Ashdin Publishing
Journal of Drug and Alcohol Research
Vol. 13 (2024), Article ID 236284, 06 pages
DOI: 10.4303/JDAR/236284

Research Article

Analysis of Drug Investigation of Anti Melanomal Effect of *Ipomoea Pes-Tigridis* L. Silver Nano Particles Produced by Green Synthesis Method through Pharmaceutical Scope

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Received: 31 January 2024; Manuscript No: JDAR-24-126398; **Editor assigned:** 02 February 2024; PreQC No: JDAR-24-126398 (PQ); **Reviewed:** 16 February 2024; QC No: JDAR-24-126398; **Revised:** 21 February 2024; Manuscript No: JDAR-24-126398 (R); **Published:** 28 February 2024; **DOI**: 10.4303/JDAR/236284

Abstract

The study aims to explore the green synthesis and therapeutic potential of silver nanoparticles (AgNPs) using the leaf extract of *Ipomoea pes-tigridis* L., a plant known for its diverse medicinal properties. Phytochemical screening was conducted to identify active compounds in the leaf extract responsible for nanoparticle synthesis and stabilization. Various analytical techniques, such as UV-visible spectroscopy, Fourier Transform Infrared (FT-IR) Spectroscopy, Scanning Electron Microscopy (SEM), particle analyzer, and X-Ray Diffraction (XRD), were employed to characterize the biosynthesized AgNPs. MTT assay was used to evaluate their cytotoxic potential. UV-visible spectrophotometry confirmed successful AgNP synthesis, with a distinct Surface Plasmon Resonance (SPR) band at 425 nm, indicative of small, spherical nanoparticles. FTIR analysis demonstrated the role of phytochemicals as capping and stabilizing agents. SEM and XRD analyses provided insights into particle size and crystal structure, revealing a face-centered cubic lattice in the AgNPs. MTT assay was performed on 2 melanoma cell lines (A375 and B-16-F10) and Normal Human Dermal Fibroblasts (NHDF). The AgNPs displayed lower IC50 values than the leaf extract in cancer cell lines and demonstrated selective toxicity by showing higher IC50 values in normal cells. The study showcases the successful, green synthesis of AgNPs using *Ipomoea pes-tigridis* L. leaf extract. These AgNPs exhibited promising cytotoxic effects on melanoma cells and lower toxicity toward normal cells. The results establish the groundwork for further investigation into using such plant-derived nanoparticles for cancer treatments.

Keywords: *Ipomoea pes-tigridis* L; AgNPs; MTT assay; Melanoma

Introduction

Nanotechnology, particularly the study of nanoparticles, has revolutionized several industries, from consumer goods and healthcare to environmental applications [1]. Due to their distinct physical characteristics, these tiny particles, less than 100 nm, are very intriguing. Among them, metallic nanoparticles such as silver nanoparticles (AgNPs) have attracted interest for their strong antimicrobial properties [2]. Historically, toxic substances were frequently used in chemical processes for producing nanoparticles. However, a move toward environmentally benign "green" synthesis is currently picking up steam and frequently uses microorganisms and plant extracts as reducing agents [3]. These biogenic techniques are economical and environmentally responsible. For instance, plant-based ingredients are being used more frequently to create AgNPs, making the procedure environmentally friendly and improving the antibacterial capabilities of the nanoparticles [4]. Plants have long been cherished as the foundation of medicinal therapy since they frequently provide a safer alternative to synthetic medications, especially for long-term treatment approaches. These botanical sources, abundant in various phytochemicals, can support health restoration independently or in combination [5]. Modern tools are now available to investigate these plant-based sources' phytochemical profiles and therapeutic effects in response to a growing interest in alternative medicine. These developments have streamlined the medication development process and made it easier to identify bio-active components [6]. Natural medicines have become popular as potential supplements or replacements for conventional pharmaceutical treatments due to their favourable safety profile, which includes few side effects and lower toxicity [7].

