anti-inflammatory drug, as shown in Table 7. At the tested concentrations of 100, 200, and 500 µg/ml, Sf-AgNPs exhibited a lower inhibition rate than diclofenac sodium, but it was greater than the Sf-AQSE. Thus, the study reveals that Sf-AgNPs can prevent or delay protein denaturation, making them a good choice for an effective anti-inflammatory agent.

3.7. Cytotoxicity screening

The MTT assay was employed to evaluate the cytotoxic impact of Sf-AgNPs on the SK-MEL-5 cancer cell line, demonstrating a strong cytotoxic effect. The study also showed that SK-MEL-5 cancer cells administered with various concentrations (6.25, 12.5, 25, 50, 100 µg/ml) of Sf-AgNPs showed a dose-dependent reduction in cell viability (Fig. 6). The cell viabilities for SK-MEL-5 cancer cells at 6.25, 12.5, 25, 50, and 100 µg/mL of Sf-AgNPs were found to be 94 %, 86 %, 77 %, 61 %, and 46 %, respectively.

The phase contrast microscopic images showing the dose-dependent cytotoxic effect of Sf-AgNPs are shown in Fig. 7. The IC_{50} value for Sf-AgNPs against the SK-MEL-5 cancer cell line was determined as 85.71 µg/ml. These findings highlight the considerable cytotoxicity of *S. foetida* seed extract-derived AgNPs against the viability and proliferation of the SK-MEL-5 cancer cell line.

According to reports, the cytotoxicity of AgNPs to cell lines is brought about by the nanoparticles being taken up by cells via pinocytosis and endocytosis. It has been reported that nanoparticles are cytotoxic if cell viability falls below 50 %. AgNPs derived from plant extracts

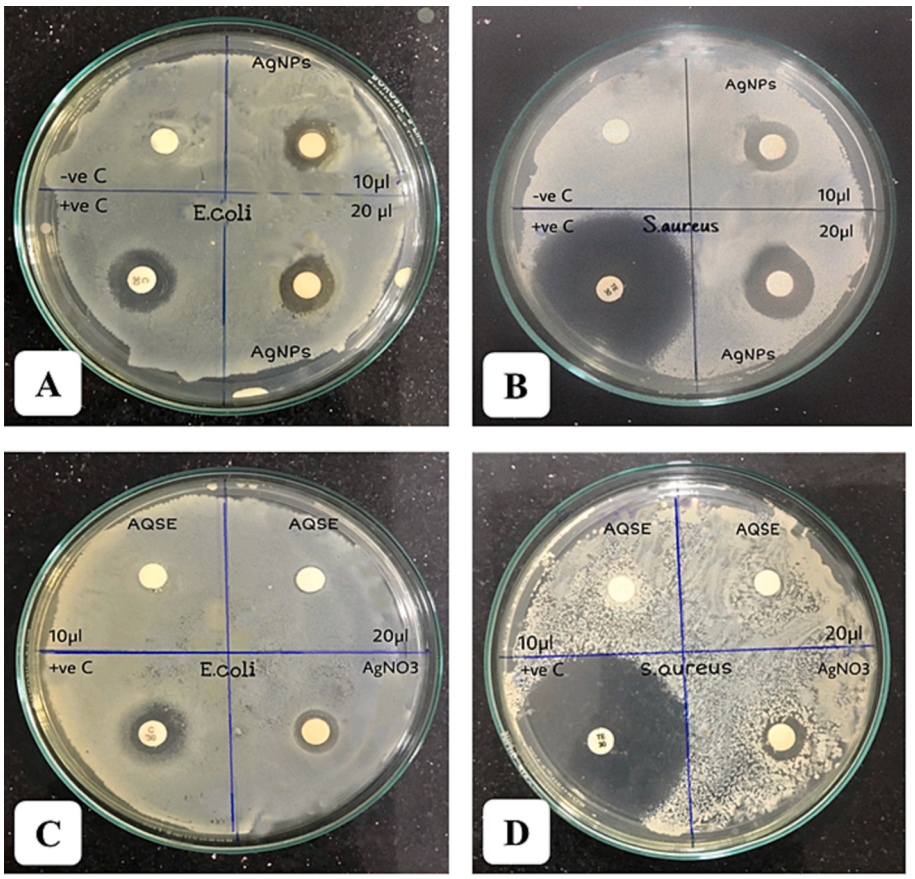

Fig. 5. Antibacterial activity of Sf-AgNPs, Sf-AQSE and $AgNO_3$. (A) Antibacterial activity of Sf-AgNPs tested against *E. coli*; (B) Antibacterial activity of Sf-AgNPs tested against *S. aureus*; (C) Antibacterial activity of $AgNO_3$ and Sf-AQSE against *E. coli*; (D) Antibacterial activity of $AgNO_3$ and Sf-AQSE against *S. aureus*; * -ve – Negative control; +ve – Positive control; AQSE - Aqueous seed extracts of *S. foetida.*

Table 5
Antibacterial activity of $AgNO_3$ and Sf-AQSE.

