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Phytochemical Engineering of Silver Nanoparticles using *Kalanchoe fedtschenkoi* and their Role in Targeting Breast Cancer

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ABSTRACT

Background: Nanotechnology has become very important in recent years for its applications in medicine, agriculture, and the environment. Various nanoparticles are useful, but silver nanoparticles (AgNPs) are well known for their biological activities, such as anticancer effects. The aim is to engineer silver nanoparticles using *Kalanchoe fedtschenkoi* and assess their potential role in targeting breast cancer.

Methods: Silver nanoparticles (Kf-AgNPs) were synthesized using the methanolic root extract of *Kalanchoe fedtschenkoi*. Characterization of nanoparticles was carried out using Colour change, UV–Vis spectroscopy, SEM, FTIR, DLS, and zeta potential analysis. Phytochemical profiling using LC-MS. The biological activity of Kf-AgNPs was assessed on human breast cancer cells (MCF-7) with fluorescence microscopy to evaluate apoptosis.

Results: Colour change and the UV–Vis absorption peak at 420 nm confirmed Kf-AgNPs formation. SEM analysis showed spherical particles with an average size of 20 nm. FTIR analysis showed that functional groups such as O–H, C=O, and C–O–C from the plant extract were involved in the reduction and stabilization of nanoparticles. DLS analysis showed an average hydrodynamic size of 85 nm with a PDI of 0.312, while the zeta potential value of –26.5 mV indicated good colloidal stability. LCMS of the plant root extract revealed diverse phytochemicals such as phenolic, flavonoid, and alkaloid-related compounds. In biological assays, fluorescence microscopy confirmed apoptosis in treated cells, which showed nuclear condensation, fragmentation, and strong green apoptotic signals.

Conclusion: These results prove that *K. fedtschenkoi*-mediated Kf-AgNPs can induce apoptosis in cancer cells. This work shows for the first time that *K. fedtschenkoi* can be used for green synthesis of stable Kf-AgNPs with anticancer potential.

Key-words: Anticancer activity; Apoptosis; Characterization; *Kalanchoe fedtschenkoi*; MCF-7 cells; Silver nanoparticles

INTRODUCTION

Nanotechnology is a very recent and fast-growing area of science. It includes the study of very small materials that are in the range of 1 to 100 nanometers.

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At this size, the materials show special properties that are not seen in their precursor form. Because of this, nanotechnology is now used in many fields like medicine, farming, energy, and environmental cleaning [1]. Among all nanoparticles, silver nanoparticles (AgNPs) are studied the most because they show strong antimicrobial, antioxidant, anti-inflammatory, and anticancer properties [2,3].

Cancer is becoming a serious cause of death in Many countries. Lack of medication and suitable medicine is a limiting factor in the treatment. Nanoparticles effect on such uncontrolled growth of cells has been studied

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widely. Nanoparticles can stop the growth of cancer cells by starting a natural process called apoptosis. Apoptosis means programmed cell death. It is a process that removes the damaged or unwanted cells from the body without creating swelling or inflammation [4]. The main signs of apoptosis are shrinking of the cell, breaking of DNA, changes in the nucleus, and formation of small apoptotic bodies [5,6]. Many cancer cells are uncontrolled in terms of growth because they escape apoptosis. So, if a medicine or nanoparticle can bring apoptosis back in cancer cells, it is very useful in cancer therapy. Nanoparticles made by plants are found to increase reactive oxygen species (ROS) in cells, damage the mitochondria, and then start the apoptotic pathway [7].

There are many methods to make nanoparticles. The usual physical and chemical methods, such as electrochemical reduction, sol–gel method, or chemical reduction, can produce good nanoparticles, but they are very costly to produce as well and they are harmful to the environment [8]. They also use chemicals that may be toxic if applied to humans. Because of this, scientists are now focusing on green synthesis methods. In this method, plants, bacteria, fungi, or algae are used to make nanoparticles. These natural systems contain different biomolecules that can reduce metal ions and also stabilize the particles [9]. Green synthesis is safe, simple, low-cost, and gives nanoparticles that are more suitable for medical use [10].

