

CYTOTOXIC EFFECT AND FREE RADICAL SCAVENGING ACTIVITY OF ANDROGRAPHIS PANICULATA STEM MEDIATED SELENIUM NANOPARTICLES

Irene Jogi¹, S.Sangeetha^{*2}

¹Department of Anatomy, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical sciences (SIMATS), Saveetha University, Chennai – 77.

²Assistant Professor, Department of Anatomy, Saveetha Dental College and Hospital, Saveetha Institute of Medical and technical Sciences (SIMATS), Saveetha University, Chennai – 77.

Corresponding Author : *Mrs.S.Sangeetha

Assistant Professor, Department of Anatomy, Saveetha Dental College and Hospital, Saveetha Institute of Medical and technical Sciences (SIMATS), Saveetha University, No. 162, Poonamalle high road, Velappanchavadi, Chennai- 600077

irenejogi2005@gmail.com¹
saneetha.sdc@saveetha.com²

ABSTRACT

Introduction: Nanotechnology is revolutionizing biomedical sciences, particularly in synthesizing selenium nanoparticles (SeNPs) using plant-based methods. These plants contain metabolites like proteins and flavonoids that act as reducing agents to generate and stabilize nanoparticles. SeNPs, known for their immunomodulatory and antioxidant properties, are significant in medicine, agriculture, and environmental applications. *Andrographis paniculata*, a traditional medicinal plant, shows promising cytotoxic and free radical scavenging activities when used in SeNP synthesis. **Materials and methods :** The study investigates the cytotoxic effect and free radical scavenging activity of selenium nanoparticles synthesized using *Andrographis paniculata*. The plant extract, prepared by filtering the leaves, was mixed with sodium selenite to produce the nanoparticles. These nanoparticles were then tested on brine shrimp nauplii to assess their toxicity at various concentrations. Observations and data calculation were performed to evaluate the mortality rate, providing insight into the potential biomedical applications of the synthesized nanoparticle. **Results:** The study evaluates selenium nanoparticles (SeNPs) synthesized using *Andrographis paniculata* for their cytotoxic and antioxidant properties. The SeNPs exhibit dose-dependent antioxidant activity, with increasing efficacy at higher concentrations, comparable to standard antioxidants. Although the standard shows greater antioxidant activity at lower doses, *A. paniculata* SeNPs demonstrate significant inhibition of oxidative agents, suggesting their potential for biomedical applications. Additionally, the SeNPs show minimal cytotoxicity and promote wound healing, while the nauplii assay reveals a time and dose-dependent cytotoxic effect, highlighting their therapeutic versatility. **Conclusion:** The study demonstrates that *Andrographis paniculata* stem extract effectively synthesizes selenium nanoparticles (SeNPs) with strong cytotoxic and antioxidant properties. These SeNPs show significant potential as therapeutic agents in cancer treatment due to their notable cytotoxic effects against various cancer cell lines. Additionally, their robust free radical scavenging activity underscores their potential as antioxidants. The dual functionality of *A. paniculata* stem-mediated SeNPs offers a promising avenue for developing novel anticancer and antioxidant therapies. Further research is essential to understand their mechanisms and assess in vivo efficacy and safety.

KEY WORDS: *Andrographis paniculata* ,Selenium nanoparticles (SeNPs), Cytotoxicity , Free radical scavenging , Antioxidant , Anti Cancer

Introduction

Many intriguing avenues in the realm of biomedical sciences are being explored by nanotechnology. Different plant parts are used in the biogenesis of selenium (Se) nanoparticles because they contain metabolites such as proteins, flavonoids, phenols, alkaloids, and other phytochemicals that function as reducing agents to generate and stabilize nanoparticles. In

addition, nanotechnology is extensively used in many other technologies, including medicine and agriculture [1]. One of the most exciting new technologies that offers a plethora of creative applications in the sciences is nanotechnology. Current research is very interested in the environmentally friendly synthesis of nanoparticles using microorganisms, algae, and plant components

. Three plants—*Vitis vinifera*, *Cissus quadrangularis*, *Piper nigrum*, *Garcinia mangostana*, *Nitraria schoberi*, *Abelmoschus esculentus*, and *Medicago sativa*, *Andrographis paniculata*, turmeric, green apple, lemon grass, red apple, black tea, and green tea. —are utilized to synthesize nanoparticles. Green synthesis of nanoparticles using plant materials through well-known phytochemical metal nanoparticles has been used in cancer therapy, targeted drug delivery, molecular imaging wastewater treatment, catalysis, biosensor development, fuel elements, cosmetics and antiseptics

