J Herbmed Pharmacol. 2024; 13(2): 260-268.

http://www.herbmedpharmacol.com doi: 10.34172/jhp.2024.49325

Journal of Herbmed Pharmacology

Anticancer activity of silver nanoparticles synthesized from aqueous leaf extract of *Solanum trilobatum* (Purple fruit pea eggplant) on human oral cancer cells

Anuradha Ganesan[1*], Gautham Kumar N[2], Prabhu Manickam Natarajan[3,4,5]

[1]Department of Oral Medicine & Radiology, SRM Dental College, Bharathi Salai, Chennai-89, India
[2]Department of Periodontics, Madha Dental College & Hospital, Kundrathur, Chennai-69, India
[3]Department of Periodontics College of Dentistry, Ajman University, Ajman, UAE
[4]Department of Clinical Sciences, Ajman University, Ajman, UAE
[5]Center of Medical and Bio-allied Health Sciences and Research, Ajman University, Ajman, UAE

ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 24 October 2023
Accepted: 25 January 2024

Keywords:
Silver nanoparticle
Solanum trilobatum
Antineoplastic agents
Mouth neoplasms
Aqueous extracts

ABSTRACT

Introduction: This research explores the capability of silver nanoparticles (AgNPs) produced via friendly methods by using *Solanum trilobatum* leaf extract. The choice of *S. trilobatum* was supported by its diverse phytochemical makeup, which comprises recognized bioactive elements known for their anti-inflammatory and anti-cancer attributes. This research explores the effect of AgNPs derived from this plant as a promising eco-friendly strategy in oral cancer treatment.

Methods: The synthesis of AgNPs involved employing *S. trilobatum* leaf extract, with observable color changes and spectral analyses confirming the unique characteristics of the nanoparticles. Fourier transform infrared spectroscopy detected distinct functional groups, whereas scanning electron microscopy (SEM) confirmed the presence of biocapped nanoparticles exhibiting various shapes and sizes spanning from 100 to 300 nm.

Results: Cytotoxicity assessments via the 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay revealed a half-minimal inhibitory concentration (IC50) value of 3.715 ± 0.242 µg/mL against the oral cancer (KB) cell line, indicating a significant inhibition. Acridine orange/ethidium bromide (AO/EtBr) and reactive oxygen species (ROS) assays further supported the AgNPs' anti-cancer potential, affirming their promise for oral cancer therapy.

Conclusion: AgNPs synthesized from *S. trilobatum* leaf extract showed substantial potential as a therapeutic agent for oral cancer. This research adds to the increasing evidence endorsing the use of environmentally friendly synthesized nanoparticles in medical treatments.

Implication for health policy/practice/research/medical education:
Findings from this study unveiled the prospective significance of eco-friendly synthesis, employing silver nanoparticles derived from *Solanum trilobatum* leaf extract, showcasing its promise as a safe and potent anti-cancer agent without notable toxicity concerns for future applications.

Please cite this paper as: Ganesan A, Kumar NG, Natarajan PM. Anticancer activity of silver nanoparticles synthesized from aqueous leaf extract of *Solanum trilobatum* (Purple fruit pea eggplant) on human oral cancer cells. J Herbmed Pharmacol. 2024;13(2):260-268. doi: 10.34172/jhp.2024.49325.

Introduction

Nanotechnology, a fast-growing field with diverse applications, is very promising, especially in health care (1). First introduced by Feynman in 1959, nanotechnology encompasses the creation, handling, and visualization of nanostructures sized between 1 to 100 nm. The enhanced properties of nanoscale particles and their better atomic interactions on their surfaces have given successful results in comparison with conventional systems (2). Silver nanoparticles (AgNPs) stand out as highly promising metal nanomaterials, garnering significant focus due to their unique physicochemical characteristics

***Corresponding author**: Anuradha Ganesan,
Email: anug77@yahoo.com

and exceptional antibacterial capabilities in recent years (3,4). AgNPs possess notable properties due to their diversity in the form of magnetic and optical polarization, catalysis, electrical conductivity, surface-enhanced Raman scattering (SERS), and most importantly antimicrobial and cytotoxic activities (5). Numerous approaches, encompassing both physical and chemical methods, have been explored in the synthesis of AgNPs. However, the environmental risks posed by their by-products and the significant expenses involved in manufacturing are pivotal concerns. As a result, environmentally sustainable techniques employing diverse biomolecules such as microorganisms, plants, membrane templates, viruses, DNA, and diatoms have been investigated for the eco-friendly production of AgNPs.

Having known about various methods of producing eco-friendly AgNPs, the most stable, easily accessible, cost-effective, and highly biocompatible method is the use of plant-mediated synthesis. The use of plant extracts is preferred as they are rapid with single-step methods used during the biosynthesis process. Current advancements have provided numerous methods to obtain increased productivity of nanoparticles that possess variable shapes, sizes, and stability. The properties relevant to the mechanical, magnetic, and chemical nature of the obtained nanoparticles relate to the shape and size, further surface area, and surface charge. These parameters are determined by adopting various strategies such as UV-vis spectroscopy (UV-vis), Fourier transform infrared (FTIR) analysis, scanning electron microscopy (SEM), transmission electron microscopy (TEM), etc (6,7).

