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Research Article

Selenium nanoparticles synthesized via green methods from Calluna vulgaris extract: Exploring their antioxidant and antibacterial activities

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Abstract: This study introduces a sustainable and environmentally friendly method for synthesizing selenium nanoparticles (SeNPs) by using Calluna vulgaris as a reducing agent. The process involves the addition of Na2SeO3 to a C. vulgaris aqueous solution, followed by reduction with ascorbic acid. UV-Vis spectroscopy confirmed SeNP formation, with a distinct absorption peak at 289 nm. Morphological analysis via Scanning Electron Microscopy (SEM) revealed spherical nanoparticles below 100 nm, as corroborated by Transmission Electron Microscopy (TEM) images displaying sizes ranging from 42.91 to 66.93 nm. Energy Dispersive Spectroscopy (EDS) confirmed the presence of selenium. Antibacterial assessments demonstrated the efficacy of *C.vulgaris* Selenium Nanoparticles (Cv-SeNPs) against gram-positive (*Enterococcus faecalis*, Staphylococcus aureus) and gram-negative bacteria (Escherichia coli). Cv-SeNPs exhibited notable antibacterial activity, particularly against E. Faecalis. In terms of antioxidant activities, Cv-SeNPs exhibited significant scavenging potential against DPPH and ABTS radicals, with low IC50 values of 24.72 and 16.87 µg/mL, respectively. The scavenging activities increased with concentration, reaching 86.6% for DPPH and 99.7% for ABTS at specific concentrations. The inclusion of ascorbic acid as a capping agent further augmented the free radical scavenging capabilities, indicating a synergistic relationship between selenium nanoparticles and capping agents. This research underscores the dual functionality of Cv-SeNPs as effective antibacterial agents and potent antioxidants. The green synthesis methodology utilizing C. vulgaris offers a sustainable approach for producing selenium nanoparticles with desirable characteristics, suggesting potential applications in medicine and industry. Further research on biomedical and industrial uses of Cv-SeNPs is needed.

1. INTRODUCTION

In recent years, nanotechnology has emerged as an incredibly promising and rapidly advancing field, owing to its extensive applications in applied sciences and technology (Yesilot & Aydin, 2019). Nanoparticles exhibit unique characteristics attributed to their high surface energy and significant surface-to-volume ratios. The popularity of metallic nanoparticles has soared due to their diverse applications across various scientific domains, encompassing physics, chemistry, materials science, and biomedical sciences (Raveendran *et al.*, 2003).

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Nanoparticles can be synthesized through three distinct methods: physical, chemical, and biological (commonly known as green synthesis). Physical methods necessitate expensive equipment, high temperatures, and high pressure. Chemical synthesis involves the use of toxic substances that pose environmental and health risks (Parveen *et al*., 2016). Additionally, these chemical methods are costly, toxic, and may be absorbed onto the nanoparticle surfaces (Adibian *et al.*, 2022). Due to the drawbacks associated with physical and chemical synthesis, there has been a shift towards green synthesis—an environmentally friendly and more cost-effective approach. Biological sources, such as plants, bacteria, fungi, and algae, containing natural molecules, are employed in green synthesis for nanoparticle production (Mittal *et al.*, 2013; Parveen *et al.*, 2016). This method offers a more sustainable and affordable alternative. Nanoparticles synthesized using plant extracts exhibit increased stability and a greater diversity in terms of shape and size compared to those produced by other organisms (Nazir *et al*., 2018).

Among the nanoparticles studied in recent years, SeNPs have attracted great attention due to their extraordinary physicochemical properties and potential biological activities (Barzegarparay *et al*., 2023). Selenium, an essential trace element, plays a vital role in maintaining human health and has been associated with a range of biological activities, including antioxidant and antibacterial properties (Roman *et al*., 2014; Mocchegiani *et al.*, 2009). The development of SeNPs from plant extracts offers a sustainable and biocompatible approach to harnessing the benefits of selenium (Khurana *et al*., 2019).

