

In Vitro Controlling Of Wound Pathogens Using Andrographis Paniculata Stem Mediated Selenium Nanoparticles

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Abstract

Introduction: Wound infections, caused by microorganisms such as *Staphylococcus aureus* and *Escherichia coli*, impede healing and damage tissue. Developing eco-friendly nanoparticle synthesis methods is crucial, with selenium nanoparticles (SeNPs) showing promise in biomedicine due to their low toxicity and high biocompatibility. SeNPs, produced by microorganisms or chemical methods, exhibit significant antioxidant and anti-inflammatory properties. The medicinal herb *Andrographis paniculata*, rich in polyphenols, flavonoids, and diterpenoids, has been traditionally used to treat various infections and inflammatory conditions, enhancing immune responses and offering potential therapeutic benefits.

Aim: In vitro controlling of wound pathogens using *Andrographis paniculata* stem mediated selenium nanoparticles has been evaluated

Methods: Preparation: *Andrographis paniculata* stem extract was mixed with sodium selenite solution to synthesize SeNPs. The agar well diffusion method was carried out against *Staphylococcus aureus*, *Pseudomonas sp*, *Escherichia coli*.

Results: *Andrographis paniculata* stem-mediated selenium nanoparticles (SeNPs) show significant antibacterial activity against *Staphylococcus aureus*, with effectiveness increasing at higher concentrations. SeNPs also exhibit moderate, consistent activity against *Pseudomonas sp.* and *Escherichia coli*. Further research could explore their mechanism, stability, and potential in antimicrobial therapies.

Conclusion: The SeNPs synthesized from the stem extract of *Andrographis paniculata* showed excellent antibacterial potential; hence further studies are needed to assess the efficacy and safety for dental uses.

Keywords: Seleniumnanoparticles(SENPS), *Andrographis Paniculata*, Antibacterial Activity, *Staphylococcus aureus*, *Pseudomonas sp*, *Escherichia coli*

INTRODUCTION

The invasion of a wound by multiplying microorganisms to the point where the host has a localized, systemic, or both kind of response is known as wound infection. Microorganisms in the wound hinder the healing process and cause harm to nearby tissue(1). *Proteus* species, *Streptococcus spp.*, *Enterococcus spp.*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Escherichia coli* are common bacterial pathogens linked to wound infection(2). These organisms have the capacity to colonize damaged skin and show a built-in resistance to numerous antibiotics and antiseptics, allowing them to live for extended periods of time and even reproduce in the absence of nourishment (3).

The demand to create ecologically safe nanoparticle synthesis methods that avoid using hazardous compounds in the synthesis methodology is currently increasing(4,5). It is only recently that material scientists have begun to view microorganisms like bacteria and yeast as potential eco-friendly nanofactories, despite the fact that many biotechnological applications like the remediation of toxic metals use them (the detoxification often occurring via reduction of the metal ions/formation of metal sulfides).(3,6)

Among the nanomaterials, selenium nanoparticles (SeNPs) have drawn consideration and are broadly acknowledged in biomedicine and food science because of their low poisonousness and high biocompatibility. Captivatingly, selenium is a vital variable in the development of selenoproteins, which are imperative cancer prevention agents like thioredoxin reductase, glutathione peroxidase, and deiodinase (3,6,7). Selenium is one of the minor elements, which is fundamental for the upkeep of human wellbeing, with around 40–300 mg as day by day dietary enhancement for a grown-up(8).

The size of the SeO particles is an important factor that determines their potential chemical or biological activity. Nano-Se particles smaller than 100 nm have greater antioxidant activity than larger particles (9). Nano-Se particles of size 5–200 nm can directly scavenge free radicals in vitro depending on their size. Microorganisms can produce SeO nanoparticles ranging from 30 nm (7) to 500 nm depending on the reducing species and conditions. The microbial production of nanoparticles can, therefore, be seen as an opportunity not only for bioremediation but also in nanobiotechnology due to the multiple applications of this element. Selenium (Se), a chalcogen element that is present in trace amounts in the Earth's core, and due to its scarcity, has been classified by the American Physical Society and the Materials Research Society as a critical element (10). (11) Indeed, selenium is widely known as an essential element for life, found in amino acids and proteins.