The word “selenium” originates from the Greek word “Selene” which means moon goddess. Selenium (Se) has unique properties and great potential in the fields of physics, chemistry, and biology.[2] Selenium nanoparticles have advantages over other nanomaterials because of the promising role of selenium in the stabilization of the immune system and activation of the defense response [3]. The use of SeNPs and their supplements not only has pharmacological significance but also boosts and prepares the body’s immune system to fight virus [4]. Selenium is reported as an important element and acts as the cofactor and coenzyme of catalytic-active sites of various selenoproteins and enzymes in the human body and provides protection to cells and tissues from oxidative injuries and stress.[5] [6]. Selenium also plays an important role in iodine and peroxides metabolism in the body, regulates the level of iodine and free radicals, and can enhance disease resistance.[7] [8]

Andrographis Paniculata Nees is a plant that has been effectively used in traditional Asian medicines for centuries. The plant belongs to the family of Acanthaceae. The common name is Kalmegh or *Bhuminim* [9]. It is an annual, branched, erect - measuring ½ to 1 meter in height. This plant is of Indian origin and is been used for over a decade in Ayurvedic Medicine purposes. Mainly it is available in India and Sri Lanka. The leaves measure about 7.5 cm long and 2.5 cm wide. The flowers from this plant are white in color. The seeds are small and yellowish-brown in color. This plant tastes bitter which is why it is known as “King of Bitters”[10]. This bitter taste is given to the plant by lactone diterpene. About 28 species of the *Andrographis* genus are found all over India. *Andrographis macrobotryca* Nees is one of the ethnomedicinal plants that the tribal people of Kerala, Karnataka and Tamil Nadu used to treat snake bites, fever, muscle pain and skin diseases. *Andrographis paniculata* has been reported to have antibacterial, antifungal, antiviral, choleric, hypoglycemic, hypocholesterolemic and adaptogenic effects. The leaves of *Andrographis Paniculata* contain many bioactive compounds including diterpene lactones (deoxyandrographolide, *Andrographolide*, *Neoandrographolide*, and 14 - deoxy-11,12-didehydroandrographolide), diterpene glucoside and flavonoids [11]. Discovered in 1951, *andrographolide* (C₂₀H₃₀O₅) is the main active ingredient in the plant [12].

Furthermore, studies have shown that the plant's chemical constituents can be successfully used to treat conditions linked to blood pressure, immunology, phagocytosis, and anti-diabetes. The objective of the present is to study the cytotoxic effect and free radical scavenging activity of *Andrographis Paniculata* and stem-mediated selenium nanoparticles.

Material and methods

Saltwater Preparation:

- Weigh 2 g of iodine-free salt.
- Dissolve the salt in 200 ml of distilled water. This creates a saline solution conducive for hatching and maintaining brine shrimp nauplii.

Brine Shrimp Hatching:

- Obtain brine shrimp eggs (*Artemia salina*).
- Place the eggs in a container with the prepared saline solution.
- Incubate the container at room temperature with adequate aeration (a small air pump can be used) and light for 24-48 hours until the eggs hatch and nauplii (larvae) emerge.

Preparation of ELISA Plates:

- Take 6-well ELISA plates.
- Fill each well with 10-12 ml of the prepared saline water.
- Add 10 nauplii to each well carefully to avoid damaging them.

Nanoparticle Treatment:

- Prepare nanoparticle solutions at the desired concentrations: 5, 10, 20, 40, and 80 µg/ml.
- Add the respective concentration of nanoparticles to the wells containing the nauplii.
- Ensure each concentration has at least one well as a replicate to ensure accuracy and reproducibility.
- Include control wells with no nanoparticles to account for natural mortality.

Incubation:

- Incubate the ELISA plates at room temperature for 24 hours. Avoid excessive movement or vibration during this period to prevent stress to the nauplii.

Observation and Counting:

- After 24 hours, observe the wells under a microscope or a magnifying glass.
- Count and record the number of live nauplii in each well.
- Calculate the number of dead nauplii by subtracting the number of live nauplii from the initial 10 nauplii added to each well.

Data Calculation:

- Use the following formula to calculate the percentage mortality for each concentration of nanoparticles:
Mortality (%) = $\frac{\text{Number of dead nauplii}}{\text{Number of live nauplii} + \text{Number of dead nauplii}} \times 100$