These nanoparticles show promising properties in the fields of medicine, biotechnology, and environmental science. Additionally, the production of selenium nanoparticles using natural resources (plant extracts and microorganisms) has attracted much attention by promoting sustainability and minimizing environmental hazards associated with traditional methods (Cittrarasu *et al*., 2021). One such natural source of interest for nanoparticle synthesis is *Calluna vulgaris*, commonly known as heather. Heather, a plant with widespread use in traditional medicine, is employed for treating a variety of conditions, including rheumatism, arthritis, eye diseases, kidney stones, inflammation of the bladder and kidneys, bronchitis, diarrhea, eczema, high blood pressure, increased irritability, anxiety, and sleep disorders. Extensive research has unveiled numerous pharmacological effects associated with heather, encompassing anti-inflammatory, antiseptic, sedative, diuretic, antiviral, cytotoxic, antiproliferative, antibacterial, cardioprotective, hepatoprotective, cytotoxic, and antioxidant properties (Kaunaite *et al*., 2022). *C. vulgaris* is an indigenous plant species found in many regions and has been recognized for its rich phytochemical composition, including flavonoids, phenolic acids, and other bioactive compounds (Starchenko *et al*., 2020). These compounds not only impart therapeutic properties to *C. vulgaris* but also serve as effective reducing and stabilizing agents for the green synthesis of nanoparticles (Mustapha *et al*., 2022; Shafey *et al*., 2020).

This study aims at the green synthesis of SeNPs using *C. vulgaris* extract as a reducing and capping agent. The utilization of *C. vulgaris* extract offers a sustainable and cost-effective approach, reducing the need for hazardous chemicals in the nanoparticle synthesis process. Furthermore, the phytochemicals present in *C. vulgaris* are expected to play a vital role in determining the properties and activities of the synthesized SeNPs. One of the primary objectives of this study is to investigate the antioxidant activity of SeNPs synthesized from *C. vulgaris* extract. Antioxidants play a crucial role in protecting cells and organisms from oxidative stress-induced damage by neutralizing harmful free radicals. Given the growing interest in natural antioxidants, understanding the potential of SeNPs synthesized from *C. vulgaris* extract as effective antioxidants holds substantial importance in the field of health and wellness. In addition to their antioxidant potential, SeNPs have also exhibited remarkable antibacterial properties. The development of effective antibacterial agents is crucial in combating the rising threats of antibiotic-resistant bacteria. Therefore, this study aims to evaluate the antibacterial activity of SeNPs synthesized from *C. vulgaris* extract against a range of pathogenic bacteria, shedding light on their potential as an alternative or adjunctive therapy in bacterial infections.

2. MATERIAL and METHODS

2.1. Preparation of Heather (*C. vulgaris L.*) Extract

Dried heather (Ecodab, Batch no# P16S06) was obtained from a local market. The 5-gr weighed heather was ground into a powder using a mortar and pestle.100 mL of distilled water (dH_2O) was added to the powdered heather and mixed thoroughly. The mixture was boiled in a microwave oven for 1 minute (1200 W, 50 Hz). After cooling, the obtained extract was filtered through Whatman No. 1 filter paper to obtain the heather aqueous extract. For the synthesis of selenium nanoparticles, heather extract was freshly prepared and used.

2.2. Synthesis of Selenium Nanoparticles (Cv-SeNP)

In the production of selenium nanoparticles, the green synthesis method used by Wang *et al.* (2018) was modified and used. 20 mL of 50 mg/mL *C. vulgaris* extract was added dropwise to the prepared 80 mL of 10 mM Sodium Selenite (Na_2SeO_3) (Bostonchem, Boston, MA, Cas #10102-18-8) solution. The mixture was stirred at room temperature for 2 hours. Subsequently, 2 mL of freshly prepared L-Ascorbic acid (0.2 M) (Carlo Erba, France, Cas#50-81-7) was added dropwise to the mixture. Stirring was continued until a color change was observed.

