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Green Synthesis of Selenate Nanoparticles Using Vitis vinifera Extract: Preparation, Characterization, and Antimicrobial Efficacy

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Abstract

Background

The present work aimed to environmentally synthesize Selenate nanoparticles (SeO^{2-}_4) using VV (Vitis vinifera) extract and assessed their antimicrobial efficiency against Enterococcus faecalis, Streptococcus mutans, and Staphylococcus aureus.

Methodology

SeO^{2-}_4 was created by combining VV extract with a sodium selenium (Na2SeO4) solution. EDX (Energy-Dispersive Electron Microscopy), FTIR (Fourier Transform Infrared) analysis, SEM (Scanning Electron Microscopy), UV-visible spectroscopy, and were used to characterize the NP (Nano Particles). The agar disc diffusion technique was applied to determine the antimicrobial activity against E. faecalis, S. mutans, and S. aureus.

Results

The green production of SeO^{2-}_4 using VV extract produced nanoparticles of varying sizes and morphologies. Up to 10 nm-long conical and circular NPs were revealed by SEM examination. Particles of oxygen (O) and selenium (Se) were verified by EDX analysis. SeO^{2-}_4 synthesis was shown by UV-visible spectroscopy, having a peak at 290 nm. At higher concentrations, such as 15 mm at 100 µL, these NPs showed greater inhibitory zones and good antibacterial action against S. aureus. Conversely, they demonstrated no activity against E. faecalis and only 12 mm of activity at 100 µL against S. mutans.

Conclusions

NP may be produced efficiently by employing VV extract in the environmentally friendly synthesis of SeO^{2-}_4 - NP. Its high antibacterial action against S. aureus suggests that certain antimicrobial treatments may be useful in treating related illnesses.

Categories: Public Health, Dentistry, Infectious Disease

Keywords: staphylococcus aureus, antimicrobial potential, Vitis vinifera extract, Selenate nanoparticles, green synthesis

Introduction

The field of nanotechnology has emerged as a cutting-edge technology with many applications in the food processing, chemical, pharmaceutical, and mechanical sectors [1]. Devices, energy generation, optics, medicine delivery, and ecological research are among the interesting applications of nanotechnology [2]. People's quality of life has been greatly enhanced by the application of nanotechnology to address many pressing problems that people encounter daily. These problems include the need for more dependable energy sources, the effects of climate alteration, and developments in industries such as healthcare, beauty, and textiles. Metal oxide NPs have garnered significant attention in the last 10 years because of their numerous uses in a variety of technological disciplines, [3]. SeNPs are used in xerography, photometers, photocopying, photocells, and because of their X-ray sensing, photoelectric, special semiconducting, and qualities [4]. Scientists are focusing their attention on developing quick and environmentally safe technologies for nanomaterial synthesis because conventional approaches for the synthesis of NPs involve several difficult processes like the use of hazardous chemicals, lengthy processing times, and high costs [5]. SeO^{2-}_{4}-NPs have attracted a lot of attention compared to other NPs because of their distinct electronic, optical, and medicinal properties [6]. They are also highly biocompatible, bioavailable, and low toxicity, which makes them appropriate for a variety of biological uses, like the development of biological membranes and other related uses [4].

Green synthesis aims to create NPs by extracting phytochemicals like sucrose, flavonoid alkaloids, terpenoids, and polyphenols—from algae, fungus, bacteria, plants, as well as other creatures. These phytochemicals serve as both stabilizing and reducing agents. Due to these advantages—easy availability, low cost, and biocompatibility—green NP synthesis using plant extracts is emerging as a potential trend in green chemistry. SeO^{2-}_{4}-NPs have been produced with a range of plant parts, like peels, seeds, fruits, stems, roots, and leaves [8,9]. The findings indicated that there is a great deal of promise for using plant extracts as decreasing agents while making NPs. The generation of SeO^{2-}_{4}-NPs from plants has been reported in earlier studies, but little is known about their biological activities, including antibacterial, larvicidal, protein kinase, and anticancer effects [13].