Solanum trilobatum, a well-known tropical plant from the family *Solanaceae* is abundant in China, Vietnam, Thailand, India, Indochina to Indonesia. *S. trilobatum* stands as a significant reservoir of numerous pharmacologically vital compounds, including the steroidal hormone solasodine, solasonine, diosgenin, and a range of beneficial alkaloids. Researchers have extensively examined this plant for its diverse pharmacological functions, such as hepatoprotective properties, anti-inflammatory effects, antimicrobial actions, hemolytic activity, immunomodulation, and antibacterial potential, among others. *S. trilobatum* plant extracts have been skillfully integrated into diverse formulations and tailored to address a spectrum of health conditions like rheumatism, pain management, detoxification, hepatitis, and cirrhosis. Plants of the same family have been vastly elucidated for various therapeutic applications and possess phyto-insecticidal and cytotoxic properties (8,9).

On the other hand, oral cancer is still a challenge worldwide and is a leading cause of death, with its prevalence very common in developing countries, where the use of tobacco is considerably high. Current treatment approaches include radiation, chemotherapy, immunotherapy, and hormone-based therapies along with surgical procedures. Though we have all these advancements, the success rate in treating oral cancer and the various complications due to treatment remain a major concern. The conventional treatments do not still provide a complete cure to the ailment but instead, leave traces of short- to long-term side effects. With all these pitfalls in the current scenario, it is important for an alternative medicinal strategy with minimal side effects (10,11). In recent decades, many researchers have done extensive research studies on utilizing and identifying different medicinal plants for alternative treatment strategies. Medicinal plants have various types of components that exhibit different kinds of biological activities in treating various types of disease conditions (12).

Solanum trilobatum is a well-known medicinal plant that is applied to various traditional medicines. Some studies on the cytotoxic potential of *Solanum* species are available in the current literature but no reports are available on its anti-cancer potential in oral cancer. Therefore, the focus of this investigation was to utilize the aqueous leaf extract of *S. trilobatum* for the eco-friendly production of AgNPs and assess their in-vitro anticancer effects on human oral cancer cells.

Materials and Methods

Processing of leaves aqueous extract

Solanum trilobatum leaves were purchased from a utilized center in Tenkasi, Tamil Nadu, India, and were identified by a Professor of Botany at a government University in Tenkasi, Tamil Nadu. A voucher specimen (No. VSN: 4548) was deposited there. The procedure involved taking the leaves, washing them with tap water, and subsequently rinsing them with double distilled water (DDH2O) before finely chopping them into small pieces. The leaves were dried in the shade and powdered. Ten grams of the powdered plant sample was added to 100 mL DDH$_2$O and bubbled at 80°C for 10 minutes. The gathered extract underwent filtration using Whatman no. 1 filter paper, with the resulting filtrate collected in a 250 mL Erlenmeyer flask (Figure 1a). The extractions were stored at room temperature to be used for further studies. Then, 50 mL of *S. trilobatum* leaf extract was added to 50 mL of 1 mM silver nitrate (AgNo3) solution at room temperature and the reduction of AgNPs was observed (Figure 1b).

Biosynthesis of silver nanoparticles

The prepared plant extract and aqueous silver nitrate solution were mixed in a 1:20 ratio and incubated until the color change was observed (15-20 minutes). Then, the extract was washed by centrifugation at 7000 rpm for 20 minutes followed by resuspension of the pellet obtained in 1x phosphate buffered saline (PBS) to clear any uncoordinated biological molecules. The dissolved pellet was refrigerated for further analysis.

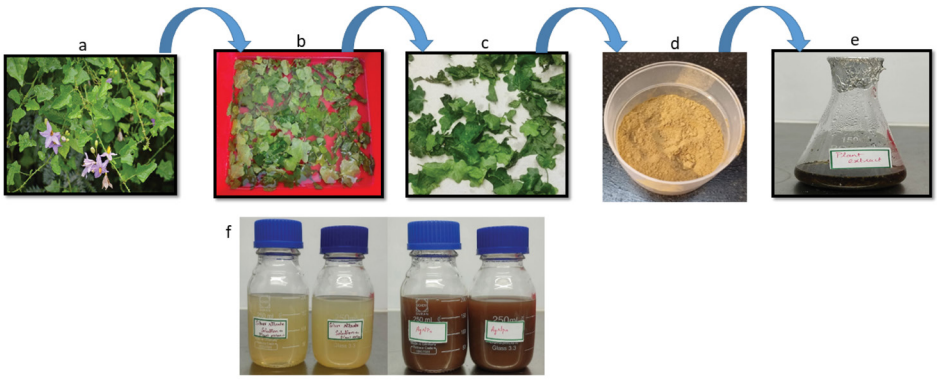

Figure 1. Green synthesis of silver nanoparticles (AgNPs) using *Solanum trilobatum* leaves extract. (a) Fresh leaves procured, (b) leaves washed with tap water, followed by double distilled water, (c) dried in the shade, and (d) powdered; (e) *Solanum trilobatum* leaf extract collected in a 250 mL Erlenmeyer flask; (f) Colour change before AgNPs synthesis and after AgNPs synthesis.

Analysis and characterization of the synthesized AgNPs

The color change was recorded as a preliminary confirmation of AgNPs synthesis before proceeding into UV-vis spectroscopic analysis. Based on their surface plasmon resonance (SPR), shape, size, and distribution, the preliminary characterization of AgNPs was conducted.

