ORIGINAL ARTICLE

Herbal-mediated preparation of nano-sized particles of selenium, its characterization, and evaluation of its antimicrobial activity

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ABSTRACT

This study aimed to synthesize selenium nanoparticles (SeNPs) using stevia plant leaves and to evaluate the antimicrobial effect of nano-sized particles of selenium. A new organic synthesis of nano-sized selenium particles was done in this study. In this study, stevia leaves for the synthesis of nano-sized particles along with sodium selenite were done. This mixture was mixed homogeneously using an orbital shaker overnight. The color change indicated the formation of SeNPs. The SeNPs were evaluated for their size and shape and their antimicrobial property was assessed. SeNPs showed excellent antimicrobial activity against *Candida albicans* at 100 μL concentration. At 25 μL and 50 μL, *C. albicans* showed intermediate susceptibility to SeNPs which also indicated a good antimicrobial potential. Following *C. albicans*, SeNPs showed good antimicrobial potential against *Streptococcus mutans* at all concentrations. SeNPs possessed good antimicrobial activity against most common oral pathogens.

Key words: Green synthesis antimicrobial, nanoparticles, selenium

INTRODUCTION

The emergence of nanotechnology in recent times has led the way for the advancement of treatment options for diseases of varying pathophysiology.[1-3] Nanotechnology comprises particles of size <100 nm.[4] Nanoparticles increase the efficacy of ionized drugs and enhance the water-solubility of compounds to penetrate. The versatility of drug delivery systems can also be increased by characterizing the surface of nanoparticles with targeting ligands. Selenium being a metalloid possesses both nonmetal and metal properties. Selenium also is a photo-electrically active semiconductor.[5] Selenium is extremely essential for humans owing to the fact that it enhances the action of the, glutathione peroxidase, seleno-enzyme and inhibits free radicals from causing damage to cells and tissues.[5] The ability of selenium nanoparticles (SeNPs) to enhance the biological and photoelectrical properties of its nonnanocounterpart has been the primary reason of increased interest in its properties.[4]

SeNPs have shown reduced toxicity in comparison to varied selenium species. SeNPs have been also been used in various pathological conditions in the past.[6-8] SeNPs also have increased bioavailability and biological activity.[9]

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Stevia rebaudiana Bertoni is found in America belonging to the composite family. It is known to cure hypertension, hyperglycemia, and rotavirus infection.

Various plant sources such as *Clausena dentata* leaf extract,[10] hawthorn fruit extract,[11] and fenugreek seed extract[10,11] have been used in the past to synthesize SeNPs. Our research and knowledge have resulted in high-quality publications from our team.[12-23] In this study, nano-sized particles of selenium were prepared and their antibacterial properties were evaluated.

Rationale

The rationale of this study was to synthesize nano-sized particles of selenium with effective antimicrobial properties which could be utilized in future dental applications.

MATERIALS AND METHODS

The primary investigator (DK) was involved in the preparation and testing of nano-sized SeNPs. The steps involved are mentioned as follows:

Synthesis of stevia extract

Leaves of the stevia plant were collected and were cleaned with distilled water multiple times, after which they were dried in an incubator. Coarse particles were prepared using a mortar to grind the leaves. 100 ml purified water was used to mix 1 g of coarse powder. It was then heated at 60°C–70°C on a heating mandrel for 10–15 min. Whatman Filter paper number 1 was used to filter the solution. A conical flask was used to collect the supernatant and the residue was discarded [Figures 1 and 2].

Preparation of nano-sized particles of selenium

Nano-sized particles of selenium were prepared by the reducing sodium selenite solution. Extract of stevia plant was used as a reducing agent. To the solution of 30 milli molar (0.519 grams) sodium selenite and water, 50 ml of filtered stevia extract was added and allowed to mix overnight on an orbital shaker. A ultraviolet–visible (UV-vis) spectrophotometer was used to analyze the preparation of the nano-sized particles. The preparation of the nano-sized particles was indicated by a change in the color of the solution. The selenium nano-sized particles were filtered from the solution by centrifugation for 20 min.

Characterization of nano-sized particles of selenium

The UV-vis absorption peak of the synthesized nano-sized particles was evaluated using UV-vis spectroscopy. 330–660 nm was selected as the range of scanning. UV-vis spectroscopic analysis depends on the conversion of the solution of stevia selenium to SeNPs. The visual change in the color will indicate the formation of NPs.

Transmission electron microscopy (TEM) was used to analyze the physical characteristics `of the nano-sized particles [Figure 3].

