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Biosynthesis of Selenium Nanoparticles from *Annona muricata* Fruit Aqueous Extract and Investigation of their Antioxidant and Antimicrobial potentials

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Abstract

The focus of the research was to synthesize biogenic selenium nanoparticles (SeNPs) from *A. muricata* in an environmentally friendly, non-toxic, biocompatible, and affordable way. The successful synthesis of SeNPs was preliminarily confirmed by the development of brick-red color. The DLS pattern in our investigation revealed that the synthesized SeNPs were nanosized, with sizes ranging from 80 to 120 nm. The zeta potential for SeNPs was found to be -26 mV and indicated that SeNPs were negatively charged. The micro-morphology of SeNPs was determined by SEM analysis and found that they were spherical in shape and size of 120 – 160 nm. EDX showed that SeNPs contain selenium (86.22%), carbon (7.30%), and oxygen (3.51%). In antioxidant activity, IC_{50} values of SeNPs were determined as 58.98 ± 0.70 and 66.10 ± 1.01 µg/mL in DPPH free radical scavenging and ABTS free radical scavenging activities, respectively. In antibacterial activity, SeNPs exhibited potential antibacterial activity by the zone of inhibition assay. The strong antimicrobial activity of SeNPs was observed against Gram-positive bacteria related to Gram-negative bacteria. In the micro-well dilution technique, MIC and MBC of SeNPs were found best against Gram-positive bacteria related to Gram-negative bacteria. The as-synthesized SeNPs could be highly beneficial as antioxidant and antibacterial agent in the biomedical field.

Keywords: *Annona muricata*, Green synthesis, Selenium nanoparticles, Antioxidant activity, Antimicrobial activity.

Introduction

Nanotechnology has the potential to develop and benefit humanity through a broad array of applications in medical, food science, energy, the environment, information technology, and aerospace innovations (1,2). Metallic nanoparticles have captivated the interest of scientists over the last few decades, and they are now widely used in biological and engineering fields. In the recent scenario, trace element selenium (metalloid) has attained a lot of interest from researchers (3). Selenium is found not only in organic and inorganic molecules, but also as a key component in the amino acids selenocysteine and selenomethionine, as well as in selenoproteins, which are found in all Domains of life. Second, selenocysteine is the 21st proteinogenic amino acid. Third, selenium is a micronutrient with a wide range of roles, especially in mammalian essential processes regulation (4). Therefore, the present study focused to provide insight into the uniqueness of selenium element in the nanoparticles synthesis and explore their properties and functions.

In materials science, “green” synthesis has gotten a lot of attention as a dependable, long-lasting, and environmentally friendly method for making a variety of materials/ nanoparticles, such as metal/metal oxide nanomaterials, hybrid materials, and bioinspired materials. The green synthesis is seen as a significant tool for reducing the negative consequences of typical nanoparticle synthesis methods used in laboratories and industries (5,6).

This study investigated the role of phytochemicals of *Annona muricata* fruit aqueous extract as reducing and stabilizing agents in selenium nanoparticles (SeNPs) synthesis. The as-synthesized SeNPs were characterized for ensuring their bio-potentials including antioxidant and antibacterial.

Materials and Methods

Chemicals and reagents: Mueller-Hinton broth medium, agar agar, and sodium selenite (99%) was obtained from HiMedia, Mumbai, India. The plasticware required for the assays were procured from Tarsons, Bengaluru, India. The glassware was obtained from Borosil in Mumbai, India.

Synthesis of selenium nanoparticles: *Annona muricata* (soursop) fruits were obtained from local market in Vijayawada, Andhra Pradesh. Fruits were gently cleaned with distilled water and pulverised in a blender. The aqueous fruit extract was filtered using a muslin cloth and Whatman no.1 filter paper and used for selenium nanoparticle synthesis (SeNPs).

Briefly, 100 mL of *A. muricata* aqueous fruit extract was mixed with 50 mM of sodium selenite for 12 hrs at room temperature and 50-100 rpm. Any change in color to brick red in the reaction mixture was detected. The biogenic selenium nanoparticles (SeNPs) were collected at 15,000 rpm and pellet was washed with distilled water at 15,000 rpm. The dried-out pellet was crushed into powder using mortar and pestle, and obtained brick red colored powder SeNPs were used for different characterization and bio-potential analysis. (7)

Characterization of selenium nanoparticles: At a wavelength range of 200 – 800 nm, the absorption spectrum of SeNPs was recorded using a UV–Vis spectrophotometer with double beam (Shimadzu, Japan). The DLS (dynamic light scattering) technique was used to determines particle size (Bruker, USA). The stability or charge of SeNPs was determined from zeta potential (ZP) of a particle (Bruker, USA). The size and shape of SeNPs was determined by scanning electron microscope (FEI Quanta, USA). The chemical composition of SeNPs was determined by scanning electron microscope (SEM)-energy Dispersive X-Ray analysis (EDX) analysis (FEI Quanta, USA) (7).