2.3. Characterization of Selenium Nanoparticles

The UV-Vis spectra of the synthesized selenium nanoparticles were recorded using a Shimadzu UV-1801 UV-VIS spectrophotometer. The spectrum of the SeNP solution was measured in the wavelength range of 200-600 nm at 25^{o}C. The particle size and morphology were observed using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) (JEOL JEM-1400 Plus). The elemental composition of the SeNP was determined using energy-dispersive X-ray spectroscopy (EDS) attached to the SEM (JEOL JSM-7100-F). The SEM, TEM, and EDS analysis were carried out at the Science and Technology Application and Research Center of Canakkale Onsekiz Mart University (COBILTUM).

2.4. DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay

The antioxidant potential of selenium nanoparticles was assessed employing the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, following the procedure outlined by Saranya *et al.* (2023) with slight modifications. A 0.1 mM DPPH (abcr GmbH, Germany, Cas# 1898-66-4) solution was prepared using methanol. Selenium nanoparticles (SeNPs), synthesized at varying concentrations (0-5000 µg/mL), were mixed with the DPPH solution in a 1:1 ratio. Following the addition of the DPPH solution, the samples were left in the dark at room temperature for 30 minutes. Subsequently, the absorbance of each sample was measured at 517 nm using a UV-Vis spectrophotometer. DPPH served as the control, and methanol was employed as the blank. Ascorbic acid was utilized as the reference compound. IC_{50} values were obtained using GraphPad Prism software. The experiments were conducted in triplicate, and the free radical scavenging activity was expressed as a percentage of inhibition, calculated using the formula:

$$DPPH\ scavenging\ activity\ (\%) = \frac{(\text{Abs of control} - \text{Abs of sample})}{\text{Abs of control}} x100$$

2.5. ABTS (2,2-azinobis- (3-ethylbenzothiazoline-6-sulphonic acid)) Assay

The ABTS scavenging activity was determined by the method described by Re *et al.* (1999) with minor modifications. Briefly, ABTS* working solution was prepared by adding sodium persulfate (2.45 mM) to the prepared ABTS stock solution (7.4 mM) (PanReac Applichem GmbH, Darmstadt, Germany, Cas no#30931-67-0). To achieve an absorbance of 0.7 units at 734 nm, 1 mL of the ABTS* solution was diluted by combining it with 76 mL of methanol, as measured using a spectrophotometer. Selenium nanoparticles (500 µL) and control (methanol

(500 µL) were mixed with 500 µL of ABTS* solution and allowed to react. Absorbance was taken at 734 nm after 15 min using a spectrophotometer. IC_{50} values were obtained using GraphPad Prism software. Experiments were performed in triplicate and free radical scavenging activity was expressed as percent inhibition determined using the following formula:

$$ABTS\ scavenging\ activity\ (\%) = \frac{(\text{Abs of control} - \text{Abs of sample})}{\text{Abs of control}} x100$$

3. FINDINGS

3.1. Preparation and Characterization of Cv-SeNPs

An allotted volume of Na_2SeO_3 solution (10 mM) was introduced into an aqueous solution of *C. vulgaris* (50 mg/mL) and thoroughly mixed. Subsequently, a specific quantity of ascorbic acid was added to facilitate the reduction of the SeO_3^{2-} precursor to its atoms. Throughout the synthesis process, the augmented Se^o atoms aggregated into selenium nuclei, rapidly expanding as the redox reaction advanced, culminating in the generation of selenium nanoparticles (Wang *et al*., 2018). Following the reaction, a color change to ruby red was observed due to the formation of SeNP (Figure 1).