Grapes, scientifically known as Vitis vinifera (VV) are known for their richness in polyphenolic compounds, which have been demonstrated to possess antibacterial effects. Research shows that grape extracts have strong antibacterial properties against a range of microorganisms. This might be explained by the variations between gram-positive & negative bacteria's cell walls. The findings underscore the potential of grape extracts and their

polyphenolic components as natural antibacterial agents, with implications for further research in developing supplements or applications in the field of antibacterial interventions [14]. Utilizing grape extract alongside peel-based synthesis provides a comprehensive approach to SeO^{2-}_{4}-NPs production, ensuring versatility and enabling the tailoring of NP properties to suit a wider array of applications. This dual approach harnesses the full potential of the grape, minimizing waste and maximizing the utility of its components in nanotechnology[4].

This study aimed to produce SeO^{2-}_{4}-NPs using VV fruit extract in an environmentally friendly manner, analyze and confirm the properties of the synthesized NPs, and assess the effectiveness of the SeO^{2-}_{4}-NPs against common oral microbes. The goal was to contribute to the expansion of sustainable NP production and explore potential applications in antimicrobial research.

Materials & Methods

The study received permission from the "Saveetha Dental College and Hospitals" Review Board in Chennai (approval number: SRB/SDC/ENDO-2107/22/020).

Preparation of AC fruit extract

The AC fruit was cut into clean, fresh pieces after being purchased at a local fruit store. Following crushing, a Whatman number 42 filter was used to filter the pulp. An further hour of centrifugation was performed using a hand centrifuge on the fruit juice that had been filtered in order to obtain a clear extract. This extract was maintained cold (Fig. 1)

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Figure 1: Grape extract formation from fruit (a) to the formation of Grape - Sodium Selenate concentrate (b).

Preparation of SeO^{2-}_4 solution

Grape extract acted as a mediator in the green synthesis process used to create SeO^{2-}_4-NPs.

Preparation of Diluted SeO^{2-}_4 Solution

This required the weighing, transport, and diluting of 3.46 g of Sodium Selenate (20 mmol) into a 50 mL beaker. While the temperature was kept between 50°C and 60°C, the resultant solution was stirred.

Addition of Grape Extract to SeO^{2-}_4 Solution

One milliliter of VV fruit extract has been added to the diluted SeO^{2-}_4 solution. SeO^{2-}_4-NPs were generated in suspended form within the solution. To guarantee that all of the ingredients were evenly mixed, the mixture was gently swirled. Aqueous SeO^{2-}_4 solution was mixed with VV broth, and the reaction mixture's colour progressively changed. The experiment produced a clear solution once the NPs were created.

Orbital Shaking and Centrifugation

After the solution was centrifuged for 10mins at 8,000rpm to separate the NPs from the

residual solution, it was agitated in an orbital shaker to produce homogenous NPs. The residue was a plant extract mediated SeO^{2-}_{4}-NPs, and the supernatant from the resultant mix was used for characterization. In the same test tube, they were allowed to dry at ambient temperature. One of the earliest signs that metal salts are being reduced to NPs is the visual observation of a change in colour in a solution. This is the NP synthesis endpoint indication.

Characterization of NPs

The NPs were defined by FTIR, SEM, UV-Vis spectroscopy, and EDX. Using the agar disc diffusion technique, antimicrobial activity against common oral bacteria including Staphylococcus aureus, Streptococcus mutans, and Enterococcus faecalis was examined at varying doses.

Various characterization approaches were employed to assess the characteristics and efficacy of the synthesized SeO^{2-}_{4}-NPs against E. faecalis, S. mutans, and S. aureus.

UV-Vis Spectroscopy

UV-Vis spectrometry ("M/S Perkin Elmer, Lambda 25, Waltham, MA, USA") was used to evaluate the optical characteristics and conformation of SeO^{2-}_{4}-NPs at room temperature. Between 200 and 900 nm, the spectrum analysis was carried out with a resolution of 1 nm [19].

SEM

SEM ("JEOL USA Inc., Peabody, MA, USA") was applied to analyze the synthetic NPs' surface morphology. A tiny coating of conductive material was applied to the produced NPs before they were placed on a sample holder and examined using a high-resolution SEM [20].