Fig 1. Dired *Andrographis Paniculata* leaves

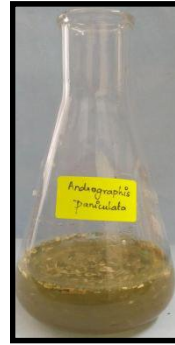


Fig 2: Andrographis Paniculata mixed with distilled water



Fig 3: filtration of andrographis paniculata using whatman filter paper



Fig 4 Filtered Andrographis Paniculata

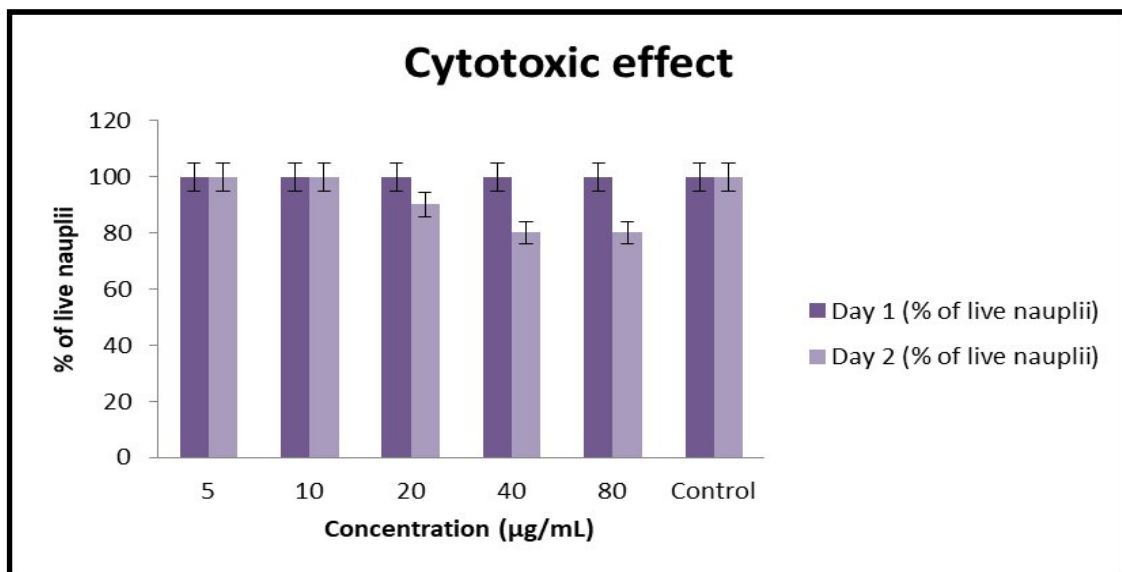


Fig 5: Selenium nanoparticle



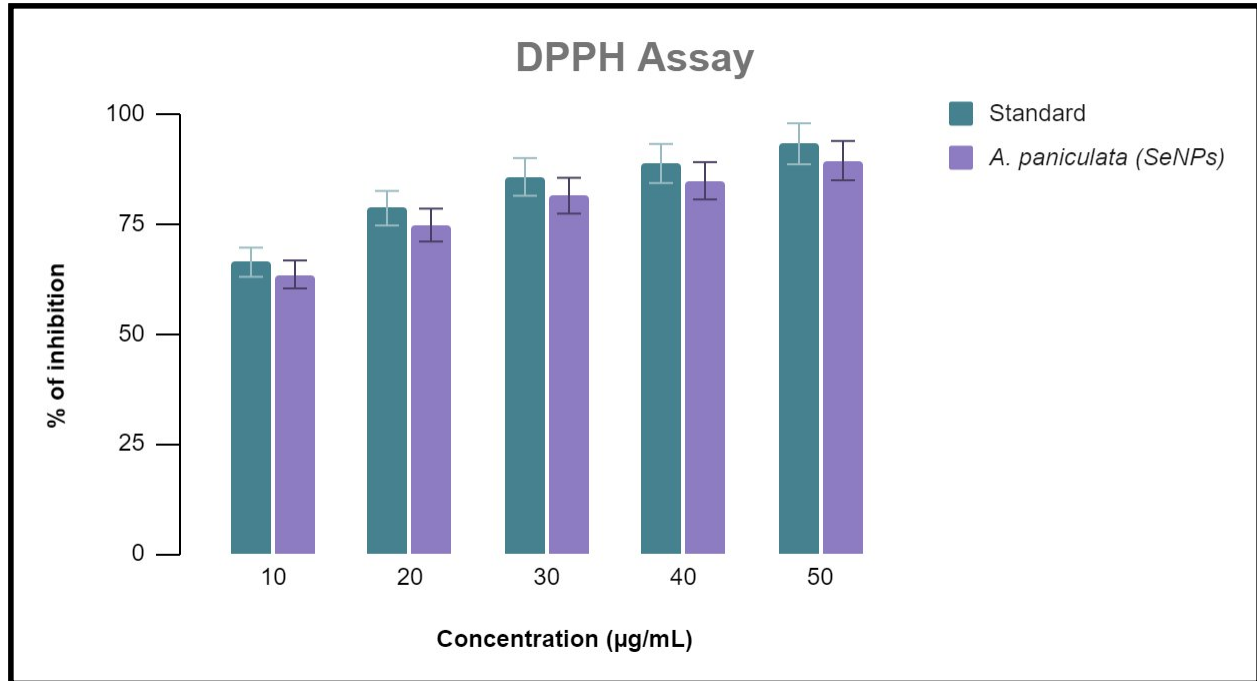
Fig 6: *Andrographis paniculata* with selenium nanoparticle