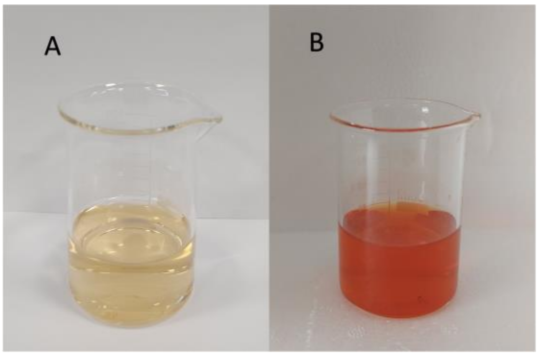

Figure 1. *C. vulgaris* extract **(A),** Selenium nanoparticles (Cv-SeNP) synthesized with *C. vulgaris* extract **(B).**

Along with these color changes, 200-600 nm wavelength scans were made in UV spectroscopy. UV-vis spectroscopy is an important method that reveals the formation and stability of SeNPs in aqueous solution by color change. Typically, the characteristic wavelength for synthesized selenium nanoparticles falls within the range of 200–300 nm (Barzegarparay *et al*., 2023; Zeebaree *et al*., 2020). By Uv-Vis measurement of Cv-SeNP, a shoulder absorption peak was observed at 289 nm, as illustrated in Figure 2. Similarly, few studies have reported the absorption peak of SeNPs to be approximately at 260-280 nm (Rajasekar & Kuppusamy, 2021; Shin *et al*., 2022; Gangadoo *et al*., 2017).

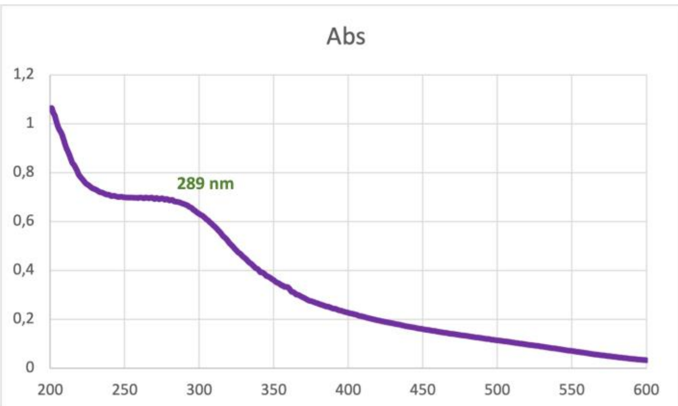

Figure 2. UV-Spectra analysis of SeNPs synthesized using *C. vulgaris* extract

The surface morphologies and sizes of SeNPs obtained by the green synthesis method were analyzed using SEM. The morphological analysis using SEM revealed that the nanoparticles exhibited a spherical structure with a size below 100 nm. The SEM image of SeNPs is shown in Figure 3A. EDS was used to determine the amount of elemental compounds and the purity of the nanoparticles. The highest peak in nanoparticles synthesized using *C. vulgaris* leaf extract was observed in selenium (15.2%) (Figure 3B). Other peaks observed with selenium in the EDS spectrum were carbon, oxygen, sodium, and very low amounts of copper and chlorine.

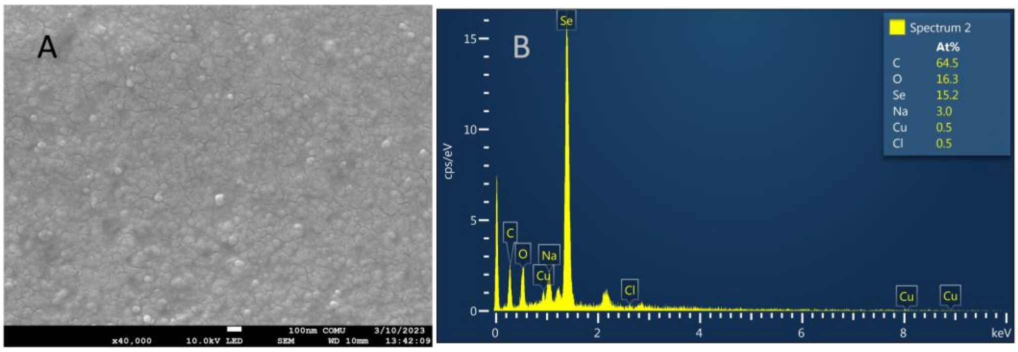

Figure 3. Scanning electron micrograph of synthesized Cv-SeNPs **(A)**, Identification of elemental composition using EDS spectra **(B)**