EDAX

To ascertain the synthesized NPs' elemental composition, EDAX analysis was performed. After positioning the NPs on an appropriate substrate, they were examined using an EDAX detector-equipped SEM (Bruker Germany, D8 Advance Diffractometer, Leipzig, Germany) [21].

Antibacterial Testing

To test the antibacterial activity of several dosages of SeO^{2-}_{4}-NPs against oral pathogens such as S. mutans, S. aureus, as well as E. faecalis the agar well diffusion technique was applied to

MHA ("Mueller-Hinton agar") plate. The MHA was made using pH 7.0 double-distilled water and subcultured for 15 mins at 121°C to sterilize it. After sterilizing the MHA, it was "added to the petri dish and let to solidify at room temperature under a laminar flow. A microbial culture solution was utilized to saturate a sterile cotton swab, and the result was an inoculum of 106 cfu/mL of newly cultured bacteria on MHA plates. Three 9 mm diameter wells were then drilled into the medium of MHA and filled with different quantities (25 µL, 50 µL, and 100 µL) of the finished NP solution using a micropipette. After that, the solution was left to permeate the medium for four hours at room temperature. The culture plates were then kept at 37°C for a further 24 hours of incubation. Following incubation, the zone of inhibition on each plate was measured to determine its diameter (mm) [19]. To test the antibacterial activity of various doses of SeO^{2-}_{4}-NPs against oral pathogens like E. faecalis, S. mutans, and S. aureus, the agar well diffusion technique was applied on MHA plate. After preparing the MHA with "double-distilled water" (pH 7.0) and sterilizing it in an autoclave for fifteen minutes at 121°C, the MHA was transferred onto a petri dish and allowed to solidify in a laminar flow at room temperature. A microbial culture solution was utilized to saturate a sterile cotton swab, and the result was an inoculum of 106 cfu/mL of newly cultured bacteria on MHA plates. Three 9 mm diameter wells were then drilled into the medium of MHA and filled with different quantities (25 µL, 50 µL, and 100 µL) of the finished NP solution using a micropipette. Following that, the solution was given four hours to diffuse to the medium at room temperature. The culture plates were then incubated at 37°C for a further 24 hrs. Following incubation, the zone of inhibition" on each plate was determined to determine its diameter (mm) [19].

FTIR Spectroscopy

FTIR analysis ("Thermo Nicolet, Avatar 330, Waltham, MA, USA") was conducted to examine whether functional groups were present in the synthesized NPs. For FTIR analysis, the NPs were crushed into a pellet and combined with potassium bromide (KBr) [22].

Results

UV-Vis spectroscopy

The "UV-Vis spectroscopy of green synthesis SeO^{2-}_{4}-NPs provided insight into the optical characteristics of the as-prepared SeO^{2-}_{4} nanostructure" sample. At 325 nm, there was a clear and noticeable single peak with an absorbance value of 3.000. (Figure 2). Strong evidence of the presence of SeO^{2-}_{4}-NPs and convincing evidence of the effectiveness of the NP manufacturing process is provided by the correlation between the observed peak and the

characteristic properties of these particles.