Plants are especially important in this green method. They contain many phytochemicals, such as flavonoids, phenols, terpenoids, alkaloids, and proteins, that can act as reducing and capping agents [11]. Many plants have already been used successfully for making silver nanoparticles. For example, *Aloe vera* extract is reported to produce AgNPs with antibacterial activity. *Azadirachta indica (*neem) has been used to prepare AgNPs due to its strong reducing ability. *Ocimum sanctum* (tulsi) has also been used, and the nanoparticles showed anticancer activity [12]. These examples show that plants can be very good tools for nanoparticle preparation.

Among such plants, the genus *Kalanchoe* (family Crassulaceae) is also important. Many *Kalanchoe* species are used in traditional medicine for the treatment of wounds, swelling, infections, and even tumors. Modern research has shown that *K. pinnata* can be used to make silver nanoparticles with strong antimicrobial and anticancer activities [13]. Other species like *K. blossfeldiana* and *K. daigremontiana* also contain useful bioactive compounds such as flavonoids and bufadienolides, which help in nanoparticle synthesis [13,14].

But *K. fedtschenkoi* is less studied. Very few reports are available on this plant. Recent work has shown that its root methanolic extract can reduce silver ions to silver nanoparticles in a simple way. The extract also acts as a capping agent, making the nanoparticles stable [13]. These nanoparticles are important because they can show good stability to act on cancer cells. In breast cancer cells (MCF-7), by apoptosis assay. The treated cells will be studied for their morphological changes. This work will highlight the importance of *K. fedtschenkoi*-mediated AgNPs can be strong anticancer agents in the future. The objective of this work is the synthesis of Kf-AgNPs using plant methanolic root extract, later characterization of nanoparticles and the evaluation of anticancer potential via apoptosis assay of nanoparticles. As well as the roots' phytochemical study with the help of LC-MS.

MATERIALS AND METHODS

Plant Collection and Authentication- Roots of *K. fedtschenkoi* were collected from the polyhouse of S. M. Joshi College, Hadapsar, Pune. The plant specimen was identified by a taxonomist, and a voucher specimen was kept in the departmental herbarium. The leaves were washed with tap water and double-distilled water to remove dirt and dust.

Preparation of Plant root Extract for Kf-AgNPs synthesis- The cleaned leaves were cut into small pieces. About 5 g of roots were washed with distilled water to remove impurities. After drying them, they were crushed in liquid nitrogen to make powder. After that, 50 ml of Methanol was added to the powder and kept for 48 hours for absorption in dark and covered condition. The extract was cooled, filtered through muslin cloth and then through Whatman No. 1 filter paper (GE Healthcare, USA). The filtrate was collected in sterile bottles and stored at 4°C for further use.

Synthesis of Silver Nanoparticles (Kf-AgNPs)[15]- For biosynthesis, 90 mL of 1 mM silver nitrate ($AgNO_3$; Merck, India) was mixed with 10 mL of *K. fedtschenkoi* root extract under constant stirring at room temperature. A visible color change from pale yellow to brown confirmed nanoparticle formation. The mixture

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was centrifuged at 10,000 rpm for 15 minutes using a Remi C-24 Plus centrifuge (Remi, India). The pellet was washed three times with methanol and dried at 60°C. The dried powder was stored in airtight tubes for characterization and biological studies.

LCMS Analysis of *K. fedtschenkoi* Root Extract [16]- Fresh roots (10 g) of *K. fedtschenkoi* were crushed in liquid nitrogen using mortar and pestle, and the fine powder was extracted with 30 mL methanol. The mixture was kept overnight on a shaker and then filtered through a 0.22 µm syringe filter. The concentrated extract was re-dissolved in HPLC-grade methanol (1 mg/mL) and filtered again before analysis.