In the chemical technique, nano Se particles require different reductants using Se precursor salts. In this method, sodium selenite and ascorbic acid are used to produce Se nanoparticles which can be observed in a light orange color (12). Ascorbic acid is also known for its functionality as antioxidative and immunostimulation roles. Furthermore, nano Se form (35–70 nm) was formed chemically in ambient circumstances with organic acids such as acetic and oxalic acid and polyvinyl alcohol (PVA) as a binding material (13,14).

Nano-selenium is a potential drug delivery carrier for antioxidant or anti-inflammatory effects due to its low toxicity, strong biocompatibility, and antioxidation capacities (14). Selenium (Se) is integrated into Selenoproteins as selenocysteine, where it regulates biological systems such as the antioxidant system (15). Various research shows that SeNPs have a wide range of medicinal uses, ranging from antioxidant activity to anticancer effects, owing to their redox-balancing function (16).

The medicinal herb *Andrographis paniculata* has a very bitter flavor. It has been used for ages to treat a wide range of chronic and infectious disorders, including fever, herpes, sore throats, and respiratory infections. Its primary components are polyphenols, flavonoids, and diterpenoids. Andrographolide is the main diterpenoid in *A. paniculata*, accounting for around 4%, 0.8–1.2%, and 0.5–6% of the dried whole plant, stem, and leaf extracts, respectively (17)(18).

Indian-origin plant kalmegh has been utilized for Ayurvedic medicine for ten years. It is mostly found in Sri Lanka and India. West Bengal, Orissa, Bihar, Jharkhand, Andhra Pradesh, Assam, Kerala, Karnataka, and Uttar Pradesh are among the states where the plant's wild variant can be found. Although it may be grown across India, the plains are better suited for commercial and high-production agriculture. The annual plant kalmegh grows to a height of three feet. The leaves measure 2.5 cm in width and 7.5 cm in length. The plant produces white flowers. The seeds have a yellowish brown color and are tiny in size. The plant known as the "king of bitters" is extremely bitter because of its taste.

For millennia, *A. paniculata* has been used successfully as a medicinal herb in numerous traditional systems, where it has been used to treat skin infections, diabetes, fever, and common cold symptoms (19). For more than 20 years, it has been documented that *A. paniculata* and its constituent diterpene lactones exert immunomodulatory actions via a variety of mechanisms (20). Many pharmacological benefits of *A. paniculata*, including its anti-inflammatory, anti-infectious, and anti-cancer activity, have been attributed to its immunostimulant qualities.

AIM

This study aims to produce and analyze selenium nanoparticles (SeNPs) utilizing stems from *Andrographis paniculata*. The study compares the effectiveness of these SeNPs with conventional antimicrobial drugs and assesses their antibacterial activity against *Pseudomonas* sp., *Escherichia coli*, and *Staphylococcus aureus*. The final goal of this study is to investigate these SeNPs' potential as supplemental or alternative antibacterial agents for the treatment of wounds.

MATERIALS AND METHODS

The process began with obtaining approximately 100g of dried leaf powder, which was then combined with water and filtered through a filter cup. The plant extract was then obtained, and after the solution was left undisturbed until it was filtered, a solution of sodium selenite solution—the nanoparticle used in our study—was mixed with the plant extract and *Andrographis paniculata* with sodium selenate was obtained.