Sl. No.	Name of bacterial species	Zone of inhibition (mm)			
		Positive control (PC)	$AgNO_3$ (5 µl)	Sf-AQSE (10 µl)	Sf-AQSE (20 µl)
1	*Escherichia coli*	16.67 ± 0.89	10.00 ± 0.67[a]	Nil	Nil
2	*Staphylococcus aureus*	35.67 ± 0.44	12.17 ± 0.78[a]	Nil	Nil

*Values are mean ± SD (n = 3); [a] $p \leq 0.05$ versus positive control.

Table 6
Inhibition of proteinase activity exhibited by Sf-AQSE and Sf-AgNPs.

Treatment	% of inhibition		
	50 µg/ml	100 µg/ml	200 µg/ml
Sf-AQSE	9.47 ± 0.45[a]	23.17 ± 0.76[a]	53.50 ± 0.87[a]
Sf-AgNPs	17.17 ± 0.76[b]	27.27 ± 0.46[b]	63.43 ± 0.60[b]
Diclofenac sodium	24.17 ± 0.56[c]	35.73 ± 0.49[c]	72.60 ± 0.40[c]

*Values are mean ± SD (n = 3); Superscripts in the same column marked with different letters show significant statistical differences ($p \leq 0.05$).

increase the generation of free radicals, causing DNA damage and ultimately cell death. Remarkably, AgNPs generate hydroxyl radicals, rendering them particularly lethal to cancer cells. This oxidative stress caused by nanoparticle-induced free radicals leads to apoptosis through caspase-mediated and mitochondria-dependent pathways. This interaction underscores AgNPs' promise as potential agents for targeted

Table 7
Inhibition of protein denaturation by Sf-AQSE and Sf-AgNPs.

Treatment	% of inhibition		
	100 µg/ml	200 µg/ml	500 µg/ml
Sf-AQSE	19.10 ± 0.96[a]	39.90 ± 0.79[a]	58.93 ± 0.90[a]
Sf-AgNPs	22.57 ± 0.60[b]	42.83 ± 0.76[b]	69.80 ± 1.31[b]
Diclofenac sodium	28.67 ± 0.44[c]	59.83 ± 0.56[c]	73.77 ± 0.51[c]

*Values are mean ± SD (n = 3); Superscripts distinguished by different letters in the same column indicate significant statistical differences ($p \leq 0.05$).

cancer therapy [41–43].

Nanoparticles are used in a variety of interesting ways in medicine and pharmacy, which include medical imaging, filters, nanocomposites, drug delivery systems, and cancer treatment formulations. [38]. The synthesis of metal nanoparticles using plants is of immense significance to the nanobiotechnology research community since it offers various benefits over chemical, physical, and microbiological techniques. This method is well-suited for large-scale industrial applications owing to its speed, reproducibility, ecological friendliness, environmental compatibility, and cost-effectiveness. The use of biological extracts obtained from various plant components, particularly for the synthesis of AgNPs, has piqued the interest of many researchers [44]. The current study also established that the *S. foetida* seed-derived AgNPs have strong *in vitro* antioxidant, antibacterial, anti-inflammatory, and cytotoxic effects. Therefore, the results highlight the effectiveness of Sf-AgNPs in many biomedical applications, such as antibacterial, anti-inflammatory, antioxidant, and anticancer therapy, with prospective uses in the development of nanomedicine. They can also be incorporated into wound

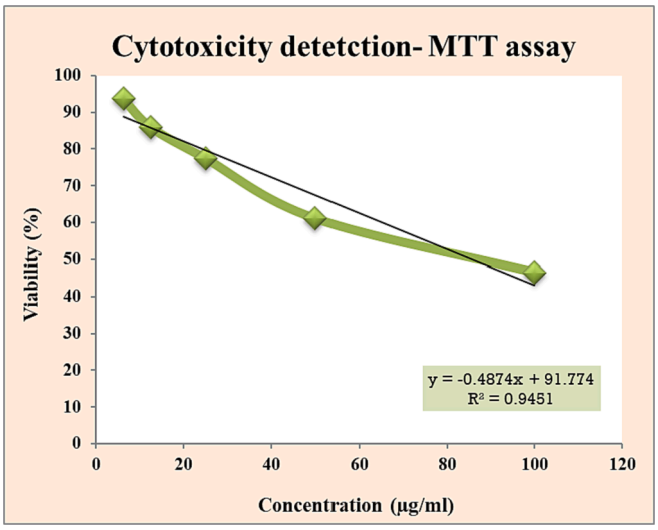

Fig. 6. Cytotoxic effect of Sf-AgNPs on SK-MEL-5 cancer cell line using MTT assay.

dressings and bandages. Additionally, these nanoparticles can be applied in various medical devices, such as catheters, prostheses, and vascular grafts, contributing to applications in tissue engineering. Moreover, their potential extends to diagnostic processes, including anti-permeability factors and bio-sensing. They may also have applications in dental care, such as in dental fillings, coatings, and materials [45]. While previous research has explored AgNP synthesis using various plant sources, this work is significant as it marks the first instance of synthesizing AgNPs from *S. foetida seeds*, using a green synthesis approach. The study's comprehensive characterization methods and exploration of the *in vitro* effects of Sf-AgNPs on antioxidant, antibacterial, anti-inflammatory, and cytotoxic activities further highlight its original contribution to the field.