Antibacterial activity

The following microbes were used to test the antimicrobial effect of SeNPs: *S. Candida albicans, Staphylococcus aureus, Faecalis,* and *Streptococcus mutans.* Agar well diffusion technique was used to check the antimicrobial effect of nano-sized particles of selenium. The varying quantities of dilutions of SeNPs (25 µL, 50 µL, and 100 µL) were added to agar plate wells. A temperature of 37°C was used to incubate the agar plates for 1 day. The zone of inhibition was identified using a caliper [Figure 4].

RESULTS

Nano-sized particles synthesis

The extract of stevia along with sodium selenite solution revealed a change of color from dull yellowish to dark purplish. The formation of SeNPs was confirmed by this transition.

Ultraviolet–visible spectroscopy

The surface plasmon resonance (SPR) shows an unusual occurrence to noble metal nanoparticles that generate intense electromagnetic fields on the surface of the particles and thus the radioactive properties such as absorption and scattering are increased.[24] Hence, UV spectroscopic analysis

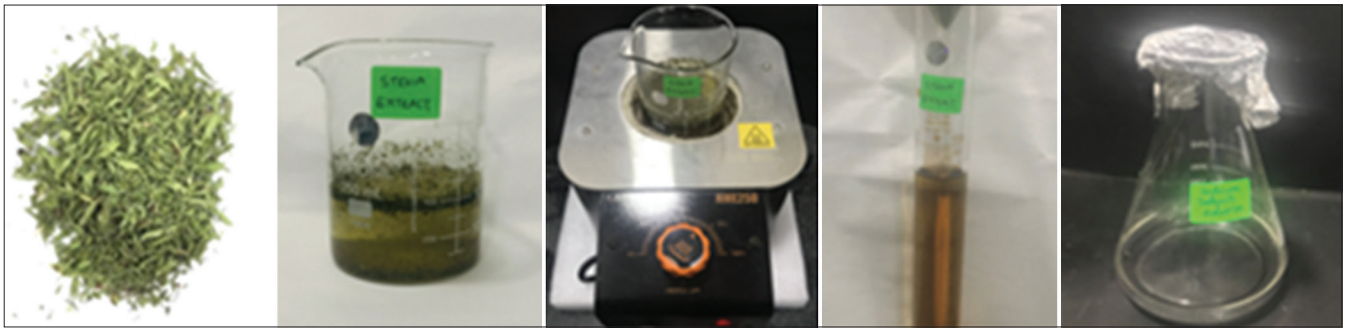

Figure 1: Preparation of stevia extract: Stevia plant leaves were cleaned with water and then allowed to dry 1 g course powder was mixed with 100 ml water and heated at 60°–70° for 10–15 min. Purification was done and the supernatant was collected

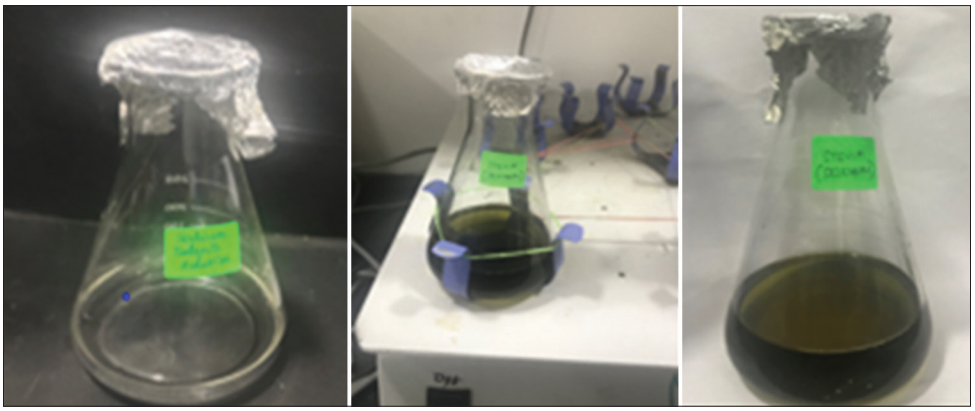

Figure 2: Preparation of selenium nanoparticles: Selenium nanoparticles were prepared by addition of 30 milli molar of Sodium Selenite into 50 ml of distilled water and 50 ml of Stevia extract

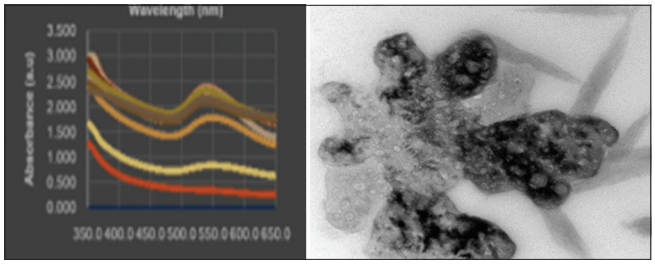

Figure 3: Morphology of nanosized particles of selenium

confirmed the formation of selenium particles in this study. At 550 nm, a sharp peak was observed which correlated to the SPR band of the nano-sized particles.