TEM analysis and energy dispersive X-ray spectroscopy (EDX)

The characterization of the synthesized AgNPs' morphology and size involved TEM analysis. Initially, AgNPs were infused with methanol, followed by dehydration and thorough grinding to create a powdered form. This powder was then transformed into a thin film on carbon-coated copper TEM grids. To enhance stability, the film underwent treatment with 1% osmium tetra-oxide and was left to stand for 2 hours using Hitachi H-600 TEM equipment.

Additionally, another thin film sample was produced on a carbon-coated copper grid and dried to facilitate analysis at various resolutions utilizing an SEM (JEOL-6390). The elemental composition of the reduced ion AgNPs was examined via EDX. For EDX analysis, the samples were prepared on a carbon-coated copper grid using equipment from Bruker AXS Inc. USA, specifically the Quantax-200.

UV-vis spectrophotometry

Metal nanoparticles get absorbed into the visible spectrum due to the SPR. Thus, UV-vis spectroscopy is a preliminary method to study the formation of metal nanoparticles and proves to be a very useful tool to analyze nanoparticles. The biological lessening of silver ion (Ag+) ions by using the leaves extracts of *S. trilobatum* was checked from time to time by the selection of 1 µL aliquots, and the UV-vis absorbance spectrum was captured on the UV-visible spectrophotometer at a wavelength range of 220-700 nm.

Scanning electron microscopy

SEM analysis was conducted to study the topological analysis of biosynthesized AgNPs. Accelerated voltage was maintained to 10 kV, and the working distance adjustment was done to around 3 mm. The required contrast and brightness were adjusted per the optimal values to distinguish the background.

FTIR spectroscopy

FTIR spectral analysis of AgNPs provides the details on the development of crystalline nanoparticles and determines the functional groups present in the sample. The profile is determined based on the absorption spectrum in the form of peaks that represent the presence of components in a higher concentration that show the presence of different bond types and functional groups (e.g., ketones, alkanes, halides, and amines), which absorbs infra-red (IR) radiation of different wavelengths. FTIR analysis was done using the instrument Shimadzu FTIR 8400 ranging from 4000 to 400 cm^{-1}.

Cell culture maintenance

The KB oral cancer cell lines were obtained from the National Centre for Cell Sciences (NCCS), Pune, India, and maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 µg/mL) under conditions of 5% CO_2 at 37 °C.

3-[4,5-Dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay

The MTT assay in this experiment was performed by seeding KB cells at a density of 1×10^4 cells/mL in 96-well plates. After 24-hour incubation, the cells were exposed to varying concentrations (50 to 500 µg/mL) of the samples for 24 hours at 37 °C. Following incubation, the MTT dye (5 mg/mL in PBS) was added, and after a 4-hour incubation at 37 °C, formazan precipitates were dissolved in 100 µL of concentrated dimethyl sulfoxide (DMSO). Cell viability was measured at 540 nm using a multi-well plate reader, and the results were expressed as percentages

relative to the control. IC50 values were computed from dose-responsive curves representing 50% cytotoxicity compared to vehicle control cells, with experiments conducted in triplicate.

Measurement of apoptotic induction using acridine orange/ethidium bromide (AO/EB) dual staining method

To assess apoptotic induction, KB cells were treated with glucose NPs at concentrations of 10 and 12.5 µg/mL for 24 hours. Following treatment, cells were detached, washed with cold PBS, and stained with a mixture of AO (100 µg/mL)/EB (100 µg/mL) at a ratio of 1:1 for 5 minutes at room temperature. Fluorescence microscopy at 20x and 40x magnifications was used to observe stained cells and count apoptotic cells relative to the total cell count.

DAPI (4′,6-diamidino-2-phenylindole) staining for nuclear apoptosis

For DAPI staining to examine nuclear apoptosis, KB cells (5×10^4) were seeded in 6-well plates, treated with test samples (2 µg and 4 µg), and incubated for 24 hours. Cell morphology was observed using phase contrast microscopy. Further, the fixed cells were permeabilized and incubated with DAPI (0.5 µg/mL). Then, the apoptotic nuclei were visualized under a fluorescent microscope with excitation at 359 nm and emission at 461 nm wavelengths.

Detection of reactive oxygen species (ROS) using carboxy-2′,7′-dichlorodihydrofluorescein diacetate (carboxy-H2DCFDA)

After 24-hour treatment with 2 and 4 µg concentrations of the samples, carboxy-H2DCFDA (1µM) was added to a culture medium for 30 minutes at 37 °C. Subsequently, the cells were washed, protected from light, and visualized under a fluorescence microscope.

These experiments were conducted in accordance with the described methodologies to evaluate the cytotoxic, apoptotic, and ROS-inducing effects of the tested samples on KB oral cancer cells.

Results

AgNP synthesis was successfully achieved using aqueous leaf extracts of *S. trilobatum*. After storage at room temperature, when 50 mL of 1mM $AgNO_3$ solution was mixed with the leaf extract, a reduction of $AgNP_3$ was noted through visual color changes in less than 20 minutes.

Analysis and characterization of biosynthesized silver nanoparticles

AgNPs (TEM) analysis and EDX

TEM analysis revealed that the AgNPs synthesized from aqueous leaf extracts of *S. trilobatum* had spherical shape morphology and an average particle size of 20 nm (Figure 2). The EDX analysis of elemental composition exhibited

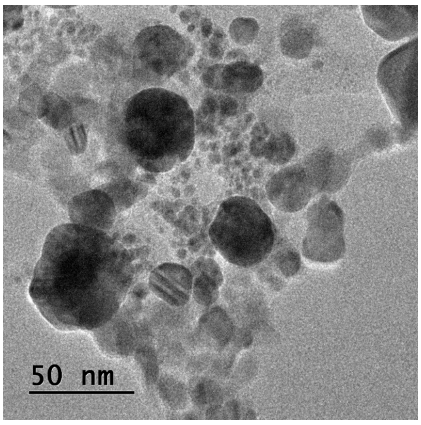

Figure 2. Transmission electron microscopic (TEM) micrograph of biosynthesized AgNPs at 50 nm scale.

a prominent signal solely from the silver (Ag) region, affirming the pure crystalline nature of the particles composed entirely of silver (Figure 3).