Ipomoea pes-tigridis L., often called morning glory or tiger foot, is a climbing or sprawling herbaceous plant in the *Convolvulaceae* family. It thrives predominantly

in tropical and subtropical zones and possesses diverse bioactive molecules, including ergoline and indolizidine alkaloids, nor-tropane alkaloids, flavonoids, glycolipids, lignin, and triterpenes. This plant has a spectrum of pharmacological attributes, including psychotropic, uterotonic, and haemostatic effects [8]. It is traditionally utilized for many medical conditions, ranging from peptic ulcers, rheumatoid arthritis, and joint ailments to inflammations, sexually transmitted diseases, and gout [9]. The plant also serves as a diuretic, laxative, analgesic, and natural remedy for poisonous insect stings and snake bites. Its ability to cleanse the body effectively mitigates acne scars and sores. *Ipomoea pes-tigridis* L., combined with chemicals that work synergistically to confer its curative qualities, is an important aspect of folk medicine. Investigating this plant species may be essential for discovering novel natural compounds with antioxidant and anti-cancer properties. A thorough understanding of the phytochemical composition of *Ipomoea pes-tigridis* L. and its pharmacological functions is essential for creating advanced natural treatment methods. Such a study can potentially find anti-cancer medicines with fewer adverse effects than conventional chemotherapy. Additionally, its natural antioxidant activity may help avoid or treat diseases linked to oxidative stress [10-12].

Materials and Methods

Plant material

Ipomoea pes-tigridis was collected from the Osmania University campus, Telangana, in September.

Reagents and chemicals

All the chemicals and reagents were procured from Sigma Aldrich (laboratory grade).

Preparation of extracts

The aerial parts of Ipomoea pes-tigridi L. were harvested and dried in the shade. The dried leaves were ground into a fine powder and underwent a defatting process using n-hexane. The powder was then subjected to hydroalcoholic extraction through maceration with 70% ethanol. The resulting extracts were filtered, and the solvent was removed through evaporation with a rotary evaporator, yielding a solid extract. The yield percentage was calculated and recorded [13].

Phytochemical screening

Preliminary phytochemical analysis of the ethanolic extract of *Ipomoea pes-tigridis* L. was performed according to the established protocols [14].

Biosynthesis of AgNps

Various concentrations of plant extracts were prepared from the stock solution (1 gm/L). $AgNO_3$ solution was prepared using double distilled water. The extracts were added to the $AgNO_3$ solution in a 250 ml beaker, and the reaction was allowed to occur at various parameters. $AgNO_3$ solution concentration was kept constant throughout the experiments.

To optimize the synthesis of Silver Nanoparticles (AgNPs), we explored various conditions, including different concentrations of plant extract (0.10%, 0.20%, 0.40%, 0.80%, 1.00%) and silver nitrate ($AgNO_3$) (0.5 mM, 1 mM, 3 mM, 5 mM, 7 mM, 10 mM). These were mixed in varying ratios and subjected to reaction times of 15 minutes, 30 minutes, 60 minutes, 90 minutes, and 120 minutes at temperatures of 25°C, 60°C, 80°C, and 100°C. Post-reaction, each sample was centrifuged at 11,000 rpm for 15 minutes to separate the AgNPs, which settled as a pellet at the bottom. The pellet was subsequently washed with deionized water, a step repeated 2 times-3 times to ensure purity, before drying the samples in an oven at 50°C for further characterization. The optimized conditions were subsequently employed for bulk production of Ag-NPs, leveraging their efficiency in rapid ion reduction and nanoparticle stabilization [15,16].

Characterization of AgNPs

Several analytical techniques were employed to evaluate the synthesized silver nanoparticles (AgNPs) characteristics. Ultraviolet-Visible (UV-Vis) spectroscopy served as the initial assessment tool. For this analysis, 3 mL of AgNP solution was introduced into UV-transparent cuvettes and analyzed at room temperature (25°C). The spectroscopy was conducted over a wavelength range of 200 nm-800 nm with a resolution of 1 nm, following established protocols. Fourier Transform Infrared (FTIR) spectroscopy, employing a BRUKER OPTICS, Germany (Model TENSOR 27), was used to identify the functional groups on the biosynthesized AgNPs. Spectra were collected from 600 cm^{-1} to 4000 cm^{-1} with a 5 cm^{-1} transmittance setting. Morphological evaluation was performed using Field Emission Scanning Electron Microscopy (FESEM), utilizing a FEI (Model: Apreo LoVac, with retractable STEM 3+ Detector, DBS Detector). For this, the AgNP samples were adhered to carbon conductive tape to facilitate high-resolution imaging. The particle size, volume, and distribution were measured using a particle size analyzer. Further insights into the particle size were assembled through X-ray diffraction (Model: Miniflex) for the diameter of nanoparticles using the Scherrer equation [15,16].