TEM images of Cv-SeNPs, as depicted in Figure 4, reveal a uniform spherical structure, aligning with the symmetrical single peak observed in the UV-Vis analysis. The sizes of the Cv-SeNPs range from 42.91 to 66.93 nm.

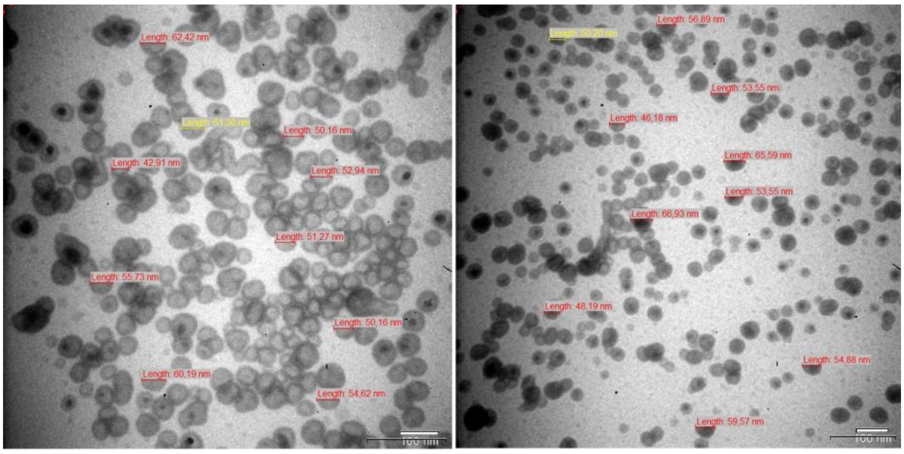

Figure 4. Transmission electron micrograph of synthesized Cv-SeNPs

3.2. Antibacterial Activity of Cv-SeNPs

In earlier research, various mechanisms for the antimicrobial effects of nanoparticles have been suggested by researchers. One crucial factor influencing this activity is the size of the nanoparticles. The small size allows nanoparticles to traverse cell walls and membranes, leading to cell lysis. Additionally, nanoparticles disrupt the respiratory cycle and the generation of ATP, hindering cell division, and ultimately resulting in the death of microbial cells (Zonaro *et al*., 2015). The antimicrobial activities of Cv-SeNPs were evaluated against three microorganism strains: gram-positive bacteria (*Enterococcus faecalis*, *Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli*) by disk diffusion method. Additionally, a Penicillin/streptomycin double antibiotic disk was used as a positive control. It was determined that the positive control antibiotic showed a 22 mm diameter inhibition zone against the test microorganisms. It was observed that Cv-SeNPs synthesized by the green synthesis method were effective against all test microorganisms (Figure 5). Disc diameters are included when calculating the results. At a concentration of 1500 µg/mL, the antibacterial efficacy against the three pathogenic microbes followed the order*: E. faecalis* > *S. aureus* > *E. coli*. The associated antibacterial zone diameters were 12.13 mm, 10.14 mm, and 10.09 mm, respectively.

