RESEARCH ARTICLE

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Environmentally friendly rapid green synthesis of SeNPs using grapefruit (*Citrus paradisi*) leaves extract, and their antimicrobial potential

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Abstract

The utilisation of plant biomass in the production of nanoparticles is gaining popularity because of its associated benefits. Selenium nanoparticles (SeNPs) are highly valuable due to their involvement in numerous biological functions. In this study, SeNPs were rapidly synthesized using the environmentally friendly and low-cost green synthesis approach using *Citrus paradisi* (Grapefruit) leaves extract. The synthesized SeNPs were characterized using TEM, AFM, DLS, UV-vis, XRD, and EDX data. The data revealed that SeNPs had a spherical and uniform shape, with an average size of 45 nm, a surface charge of -20.54 mV, and a peak absorbance wavelength of 326 nm. The inhibitory impact of SeNPs on harmful strains and cancer cells was investigated using the microdilution method. The development of bacteria was effectively inhibited at concentrations ranging from 4 to 16 µg ml^{-1}.

Keywords: Antimicrobial, Anticancer, Extract, *Citrus paradisi*, Green synthesis, SeNPs

Type: Research Article
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INTRODUCTION

Nanotechnology is a crucial interdisciplinary field that encompasses various scientific and technological fields including biomedicine, agriculture, pharmaceuticals, environmental science, chemical engineering, textile technology, electronic physics, and information technology. In this sector, especially the vast diversity of synthesis methods of nanoparticles and some advantages such as environmental sensitivity and inexpensive cost are among the most attractive features of this field (Kumar et al., 2017; Alagesan & Venugopal, 2019; Hashem & Salem, 2022)this study aimed to biosynthesize SeNPs using aqueous extract of Urtica dioica leaf through green and ecofriendly method. Moreover to fully characterize SeNPs using different techniques, and to evaluate it for antimicrobial activity as well as anticancer activity. Main Methods and Major Results: SeNPs were biosynthesis using aqueous leaf extract of U. dioica (stinging nettle. The benefits of synthesizing nanoparticles using plant biomass include ease of method, cost-effectiveness, environmental friendliness, biocompatibility, high product yield, easy resource accessibility, and increased appeal for research in this area (Anu et al., 2017; Chellapandian et al., 2019; Gunti et al., 2019; Alipour et al., 2021; Hashem & Salem, 2022; Mariadoss et al., 2022)phytofabricated selenium nanoparticles (PF-SeNPs. Extracts from plant sources contain bioactive components like alcohol, phenolic compounds, and flavonoids, which have active groups that contribute to reduction, stability, and coating properties (Alipour et al., 2021; Perumal et al., 2021; Vu et al., 2022). Nanoparticles produced by plants are valuable products used in several fields, particularly in biomedical applications, due to their biocompatible architectures (Ndwandwe et al., 2021; Perumal et al., 2021; Adibian et al., 2022).

Selenium (Se) is a vital element involved in antioxidant systems and several biological activities including catalase and superoxide dismutase. SeNPs have a competitive edge over selenium salts as a selenium source due to their reduced toxicity compared to the numerous types of selenium salts present in nature. Selenium (Se) is a vital element involved in antioxidant systems and several biological activities including catalase and superoxide dismutase. SeNPs have a competitive edge over selenium salts as a selenium source due to their reduced toxicity compared to the numerous types of selenium salts present in natüre (Ranjitha & Rai, 2021; Chen et al., 2022)synthesis of selenium at the nanoscale level is important. Selenium nanoparticles (SeNPs. Alcohol/phenol amine groups of phytochemicals in plant extracts are bioactive organic molecules that reduce SeO_3^{-2} to form, stabilize, and coat SeNPs. (Anu et al., 2017; Ezhuthupurakkal et al., 2017; Alagesan & Venugopal, 2019; Cittrarasu et al., 2021; Pon Matheswari et al., 2022).