Breadth, $B = K\lambda / I \cos \theta$

λ=the wavelength of the X-ray radiation source

θ=the half diffraction angle–Bragg angle

K=the Scherrer constant

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay

The MTT assay was used to determine the cytotoxic potential of crude extract and AgNps of *Ipomoea pes-tigridis* L. Mouse melanoma cells (B-16-F10) and human melanoma cells (A375), and normal human dermal fibroblast (NHDF) as control were obtained from the American Type Culture Collection (ATCC) were incubated with the test samples in a 96-well plate under standard conditions (37°C, 5% CO_2, 72 hours). After treatment with MTT salt (20 µl, 2 mg/ml,

phosphate-buffered saline) and subsequent incubation for 3 hours under the same conditions, the purple formazan produced by the reduction of MTT salt with mitochondrial enzymes was extracted with DMSO (100 µl). The intensity of the colored formazan was measured using a spectrophotometer (540 nm) in triplicates, and the intensity of the formazan represented the viability of cells. This intensity could be proportional to the quantity of living cells and expressed as IC50 values. The obtained values were compared with the standard (Doxorubicin) and blank [17].

Results and Discussion

Preliminary phytochemical screening

The leaf extract of *Ipomoea pes-tigridis* L. contains a variety of phytochemicals, including alkaloids, saponins, flavonoids, tannins, amino acids, and carbohydrates that contribute to the environmentally friendly synthesis of silver nanoparticles (AgNPs). Alkaloids and tannins primarily act as reducing agents that convert silver ions into nanoparticles. Saponins, flavonoids, and amino acids serve dual roles, both reducing silver ions and stabilizing the formed nanoparticles. Carbohydrates mainly act as stabilizing agents, preventing the aggregation of the nanoparticles. These phytochemicals work together to efficiently form and stabilize AgNPs, making them well-suited for various applications [18-21].

Biosynthesis of AgNPs

To optimize the route of nanoparticle synthesis, we have tested various concentrations of $AgNO_3$, with different quantities of leaf extract of *Ipomoea pes-tigridis* L., at various temperatures and periods. After 60 min, the dissolution of the 0.2% concentration leaf extract with 1 mM of $AgNO_3$ at 40°C caused a rapid change in color from light yellowish to dark brown, indicating the fast reduction of Ag+ to AgO in $AgNO_3$ solution (Figure 1).

Figure 1: Confirmation of biosynthesized silver nanoparticles (AgNPs) based on the colour change

UV-visible spectroscopy

UV-visible spectrophotometry confirmed that silver nanoparticles (AgNPs) had been successfully synthesized in the solution. This method showed an SPR absorption band with a wavelength between 430 nm and 436 nm. The newly synthesized AgNPs, silver nitrate, and measurements from *Ipomoea pes-tigridis* L. leaf extract were all included in the spectral data. At 425 nm, a distinct, single absorption peak was seen that was unique to the AgNPs.

The characteristics of the SPR bands can reveal information about the size, distribution, and shape of the nanoparticles. Smaller, spherical nanoparticles are typically present in mixtures when a single, clearly defined SPR band appears at shorter wavelengths. The occurrence of several SPR bands, on the other hand, at longer wavelengths would suggest larger, anisotropic nanoparticles. Particularly, the symmetry of the SPR band shows that the solution does not have considerable particle aggregation. Therefore, the distinct, symmetric signal at 425 nm probably proves that small, spherical AgNPs were successfully formed with little agglomeration in the solution (Figure 2).