Figure 1: Dried leaf powder

Figure 2: Dried leaf mixed with water

Figure 3: Filtration

Figure 4: Plant Extract

Figure 5: Sodium selenate solution

Figure 6: *A.paniculata* with sodium selenate

Figure 7: *A.paniculata* SeNPs

The antimicrobial activity of selenium nanoparticles was evaluated using the agar well diffusion technique. Mueller Hinton agar plates were prepared and sterilized using an autoclave at 121°C for 15- 20 minutes. After sterilization, the medium was poured onto the surface of sterile Petri plates and allowed to cool to room temperature. The bacterial suspension (*Staphylococcus aureus*, *Pseudomonas* sp, *Escherichia coli*) was spread evenly onto the agar plates using sterile cotton swabs. Wells of 9 mm diameter were created in the agar plates using a sterile polystyrene tip. The wells were then filled with different concentrations (25, 50, 100 µg/mL) of CuONPs. An antibiotic (e.g., Bacteria-Amoxycillin, Fungi-Fluconazole) was used as a standard. The plates were incubated at 37°C for 24 hours and 48 hours for fungal cultures. The antimicrobial activity was evaluated by measuring the diameter of the inhibition zone surrounding the wells. The diameter of the zone of inhibition was measured using a ruler and recorded in millimeters(mm) and the zone of inhibition was calculated.