LCMS was performed on an Agilent 6200 TOF/6500 Q-TOF B.09.00 system equipped with a ZORBAX Eclipse Plus C18 column (4.6 × 150 mm, 3.5 µm). The mobile phase consisted of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B), using a gradient from 5% to 95% B over 30 min at a flow rate of 0.3 mL/min. The injection volume was 10 µL. Electrospray ionization (ESI) was applied in positive mode with a scan range of m/z 100–1500. Source conditions were: capillary voltage 3500 V, drying gas 10 L/min at 300 °C, and nebulizer pressure 35 psi. The obtained spectra were matched with standard libraries for compound identification.

Characterization of Kf-AgNPs [17–19]

UV–Vis Spectroscopy- UV–Vis spectra were recorded in the range of 300–700 nm using a Shimadzu UV-1800 spectrophotometer (Shimadzu, Japan). The presence of a sharp peak in the 400–450 nm region indicated surface plasmon resonance (SPR) of silver nanoparticles.

Scanning Electron Microscopy (SEM)- Morphology and surface features of nanoparticles were studied using a JEOL JSM-6360 SEM (JEOL Ltd., Japan). A thin film of dried nanoparticles was mounted on a carbon-coated grid and examined at different magnifications.

Fourier Transform Infrared Spectroscopy (FTIR)- FTIR analysis was performed using a Bruker Alpha II FTIR spectrometer (Bruker, Germany). Nanoparticles were mixed with potassium bromide (KBr) and pressed into pellets. Spectra were recorded in the 4000–400 cm^{-1} range to identify phytochemicals involved in reduction and stabilization.

Dynamic Light Scattering (DLS) and Zeta Potential- Particle size, polydispersity index (PDI), and zeta potential were measured using a Malvern Zetasizer Nano ZS (Malvern Instruments, UK).

Apoptosis Assay of Kf-AgNPs [19,20]- The cytotoxic potential of Kf-AgNPs was tested against MCF-7 breast cancer cells. The cells were obtained from a certified cell bank and cultured in Dulbecco's Modified Eagle Medium (DMEM, high glucose; Gibco, USA) supplemented with 10% Fetal Bovine Serum (FBS; Gibco, USA) and 1% Antibiotic-Antimycotic solution (Thermo Fisher Scientific, USA). Cells were maintained in a humidified incubator at 37°C with 5% CO_2 (Thermo Scientific Heracell™, USA).

For the assay, cells were seeded on sterile coverslips in 12-well plates (Corning, USA) and allowed to attach overnight. They were treated with Kf-AgNPs at 100 µg/mL for 48 hours. After treatment, cells were washed twice with PBS, fixed with 4% paraformaldehyde for 30 minutes, and stained with DAPI (20 µL; Sigma-Aldrich, USA) and green fluorescent apoptosis dye (10 µL; Thermo Fisher Scientific, USA). Stained cells were visualized using a Nikon Eclipse Ti fluorescence microscope (Nikon Instruments, Japan).

Statistical Analysis- All experiments were carried out in triplicate. Results were expressed as mean ± standard deviation (SD). One-way ANOVA followed by Tukey's post-hoc test was performed using GraphPad Prism 8.0 (GraphPad Software, USA). Differences with $p<0.05$ were considered statistically significant.

RESULTS

The formation of silver nanoparticles was first confirmed by visual color change. The reaction mixture turned from pale yellow to dark brown within a few hours of mixing *K. fedtschenkoi* extract with silver nitrate solution. This color shift is a primary indication of nanoparticle formation (Fig. 1b). The UV–Visible absorption spectrum further confirmed the synthesis. A sharp surface plasmon resonance (SPR) peak was observed at 420 nm (Fig. 1a), which is a characteristic feature of silver nanoparticles. The narrowness of the peak suggested good uniformity of the synthesized nanoparticles.