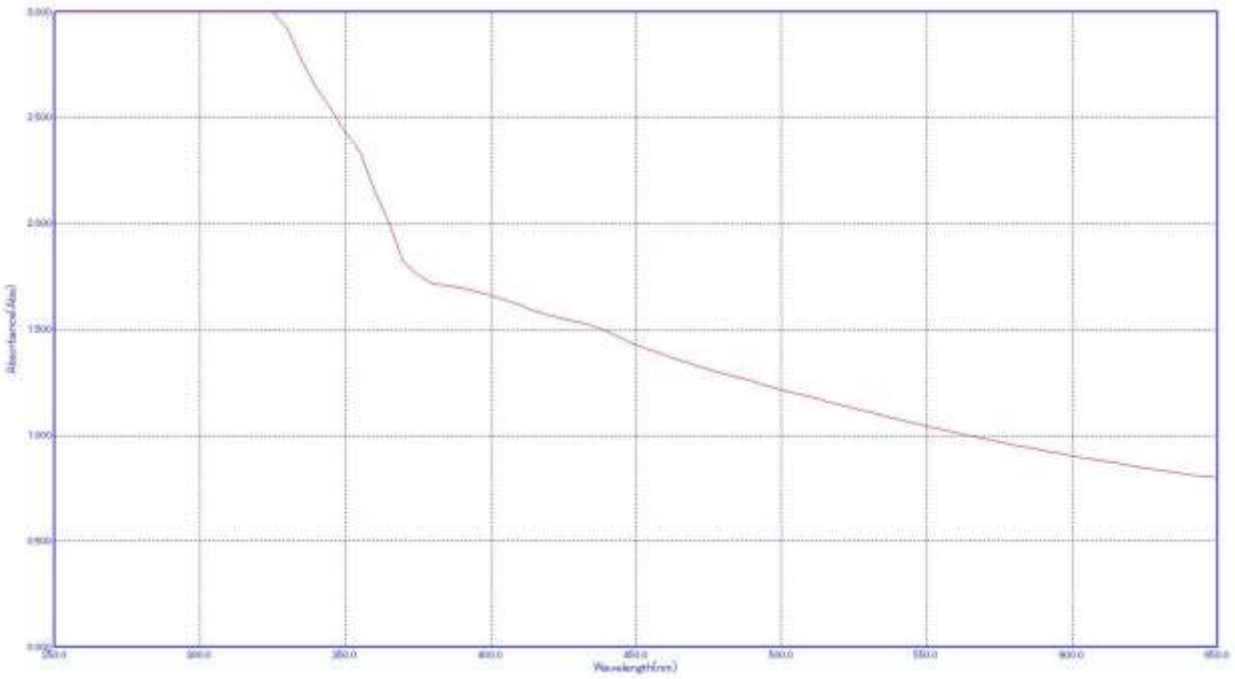

Figure 2: UV-visible spectroscopy showing a single peak with an absorbance of 3.000 and a wavelength of 325 nm.

SEM analysis

Subsequently, a white powder formed when the fluid was dried in a hot air oven. Using SEM, the morphology of the SeO^{2-}_{4}-NPs was examined. At 5,500× magnification, they showed an amorphous mass with conical forms up to 10 nm in size (Fig. 3). The average particle size was calculated by taking measurements of many NPs and averaging them.

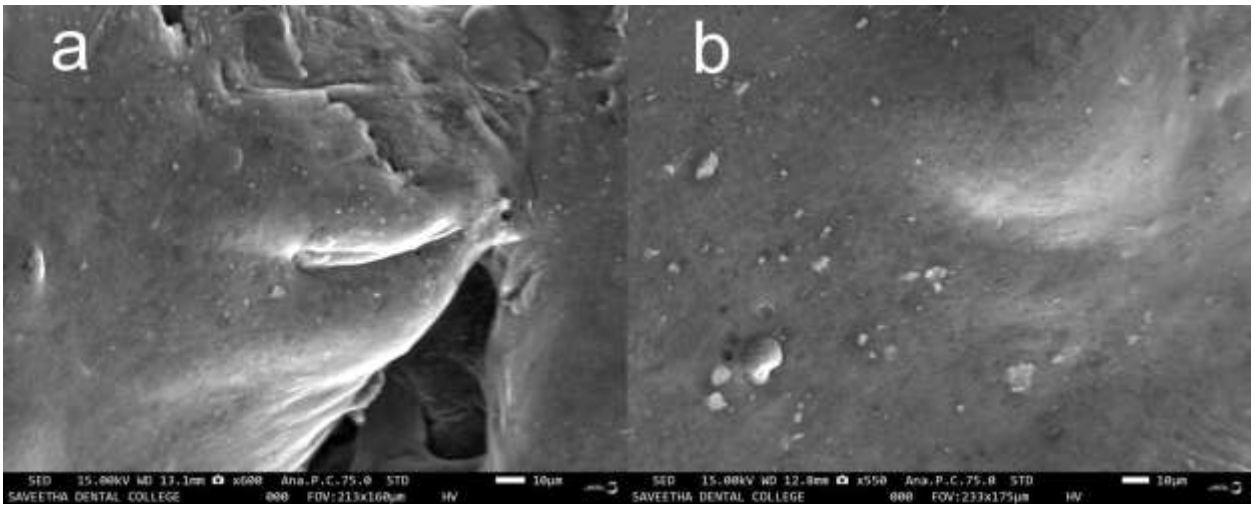

Figure 3: SEM of samples display nanoparticles of circular shape at 600× (a) and 5,500× (b) magnification.