UV-visible spectroscopy

The UV-visible spectrophotometry of the $AgNP_3$ solution presented a strong optical density peak at around 500 nm (Figure 4a), which is the typical SPR peak of AgNPs and thus it confirms the formation of AgNP synthesis.

FTIR spectroscopy

FTIR analysis (Figure 4b) clearly shows that the participation of polyphenols available in the plant extract is mostly responsible for the biological reduction of silver ions (Ag+) into silver nanoparticles (Ag0) along with other bio-compounds such as flavonoids, phenols, alcohols, aromatics, and alkaloids. They also play a major role in stabilizing AgNPs.

Scanning electron microscopy

SEM images of AgNPs prepared from the *S. trilobatum* leaf extract showed that the sizes of the primary synthesized particles were in the nano-size range (Figure 5).

MTT assay

An in vitro cytotoxicity assay was done to detect the potentially toxic compounds that affect the basic cell morphology and functions. Cell proliferation was evaluated by MTT assay with varying concentrations (ranging from 1–8 µg/mL). The rate of absorbance was determined at 525 nm, depicting viable control cells which were untreated. However, the viability gradually decreased with increasing concentrations of the AgNPs (Figure 6a). The IC_{50} of AgNPs was 3.715 ± 0.242 µg/mL, particularly against the oral cancer cells.

Measurement of apoptotic induction using AO/EB dual staining method

Oral cancer (KB) cells, treated with 2 and 4 µg/mL

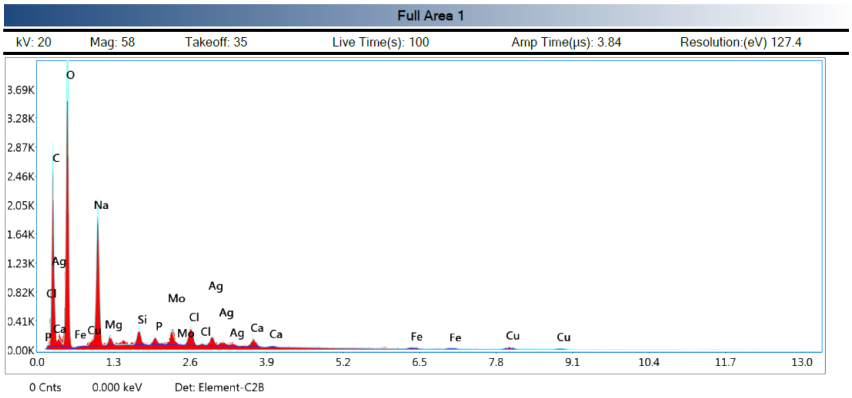

Figure 3. Energy-dispersive X-ray spectroscopy (EDX) spectrum of synthesized silver nanoparticles.

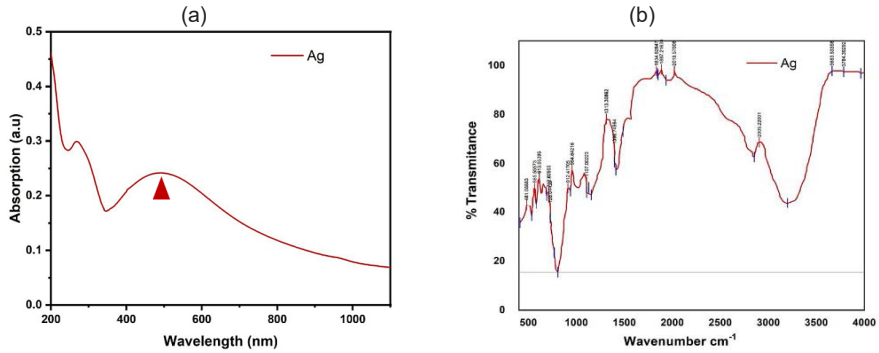

Figure 4. Nano-characterization of silver nanoparticles (AgNPs); (a) ultraviolet-visible spectroscopy analysis of AgNPs showed surface plasmon resonance peak at 500 nm; (b). FTIR (Fourier transform infrared analysis) spectrum of the AgNPs synthesized by the reduction of silver nitrate with the *S. trilobatum* leaf extract.

for 24 hours and stained with dual dye AO/EB, were analyzed by fluorescence microscopy. Living cells showed green fluorescence with normal nuclear appearance. Yellow fluorescence was seen in early apoptotic cells with condensed chromatin with fragmented nuclei. Late apoptotic cells exhibited an orange fluorescence, indicating chromatin condensation or fragmentation within the cell nucleus, resulting in a uniform red-orange staining pattern (Figure 6b).