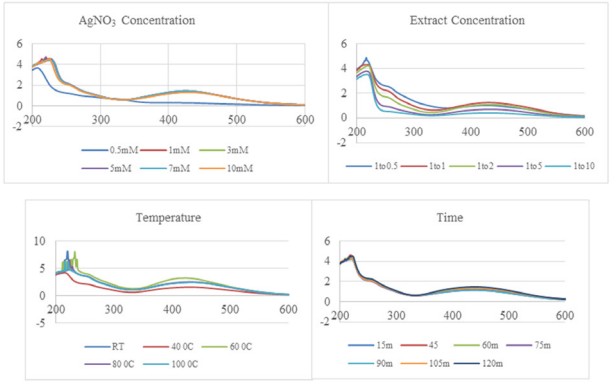

Figure 2: UV-visible spectra of AgNPs synthesized from different parameters

Fourier Transforms Infrared Spectroscopy (FTIR)

The Fourier Transform Infrared (FT-IR) spectroscopy of the *Ipomoea pes-tigridis* L. leaf extract revealed specific absorption bands indicative of various functional groups. A broad and pronounced band was observed at 3385.07 cm^{-1} and 3236.55 cm^{-1}, characteristic of hydroxyl (OH-) groups. These bands are associated with alcoholic, phenolic, and flavonoid compounds, which serve as capping agents stabilizing the nanoparticle surface.

Additionally, an absorption band at 1635.64 cm^{-1} correlates with C=C bonds in aromatic compounds. Notably, the bands at 1201.65 cm^{-1} and 1288.45 cm^{-1} signify the existence of carboxylic acid groups. These absorption features suggest a rich phytochemical composition in the leaf extract, contributing to the nanoparticle capping process. Furthermore, bands detected at 725.23 cm^{-1}, 719.45 cm^{-1}, and 767.67 cm^{-1} indicate the presence of =CH bonds in aromatic compounds in the plant extract and the biosynthesized silver nanoparticles (AgNPs). A wavenumber shift between the leaf extract and AgNPs implies an interaction between the biomolecular functional groups and silver cations. This shift is likely due to the redox processes involved in forming the AgNPs. Such findings reinforce the concept that biological molecules are instrumental in forming and stabilizing colloidal silver

nanoparticles in aqueous solutions, offering a means to control their size and minimize aggregation (Figure 3).

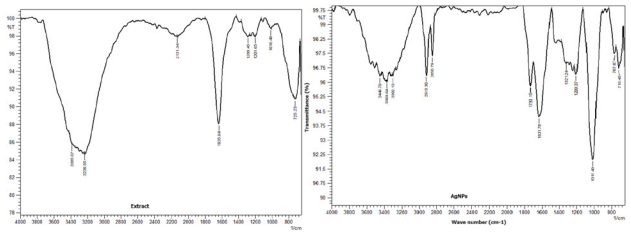

Figure 3: FT-IR of the extract and AgNPs

SEM analysis

Morphological features of the biosynthesized AgNPs were evaluated using Scanning Electron Microscopy (SEM) techniques. Figure 4 depicts the SEM micrographs obtained for the nanomaterial. The particles exhibited a spherical geometry, although a minor fraction was also observed to possess an ellipsoidal form. The micrographs revealed the uniform dispersion of the AgNPs throughout the aqueous medium. The particle size of selected AgNPs, as measured from the SEM images, ranged from approximately 52.38 nm to 144.9 nm. It is noteworthy that a considerable degree of particle agglomeration was observed. This agglomeration is likely an artifact from the dehydration process employed during sample preparation for SEM analysis. SEM images further elucidated a distinct organic layer encapsulating the AgNPs. This organic coating lends credence to the involvement of plant extract metabolites in both the nucleation and stabilization of the biosynthesized nanoparticles.