Antioxidant potential of selenium nanoparticles: Antioxidant potential of SeNPs was determined by DPPH and ABTS assays as per technique of Gunti et al. and Kalagatur et al (7,8).

Antimicrobial activity of selenium nanoparticles: In the present study, the antibacterial activity of SeNPs was determined against *Pseudomonas aeruginosa* – MTCC 741, *Staphylococcus aureus* – ATCC 13565, *Enterococcus faecalis* – MTCC 439, *Escherichia coli* – MTCC 41, *Listeria monocytogenes* – MTCC 657, *S. aureus* – ATCC 14458*, and Klebsiella pneumoniae* – ATCC 13883. Bacteria were cultured for 24 hours at 37°C in Mueller-Hinton broth medium, and their growth was measured in optical density (OD) at 600 nm using a multi-plate reader. The OD of bacterial culture to 0.5 McFarland standard (1.5 X 10^8 CFU/mL) using sterile saline, pH 7.4 (PBS), and used for various antibacterial activities. The antibacterial activity was determined by zone of inhibition and micro-well dilution assays.

Zone of inhibition assay: The zone of inhibition assay was used to investigate the antibacterial activity of SeNPs. Briefly, Mueller-Hinton agar plates were prepared in sterile conditions and

used to test SeNPs' antibacterial activities. On growth medium plates, a volume of 100 µL of 0.5 McFarland standard of overnight bacterial culture suspension was spread plated. Following that, a sterile cork borer was used to make wells in Petri plates, and different concentrations of SeNPs (100, 200, and 300 µg/well, made in PBS pH7.4) were added and incubated overnight at 37 °C. The zone of inhibition (antibacterial activity) of SeNPs against pathogens was determined using a Zone-scale approach. The antibacterial agent ampicillin was employed as a standard (9).

Micro-well dilution technique: The Clinical & Laboratory Standards Institute (CLSI) suggested the micro-well dilution technique for testing antibiotic efficacy against microbial pathogens in terms of least inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) (Barry, 2007). The lowest concentration of an antimicrobial agent that would suppress the evident growth of a bacterium after an overnight incubation is known as the minimum inhibitory concentration (MIC). Minimum bactericidal concentrations (MBCs) are defined as the lowest antimicrobial agent that will halt an organism from growing after subculture on antibiotic-free media.

Briefly, SeNPs at various concentrations and an overnight bacterial culture of 0.5 McFarland (10 µL) were introduced to a 96-well plate, and final volume was adjusted to 100 µL with Muller Hinton broth (MHB) and incubated at 37 °C for 24 hours, and followed by OD measured using a microplate reader. A minimal inhibitory concentration of SeNPs was defined when there was no increase in OD (MIC). Furthermore, 10 µL was collected from wells of 96-well plate and spread out on Muller Hinton agar (MHA) Petri plates, which were then incubated at 37 °C for 24 hours. SeNPs concentrations at bacterial growth were not detected determined as MBC. Ampicillin was a standard antibacterial agent in the study. The control wells were those that were not treated with either SeNPs or ampicillin (10).

Statistical analysis: The GraphPad Prism software was used to conduct statistical analysis (Trial version 7.0). All measurements were made independently at three times (n = 3). The mean and standard deviation were used to express the data. Tukey's multiple range tests and analysis of variance (ANOVA) in a completely randomized design were employed to compare the significant differences between the test samples. The 95% confidence limits ($p \leq 0.05$) were used as significance.

Results and Discussion

Synthesis and characterization of selenium nanoparticles: In our study, *A. muricata* aqueous fruit extract successfully synthesized the SeNPs from sodium selenite during 12 hours (**Fig. 1**). The synthesis of SeNPs was preliminarily confirmed by the development of brick-red color. Following, synthesized SeNPs were confirmed and characterized by UV-Visible spectroscopy, DLS, Zeta, XRD, SEM, and SEM-EDX analysis.

Figure 1: Synthesis of selenium nanoparticles from *A. muricata* aqueous fruit extract.

