

Anticariogenic Potential Of Selenium Nanoparticles Synthesized Using Andrographis Paniculata Stem Extract

S. Keerthivasan¹, *Mrs. S. Sangeetha²

¹Department of Anatomy, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical sciences (SIMATS), Saveetha University, Chennai -600077, India, 152301048.sdc@saveetha.com

²Assistant Professor, Department of Anatomy, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical sciences (SIMATS), Saveetha University, Chennai -600077, India.

sangeethas.sdc@saveetha.com

ABSTRACT

Introduction: Dental caries is a dynamic sugar and biofilm-associated disease of both primary and permanent teeth. Selenium nanoparticles are offering a new perspective for antimicrobial applications, while *Andrographis paniculata* is a plant known for its medicinal uses.

Aim: The anticariogenic potential of SeNPs synthesized using *Andrographis paniculata* stem extract 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 *Streptococcus mutans*, *Lactobacillus* sp., *Candida albicans*, and *Escherichia coli*.

Results: SeNPs showed considerable antimicrobial activity against *C. albicans* and *Lactobacillus* sp. The inhibition zones increased with an increase in concentration, but the SeNPs did not show any remarkable activity against *S. mutans* and *E. coli*.

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

Keywords: Dental caries, Selenium nanoparticles (SeNPs), *Andrographis Paniculata*, Anticariogenic potential, Antimicrobial Activity, *Streptococcus mutans*, *Lactobacillus* Sp., *Candida albicans*, *Escherichia coli*

INTRODUCTION

Dental caries is a sugar-driven, biofilm-mediated, multifactorial dynamic disease that results in the phasic demineralization and remineralization of dental hard tissues. Caries can occur throughout life both in primary and permanent dentitions and damage any tooth crown and, in later life, exposed root surfaces(1). Causative factors of dental caries include mainly microorganisms such as *Streptococcus mutans* and *Lactobacillus* species.(2) The limits of caries intervention and diagnosis are evolving. Currently, radiographic, tactical, and optical data are used by dentists to identify relatively advanced alterations in the tooth hard tissues.(3) Early childhood caries refers to dental caries in children under the age of six.(4) Various inorganic – water and electrolytes – and organic – proteins and peptides – components may protect teeth from dental caries(5). Since teeth are constantly bathed in saliva, the constituents and properties of this oral fluid play a very important role in the occurrence and progression of dental caries.(6)

Nano particles have an enormous impact on society. In comparison with their bulk material counterparts, nanoparticles exhibit very high surface areas. One of the fast-growing areas in today's technological developments is the design of bioinspired nanoparticles(7).(8) Selenium nanoparticles are used in various oxidative stresses.(9) Natural nanocarrier, selenium, exhibits potent antibacterial, antioxidant, anti-cancer, and anti-inflammatory properties.(10) While selenium is an essential trace element for growth and health maintenance in the diet, depending on the dosage and chemical type, it can be poisonous and seriously harm a person. It is distinct with its high level of biological, Moderate activity and low toxicity with cytotoxic property.(11) Potential applications of nanotechnology are huge and already implemented in many scientific fields such as pharmacy, diagnostics, environmental, energy, engineering, electronics, and chemical industries. Selenium nanoparticles, or SeNPs, thus provide, we believe, a fresh opportunity in nutritional supplementation since they are less toxic and possibly releasable with time after intake..(12)

They are colored bright orange-red and preparations can be done chemically, physically, or biologically. Biogenic SeNPs are stable and nonagglomerated due to covering by these biomolecules naturally. Several theories have been put out to explain the mechanism of SeNP microbiological production.(13)SeNPs' importance at cellular and tissue levels has drawn the attention of numerous researchers from all parts of the world toward their biological applications. As is already known, several abiotic stresses are able to induce an overproduction of harmful ROS and cause oxidation of critical components like proteins, lipids, and carbohydrates, which in turn may result in a wide spectrum of disorders.(14)

Andrographis paniculata (F.) Nees (Acanthaceae) originated from Taiwan, Mainland China, and India. The herb tastes extremely bitter and hence the medication is related to upper respiratory infections, liver diseases, gastrointestinal problems in children, colic pain, and common cold. More than 20 diterpenoids and over 10 flavonoids have been reported from *A. paniculata*. Active chemicals extracted with ethanol or methanol from the entire plant including the leaf and stem. The main diterpene in *A. paniculata* is andrographolide (C₂₀H₃₀O₅) that constitutes about 4%, 0.8~1.2%, and 0.5~6% of the dried whole plant, stem, and leaf extracts respectively.(15)