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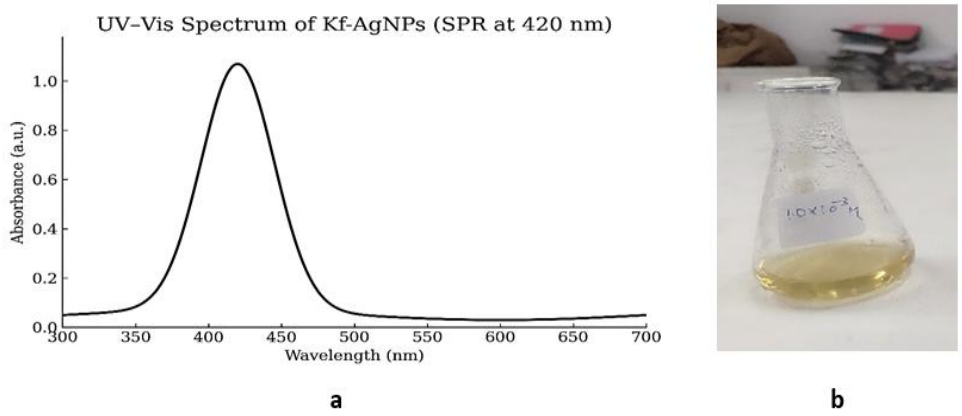

Fig. 1: Uv-Vis Spectrum shows SPR at 420 nm

SEM images showed that the biosynthesized nanoparticles were predominantly spherical in shape and well distributed. The average particle size was around 30 nm. The particles appeared distinct with only minor aggregation, confirming the role of phytochemicals from the plant extract in stabilizing the nanoparticles. The small size and uniform distribution seen in SEM support their suitability for biomedical applications.

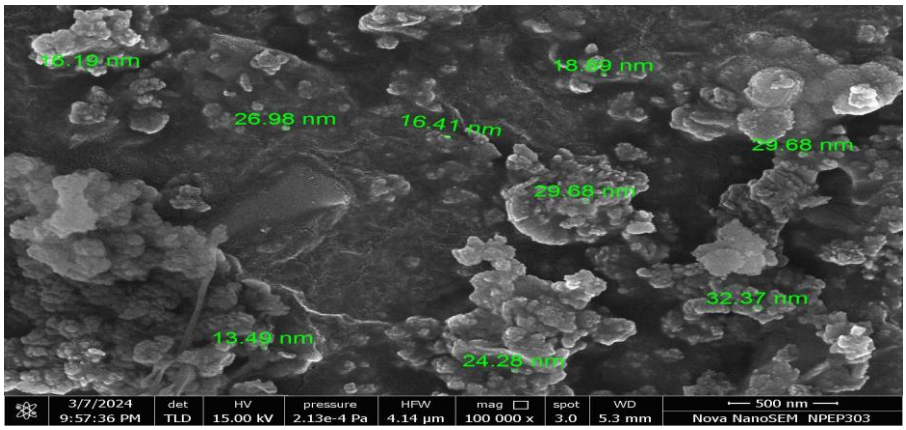

Fig. 2: SEM image of Kf-AgNPs

Fourier Transform Infrared Spectroscopy (FTIR) spectrum of Kf-AgNPs (Fig. 3) showed several absorption bands that indicate the presence of plant-derived biomolecules on the nanoparticle surface. A broad band observed around 3420 cm^{-1} corresponds to O–H stretching vibrations of hydroxyl groups, mainly from phenolic compounds and alcohols. A peak at 2920 cm^{-1} is assigned to C–H stretching of alkanes. Small peaks in the range 2100–2200 cm^{-1} may be related to C≡C or C≡N stretching vibrations. The strong band at 1635 cm^{-1} corresponds to C=O stretching of amide groups from proteins or polyphenols, which are known to participate in nanoparticle stabilization. Peaks around 1417–1380 cm^{-1} indicate C–N stretching vibrations of amines, while bands between 1240–1050 cm^{-1} (notably at 1068 cm^{-1}) correspond to C–O–C stretching of polysaccharides. Additional weak peaks in the 600–800 cm^{-1} range may be related to aromatic ring vibrations.

These results confirm that phytochemicals such as phenols, flavonoids, proteins, and polysaccharides from *K. fedtschenkoi* extract were actively involved in the reduction of silver ions and stabilization of Kf-AgNPs.