EDAX

The range of the $SeO2^{-}{}_{4}$ crystallite was continuously occupied by the EDX peaks. The successful synthesis of $SeO^{2-}{}_{4}$-NPs was validated by the analysis, which also revealed the existence of oxygen and selenium. No further peaks were visible in the analysis since the substance used to produce $SeO^{2-}{}_{4}$-NPs was inert and pure (Figure 4). A pattern resembling that found in the JCPDS ("Joint Committee on Powder Diffraction Standards"): 36-1451 database was observed at this diffraction peak position.

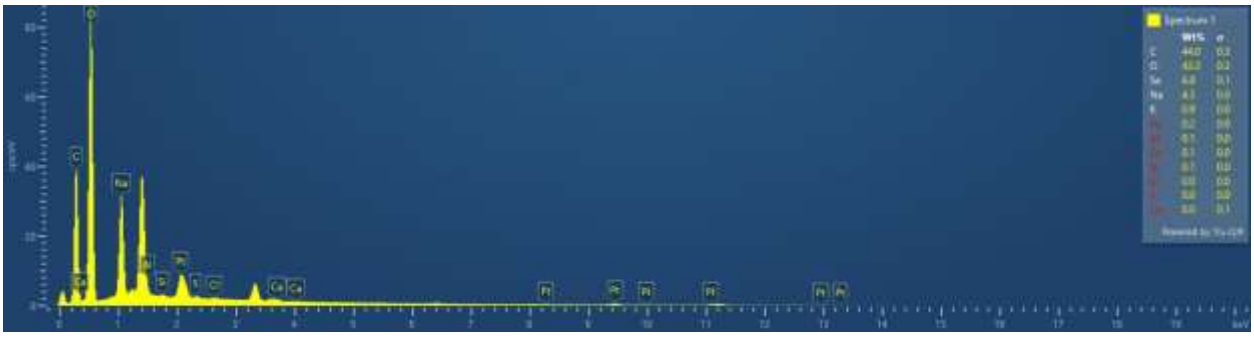

Figure 4: Energy-dispersive electron microscopy results presenting quantities of oxygen, zinc, and carbon as 44%, 43.3%, and 6.8% (Wt.%), respectively.

Wt.%: Weight percentage

Agar disc diffusion

The results showed moderate antimicrobial activity of SeO^{2-}_4-NPs solution against S. aureus at various concentrations, especially 25 µL, 50 µL, and 100 µL corresponding to a ZOI of 10 mm, 12 mm, and 14 mm, respectively. The NP also showed moderate antimicrobial activity against S. mutans at concentrations 25, 50, and 100 µL corresponding to a ZOI of 11 mm, 12 mm, and 13 mm, respectively. These measurements were made linearly. Since the well diameter was 9 mm, no ZOI was observed against E. faecalis, indicating that even at the highest tested dosages, there was no action against this strain of bacteria (Table 1).

Organism	Volume of sample			AB
	25 µL	50 µL	100 µL	
S. mutans	11 mm	12 mm	13 mm	31 mm
S. aureus	10 mm	12 mm	14 mm	41 mm
E. faecalis	9 mm	9 mm	9 mm	35 mm

Table 1: Antimicrobial efficacy of nanoparticles against E. faecalis, S. mutans, S. aureus, and control antimicrobial by the agar disc diffusion technique.

AB: control antimicrobial (0.2% chlorhexidine)

The distance measured is the diameter of the zone of inhibition noted which includes 9 mm well diameter.

FTIR spectroscopy

The biomolecules that are responsible for the SeO^{2-}_4 bioreduction and the capping/stabilization of SeO^{2-}_4-NPs were found using FTIR analysis. To ascertain if particular functional groups were present, the intense bands seen during measurements were envaulted

with standard values.