The main active ingredient of *A. paniculata* is andrographolide, which has been reported for various pharmacological properties, including anti-inflammatory, anti-tumor, anti-obesity, and anti-diabetic properties.(16) Anti-inflammatory, antiallergic, antiplatelet aggregation, hepatoprotective, and anti-HIV effects are among the many biological activities that have been documented for andrographolide.(16,17)

Diterpene lactones are the main active principles governing the biological activity, but flavonoids, xanthenes, and caffeic acid derivatives also contribute to anti-oxidant, anti-proliferative, anti-atherosclerotic, and anti-malarial activities.(18)

AIM

This work is aimed at investigating the anticariogenic potential of selenium nanoparticles synthesized using *Andrographis paniculata* stem extract. In this study, the anticariogenic potential of *Andrographis paniculata* stem extract-synthesized SeNPs was investigated against dental caries infection. This thus leads to exploring the nanomaterial for some antimicrobial properties on dental care applications.

METHODS AND MATERIALS

First the dried leaf powder was obtained for about 100g, then the sample was mixed with water and from that sample the water was filtered using the filter cup, after leaving the solution undisturbed until it gets filtered, the plant extract is obtained, then a solution of sodium selenite solution which is the nanoparticle for our study is being used, the plant extract is mixed with solution of selenate and finally the *Andrographis paniculata* with sodium selenate is obtained.



Figure 1: Dried leaf powder

Figure 2: Dried leaf mixed with water

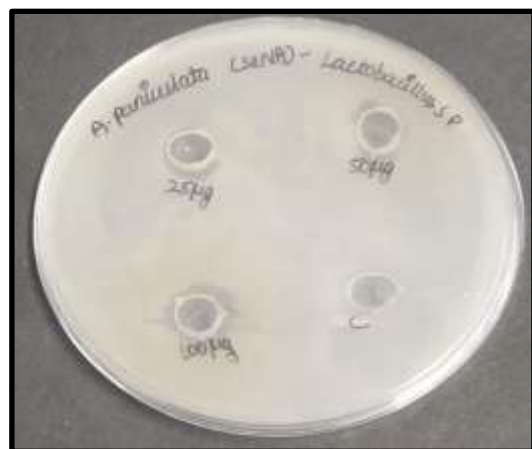
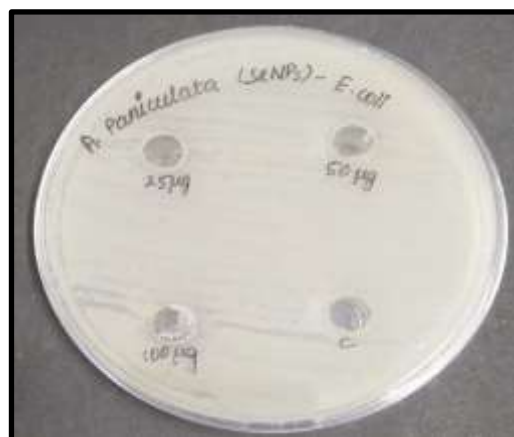
Figure 3: Filtration