UV-Visible spectroscopy was employed in the range of 200–400 nm to confirm the synthesis of SeNPs based on SPR resonance. The SPR is a resonance phenomenon induced by metal nanoparticles' conduction electrons interacting with incoming photons (11). In our study, single absorption peaks were observed at 278 nm (**Fig. 2A**). The single peak shows typical spherical morphology nanoparticles, and it may be explained by the fact that spherical particles are represented by a single SPR band, whereas anisotropic compounds are represented by many SPR bands (12). The dynamic size of the SeNPs was determined using the DLS pattern. The DLS pattern in our investigation

revealed that the synthesized SeNPs were nanosized, with sizes ranging from 80 to 120 nm (Fig. 2B). Due to the lack of numerous peaks, the DLS investigation showed that Pfb-SeNps exists in monodispersed form. The Zeta potential for SeNPs was found to be -26 mV and indicated that SeNPs were negatively charged (Fig. 2C). The phenolics, flavonoids, and tannins in *A. muricata* aqueous fruit extract may be responsible for the negative charge on SeNPs. The existence of negative electrostatic interaction causes SeNPs to form a dispersed state in the solution. In support of our results, Gunti, Dass & Kalagatur, (2019) that SeNPs synthesized from *E. officinalis* fruit extract have a negative charge too. The micro-morphology of SeNPs was determined by scanning electron microscopic (SEM) analysis and found that SeNPs were spherical in shape and size of 120 – 160 nm (Fig. 2D). Also, obtained results were similar to those of Kokila et al. and Matai et al. who revealed that plant extracts facilitated the synthesis of spherical and nano size of SeNPs (13,14). The technique of energy dispersive X-ray (EDX) microanalysis is used to determine the elemental makeup of a material. In our study, SeNPs were found to contain selenium (86.22%), carbon (7.30%), and oxygen (3.51%). The results revealed that selenium made up the majority of the produced SeNPs.

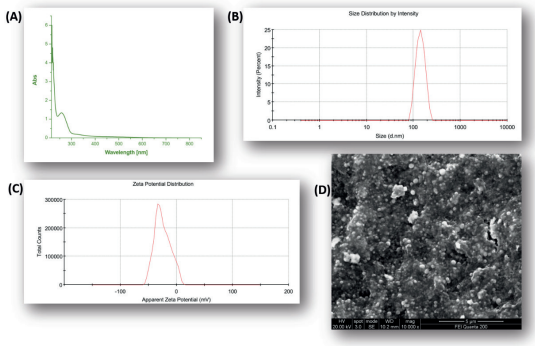

Figure 2: (A) UV-Visible spectroscopy, (B) DLS (dynamic light scattering) spectroscopy, (C) zeta potential, and (D) scanning electron microscopic images of selenium nanoparticles (SeNPs).

Antioxidant potential of selenium nanoparticles: In our study, the antioxidant activity of the as-synthesized SeNPs was evaluated by notable antioxidant assays such as DPPH and ABTS radical scavenging assays. As-synthesized SeNPs showed decent antioxidant in all the studied assays and found comparable with standard reference antioxidant ascorbic acid (Fig. 3A & B). The amount of DPPH and ABTS radicals and metal chelators were reduced in a dose-dependent manner by both SeNPs and ascorbic acid, confirming the dose-dependent nature of the antioxidant effect. The IC_{50} value of SeNPs and ascorbic acid in the DPPH radical scavenging assay was determined as 58.98 ± 0.70 and 56.30 ± 1.62 µg/mL, respectively. In ABTS radical scavenging assay, the IC_{50} values of SeNPs and ascorbic acid were determined to be 66.10 ± 1.01 and 61.92 ± 2.47 µg/mL, respectively. The study finds that selenium element and secondary metabolites of *A. muricata* aqueous fruit extract that colonized the surface of selenium nanoparticles may be responsible for SeNPs' antioxidant properties. The study concluded that SeNPs may scavenge free radicals from cells, prevent or reduce oxidative damage, and lower the risk of a variety of oxidative-related diseases (including heart disease and certain cancers) (15).

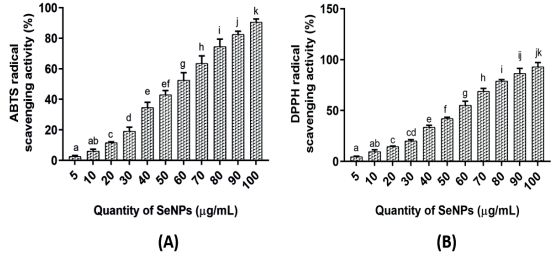

Figure 3: Antioxidant activity of biogenic selenium nanoparticles (SeNPs) determined by (A) ABTS and (B) DPPH radical scavenging activity. The experiments were repeated three times in total, with the data from each experiment used to calculate the mean ± standard deviation. One-way ANOVA was used to assess the

Table 1: Antibacterial activity of selenium nanoparticles (SeNPs) determined by the zone of inhibition assay.