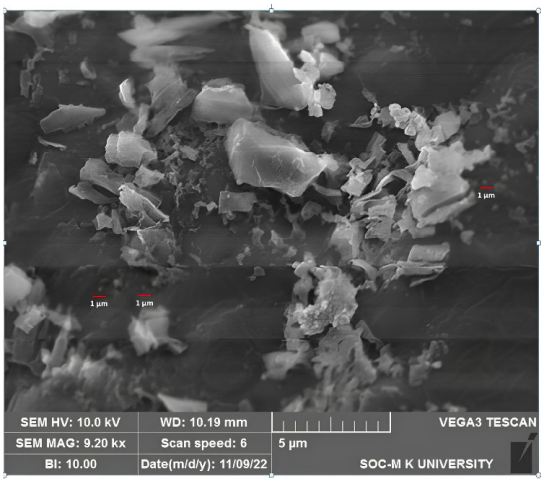

Figure 5. Scanning electron microscopy (SEM) images of silver nanoparticles formed by the reaction of 1 mM silver nitrate and aqueous extract of *Solanum trilobatum* leaf extract.

DAPI (4′,6-diamidino-2-phenylindole) staining for nuclear apoptosis

Distinct variations in nuclear morphology, including chromosome condensation and fragmentation, aid in visually distinguishing apoptotic cells stained with DAPI. Furthermore, the presence of nuclear blebbing in apoptotic cells, unlike necrotic cells which lacked this feature, provided an additional observable characteristic for differentiation. The apoptotic nuclei (intensely stained, fragmented nuclei, and condensed chromatin) were viewed under a fluorescent microscope with an excitation at 359 nm and emission at 461 nm wavelengths, respectively. Figure 7a shows nuclear apoptosis in KB cells such as shrinkage, detachment, membrane blebbing, and distorted shape induced by 2 and 4 µg/mL for 24 hours as compared with the control one. The morphology of the cell was intact in the control sample.

Detection of ROS using carboxy-H2DCFDA

Carboxy-H2DCFDA staining serves as a widely employed method for quantifying intracellular ROS levels, making

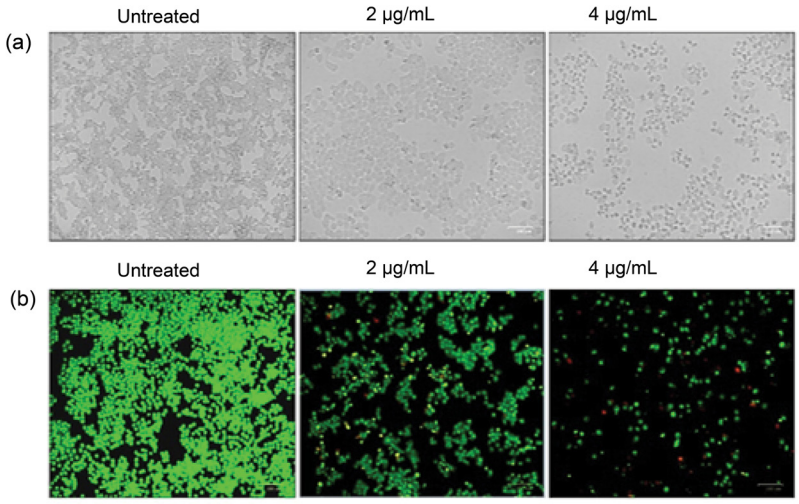

Figure 6. Cell cytotoxicity of silver nanoparticles green synthesized from leaf extracts of *Solanum trilobatum;* (a) Microscopic image of cell lysis after 3-[4, 5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay on normal and oral cancer cells after exposing to silver nanoparticles green synthesized from the leaf extracts of *Solanum trilobatum*; (b) Dual acridine Orange/Ethidium staining living cells showed green fluorescence with normal nuclear appearance.

it a key technique in oxidative stress analysis. Upon cellular uptake, this compound undergoes deacetylation by cellular esterase, transforming into a nonfluorescent form. Later, in the presence of intracellular ROS, it undergoes oxidation to produce the fluorescent compound 2,7-dichlorofluorescein, allowing for effective measurement of oxidative stress within the cell. The fluorescence intensity correlates directly with the quantity of ROS molecules present. In the present study, the cells treated with the sample showed higher ROS levels compared to the control cells and ROS was found to be dose-dependent. The cells treated with 2 and 4 µg concentrations of the sample showed a bright green fluorescence, which was found to be higher at 4 µg (Figure 7b).

Discussion

Nanoparticles have been well studied and suggested in the application of cancer treatment and other healthcare domains. Exceptional properties of nanoparticles have enabled their use in a variety of medical and commercial products (13). Nevertheless, the use of AgNPs before green synthesis has raised concerns about environmental and biological systems. Thus, an alternative source of obtaining eco-friendly $AgNO_3$ is of prime importance to exploit the wide applications of the compound in the medical field. In our case, we used *S. trilobatum,* which is easily available in India. Observation of color change was noted in the synthesis of AgNPs. In our study, the $AgNO_3$ solutions transformed color from colorless to yellow-brown and subsequently to dark brown. AgNPs were