Figure 4: Plant Extract

Figure 5: Sodium selenate solution

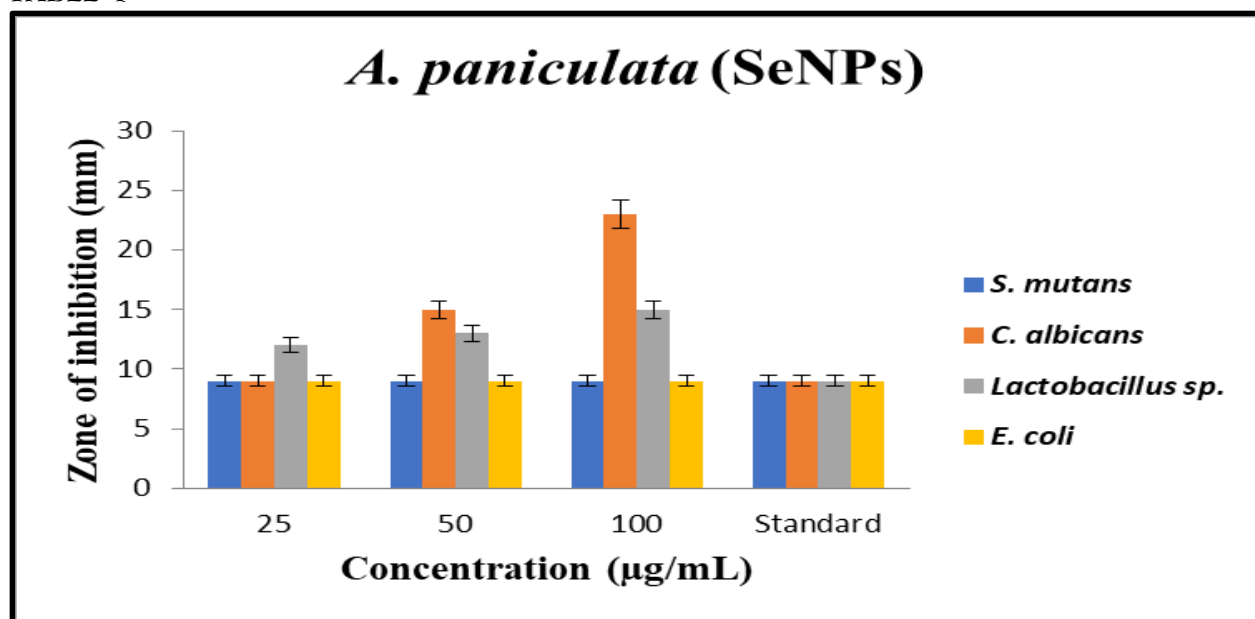
Figure 6: *A.paniculata* with sodium selenate

The antimicrobial activity of the 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 (*Streptococcus mutans*, *Lactobacillus 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 SeNPs. An antibiotic (e.g., Bacteria-Amoxyrite, Fungi-Flucanazole) 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.

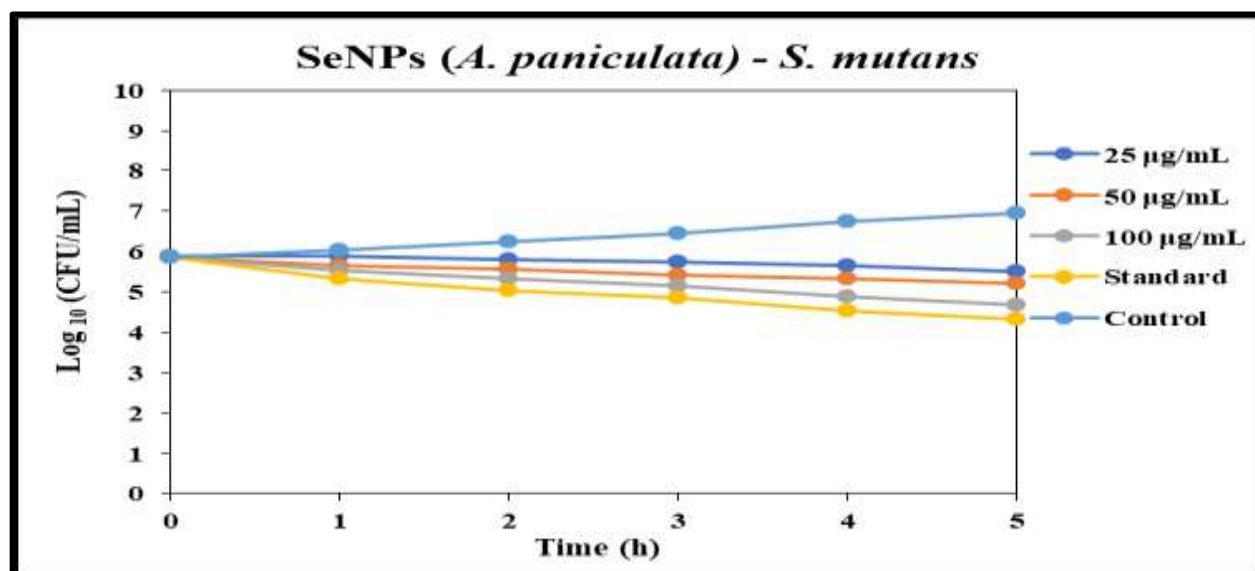
RESULTDISH 1: *Lactobacillus SP*DISH 2: *S.Mutans*DISH 3: *C.Albicans*DISH 4: *E.Coli*