With the use of FTIR investigations, the unique functional groups related to the SeO^{2-}_4-NPs were identified (Fig. 5). The peak at 835 cm-1 is suggestive of vibration/stretching of metal-oxygen bonds in SeO^{2-}_4. Carbon residues discovered during sample analysis are related to the peak at 2,928 cm-1, whereas C-O elongation is related to the peak at 1,430 cm-1. The hydrogen bonds at 1,349 and 1,585 cm-1 are produced by the stretching vibration of hydroxyl molecules.

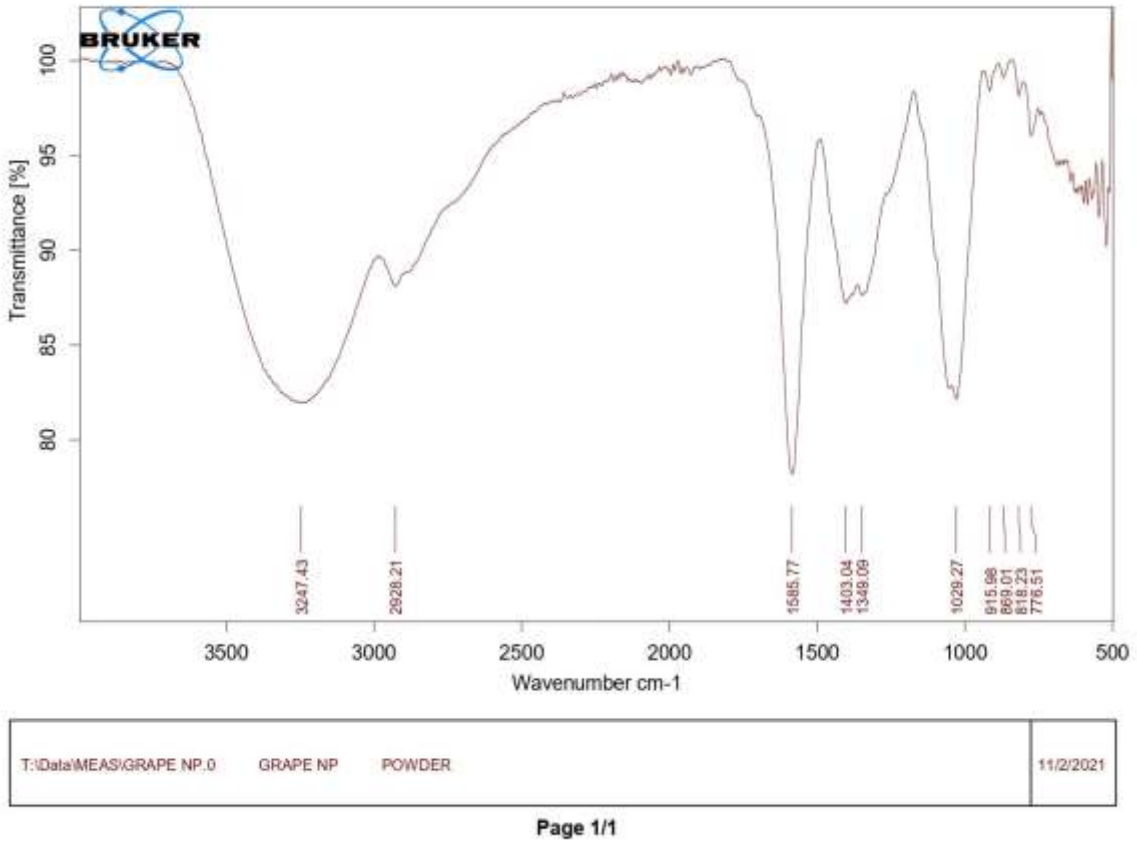

Figure 5:Fourier transform infrared results of grape-derived Selenate nanoparticles.