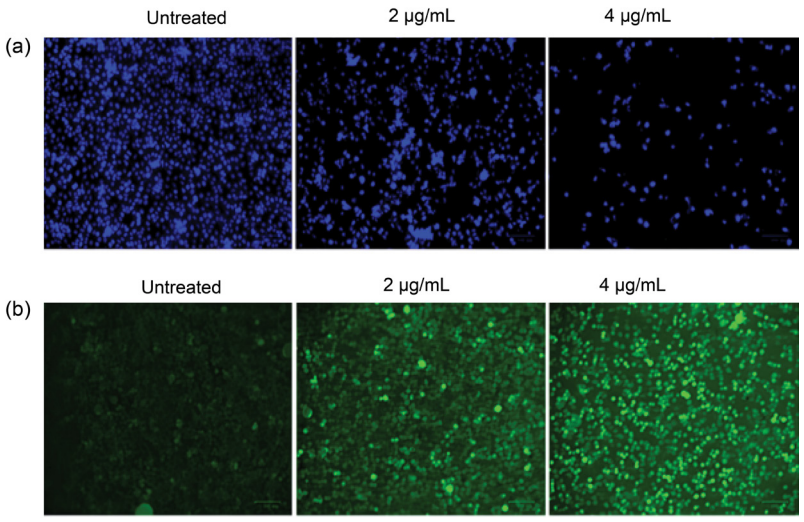

Figure 7. Nuclear apoptosis and intracellular reactive oxygen species (ROS) of oral cancer cells after exposure to silver nanoparticles green synthesized from leaf extracts of *Solanum trilobatum*. (a) 4′,6-diamidino-2-phenylindole staining shows nuclear apoptosis; (b) Carboxy-H2DCFDA staining shows bright green fluorescence showing higher ROS levels.

confirmed using SEM analysis. Previously reported studies have demonstrated the color change of the solution to be a confirmation to denote the presence of AgNPs owing to the excitation of surface plasmon vibration (14,15).

Synthesis of AgNPs was further evaluated and analyzed by UV-visible spectroscopic analysis. This is one of the gold standard methods to analyze the presence of synthesized AgNPs in a very efficient way. Extracts from plants account for the reduction of $AgNO_3$ to enable the formation of AgNPs. Silver is a compound that has free electrons, and it develops the SPR absorption band. The current method can be optimized with several other reports by establishing the temperature to higher temperatures to generate a higher absorbance peak. This would confirm the generation of a high number of nanoparticles. The analysis using the final solution gave us an understanding of Ag+ - Ag0 conversion. The wavelength band at 418 nm is thought to be due to the transitions of d-d of the Ag+ ion, disappearing totally after the green synthesis. This could be the evidence of Ag+ cation getting reduced wholly.

The FT-IR spectrum of AgNPs in the wavelengths of 400-4000 cm^{-1} showed the O-H stretching vibration of clear bands, which clarified the alcoholic and phenolic hydroxyl groups. The bands between 450 and 2000 cm^{-1} indicate C=C and C=O bonds. The strong band above 1500 cm^{-1} is related to the C-O stretching, which should be methyl groups; Ag-O bands were detected at 600-630 cm^{-1}. FTIR spectrum has indicated the presence of different other functional groups along with the phytochemicals available in the leaf of *S. trilobatum*. The vibration bands represent alkynes, amines, flavonoids, and alcohols.

The size of metallic nanoparticles is important and corresponds to cytotoxicity. Earlier reports have shown an increase in anticancer activity in decreased particle size to effectively penetrate the cells. *S. trilobatum* belongs to the family Solanaceae and is used for its antimicrobial and antioxidant properties in traditional medicines. The green biosynthesis of antimicrobial and antioxidant AgNPs has progressed the traditional use of the leaf extract with stable AgNPs that would possess higher antioxidant potency (16). Results from the cytotoxic analysis using MTT assay in our study have shown AgNPs obtained from *S. trilobatum* to be potentially cytotoxic. Also, its activity against cancer cells reduced the viability of oral cancer cells in a dose-dependent manner with the presence of $AgNO_3$, *S. trilobatum* leaf extract, and AgNPs. The rate of absorbance was determined at 525 nm, which depicted untreated viable control cells. However, the viability gradually decreased with increasing concentrations of AgNPs.

The result of green biosynthesized AgNPs on ROS accumulation in oral cancer cells was assessed along with the DNA staining (DAPI). A notable increase was observed in ROS deposit on the biosynthesized AgNPs-supplemented oral cancer cells in comparison to the control (untreated cells). This proved that the green synthesized AgNPs showed an increase in ROS proportions leading to oxidative stress and contributing to cell injury (17). Programmed cell death (apoptosis) is regulated by numerous factors that can be stimulated by high accumulation of ROS.

All the rounded cells indicate the induction of the apoptotic pathway, and many reports have mentioned these alterations as morphologic hallmarks of apoptosis (18). Diverse types of integrin compounds are known to have a predominant role in the crosstalk between cell and matrix. These processes were performed through focal adhesion and linked kinases. Once the trigger happens for the initiation of apoptosis, all kinds of focal adhesion and linked kinases (19) will be deactivated. All these prove that the synthesized nanoparticles have the potential to induce cell death in oral cancer cells by initiating the process of apoptosis. To further validate the process of apoptosis, the experiments were performed with AO/EB dual staining. The concept behind this staining is a clear indication to understand the exact impact of apoptosis in cancer cells. AO tends to integrate into both the normal and early apoptotic cells where the cell membrane is intact. This results in the formation of a fluorescent green color after linking to the DNA. Moreover, EtBr stain has the property to attach only to the damaged cells and this reaction gives the orange-red color fluorescence when attached to the DNA (20).