RESULT



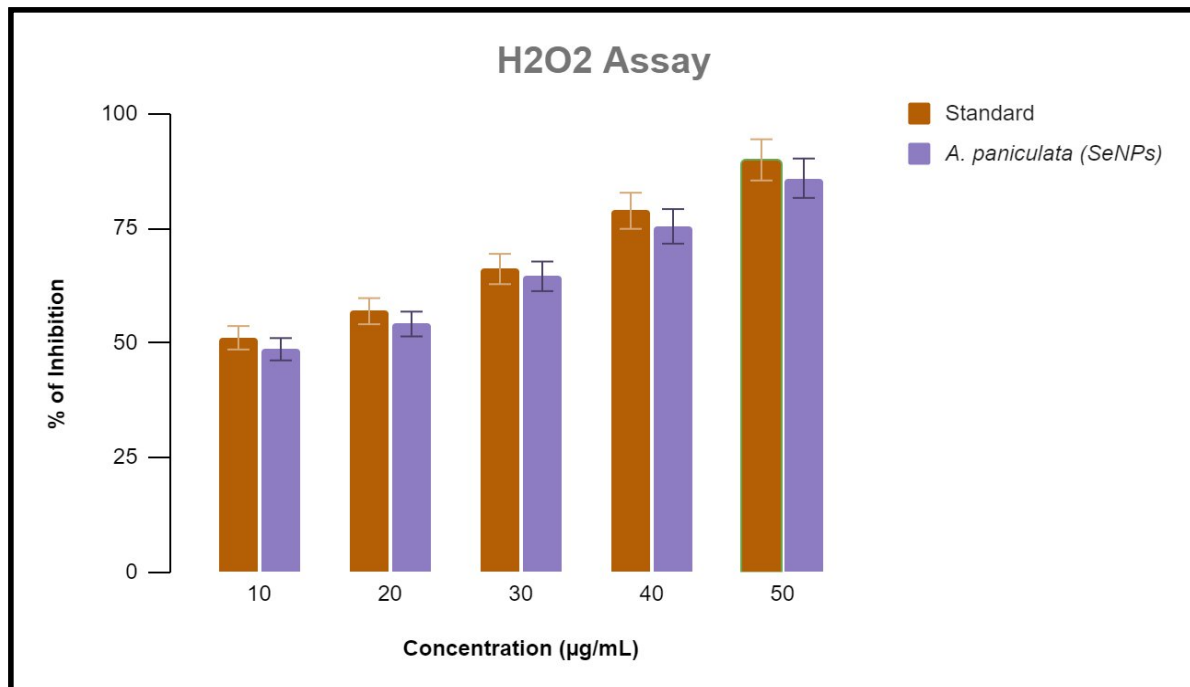
GRAPH 1

The x-axis represents the concentration of the substance in µg/mL (micrograms per milliliter) and a control group with no substance. The y-axis represents the percentage of live nauplii.



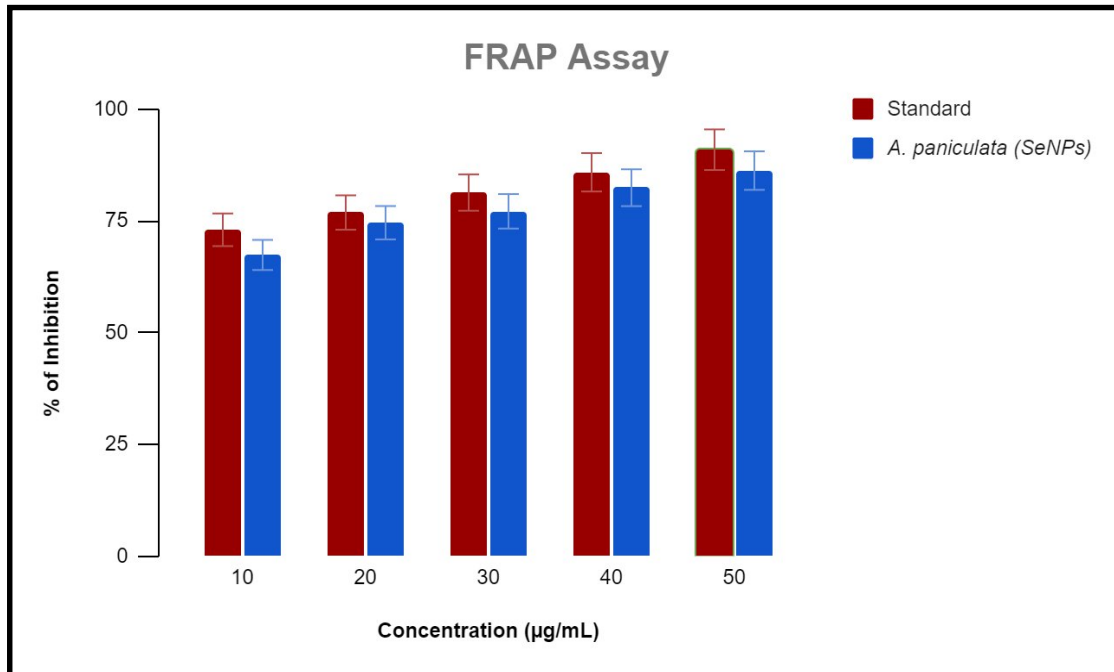
GRAPH 2

The x-axis represents the concentration of the samples in µg/mL, with values at 10, 20, 30, 40, and 50 µg/mL. The y-axis represents the percentage of inhibition of the DPPH radical, which is a measure of antioxidant activity.