RESULT



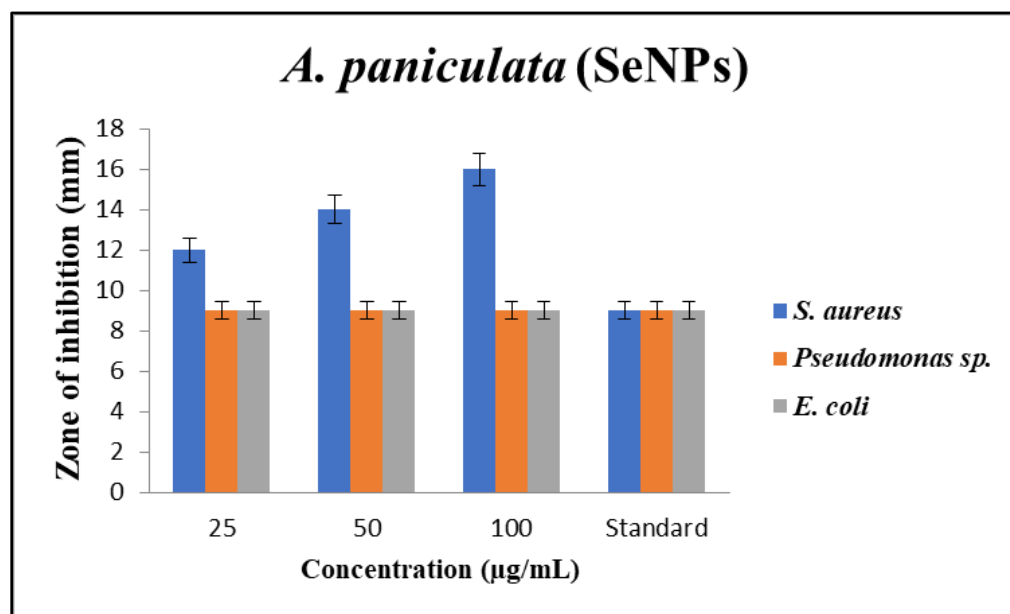
PLATE-1
E.coli

PLATE-2
S. aureus

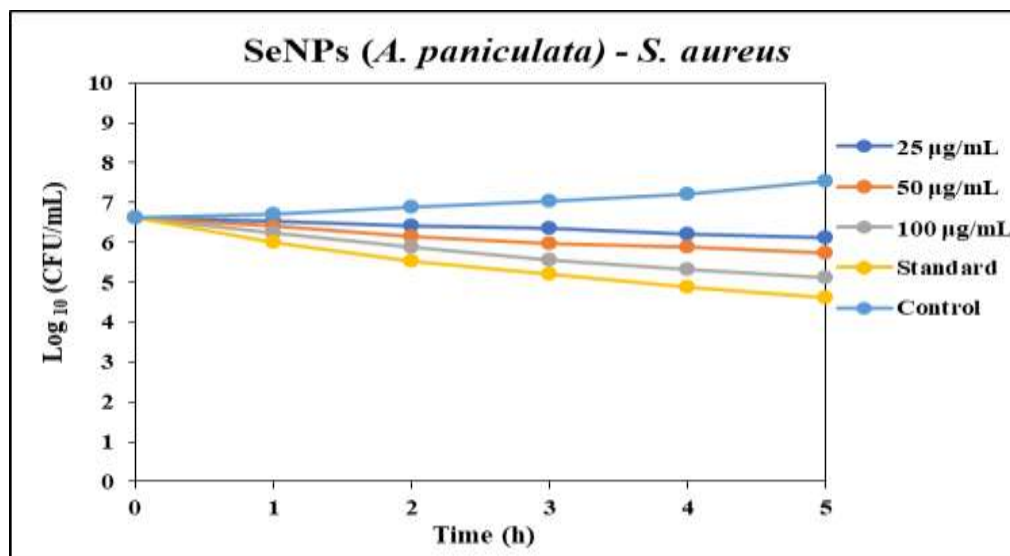
PLATE-3
Pseudomonas sp

TABLE-1

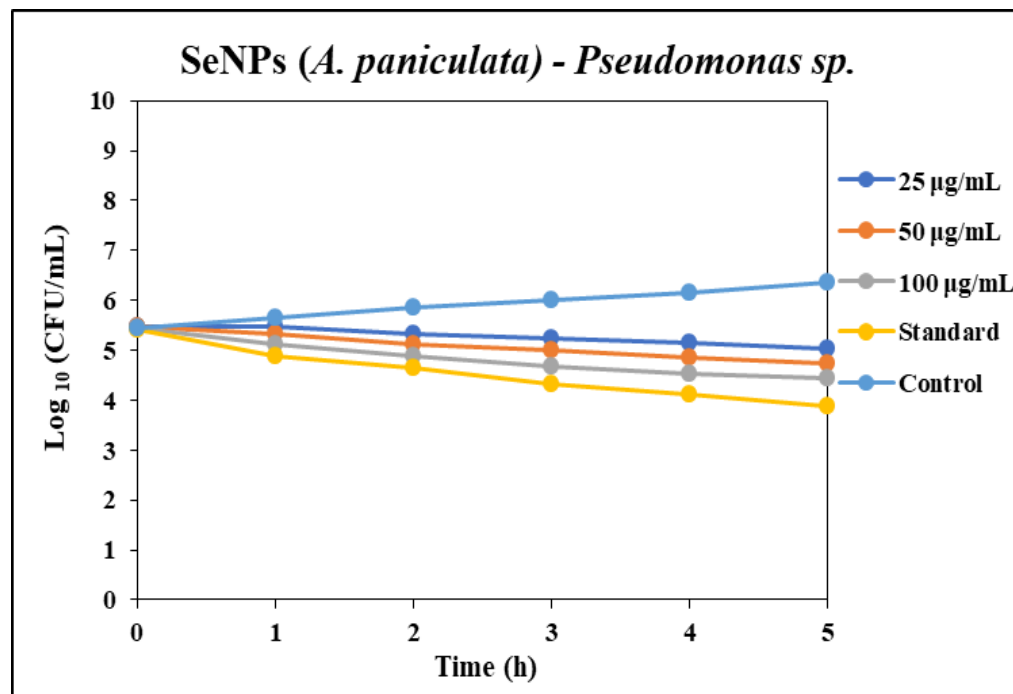
	25	50	100	Standard
<i>S. aureus</i>	12	14	16	9
<i>Pseudomonas sp.</i>	9	9	9	9
<i>E. coli</i>	9	9	9	9