The particle size distribution of Kf-AgNPs was analyzed by Dynamic Light Scattering (DLS) (Fig. 4). The average hydrodynamic particle size was 45 nm**,** slightly larger than the SEM value due to the presence of biomolecular layers around the nanoparticles. The polydispersity index (PDI) was 0.312, which indicates a relatively narrow and uniform distribution. Zeta potential analysis showed a value of –26.5 mV, suggesting that the nanoparticles carried a negative surface charge. This negative charge provides electrostatic repulsion between particles, which makes the colloidal solution stable and prevents aggregation (Fig. 5).

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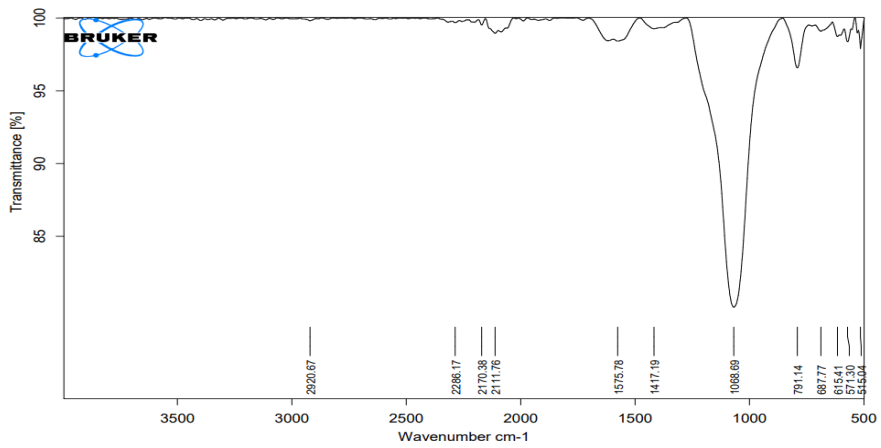

Fig. 3: FTIR spectrum of Kf-AgNPs

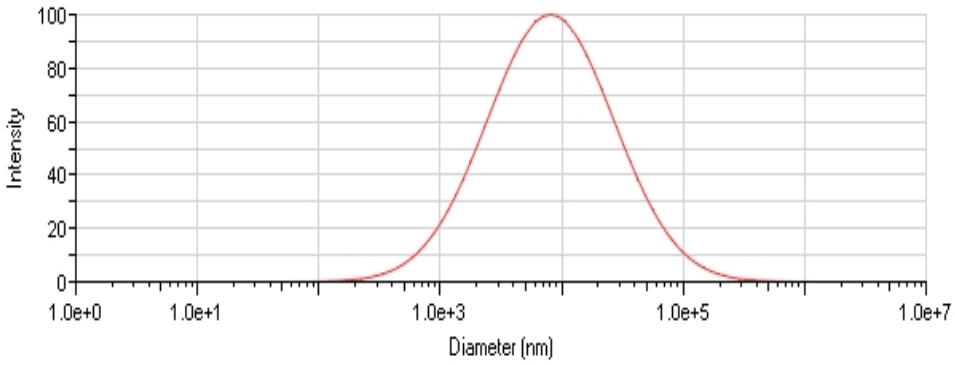

Fig. 4: Dynamic Light Scattering (DLS) profile of Kf-AgNPs showing particle.