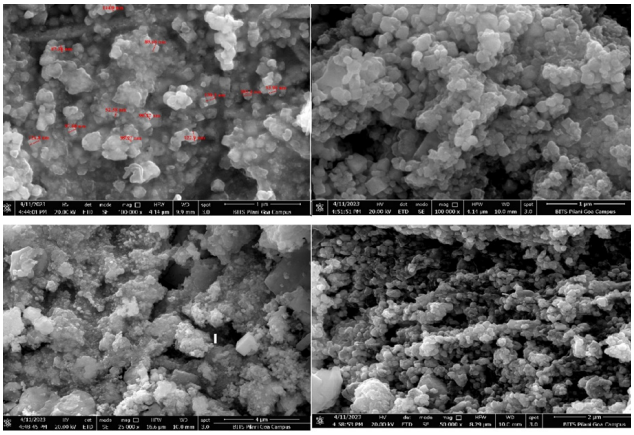

Figure 4: SEM analysis of synthesized AgNPs

Particle size analysis: The average diameter of the particles was assessed using a particle analyzer. AgNPs exhibited a size range from a minimum of 10.6 nm to a maximum of 251 nm in diameter. The formed AgNPs' average diameter was 164.3 nm, based on data collected from 3 independenrials (Figure 5).

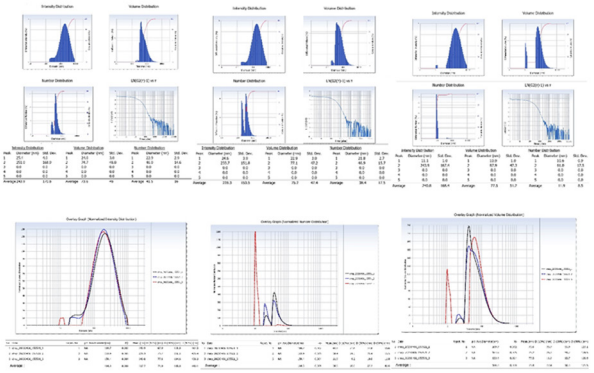

Figure 5: Particle size analysis of AgNP

XRD (X-Ray Diffraction): The crystal structure of silver nanoparticles (AgNPs) was investigated using X-ray diffraction (XRD) analysis, and the corresponding diffraction pattern is presented in Figure 6. Peaks were observed at 2 θ values of 31.690° and 32.150°, indicating a Face-Centered Cubic (FCC) lattice structure in the silver nanoparticles. Additionally, a peak at a 2 θ value of approximately 46.150° was observed, which is attributed to the presence of an extract. This suggests incorporating a stabilizing agent in the AgNPs sample, which is involved in the reduction, capping, and size control of the nanoparticles. Using the Scherrer equation and a Full Width at Half-Maximum (FWHM) value of 0.15768, the average crystal size of the formed silver nanoparticles was calculated to be 54.79 nm.

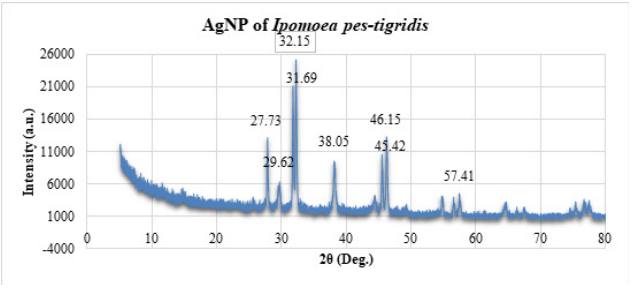

Figure 6: XRD Pattern of AgNPs synthesized from *Ipomoea pes-tigridis*

MTT assay: The results presented show IC50 values for 3 different treatments-*Ipomoea pes-tigridis* L. extract, silver nanoparticles (AgNPs), and Doxorubicin-against 3 cell lines viz., A375 and B-16-F10, which are melanoma cancer cell lines, and NHDF, which are Normal Human Dermal Fibroblasts (Table 1).