	25	50	100	Standard
<i>S. mutans</i>	9	9	9	9
<i>C. albicans</i>	9	15	23	9
<i>Lactobacillus sp.</i>	12	13	15	9
<i>E. coli</i>	9	9	9	9

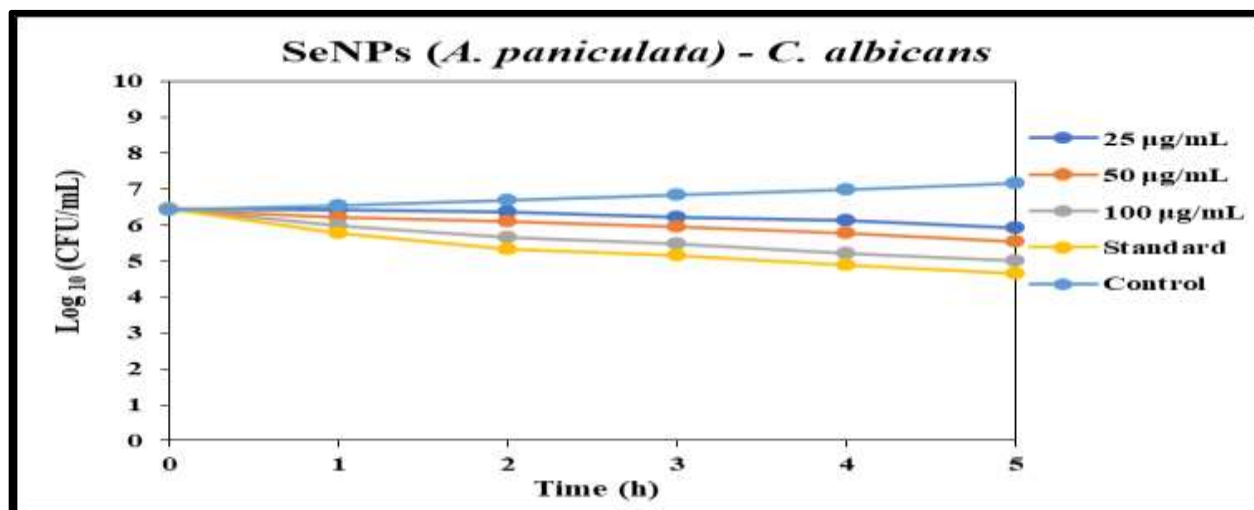
TABLE- 1



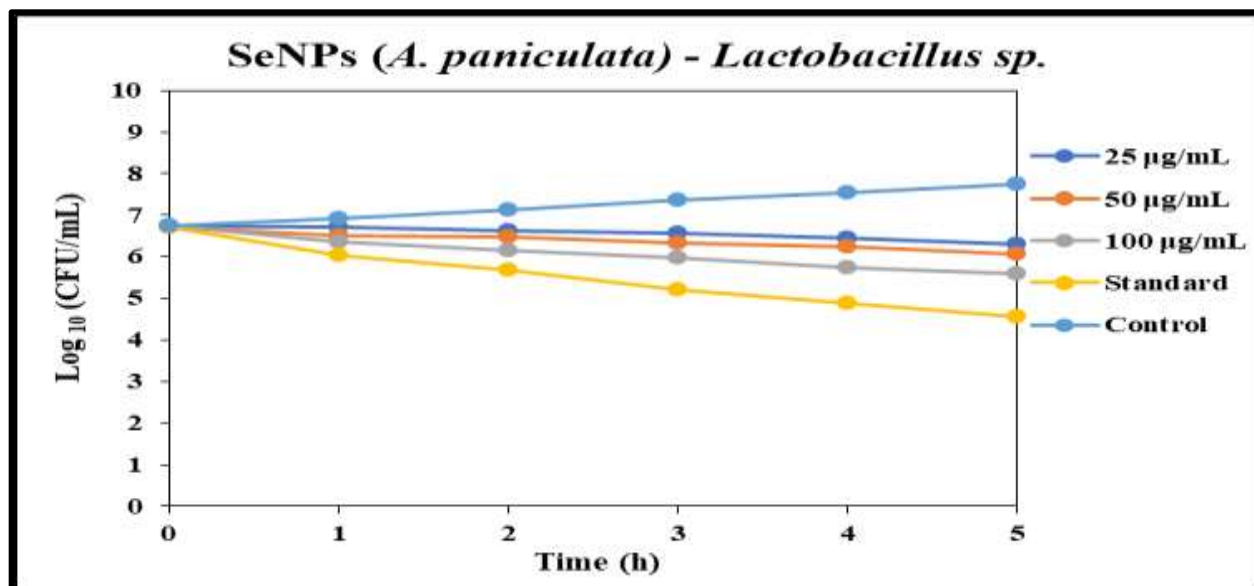
GRAPH 1: The bar graph shows the anticariogenic activity of selenium nanoparticles made from *Andrographis paniculata* extract. The data indicates the concentration on the x axis and the zone of inhibition in millimeters on the y axis