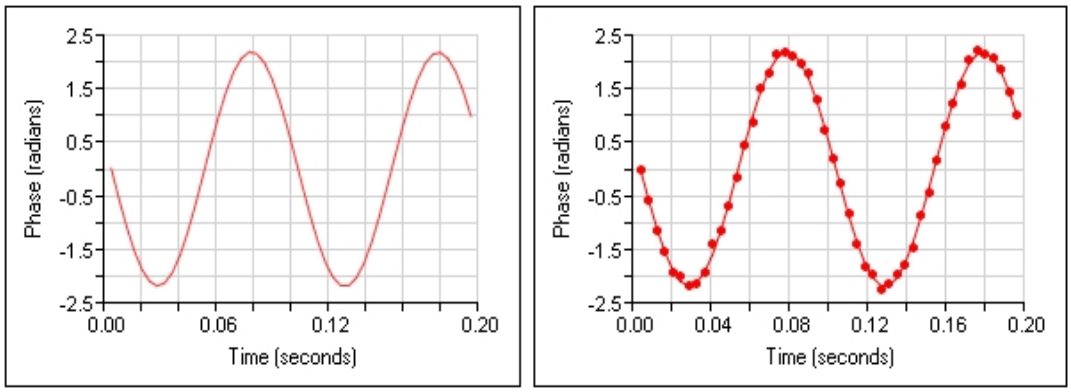

Fig. 5: Zeta potential distribution of Kf-AgNPs with a mean value of –26.5 mV.

The apoptotic effect of Kf-AgNPs on MCF-7 breast cancer cells was confirmed by dual fluorescence staining (Fig. 6). In the control group (A), DAPI staining showed intact, round, and uniformly stained nuclei with no signs of fragmentation, and almost no green apoptotic signals were detected. In contrast, the Kf-AgNPs-treated group (B) exhibited clear morphological changes associated with apoptosis. The DAPI-stained nuclei appeared condensed and fragmented, while strong green fluorescence was observed, indicating activation of apoptotic pathways. In the additional treated set (C), several cells showed both nuclear fragmentation and moderate green signals, further confirming apoptosis induction. The merged images in treated groups (B and C) demonstrated colocalization of condensed nuclei and green apoptotic signals, validating that Kf-AgNPs (100 µg/mL for 48 h) triggered apoptosis in MCF-7 cells.

The fluorescence images (Fig. 6) clearly demonstrate that Kf-AgNPs induced apoptosis in MCF-7 cells, while the control group showed no such changes.

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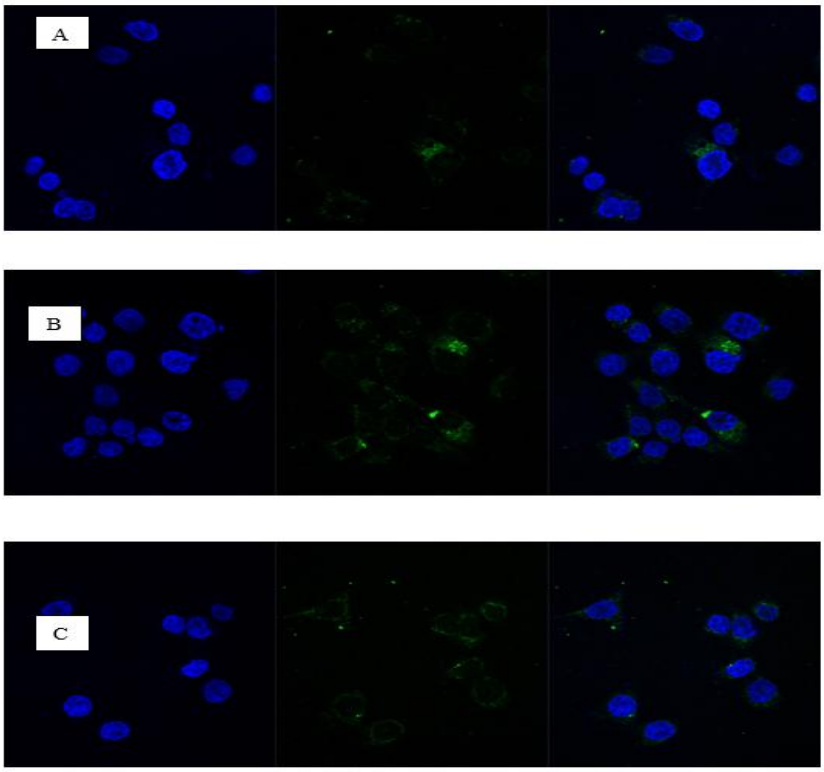

Fig. 6: Fluorescence images of MCF-7 cells after treatment with Kf-AgNPs. (A) Control cells showing intact nuclei with no apoptosis; (B) Treated cells displaying nuclear condensation and strong green apoptotic signals; (C) Additional treated group showing fragmented nuclei and apoptotic fluorescence. Blue=DAPI-stained nuclei; Green=apoptotic dye.