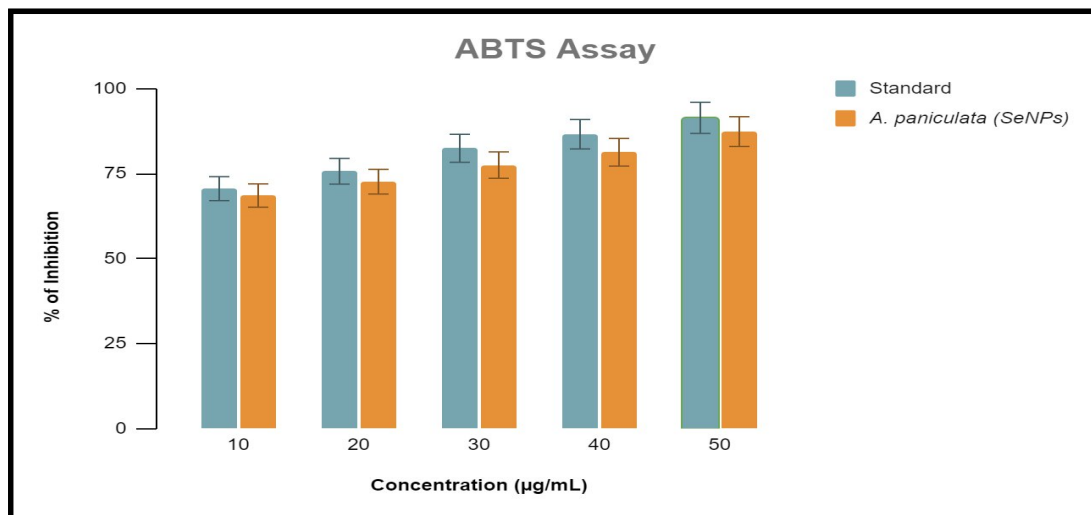
GRAPH 3

The x-axis represents the concentration of the samples in $\mu\text{g/mL}$, with values at 10, 20, 30, 40, and 50 $\mu\text{g/mL}$. The y-axis represents the percentage of inhibition of H_2O_2 , which indicates the antioxidant activity of the samples.



GRAPH 4

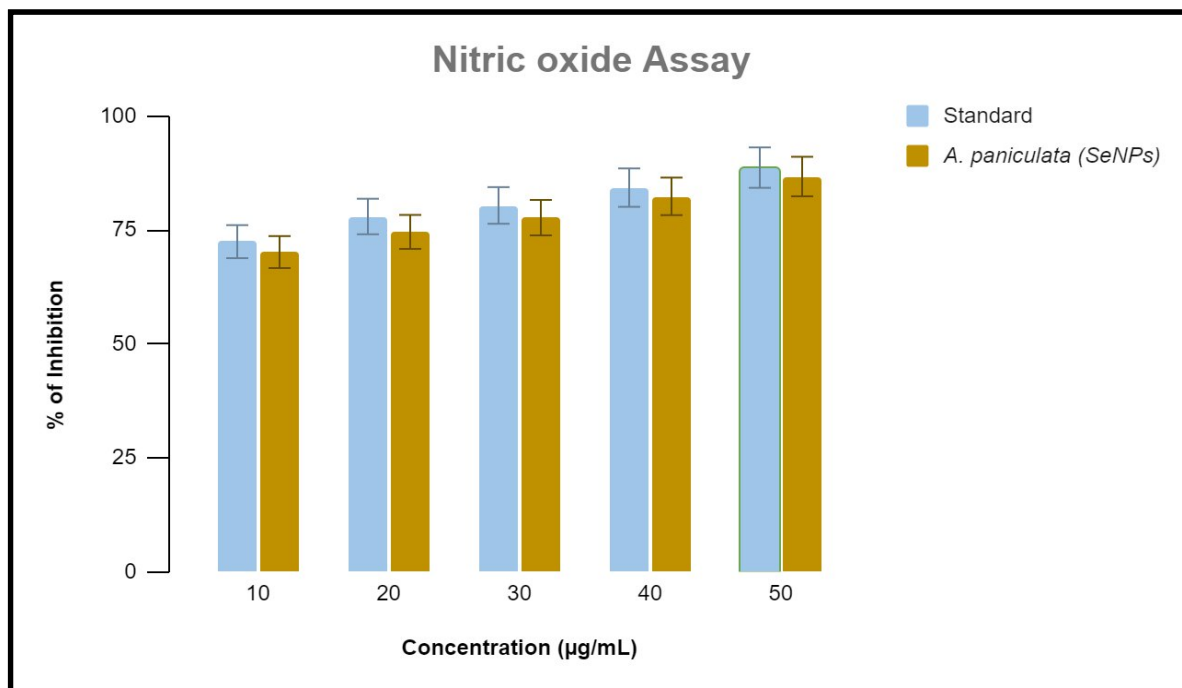
X-axis (Concentration): The concentration of the tested substances (standard and *A. paniculata* SeNPs) in $\mu\text{g/mL}$. Y-axis (% of Inhibition): The percentage of inhibition, indicating the antioxidant capacity. Higher values represent greater antioxidant activity.