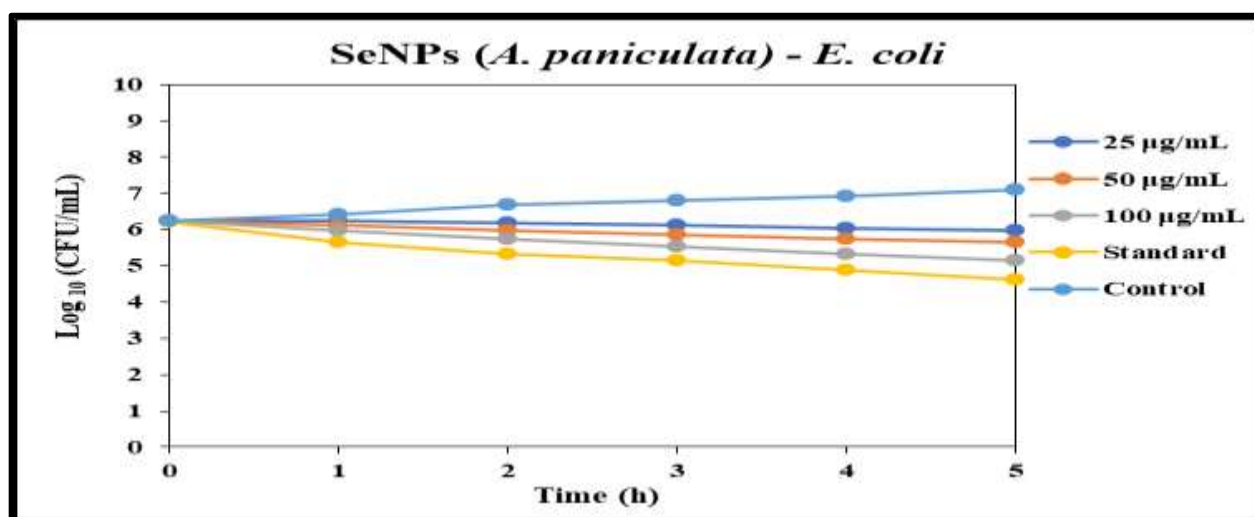
GRAPH 2: Time kill curve assay chart showing SeNPs(*A. paniculata*)-*S. mutans*



GRAPH 3:Time kill curve assay chart showing SeNPs(*A.paniculata*)-*C.albicans*



GRAPH 4:Time kill curve assay chart showing SeNPs(*A.paniculata*)-*Lactobacillus* sp.



GRAPH 5:Time kill curve assay chart showing SeNPs(*A.paniculata*)-*E.coli*

DISCUSSION

The antibacterial activity of *A. paniculata* SeNPs against *S. mutans*, *C. albicans*, *Lactobacillus* sp., and *E. coli* is portrayed in the bar chart herein given, where the activity is expressed in terms of the zone of inhibition in millimeters at concentrations of 25, 50, and 100 µg/mL, based on a standard control. In the case of *S. mutans*, the zone of inhibition was the same at all concentrations: 9 mm, as with the standard control. In contrast, for *C. albicans*, there is a marked increase in the zone of inhibition from 9 mm at 25 µg/mL to 15 mm at 50 µg/mL and 23 mm at 100 µg/mL, thus significantly exerting antimicrobial activities whose standard control remains at 9 mm. The inhibition zone for *Lactobacillus* sp. Slightly increases from 9 mm at 25 µg/mL to 13 mm at 50 µg/mL and 15 mm at 100 µg/mL while the standard control was around 12 mm. In *E. coli*, the zone of inhibition just remains at 9 mm across all concentrations just like that of the standard control.