Table 1: IC50 values of *Ipomoea pes-tigridis* L. extract, AgNPs, and Doxorubicin in MTT

Treatment	*Ipomoea pes-tigridis* L.	AgNPs	Doxorubicin
A375	12.02 ± 3.14	10.48 ± 2.34	0.14 ± 1.18
B-16-F10	18.25 ± 1.82	12.11 ± 1.65	0.73 ± 1.93
NHDF	136.42 ± 2.92	124.57 ± 3.14	112.3 ± 2.54

For the A375 cell line, when compared to the Doxorubicin (IC50=0.14 ± 1.18), AgNPs of *Ipomoea pes-tigridis* L. (IC50=10.48 ± 2.34) showed better cytotoxic potential than the *Ipomoea pes-tigridis* L. extract (IC50=12.02 ± 3.14). Similarly, in the B-16-F10 cell line, the extract has an IC50 value of 18.25 ± 1.82, and its AgNPs show an IC50 value of 12.11 ± 1.65. In comparison, Doxorubicin showed an IC50 value of 0.73 ± 1.93. The AgNPs exhibit slightly lower IC50 values in both cancer cell lines than the extract, suggesting they may be more effective at inhibiting cell growth in these melanoma cell lines.

In the case of the NHDF cell line, which serves as a non-cancerous control, the extract and the AgNPs show significantly higher IC50 values (136.42 ± 2.92 and 124.57 ± 3.14, respectively). It is a positive sign as it indicates lower toxicity to normal cells, implying some level of selective toxicity toward the cancer cells.

Summary

The study investigates the biosynthesis, characterization, and potential medical applications of silver nanoparticles (AgNPs) synthesized using leaf extract from *Ipomoea pes-tigridis* L. This plant extract contains a rich mix of phytochemicals, including alkaloids, saponins, and flavonoids, that reduce silver ions and stabilize the resulting nanoparticles. Optimized conditions for nanoparticle synthesis involved a 60-minute reaction with a 0.2% leaf extract and 1 mM of $AgNO_3$ at 40°C, which led to a significant color change, signifying the rapid reduction of silver ions. UV-visible spectrophotometry revealed a single, distinct Surface Plasmon Resonance (SPR) band at 425 nm, suggesting the successful formation of small, spherical AgNPs. Fourier Transform Infrared (FTIR) spectroscopy further confirmed the presence of functional groups in the leaf extract that act as capping agents, stabilizing the nanoparticle surface. Scanning Electron Microscopy (SEM) images showed the nanoparticles to have primarily a spherical shape, with sizes ranging from approximately 52.38 nm to 144.9 nm. Some level of particle agglomeration was noted, likely due to the dehydration process used in SEM sample preparation. Particle size analysis revealed the average nanoparticle diameter to be 164.3 nm. X-Ray Diffraction (XRD) confirmed a Face-Centered Cubic (FCC) lattice structure in the AgNPs, with an average crystal size calculated to be 54.79 nm. In cytotoxicity studies, the leaf extract and its AgNPs showed lower IC50 values in melanoma cell lines (A375 and B-16-F10) compared to a non-cancerous control (NHDF), highlighting their potential anti-cancer applications. Notably, the AgNPs demonstrated slightly more potent cytotoxicity than the extract, indicating their possible superiority in inhibiting melanoma cell growth. In summary, the research underscores the promising role of *Ipomoea pes-tigridis* L. extract in the eco-friendly synthesis and stabilization of AgNPs, which may have future applications in cancer therapy with a degree of selective toxicity.

Conclusion

In conclusion, this comprehensive study illuminates the multifaceted potential of *Ipomoea pes-tigridis* L. leaf extract in the eco-friendly biosynthesis and stabilization of silver nanoparticles (AgNPs). Through various analytical methods, including UV-visible spectrophotometry, FTIR spectroscopy, SEM, and XRD, the research robustly characterizes the synthesized nanoparticles, providing insights into their size, shape, and crystal structure. Notably, the cytotoxicity assessment *via* the MTT assay offers promising evidence for the therapeutic potential of these AgNPs, particularly in combating melanoma cell lines. These AgNPs display lower IC50 values than the raw extract in cancer cell lines, and they exhibit lower toxicity in non-cancerous cells, suggesting a level of selective toxicity. This dual attribute-efficacy against cancer cells and lower toxicity to normal cells-makes these biosynthesized AgNPs an intriguing candidate for further exploration in anti-cancer applications. The study reinforces the promise of plant-based nanotechnology as an emerging frontier in the sustainable production of nanoparticles and the development of targeted, less-toxic cancer therapies.

Acknowledgement

The authors acknowledge the Department of University for providing necessary facilities.

Conflict of Interest

Authors have no conflict of interest to declare.

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