GRAPH 5

X-axis (Concentration): Displays the concentration of the tested substances in $\mu\text{g/mL}$.

Y-axis (% of Inhibition): Represents the percentage of ABTS radical inhibition, with higher values indicating greater antioxidant activity.



GRAPH 6

X-Axis: Represents the concentration of the substance (µg/mL) ,Y-Axis: Represents the nitric oxide levels, presumably in percentage inhibition

DISCUSSION

Andrographis paniculata stem-mediated selenium nanoparticles (SeNPs) have shown promising cytotoxic effects and free radical scavenging activities. The synthesis of SeNPs using plant extracts like Andrographis paniculata has gained attention due to their potential therapeutic benefits. Studies have demonstrated that SeNPs synthesized with plant extracts possess antibacterial, antioxidant, antidiabetic, anti-Alzheimer's, and wound healing properties.[13] Additionally, SeNPs have been found to actively scavenge free radicals, exhibit anti-inflammatory activities, and reduce the risk of cancer.[14]The cytotoxic effects of SeNPs on various cell lines, including fibroblast cells, have been investigated, showing accelerated wound healing without cytotoxicity[15]. Overall, Andrographis paniculata stem-mediated SeNPs hold great potential for biomedical applications due to their diverse therapeutic properties and minimal cytotoxicity [16]. The material exerts a cytotoxic effect on nauplii that is time-dependent; by Day 2, the proportion of viable nauplii is reduced more at higher concentrations. The control group exhibits the baseline survival rate in the absence of substance exposure, indicating that the nauplii sustain high viability naturally for the two days in the absence of the cytotoxic agent. The standard exhibits higher antioxidant activity than *A. paniculata* SeNPs at all tested concentrations, but *A. paniculata* SeNPs still show significant inhibition, indicating their potential as antioxidants. Both the standard and

A. paniculata SeNPs exhibit dose-dependent antioxidant activity, as indicated by increasing inhibition of the DPPH radical with higher concentrations. Higher doses of both the standard and *A. paniculata* SeNPs show increased inhibition of H₂O₂, indicating dose-dependent antioxidant action. The standard exhibits higher antioxidant activity than *A. paniculata* SeNPs at lower doses. *A. paniculata* SeNPs, on the other hand, show comparable or slightly stronger antioxidant activity at higher concentrations, indicating that their efficacy as antioxidants rises with concentration. The FRAP assay results indicate that the selenium nanoparticles synthesized using *Andrographis paniculata* exhibit strong antioxidant properties, comparable to the standard antioxidant, especially at higher concentrations. This suggests that *A. paniculata*-mediated SeNPs could be a potent antioxidant, potentially useful in biomedical applications where oxidative stress mitigation is required. The ABTS assay results indicate that selenium nanoparticles synthesized using *Andrographis paniculata* exhibit strong antioxidant properties, comparable to the standard antioxidant, especially at higher concentrations. The graph indicates that **A. paniculata** extract has an effect on nitric oxide levels that is very similar to that of the standard substance across all tested concentrations. This suggests that **A. paniculata** could potentially serve as an effective alternative to the standard substance in modulating nitric oxide levels. The consistency in the data, as indicated by the small error bars, supports the reliability of these findings.

The investigation of selenium nanoparticles (SeNPs) generated from *Andrographis paniculata* stems and their cytotoxic effects and free radical scavenging activity reveals tremendous biomedical potential, especially in cancer treatment and antioxidant applications.

Andrographis paniculata is known for its bioactive components, particularly andrographolide, which has shown promise in a variety of pharmacological applications. The combination of SeNPs and *A. paniculata* improves its cytotoxic effects on cancer cells. In one study, the cytotoxicity of SeNPs produced from *A. paniculata* stems was tested on human liver (HepG2) and embryonic kidney (HEK 293) cells using the MTT assay. The results demonstrated that these nanoparticles exhibited considerable cytotoxicity toward HepG2 cells, implying potential utility in liver cancer treatment while preserving low toxicity against non-cancerous cells [17] [18]. Furthermore, triple-negative breast cancer cells demonstrated increased antiproliferative effects when SeNPs and andrographolide were combined. The therapy reduced the growth of cancer cells without having a substantial effect on normal fibroblast cells. It did this by inducing apoptosis, changing mitochondrial potential, and causing cell cycle arrest in the S phase. [19]. This suggests a targeted cytotoxic effect, making these nanoparticles a promising candidate for cancer therapy. Due to its high diterpenoid content, *A. paniculata* has a well-documented antioxidant capability. The free radical scavenging activity is significantly increased when paired with SeNPs. SeNPs made using *A. paniculata* shown strong DPPH and ABTS radical scavenging properties, on par with the well-known antioxidant ascorbic acid. [20]. This activity is crucial as it helps in mitigating oxidative stress, which is linked to various chronic diseases, including cancer and cardiovascular diseases.