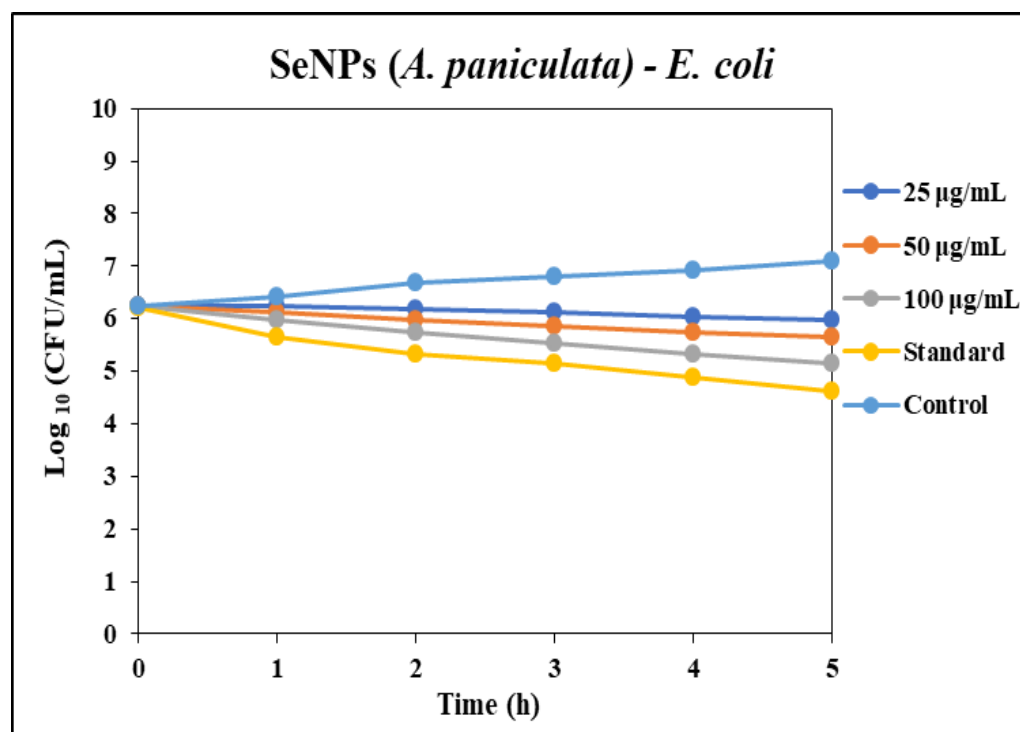
GRAPH 1: The bar graph shows the anticariogenic activity of selenium nanoparticles made from *Andrographis paniculata* extract. The data indicates the concentration on the x axis and the zone of inhibition in millimeters on the y axis



GRAPH 2: Time kill curve assay chart showing SeNPs(*A. paniculata*)-*S. aureus*



GRAPH 3:Time kill curve assay chart showingSeNPs(A.paniculata)-Pseudomonas sp



GRAPH 4:Time kill curve assay chart showing SeNPs(A.paniculata)-E.coli

DISCUSSION

Because of its potential for reducing wound infections, the production of selenium nanoparticles (Se NPs) using *Andrographis paniculata* has received a lot of interest recently. This plant, which has a wealth of medicinal qualities, works well as a reducing agent when it comes to the environmentally friendly synthesis of Se NPs. Flavonoids, phenols, and alkaloids—some of the phytochemicals found in *A. paniculata*—help

reduce selenite ions to elemental selenium, which produces nanoparticles with improved bioactivity and antibacterial qualities(21).(22)

The antibacterial activity of *Andrographis paniculata* stem-mediated selenium nanoparticles (SeNPs) against three common wound pathogens—*Pseudomonas* sp., *Escherichia coli*, and *Staphylococcus aureus*—is shown in the bar graph. The zone of inhibition at various SeNP concentrations (25, 50, and 100 µg/mL) is measured against a standard antibacterial agent. For *Staphylococcus aureus*, the inhibition zones are approximately 12 mm, 14 mm, and 16 mm at 25, 50, and 100 µg/mL, respectively. For both *Pseudomonas* sp. and *Escherichia coli*, the inhibition zone remains around 9 mm across all tested concentrations.