In addition, ROS analysis was performed to understand the mechanism of apoptosis. Mostly, all the cellular-based ROS tend to trigger the cell cycle arrest and eventually induce apoptosis in cancer cells. To note, even reports suggest that ROS can arrest the metastatic process (21,22). The chemotherapy process for cancer treatment mostly targets the ROS mechanism resulting in the induction of apoptosis (21). In our study, the synthesized nanoparticle tended to show a promising rise in the ROS in a dose-dependent manner. All these reports and data demonstrate that the nanoparticles induce apoptosis by altering the ROS levels. Few reports have discussed the importance of green synthesis and characterization and evaluated the biological activity of *S. trilobatum*-mediated AgNPs (23-25). In a recent study, researchers tried to utilize the *S. trilobatum* fruit extract in various dimensions. They evaluated the anticancer activity of the fruit extract in breast cancer cell lines. We could not find any studies in the literature on the anti-cancer activity of the leaf extracts of this plant in oral cancer cell lines. So, the present study established a simple, eco-friendly, and economical method to generate therapeutically valuable AgNPs using *S. trilobatum* leaf extract, which is predominantly available in India. Oral cancer is a significant health concern globally, and any research exploring potential treatments for this condition is highly relevant and valuable.

The study utilized an innovative method by synthesizing AgNPs from the aqueous leaf extract of *S. trilobatum*. Promising anticancer properties showed a novel approach to potential cancer treatment. Using plant extracts to synthesize nanoparticles, implies a natural and potentially eco-friendly method for developing therapeutic agents, which could be advantageous for future drug development.

This in vitro study focused on human oral cancer cells, and extrapolating these findings to human trials and clinical applications requires further research to establish safety, efficacy, and specificity to cancer cells without harming healthy cells. The study may represent an initial step, and further comprehensive research, including in vivo studies and clinical trials, is necessary to validate the effectiveness and safety of these nanoparticles as a potential treatment for oral cancer. The toxicity and biocompatibility of AgNPs in living systems must also be extensively studied to ensure they do not pose harmful effects to normal cells or tissues.

Conclusion

In the current study, the biosynthesized AgNPs from *S. trilobatum* leaf extract exhibited significant anti-cancer potential. FTIR analysis clearly showed that the participation of polyphenols available in the plant extract is mostly responsible for the biological reduction of silver ions (Ag+) into silver nanoparticles (Ag0) along with other bio-compounds such as flavonoids, phenols, alcohols, aromatics, and alkaloids. AO/EtBr and ROS assays further supported the AgNPs' anti-cancer potential, affirming their promise for oral cancer therapy. This research contributes valuable evidence to the burgeoning field of green-synthesized nanoparticles as promising candidates for medical therapeutics, particularly in the context of oral cancer intervention.

Acknowledgments

The authors wish to sincerely thank Clinbiocare Technology Pvt Ltd, Tenkasi, Tamil Nadu, for the technical support and for providing efficient laboratory services for carrying out this study.

Authors' contributions

Conceptualization: Anuradha Ganesan, Gautham Kumar N.
Formal analysis: Prabhu Manickam Natarajan.
Investigation: Anuradha Ganesan, Gautham Kumar N, Prabhu Manickam Natarajan.
Methodology: Anuradha Ganesan, Gautham Kumar N.
Project administration: Anuradha Ganesan.
Resources: Gautham Kumar N.
Supervision: Gautham Kumar N.
Validation: Anuradha Ganesan.
Visualization: Prabhu Manickam Natarajan.
Writing-original draft: Gautham Kumar N.
Writing-Review & editing: Anuradha Ganesan, Gautham Kumar N and Prabhu Manickam Natarajan.

Conflict of interests

The authors declare no conflict of interest.

Ethical considerations

This research does not entail the utilization of animal or human subjects. The authors have diligently adhered to ethical standards, ensuring the avoidance of plagiarism, misconduct, data fabrication, falsification, double publication, or redundancy.

Funding/Support

This study was funded by the authors. No external funding was received

References

1. Huston M, DeBella M, DiBella M, Gupta A. Green synthesis of nanomaterials. Nanomaterials (Basel). 2021;11(8):2130. doi: 10.3390/nano11082130.
2. Bayda S, Adeel M, Tuccinardi T, Cordani M, Rizzolio F. The history of nanoscience and nanotechnology: from chemical-physical applications to nanomedicine. Molecules. 2019;25(1):112. doi: 10.3390/molecules25010112.
3. Khan F, Shariq M, Asif M, Siddiqui MA, Malan P, Ahmad F. Green nanotechnology: plant-mediated nanoparticle synthesis and application. Nanomaterials (Basel). 2022;12(4):673. doi: 10.3390/nano12040673.
4. Mousavi SM, Hashemi SA, Ghasemi Y, Atapour A, Amani AM, Savar Dashtaki A, et al. Green synthesis of silver nanoparticles toward bio and medical applications: review study. Artif Cells Nanomed Biotechnol. 2018;46(Suppl 3):S855-72. doi: 10.1080/21691401.2018.1517769.
5. Shumail H, Khalid S, Ahmad I, Khan H, Amin S, Ullah B. Review on green synthesis of silver nanoparticles through plants. Endocr Metab Immune Disord Drug Targets. 2021;21(6):994-1007. doi: 10.2174/1871530320666200729153714.
6. Habeeb Rahuman HB, Dhandapani R, Narayanan S, Palanivel V, Paramasivam R, Subbarayalu R, et al. Medicinal plants mediated the green synthesis of silver nanoparticles and their biomedical applications. IET Nanobiotechnol. 2022;16(4):115-44. doi: 10.1049/nbt2.12078.
7. Ahn EY, Park Y. Anticancer prospects of silver nanoparticles green-synthesized by plant extracts. Mater Sci Eng C Mater Biol Appl. 2020;116:111253. doi: 10.1016/j.msec.2020.111253.
8. Pungle R, Nile SH, Kharat AS. Green synthesis and characterization of *Solanum xanthocarpum* capped silver nanoparticles and its antimicrobial effect on multidrug-resistant bacterial (MDR) isolates. Chem Biol Drug Des. 2023;101(3):469-78. doi: 10.1111/cbdd.13945.
9. Gopalakrishnan B, Selvam R, Lakshmanan G. Phytochemical screening and antibacterial activity of *Solanum trilobatum* L. and *Azadirachta indica* A. Ann Rom Soc Cell Biol. 2021;25(2):1972-81.
10. Pazhanivel K, Ganesan A. Clinical effectiveness of *Aloe vera* in the treatment of oral mucosal diseases: a double blinded randomized clinical trial. Int J Chem Biochem Sci. 2022;22:34-40.
11. Murugan AJ, Ganesan A, Aniyan Y, Kannan A, Krithika CL, Umapathy D. Formulation of topical herbal gel from the extract of purslane (*Portulaca oleracea*) leaves in two