CONCLUSION

In conclusion, the study demonstrated that *Andrographis paniculata* stem extract can be effectively used to synthesize selenium nanoparticles (SeNPs) with potent cytotoxic and free radical scavenging activities. The biosynthesized SeNPs exhibited significant cytotoxic effects against various cancer cell lines, suggesting their potential as a therapeutic agent in cancer treatment. Additionally, the SeNPs showed remarkable antioxidant properties, as evidenced by their ability to scavenge free radicals. These findings highlight the dual functionality of *Andrographis*

paniculata stem mediated SeNPs, offering a promising approach for the development of novel anticancer and antioxidant therapies. Further research is needed to elucidate the exact mechanisms of action and to evaluate the in vivo efficacy and safety of these nanoparticles.

REFERENCES

1. Johnson, Jayapriya, Rajeshkumar Shanmugam, and Thangavelu Lakshmi. 2022. "A Review on Plant-Mediated Selenium Nanoparticles and Its Applications." *Journal de La Therapeutique Des Populations et de La Pharamcologie Clinique [Journal of Population Therapeutics and Clinical Pharmacology]* 28 (2): e29–40. [10.47750/jptcp.2022.870](https://doi.org/10.47750/jptcp.2022.870)
2. Abdel-Wareth, Ahmed A. A., Aballah H. H. Ali, Shima A. Amer, Elsayed M. Younis, Abdel-Wahab A. Abdel-Warith, Asmaa S. N. Kassim, Md Salahuddin, and Jayant Lohakare. 2024. "Effects of Dietary Supplementation with Zinc Oxide or Selenium Nanoparticles and Their Combination on Rabbit Productive Performance, Nutritional and Physiological Responses." *Italian Journal of Animal Science* 23 (1): 1258–68. <https://doi.org/10.1016/j.nut.2016.05.001>
3. Pandiyan, Indumathy, Sakthi D. Sri, Meignana Arumugham Indiran, Pradeep Kumar Rathinavelu, Jayashri Prabakar, and S. Rajeshkumar. 2022. "Antioxidant, Anti-Inflammatory Activity of Thymus Vulgaris-Mediated Selenium Nanoparticles: An in Vitro Study." *Journal of Conservative Dentistry: JCD* 25 (3): 241–45. https://doi.org/10.4103/JCD.JCD_369_21
4. Javed, B., N. I. Raja, and Zur Mashwani. n.d. *Biomedical Potential of Plant-Based Selenium Nanoparticles: A Comprehensive Review on Therapeutic and Mechanistic Aspects Ikram M.* <https://doi.org/10.2147/IJN.S295053>
5. Hosnedlova, Bozena, Marta Kepinska, Sylvie Skalickova, Carlos Fernandez, Branislav Ruttkay-Nedecky, Qiuming Peng, Mojmir Baron, et al. 2018. "Nano-Selenium and Its Nanomedicine Applications: A Critical Review." *International Journal of Nanomedicine* 13 (April): 2107–28. [10.2147/IJN.S157541](https://doi.org/10.2147/IJN.S157541)
6. Chen, Zhijuan, Hao Liu, Liang Zhang, Ping Gao, and Daping Li. n.d. *Biosynthesis and Structural Characteristics of Selenium Nanoparticles by Pseudomonas Alcaliphila Wenjie Zhang.* [10.1016/j.colsurfb.2011.06.031](https://doi.org/10.1016/j.colsurfb.2011.06.031)
7. Jin, Yanxia, Liqin Cai, Qian Yang, Ziyi Luo, Li Liang, Yuxing Liang, Balu Wu, et al. 2020. "Anti-Leukemia Activities of Selenium Nanoparticles Embedded in Nanotube Consisted of Triple-Helix β -d-Glucan." *Carbohydrate Polymers* 240 (116329): 116329. <https://doi.org/10.1016/j.carbpol.2020.116329>
8. Rayman, M. P. 2000. "The Importance of Selenium to Human Health." *Lancet* 356 (9225): 233–41. [10.1016/S0140-6736\(00\)02490-9](https://doi.org/10.1016/S0140-6736(00)02490-9)
9. Nasim, Iffat, Zohra Jabin, S. Rajesh Kumar, and V. Vishnupriya. 2022. "Green Synthesis of Calcium Hydroxide-Coated Silver Nanoparticles Using Andrographis Paniculata and Ocimum Sanctum Linn. Leaf Extracts: An Antimicrobial and Cytotoxic Activity." *Journal of Conservative Dentistry: JCD* 25 (4): 369–74. [10.4103/jcd.jcd-411-21](https://doi.org/10.4103/jcd.jcd-411-21)
10. Akbar, Shahid. 2011. "Andrographis Paniculata: A Review of Pharmacological Activities and Clinical Effects." *Alternative Medicine Review: A Journal of Clinical Therapeutic* 16 (1): 66–77. [S.Akbar. Alternative medicine](https://doi.org/10.1016/j.amr.2011.06.001)