Studies show that the antimicrobial efficacy of Se NPs synthesized by this method has been demonstrated against a range of pathogens, including fungi, bacteria, and Gram-positive and Gram-negative bacteria. These nanoparticles can cause oxidative stress in microbial cells by producing reactive oxygen species (ROS), which damages cell membranes and ultimately results in cell death. In applications involving wound healing, where infection control is essential, this process is very helpful. Moreover, the bioactive components of *A. paniculata* may improve the antibacterial activity of the Se NPs, working in concert to combat pathogens like *Escherichia coli* and *Staphylococcus aureus*, which are frequently linked to wound infections.(23)(24)

Another important benefit of plant-mediated Se NPs is their biocompatibility; unlike their chemically manufactured counterparts, these nanoparticles are less likely to cause unfavorable interactions in biological systems. Because of this quality, they can be used in clinical settings, especially in topical formulations and wound dressings that try to promote healing and ward against infections.

Furthermore, selenium is essential for immune system and antioxidant defense, which can accelerate wound healing by promoting tissue regeneration and lowering inflammation.(25)(26)

To examine the size, form, and stability of the produced Se NPs, characterization methods including UV-Vis spectroscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM) have been used. According to these investigations, the nanoparticles usually have a size between 10 and 100 nm and a spherical shape, making them perfect for use in biological applications(27)(26,28). well addition to offering a cost-effective and ecologically responsible way to produce nanoparticles, the green synthesis strategy fits well with the expanding trend of employing natural ingredients in nanomedicine.

To sum up, the synthesis of selenium nanoparticles using *Andrographis paniculata* presents a fresh and practical approach to managing wound infections. Enhancing wound healing and avoiding infections is made possible by the combination of Se NPs' antibacterial qualities and the medicinal advantages of the plant. Subsequent investigations ought to concentrate on refining the synthesis procedure, comprehending the intricate mechanisms of action, and carrying out clinical trials to confirm the effectiveness and safety of these nanoparticles in wound care environments.

CONCLUSION

An important development in the realm of nanomedicine, particularly for the treatment of wound infections, is the manufacture of selenium nanoparticles (Se NPs) utilizing *Andrographis paniculata*. This method makes use of the plant's wide range of bioactive chemicals, which not only make the synthesis of Se NPs more environmentally friendly but also strengthen their antibacterial capabilities. These nanoparticles are excellent agents against common wound infections like *Staphylococcus aureus* and *Escherichia coli* because of their capacity to produce reactive oxygen species (ROS) and damage microbial cell integrity.

Additionally, plant-mediated Se NPs' biocompatibility reduces the possibility of negative reactions, which qualifies them for clinical use in wound treatment. Selenium's immune-modulating and antioxidant qualities encourage tissue regeneration and lower inflammation, which aid in the healing process even more.

The improvement of synthesis techniques and a greater comprehension of the mechanisms underlying the antibacterial properties of Se NPs ought to be the top priorities of future study. To confirm the safety and effectiveness of these nanoparticles in practical applications, clinical trials are necessary. All things considered, the creative synthesis of selenium nanoparticles using *Andrographis paniculata* offers great potential for improving wound healing techniques and thwarting infections, opening the door to more potent and all-natural treatment solutions in the medical field.

FUTURE SCOPE

Future research on *Andrographis paniculata* stem-mediated selenium nanoparticles holds significant promise. Studies can focus on testing these nanoparticles against a wider range of wound pathogens, including antibiotic-resistant strains. Optimizing and standardizing the synthesis process for consistent and scalable production is crucial. Exploring synergistic effects with other antimicrobial agents and developing multifunctional nanocomposites can enhance their efficacy. Additionally, advancements in formulating SeNPs into wound dressings, coatings, and topical applications like creams and gels can lead to more effective and sustained antimicrobial therapies, improving the management of wound infections and promoting faster healing.

CONFLICT OF INTEREST : There is no conflict of interest

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