concentration and its evaluation. Int J Chem Biochem Sci. 2022;22:110-8.
12. Hembram KC, Kumar R, Kandha L, Parhi PK, Kundu CN, Bindhani BK. Therapeutic prospective of plant-induced silver nanoparticles: application as antimicrobial and anticancer agent. Artif Cells Nanomed Biotechnol. 2018;46(Suppl 3):S38-51. doi: 10.1080/21691401.2018.1489262.
13. Jain N, Jain P, Rajput D, Patil UK. Green synthesized plant-based silver nanoparticles: therapeutic prospective for anticancer and antiviral activity. Micro Nano Syst Lett. 2021;9(1):5. doi: 10.1186/s40486-021-00131-6.
14. Adam G, Houng HT, Lischewski M, Khoi NH. A new steroidal alkaloid from *Solanum hainanense*. Phytochemistry. 1978;17(6):1070-1. doi: 10.1016/s0031-9422(00)94289-4.
15. Hussain T, Gupta RK, K S, Khan MS, Hussain MD, Arif MD, et al. Evaluation of antihepatotoxic potential of *Solanum xanthocarpum* fruit extract against antitubercular drugs induced hepatopathy in experimental rodents. Asian Pac J Trop Biomed. 2012;2(6):454-60. doi: 10.1016/s2221-1691(12)60075-6.
16. Sarojini K, Arivarasu L, Rajeshkumar S, Lakshmi T. Green synthesis of *Solanum trilobatum* mediated selenium nanoparticles and its anti-inflammatory and anti-microbial activity. Plant Cell Biotechnol Mol Biol. 2020;21(25-26):75-81.
17. Ramar M, Manikandan B, Marimuthu PN, Raman T, Mahalingam A, Subramanian P, et al. Synthesis of silver nanoparticles using *Solanum trilobatum* fruits extract and its antibacterial, cytotoxic activity against human breast cancer cell line MCF 7. Spectrochim Acta A Mol Biomol Spectrosc. 2015;140:223-8. doi: 10.1016/j.saa.2014.12.060.
18. Ganesan A, Kumar NG. Assessment of lipid peroxides in multiple biofluids of leukoplakia and oral squamous cell carcinoma patients-a clinico- biochemical study. J Clin Diagn Res. 2014;8(8):ZC55-8. doi: 10.7860/jcdr/2014/10200.4768.
19. Saraste A, Pulkki K. Morphologic and biochemical hallmarks of apoptosis. Cardiovasc Res. 2000;45(3):528-37. doi: 10.1016/s0008-6363(99)00384-3.
20. Persad S, Attwell S, Gray V, Delcommenne M, Troussard A, Sanghera J, et al. Inhibition of integrin-linked kinase (ILK) suppresses activation of protein kinase B/Akt and induces cell cycle arrest and apoptosis of PTEN-mutant prostate cancer cells. Proc Natl Acad Sci U S A. 2000;97(7):3207-12. doi: 10.1073/pnas.97.7.3207.
21. Ribble D, Goldstein NB, Norris DA, Shellman YG. A simple technique for quantifying apoptosis in 96-well plates. BMC Biotechnol. 2005;5:12. doi: 10.1186/1472-6750-5-12.
22. Akhtar MJ, Ahamed M, Kumar S, Khan MM, Ahmad J, Alrokayan SA. Zinc oxide nanoparticles selectively induce apoptosis in human cancer cells through reactive oxygen species. Int J Nanomedicine. 2012;7:845-57. doi: 10.2147/ijn.s29129.
23. Liou GY, Storz P. Reactive oxygen species in cancer. Free Radic Res. 2010;44(5):479-96. doi:10.3109/10715761003667554.
24. Manimegalai S, Rajeswari VD, Parameswari R, Nicoletti M, Alarifi S, Govindarajan M. Green synthesis, characterization and biological activity of *Solanum trilobatum*-mediated silver nanoparticles. Saudi J Biol Sci. 2022;29(4):2131-7. doi: 10.1016/j.sjbs.2021.11.048.
25. Lateef A, Prabhudas SK, Natarajan P. RNA sequencing and de novo assembly of *Solanum trilobatum* leaf transcriptome to identify putative transcripts for major metabolic pathways. Sci Rep. 2018;8(1):15375. doi: 10.1038/s41598-018-33693-4.