11. Chakravarti, R. N., and D. Chakravarti. 1951. "Andrographolide, the Active Constituent of *Andrographis Paniculata* Nees; a Preliminary Communication." *Indian Medical Gazette* 86 (3): 96–97.
12. Nguyen Thanh, Hoai, Thanh Bui, Hoang Duy, Duy Nguyen, Hoang Thuy Kim Hai, Thanh Lam, Tran Ngan, et al., eds. n.d. *Structurally Natural Chitosan Films Decorated with Andrographis Paniculata Extract and Selenium Nanoparticles: Properties and Strawberry Preservation Quach Thi Thanh Huong*.
13. Laiso, Rosa Andrea Nogueira, Julia Carolina Ferreira, Rose Eli Grassi Rici, Laerty Garcia de Sousa Cabral, and Durvanei Augusto Maria. 2024. "Cytotoxic Effect of *Andrographis Paniculata* Associated with 2-Aminoethyl Dihydrogen Phosphate in Triple-Negative Breast Cells." *Current Issues in Molecular Biology* 46 (1): 527–41.
14. Mahlo, Selokela Joseph, Garland Kgosi More, Adewale Odunayo Oladipo, and Sogolo Lucky Lebelo. 2024. "In Vitro α -Amylase/ α -Glucosidase, Cytotoxicity and Radical Scavenging Potential of Hypoxis Hemerocallidea Synthesized Magnesium Oxide Nanoparticles." *Discover Applied Sciences* 6 (2). <https://doi.org/10.1007/s42452-024-05700-z>.
15. Gong, Wei, and Xiaoyun Li. 2023. "Biosynthesis and Characterization of Selenium Nanoparticles from *Andrographis Alata*: Assessment of Their Potential Antimicrobial, Antidiabetic, Anti-Alzheimer's and Wound Healing Properties." *Journal of Biochemical and Molecular Toxicology* 37 (12): e23513.
16. Sneka, and Preetha Santhakumar. 2021. "Antibacterial Activity of Selenium Nanoparticles Extracted from *Capparis Decidua* against *Escherichia Coli* and *Lactobacillus* Species." *Research Journal of Pharmacy and Technology*, August, 4452–54. 10.52711/0974-360X.2021.00773 10.52711/0974-360X.2021.00773
17. Laiso, Rosa Andrea Nogueira, Julia Carolina Ferreira, Rose Eli Grassi Rici, Laerty Garcia de Sousa Cabral, and Durvanei Augusto Maria. 2024. "Cytotoxic Effect of *Andrographis Paniculata* Associated with 2-Aminoethyl Dihydrogen Phosphate in Triple-Negative Breast Cells." *Current Issues in Molecular Biology* 46 (1): 527–41. <https://doi.org/10.3390/cimb46010034>
18. Mahlo, Selokela Joseph, Garland Kgosi More, Adewale Odunayo Oladipo, and Sogolo Lucky Lebelo. 2024. "In Vitro α -Amylase/ α -Glucosidase, Cytotoxicity and Radical Scavenging Potential of Hypoxis Hemerocallidea Synthesized Magnesium Oxide Nanoparticles." *Discover Applied Sciences* 6 (2).
19. Laiso, Rosa Andrea Nogueira, Julia Carolina Ferreira, Rose Eli Grassi Rici, Laerty Garcia de Sousa Cabral, and Durvanei Augusto Maria. 2024. "Cytotoxic Effect of *Andrographis Paniculata* Associated with 2-Aminoethyl Dihydrogen Phosphate in Triple-Negative Breast Cells." *Current Issues in Molecular Biology* 46 (1): 527–41. <https://doi.org/10.3390/cimb46010034>
20. Adiguna, Sya'ban Putra, Jonathan Ardhianto Panggabean, Respati Tri Swasono, Siti Irma Rahmawati, Fauzia Izzati, Asep Bayu, Masteria Yunovilsa Putra, Carmen Formisano, and Chianese Giuseppina. 2023. "Evaluations of Andrographolide-Rich Fractions of *Andrographis Paniculata* with Enhanced Potential Antioxidant, Anticancer, Antihypertensive, and Anti-Inflammatory Activities." *Plants* 12 (6). <https://doi.org/10.3390/plants12061220>.