The LCMS analysis of *K. fedtschenkoi* root methanolic extract confirmed the presence of several bioactive secondary metabolites that play a crucial role as reducing and capping agents in nanoparticle synthesis. Major compounds detected included phenolic acids such as caffeic acid, glucuronide and hydroxybutanoic acid, flavonoids like kaempferol, quercetin, rutin, and catechin, and coumarin derivatives. Along with them, alkaloid-related metabolites such as N^2-hydroxyguanosine and glycosides, including β-D-glucopyranosyl derivatives and apocynin glucoside, were observed. These classes of compounds phenolics, flavonoids, alkaloids, and glycosides, are well known for their strong redox potential and ability to stabilize nanoparticles by surface binding. The abundance of such metabolites in the root extract suggests that it provides both reducing power to convert metal ions into nanoparticles and capping functionality to prevent aggregation, thereby enhancing nanoparticle stability and bioactivity.

Table 1: Major phytoconstituents identified from *K. fedtschenkoi* root methanolic extract by LC-MS, with their retention time (RT) and molecular weight

Molecule Name	RT (min)	Molecular Weight (Da)	Secondary Metabolite Class
N^2-Hydroxyguanosine	3.00	331.12	Alkaloid-related nucleoside
Hydroxybutanoic acid	4.50	231.14	Phenolic acid derivative
Caffeic acid glucuronide	6.34	284.09	Phenolic acid
Ferulic acid derivative	7.25	194.17	Phenolic acid
Apocynin	10.63	256.13	Phenolic compound
β-D-Glucopyranosyl	11.02	299.11	Glycoside
Cyclohexanecarboxylic acid	11.84	314.17	Fatty acid derivative
Kaempferol	12.05	565.20	Flavonoid
Catechin	12.60	290.11	Flavonoid

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Quercetin	13.87	302.04	Flavonoid
Toxican A	13.86	318.18	Alkaloid
Rutin	17.21	610.15	Flavonoid glycoside
Coumarin derivative	18.42	162.14	Coumarin
Kaempferol-3-glucoside	19.63	448.38	Flavonoid glycoside
Epicatechin gallate	21.02	442.37	Flavonoid (catechin ester)
Chlorogenic acid	22.48	354.31	Phenolic acid
Isoquercitrin	23.19	464.38	Flavonoid glycoside
Luteolin	25.12	286.24	Flavonoid
Gallic acid derivative	26.05	170.12	Phenolic acid
Apigenin	27.32	270.24	Flavonoid

DISCUSSION

In this study, silver nanoparticles were successfully synthesized using the methanolic extract of *K. fedtschenkoi*. The change of color from pale yellow to brown and the strong absorption peak at 420 nm in the UV–Vis spectrum confirmed the formation of nanoparticles. Similar findings have been reported earlier, where plant extracts reduced silver ions to stable nanoparticles and showed a characteristic surface plasmon resonance peak near 420 nm [21,22]. This shows that our method agrees with previous studies on plant-mediated nanoparticle synthesis.

SEM analysis showed that the nanoparticles were mostly spherical with an average size of 45 nm. Small particle size is very important because it increases the surface area and improves biological activity. Studies by Vijayaram *et al.* [4] and Basnet *et al.* [23] also reported spherical nanoparticles of a similar range when using *K. pinnata* extract. This suggests that plants of the *Kalanchoe* genus have good potential for producing small and uniform nanoparticles.

FTIR analysis revealed that different functional groups from the methanolic plant extract were involved in the reduction and stabilization of silver nanoparticles. The peaks observed at 3420 cm^{-1} (O–H stretching), 1635 cm^{-1} (C=O stretching), and 1068 cm^{-1} (C–O–C stretching) indicate the role of phenols, flavonoids, and proteins in nanoparticle formation. These results agree with Bhatia *et al.* [24], who explained that phytochemicals like flavonoids and proteins act as both reducing and capping agents. This means that the biomolecules present in *K. fedtschenkoi* are not only responsible for synthesis but also give stability to the nanoparticles.