The use of SeNPs synthesized with *Andrographis paniculata* stem extract in preventive oral health strategies opens up an exciting perspective; however, further study is needed to identify the possibilities and constraints. The study of Tuan kub on antimicrobial activity of *Andrographis paniculata* herbal gel against acne-associated pathogens had a significant effect contributed by the *Andrographis paniculata* stem extract during the experiment that could be useful in dental caries researches.(19) and They may also show anti-inflammatory activities useful against gum infection. These relevant/vector attributes have led researchers to investigate SeNPs as a very promising agent against multidrug-resistant bacteria and other microbial pathogens.(20)In the study of Vilan T Sulthan on Oral Candidiasis: A Disease of Opportunity Out of the nine oral bacteria included in the search method, only four of these oral bacteria recorded antimicrobial activity of SeNPs, namely: *C. albicans*, *S. mutans*, *E. faecalis*, and *P. gingivalis*. As such, these four microorganisms are associated with certain oral pathogenic diseases; hence, the published data are indicative of the therapeutic potential of SeNPs. These microbes have been related to a diversity of oral symptoms that include dental caries, mucosal lesions of immunocompromised patients, superinfections in periodontitis, and chronic root canal infections. In the past years, there has been a disturbing trend toward higher incidence and severity of all forms of oral candidiasis. Among the recommended treatments for such conditions is nystatin.(20,21)

The study by Menichetti on Effect of Size, Shape and Surface Functionalization on the Antibacterial Activity of Silver Nanoparticles explained that the degree of contact between biological entities and nanoparticles would determine antibacterial activity. The proximity of the spherical SeNPs to the bacteria surface has been the reason for its potent antibacterial efficacy against a wide array of oral pathogens as reflected in studies identified within this scoping review. Specifically, Se nanorods were utilized in electrochemical sensors, while the application of spherical and cubic-shaped SeNPs was in the enhancement of antioxidant, anti-cancer, and antibacterial activity.(22)It breaks down biofilms, and the antioxidant action protects against the oxidative stress associated with gum inflammation(22,23). These combined traits may result in protection against periodontal diseases and a better fight against microorganisms causing caries. Nevertheless, little has been conducted before to investigate the existing studies on the anti-cariogenic potential of SeNPs synthesized from *A. paniculata* stem extract. Future research on the synthesis process should demonstrate scalability and reliability as size, shape, and surface characteristics depend on the larger effectiveness of such nanoparticles(24,25).Both in vitro and in vivo assays for these SeNPs against oral pathogens and on dental biofilms' formation and disruption(24,26).Moreover, the potential cytotoxicity and side effects of these compounds must be assessed before their utilisation in dentistry(27).Although SeNPs synthesized with the *A. paniculata* stem extract showed very promising potential to be used as an anticariogenic agent, this would require further studies for its efficacy and safety assessment of the synthesized SeNPs. For this reason, subsequent research should focus on developing a reliable synthesis methodology at first instance, followed by testing the efficiency of SeNPs against cariogenic bacteria and biofilms, and then assessment of their oral use safety.

CONCLUSION

Andrographis paniculata was used to synthesize and characterize the selenium nanoparticles. Their unique investigation showed strong anticariogenic action against infections. Consequently, it is anticipated that polysaccharide-capped SeNPs will enhance the advantages of selenium and may exhibit synergistic activity.

FUTURE SCOPE

Future studies should concentrate on improving the environmentally friendly and scalable production of SeNPs utilizing stem extract from *A. paniculata*. A thorough characterization utilizing cutting-edge methods is necessary to comprehend their dimensions, forms, and surface characteristics. It is essential to look into functionalizing SeNPs to improve stability and biocompatibility. Studies conducted in vivo and in vitro are required to assess their effectiveness against oral infections and biofilm development. Furthermore, comprehensive safety evaluations must be carried out to guarantee their secure implementation in dentistry.

CONFLICT OF INTEREST: There is no conflict of interest

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AUTHOR CONTRIBUTION

S.Keerthivasan

Dr.Sangeetha

Dr.Rajesh Kumar

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