Discussion

Before the present research, there was a lack of analysis on the green synthesis of SeO^{2-}_{4}-NPs mediated by fruit extract of VV (grape), as older studies made use of its seed extract for the synthesis of SeO^{2-}_{4}-NP as well as Selenate NPs [16]. The severity and quantity of deaths from bacterial diseases increased sharply with the introduction of antibiotic-resistant strains. Worldwide mortality from cancer and diabetes combined is less than that of those killed by antibiotic-resistant bacterial strains [17]. This necessitates the need for the production of safer alternatives that can combat microorganisms involved in oral infections. The reduction and stability of SeO^{2-}_{4}-NPs were greatly aided by the functional groups that the FTIR analysis of the pineapple extract revealed to be present. These groups were obtained from the bioactive chemicals. In the experiment, the presence of SeO^{2-}_{4}-NPs ranging in size between 10 nm to 50 nm was verified. Zinc and oxide elements were detected by the EDAX analysis, which also verified that ZnO-NPs had formed successfully. The uniform size distribution and spherical form shown in the SEM pictures point to the effective synthesis of precisely defined NPs, further opening the door for their further development. This work examined the antibacterial properties of SeO^{2-}_{4}-NPs produced with VV at concentrations of 25 µL, 50 µL, and 100 µL. The ZOI diameter was measured in millimetres, and a moderate antibacterial impact was found against S. aureus and S. mutans, but no effect was observed against E. faecalis. The antibacterial activity was assessed with the agar disc diffusion technique. The presence of the extract's acidic nature is principally responsible for its antibacterial characteristics.

Due to their strong antibacterial properties, grape-derived SeO^{2-}_{4}-NPs are a promising therapy for diseases produced by S. aureus. For some uses, these NPs could be applied effectively in a range of media. When utilized in topical formulations like creams and ointments, they may stop S. aureus from developing on the skin, potentially providing a treatment for cutaneous infections [19]. Oral infections can be avoided by adding these NPs to mouthwash and toothpaste, which can help fight S. aureus colonization in the oral cavity [20-24]. Additionally, the prevention and management of S. aureus-associated infections in hospital settings may benefit from their inclusion in wound dressings and medical device coatings [23-27]. SeO^{2-}_{4}-NPs generated from grapes had better antibacterial activity than other NPs, opening up new therapeutic options to treat pathogen-related illnesses. Chemical methods can be substituted with VV extract-based green SeO^{2-}_{4}-NP synthesis. ZnO-NPs' production of ROS can cause oxidative stress in bacteria, which prevents DNA replication and protein synthesis [29].

Limitations of this study involve its in-vitro nature due to which it cannot capture the true essence of the complexities that might arise when these NPs are used in a day-to-day clinical scenario. An extensive study suggested an impact of synthesis temperature on the size as well as shape of

SeO^{2-}_{4}-NPs which needs to be taken into consideration [30]. There is a need for more tests on antimicrobial activity, not only against common oral microbes but also on different bacterial organisms such as S. pyogenes, K. aerogenes, and P. aeruginosa as well as localized infections of other parts of the body along with systemic infections. Cytotoxicity testing is also necessary for the further development of the product [31-33]. At last, there is a constant requirement for the expansion of an eco-friendly and commercially viable approach that explores the potential of such natural agents that aid in the synthesis of NPs under exploration.

Conclusions

The synthesis of VV fruit extract-derived SeO^{2-}_{4}-NPs is quick and easy, with the fruit extract acting as both dropping and stabilizing agents for the manufacturing of stable NPs. SeO^{2-}_{4}-NPs have promising antibacterial properties against S. aureus and S. mutans providing an opportunity for its use in various medical avenues. Further laboratory studies and clinical testing followed by product development are essential to incorporate the SeO^{2-}_{4}-NPs for patient use specifically in oral hygiene products.

Additional Information

Disclosures

Human subjects: The absence of human subjects or tissue was confirmed by all authors in this study.

Animal subjects: The absence of animal subjects or tissue was confirmed by all authors in this study. Conflicts of interest: The following information is disclosed by all authors in compliance with the ICMJE standard disclosure form: Payment/services info: According to all the authors, no organization offered them funds to support the work they submitted. Financial relationships: Each of the writers has declared that neither presently nor over the last three years, they have any financial connections to any groups that would be interested in the work they have given. Other relationships: According to the declarations of all authors, no additional relationships or activities could be seen as having impacted the work provided.

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