Bacterial culture	Zone of inhibition (mm)			
	SeNPs			Ampicillin
	100 µg/well	200 µg/well	300 µg/well	100 µg/well
Gram-negative bacteria				
E. coli – MTCC 41	07.10 ± 0.14	09.62 ± 0.31	14.35 ± 0.72	16.57 ± 0.31
P. aeruginosa – MTCC 741	05.62 ± 0.56	08.19 ± 0.43	12.57 ± 0.04	18.82 ± 0.24
K. pneumoniae – ATCC 13883	04.24 ± 0.31	07.83 ± 0.28	11.04 ± 0.52	14.63 ± 0.41
Gram-positive bacteria				
S. aureus – ATCC 13565	09.51 ± 0.46	11.90 ± 0.56	16.97 ± 0.56	15.48 ± 0.67
E. faecalis – MTCC 439	12.20 ± 0.63	16.73 ± 0.27	23.41 ± 0.50	21.16 ± 0.88
L. monocytogenes – MTCC 657	10.04 ± 0.82	14.01 ± 0.30	20.05 ± 0.57	20.58 ± 0.29
S. aureus – ATCC 14458	09.17 ± 0.23	12.39 ± 0.49	17.91 ± 0.64	22.74 ± 0.96

Table 2: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of selenium nanoparticles (SeNPs) against pathogenic bacteria determined by micro-well dilution technique.

Bacterial culture	Antibacterial concentration			
	SeNPs		Ampicillin	
	MIC	MBC	MIC	MBC
Gram-negative bacteria				
E. coli – MTCC 41	47.04 ± 2.18	66.24 ± 1.78	21.48 ± 0.31	27.70 ± 1.89
P. aeruginosa – MTCC 741	54.78 ± 1.72	71.37 ± 1.81	22.59 ± 1.04	31.41 ± 2.56
K. pneumoniae – ATCC 13883	66.41 ± 2.92	80.23 ± 4.59	28.62 ± 1.12	34.89 ± 1.07
Gram-positive bacteria				
S. aureus – ATCC 13565	35.54 ± 2.12	64.09 ± 2.41	18.11 ± 1.06	26.67 ± 1.68
E. faecalis – MTCC 439	23.12 ± 1.89	52.21 ± 2.80	10.41 ± 1.06	18.56 ± 0.72
L. monocytogenes – MTCC 657	31.57 ± 2.59	58.95 ± 3.59	12.56 ± 0.82	21.44 ± 1.90
S. aureus – ATCC 14458	38.81 ± 3.66	62.41 ± 4.01	15.09 ± 0.94	23.89 ± 1.72

statistical data, and Tukey's test was used to determine significance (p-value ≤ 0.05) among the tested samples. Within each investigation, the bar graphs with distinct alphabetic letters were significant.\

Antibacterial of selenium nanoparticles

Zone of inhibition assay: The zone of inhibition experiment was used to evaluate SeNPs' preliminary antibacterial activity. SeNPs antibacterial activity was determined at concentrations of 100, 200, and 300 µg/well by the zone of inhibition assay. The zone of inhibition against microbes was measured in mm using the Zone-scale. As-synthesized SeNPs showed good preliminary antibacterial action against tested bacteria (Table 1).

The strong antimicrobial activity of

SeNPs was observed against Gram-positive bacteria related to Gram-negative bacteria. The zone of inhibition against Gram-positive bacteria was observed in the range of 09.17 ± 0.23 – 23.41 ± 0.50 mm. In Gram-positive bacteria, the lowest zone of inhibition was observed against *S. aureus* – ATCC 14458, and the highest zone of inhibition was noticed against *E. faecalis* – MTCC 439. The zone of inhibition against Gram-negative bacteria was observed in the range of 04.24 ± 0.31 – 14.35 ± 0.72 mm. In Gram-negative bacteria, the highest zone of inhibition was observed against *E. coli* – MTCC 41, and the lowest zone of inhibition was noticed against *K. pneumoniae* – ATCC 13883.

Micro-well dilution technique: The MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) of SeNPs against pathogenic bacteria were estimated by micro-well dilution technique. The MIC and MBC of SeNPs against bacteria were found similar to the zone of inhibition assay. The MIC and MBC of SeNPs were found best against Gram-positive bacteria related to Gram-negative bacteria (**Table 2**). In Gram-positive bacteria, the best MIC and MBC were observed against *E. faecalis* – MTCC 439. In Gram-negative bacteria, the best MIC and MBC were observed against *E. coli* – MTCC 41. The study determines that as-synthesized SeNPs could be highly applicable as antibacterial agent in biomedical field (16,17).

Conclusion

Selenium nanoparticles (SeNPs) was synthesized from *A. muricata* in an environmentally friendly, non-toxic, biocompatible, and affordable way. The synthesized SeNPs found nano in size, spherical in shape, and high stable. The synthesized SeNPs exhibited potential antioxidant activity and concluded be used as scavenger for free radicals from cells, and thus, aid in prevent or reduce oxidative damage, and lower the risk of a variety of oxidative-related diseases (including heart disease and certain cancers). The as-prepared SeNPs exhibited broad spectrum antibacterial activity and could be used as an antibacterial agent in the biomedical field.

Conflict of interest

The authors declare that no conflict of interest.

Acknowledgment

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