4. Conclusion

In this study, the AgNPs were successfully synthesized using an aqueous extract of *S. foetida* seed and characterized using UV–Vis Spectroscopy, FTIR, and TEM. The green synthesis of AgNPs with *S. foetida* seed (Sf-AgNPs) is an economical and eco-friendly approach. These Sf-AgNPs outperformed the aqueous extract of the seed in terms of their significant antioxidant potential. They have also shown significant antibacterial action against *S. aureus* and *E. coli*. Additionally, by limiting protein denaturation and inhibiting inflammatory proteinases, they demonstrated anti-inflammatory potential. Most significantly, the Sf-AgNPs confirmed dose-dependent cytotoxicity against the cancerous SK-MEL-5 cells, highlighting the possibility of using these molecules as anti-cancer agents. Thus, the findings emphasize the efficacy of AgNPs synthesized from *S. foetida* seed extract in different biomedical applications, including antioxidant, antibacterial, anti-inflammatory, and anticancer therapy. The research on AgNPs synthesized from *Sterculia foetida* seed extract opens interesting prospects, including applications in biomedical fields such as wound healing and dressing, implanted materials, tissue engineering, and anticancer therapy, along with potential use in various medical devices like catheters, prostheses, and vascular grafts. However Further study is required to investigate their mechanisms of action, conduct *in vivo* studies, optimize synthesis methods, and assess biocompatibility before clinical translation.

Declaration of generative AI and AI-assisted technologies in the writing process.

During the preparation of this work, the author(s) used https://quillbot.com and https://chat.openai.com in order to improve the language and readability. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

CRediT authorship contribution statement

S. Farsana: Methodology, Investigation, Formal analysis, Data curation, Writing – review & editing. **P.N. Ansil:** Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Formal analysis, Conceptualization, Writing – original draft, Writing – review & editing. **S. Sumalekshmy:** Methodology, Formal analysis, Validation, Writing – review & editing. **S. Soumya:**

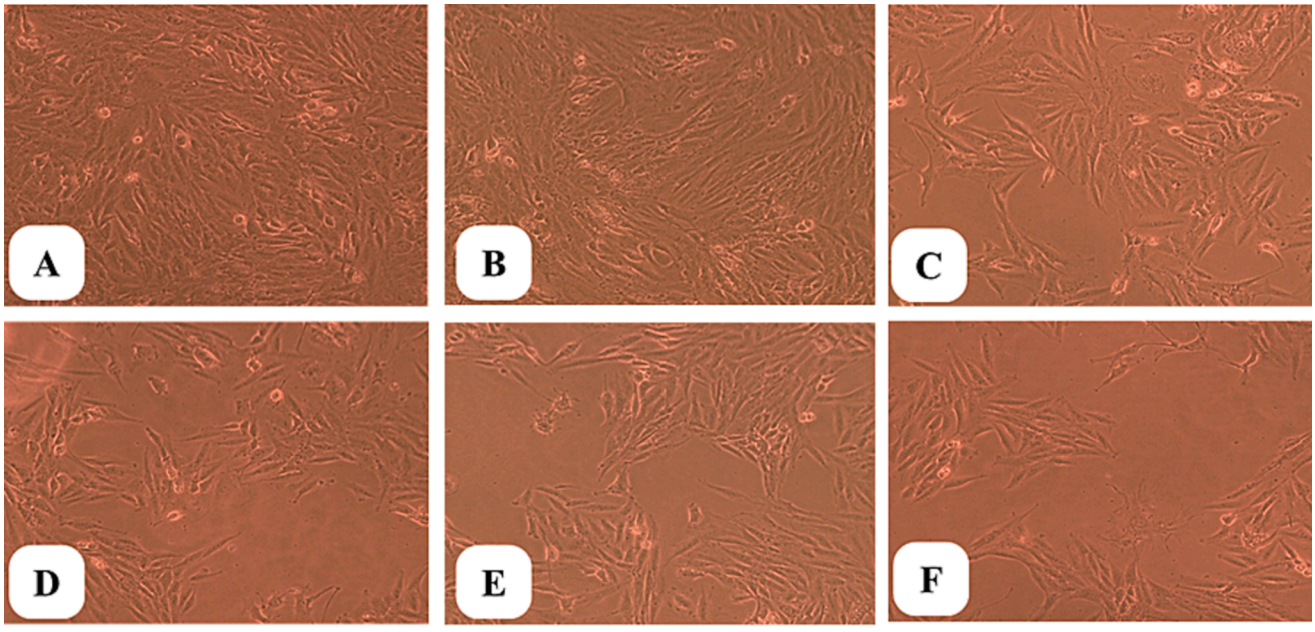

Fig. 7. Phase contrast microscopic images showing the cytotoxic effect of various concentrations of Sf-AgNPs in the SK-MEL-5 cell line. (A) Control, (B) 6.25 µg/ml, (C) 12.5 µg/ml, (D) 25 µg/ml, (E) 50 µg/ml, (F) 100 µg/ml.

Validation, Supervision, Project administration, Methodology, Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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