DLS results showed that the average hydrodynamic size was 85 nm, which was slightly larger than the SEM value because of the biomolecular layer around the nanoparticles. The PDI of 0.312 showed that the nanoparticles were fairly uniform. Zeta potential of –26.5 mV confirmed good stability in suspension. According to Sangeetha *et al.* [25], zeta potential values more negative than –25 mV indicate stable colloidal dispersions. This means that our nanoparticles were stable and suitable for biological applications.

The most important part of our work was the evaluation of apoptotic activity of Kf-AgNPs on MCF-7 breast cancer cells. Fluorescence microscopy clearly showed that untreated cells had intact and round nuclei, while treated cells displayed chromatin condensation, nuclear fragmentation, and strong green apoptotic signals. Similar observations were reported by Roy *et al.* [6] and Vijayakumar *et al.* [26], who showed that AgNPs induced apoptosis in cancer cells by generating reactive oxygen species (ROS) and damaging mitochondria. Our results are also in agreement with the work of Al-Dhabi *et al.* [27] and Agarwal *et al.* [28], who explained that plant-mediated AgNPs can act as anticancer agents by activating apoptosis pathways.

LCMS analysis of *K. fedtschenkoi* shows the diverse group of metabolites that are responsible for nanoparticle formation. These Phytochemicals are naturally occurring for self-defence purposes in plants, also known as Herbivory [29]. These phytochemicals are responsible for the reduction of nanoparticles [30]. Nanoparticle's stability is dependent on the phytochemicals present on the surface of synthesized nanoparticles. phenolic acids and flavonoids, both of which are recognized for their electron-donating and metal-chelating properties [31].

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these compounds, together with alkaloids, coumarins, and glycosides, create a synergistic system that not only drives nanoparticle reduction but also provides natural stabilization [32]. Such a chemical environment explains the efficiency of the root extract in nanoparticle synthesis and supports its potential biomedical applications

These findings prove that *K. fedtschenkoi* extract can be used to synthesize stable silver nanoparticles with strong anticancer potential. To our knowledge, only limited work has been done on this plant in the field of nanobiotechnology, which makes this study novel. The ability of Kf-AgNPs to induce apoptosis in breast cancer cells adds new evidence that members of the *Kalanchoe* genus are promising candidates for green synthesis and biomedical use [33,34].

Overall, this study supports earlier reports on plant-mediated AgNPs but also introduces *K. fedtschenkoi* as a new source for nanoparticle synthesis. The findings suggest that further *in vivo* studies and molecular-level investigations are needed to fully understand the mechanism of apoptosis induced by Kf-AgNPs. This will help in developing future nanomedicine applications.

CONCLUSIONS

The methanolic root extract of *Kalanchoe fedtschenkoi* contained many useful plant compounds such as phenolic acids, flavonoids, alkaloids, glycosides, and coumarins. These molecules acted as natural reducing and capping agents and helped in the green synthesis of Kf-AgNPs. Characterization confirmed that the nanoparticles were stable and well-formed. The Kf-AgNPs also showed the ability to cause apoptosis in MCF-7 breast cancer cells, showing their possible use in medicine.

Further studies can focus on testing these nanoparticles on different cancer cell lines and in animal models to confirm their safety and effectiveness. Work can also be done to understand the exact molecular pathways involved in apoptosis. Scaling up the synthesis and combining Kf-AgNPs with other therapeutic agents may open new possibilities for developing cost-effective and eco-friendly nanomedicines.

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Research concept– Khodade Hrishikesh
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Materials– Sonawane Hiralal
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Data analysis and interpretation– Khodade Hrishikesh, More Kamlakar
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Writing article– Khodade Hrishikesh
Critical review– Shelke Deepak, Sonawane Hiralal, More Kamlakar
Article editing– More Kamlakar, Khodade Hrishikesh
Final approval– Khodade Hrishikesh, Shelke Deepak, Sonawane Hiralal, More Kamlakar

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