Transmission electron microscopy

TEM indicated that the nano-sized particles of selenium had the shape of a sphere ranging in size of 4–45 nm.

Antimicrobial activity

The inhibition zones for SeNPs at different concentrations for various organisms are depicted in Table 1.

Table 1: Various zones of inhibition for the selenium nanoparticles

Microbes	Zone of inhibition (mm) (SeNPs')		
	Concentration (μL)		
	25	50	100
Streptococcus mutans	12	13.6	16.9
Staphylococcus aureus	11.4	11.8	11.9
Enterococcus faecalis	9.9	9.9	11.2
Candida albicans	13.3	14.2	16.1

SeNPs': Selenium nanoparticles

DISCUSSION

Through the course of this study, stevia and sodium selenite mixture was used utilized to prepare nano-sized particles. TEM revealed a resonance band of spherical SeNPs at 550 nm of size ranging from 4 to 45 nm. In similar research by Prasad *et al*., SeNPs were prepared from lemon plant leaf extract, wherein the peak was observed at 325 nm and the particles were also a sphere in shape ranging from 60 to 80 nm in size.[25] Similarly, Prasad and Selvaraj *et al*. used *Terminalia arjuna* extract for green synthesis of SeNPs, and SPR was recorded at 390 nm.[26] Geetha *et al*. reported SPR at 280 nm using pseudomonas to reduce selenite selenium.[27] He *et al*. reported maximum absorption of SeNPs prepared using a chemical reduction at 355 nm.[28] Chen *et al*. reported a peak absorbance of selenium nanorods and SeNPs at 410 nm.[29] Mishra *et al*. observed a band at 350 nm after using *Bacillus megaterium* to reduce selenite to red selenium.[30] Similarly, Shen *et al*. used selenium dioxide plus potassium borohydride without any surfactant in an ice bath and observed a peak at 380 nm.[31]

In concordance with this study, the nano-sized selenium particles derived from fenugreek seeds were spherical in shape and 50–150 nm in size.[6] Kong *et al*. used Arabic gum to synthesize SeNP of size 34 nm which were spherical in shape.[32] Li *et al*. used *Capsicum annuum* leaves to extract selenium of 80 nm in size. Yang reported SeNPs were synthesized from a polysaccharide that were spheres of 90–550 nm.[2,33]

Rangrazi *et al*. also concluded that a colloid of chitosan and selenium showed good antibacterial activity against *S. aureus*.[34] Guisbiers *et al*. also reported that SeNPs had an inhibitory effect on *S. aureus*.[35] Similarly, Rangrazi *et al*. and Khiralla and El-deeb also reported that SeNPs depicted antimicrobial activity against *Escherichia coli*.[34,36] All these results are in concordance with the results of the antimicrobial effect of nano-sized particles of selenium.

Strengths

This study highlights a very simple and cost-effective method of production of SeNPs. The preparation of SeNPs was in a very short time and it was an eco-friendly method. All these could indicate its use in many biological applications.

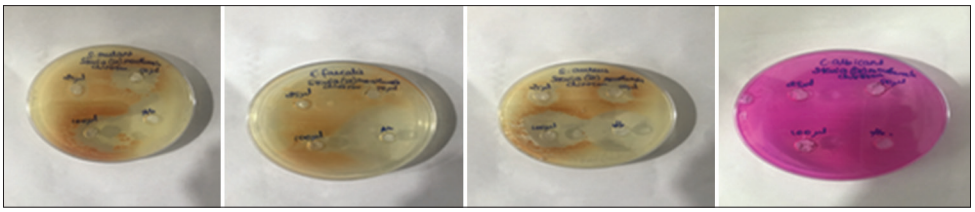

Figure 4: Inhibition zones for each organism: Antimicrobial activity of SeNP's was assessed. SeNP's: Selenium nanoparticles

CONCLUSION

Thus, the SeNPs prepared using stevia leaf extract indicated good antimicrobial potential against various plaque-producing oral pathogens.

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Conflicts of interest
There are no conflicts of interest.

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