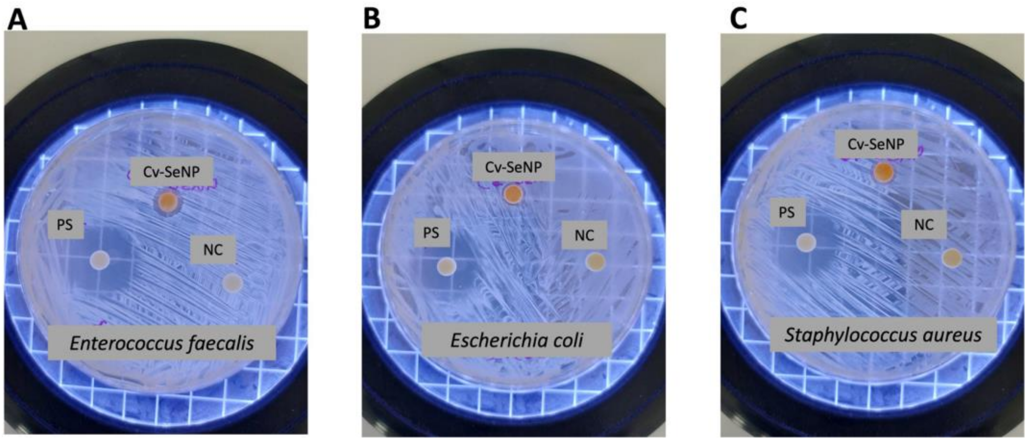

Figure 5. Antibacterial activity of Cv-SeNPs against the tested microorganisms **(A)** *Enterococcus faecalis*, **(B)** *Escherichia coli*, **(C)** *Staphylococcus aureus*

In previous studies, Se-NPs were found to have bactericidal properties against S. aureus (Shakibaie *et al*., 2015; Tran&Webster, 2011). Another study proved that Se-NPs have antibacterial properties against E. coli and S. aureus (Ali & Najmy, 2013). In a recent study, Se-NPs synthesized using Triphala extract exhibited significant antimicrobial activity against *S. mutans*, *S. aureus*, *E. faecalis*, and *Candida albicans* (Chellapa *et al*., 2020). SeNPs synthesized from *Solanum nigrum* fruit extract showed similar results against *S. typhi*, *E. coli*, *P. vulgaris*, and *V. cholerae* (Saranya *et al.*, 2023).

3.3. Antioxidant Activity of Cv-SeNPs

The antioxidative potential of Cv-SeNPs was assessed using DPPH and ABTS* assays for scavenging free radicals. Cv-SeNPs exhibited significant antioxidant activity in both DPPH and ABTS assays, demonstrating IC_{50} values of 24.72 and 16.87 µg/mL, respectively. The low IC_{50} values highlight the effective neutralization of free radicals by Cv-SeNPs. Cv-SeNPs showed 86.6% maximum DPPH scavenging activity at a concentration of 750 µg/mL (Figure 6A). The results of the present study correlate well with the previous study in which the scavenging activity of *Olea ferruginea* fruit extract-mediated SeNPs was 85.2% (Hassan *et al*., 2022).

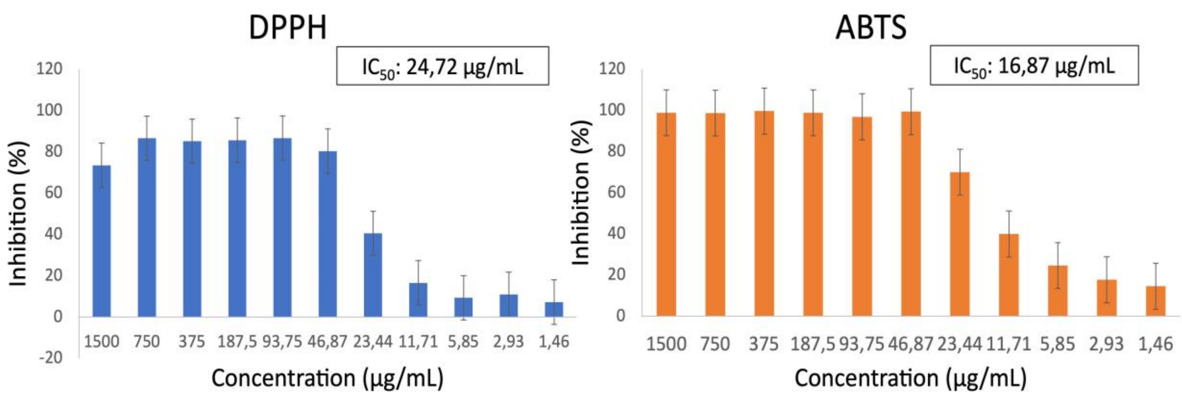

Figure 6. Antioxidant activity of the Cv-SeNPs **(A)** DPPH free radical scavenging, **(B)** ABTS radical scavenging activity

Figure 6B illustrates the antioxidant activity of SeNPs against ABTS*. Once again, the scavenging activity demonstrated an upward trend with increasing SeNP concentrations. At a concentration of 375 µg/mL, SeNPs exhibited a peak ABTS scavenging activity of 99.7%. These findings align with a prior study wherein the ABTS scavenging activity of SeNPs mediated by *Rosa roxburghii* extract was reported as 98.92% (Ge *et al*., 2023), providing additional support for the results obtained in the current investigation. The incorporation of ascorbic acid as a capping agent for SeNPs enhanced the scavenging of free radicals, indicating a collaborative improvement in antioxidant capabilities. Selenium's capacity to counteract free radicals, primarily through the upregulation of selenoenzymes like glutathione peroxidase, is well-known (Rotruck *et al*., 1973; Shin *et al*., 2022). Introducing phytomolecules (such as phenolic and flavonoids) as capping agents further fortified selenium's antioxidant properties against free radicals. The findings suggest that the synergy between selenium nanoparticles and capping agents, including ascorbic acid and phytomolecules, results in potent antioxidants, demonstrating the potential to effectively address free radicals (Kokila *et al*., 2017; Gunti *et al*., 2019).

4. DISCUSSION and CONCLUSION

This research endeavors to shed light on the unique properties of SeNPs synthesized from *C. vulgaris*, their potential applications in medicine, and their contribution to the ever-growing field of green nanotechnology. *C. vulgaris*, a plant rich in phytochemicals, represents a promising source for the eco-friendly synthesis of SeNPs. This synthesis method not only reduces the environmental impact associated with traditional chemical methods but also provides an opportunity to explore the unique attributes of *C. vulgaris* in enhancing the biological activities of the synthesized nanoparticles.

Antioxidant activity is a critical parameter in the assessment of nanoparticles for potential biomedical applications. Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) production and the body's antioxidant defense mechanisms, is implicated in numerous diseases, including cancer, neurodegenerative disorders, and cardiovascular ailments. Selenium nanoparticles have demonstrated remarkable antioxidant potential due to their ability to scavenge ROS, thus holding promise as therapeutic agents in combating oxidative stress-related diseases.

Concomitantly, the antibacterial activity of SeNPs has attracted attention in the context of addressing the escalating global challenge of antibiotic resistance. As conventional antibiotics become less effective, the search for alternative antibacterial agents intensifies. Selenium nanoparticles exhibit antibacterial properties by disrupting the bacterial cell membrane, interfering with cellular functions, and promoting oxidative stress within bacteria. These attributes make SeNPs a potential solution to combat bacterial infections, both as standalone agents and in synergistic combinations with existing antibiotics.

In summary, this research bridges the domains of nanotechnology, green synthesis, and natural product chemistry, exploring the synthesis of SeNPs using *C. vulgaris* extract and their subsequent implications in antioxidant and antibacterial activities. The outcomes of this study may contribute to the development of novel therapeutic agents and environmentally friendly nanoparticle synthesis methodologies, fostering both health and sustainability.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Ecem Erdem: Investigation, Methodology, Visualization, Writing – original draft. **Çiğdem Aydın Acar**: Investigation, Resources, Methodology, Visualization, Analysis, Supervision, Writing – review & editing.

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