Citrus paradisi, a member of the *Rutaceae* family, is a perennial species. This plant contains narirutin and naringin, flavanones, ferulic and p-coumaric acids, vanillic, gallic acids phenolic acids, carotenoids, flavonoids, terpenoids, essential oils, and ascorbic acid, which have numerous health benefits due to their pharmacologically active properties (Gupta et al., 2010; Anupama Prasad et al., 2023; Kumar et al., 2020)

The work aims to synthesize selenium nanoparticles using an extract from *Citrus paradisi* plant leaves in an environmentally friendly, cost-effective, and simple approach. The properties of the nanoparticles were characterised, and their antibacterial activities were investigated.

MATERIAL AND METHOD

Plant extract and sodium selenite solution preparation

Citrus paradisi plant leaves were collected at the end of the season in Köyceğiz, Muğla, Turkey. After rinsing with tap water and then distilled water, it was dried on blotting paper at room temperature. 10 grams of desiccated leaves were weighed, combined with 100 milliliters of distilled water, and cooked using a kettle. After filtration, the cooled extract was prepared for synthesis.

A 20 mM solution was generated using 99% pure Sigma-aldrich Na_2SeO_3 (sodium selenite) salt for the purpose of generating SeNPs using green synthesis.

Green Synthesis of SeNPs by Cp Extract

A solution of sodium selenite and Cp extract was mixed in a 5:2 ratio and the reaction took place at 55 °C and pH 6.3 for two hours. Samples were collected periodically from the synthesis medium based on color changes. The samples were diluted by a factor of 10 before being measured. The samples were analyzed for the maximum wavelength absorbance data to monitor the creation of SeNPs by assessing the color change intensity. This was done using a Perkin Elmer One UV-visible spectrophotometer (UV-vis) with measurements made in the range of 300-800 nm.

FTIR Spectra of Cp Extract

A Perkin Elmer One Fourier Transformation Infrared Spectroscopy (FTIR) was utilized to analyze the bioactive functional groups of phytochemicals involved in the bioreduction, coating, and stability of SeNPs. The frequency changes in the FTIR spectra, recorded between 4000-500 cm^{-1}, of both the Cp extract and the liquid media obtained after synthesis were informative.

Characterization of SeNPs

Maximum wavelength absorbance values were collected using UV-vis scanning to detect the production of synthesized SeNPs(. The morphological structures of the synthesized SeNPs were analyzed using a Jeol Jem 1010 Transmission Electron Microscopy (TEM) and a Park System XE-100 Atomic Power Microscopy (AFM). SeNPs' hydrodynamic size distributions were estimated using density-dependent analysis utilising a Marven Dynamic Light Scattering Spectrometer (DLS). The crystal patterns and sizes of the synthesized particles were evaluated using data obtained from a Rigaku Miniflex 600 model. The Debye-Sherrer equation in formula (1) was used to calculate the crystal nanosizes based on the FWHM values obtained from the data (Shirmehenji et al., 2021; Baran et al., 2022;)silver (4.12%

$$D = k\lambda / (\beta \cos\theta) \quad (1)$$

Regarding inequality: D represents particle size, β is half of the FWHM value at the peak, k is a shape factor (0.9), λ is the wavelength of X-ray, and θ is the diffraction angle.

The elemental composition of particles produced using Cp was analyzed using an FEI PQuanta 250 FEG Electron Disperse X-ray (EDX) to measure values. Surface charge distributions impacting stability were studied using density-dependent zeta potential data collected with a Malvern device (DLS).

Examination of the Antibacterial Potential of SeNPs on Pathogenic Strains

Antibacterial properties of SeNPs derived from an extract of Cp leaves were investigated through experimental experiments applying the microdilution method on pathogenic microorganisms. Through the applications, Minimum Inhibition Concentrations (MIC) that effectively suppressed growth were identified. Gram-positive *Bacillus subtilis* ATCC 11774 and gram-negative *Pseudomonas aeruginosa* ATCC27833 bacteria were cultivated and utilised in suitable conditions in the experimental study. Microorganism solutions were created based on the McFarland standard 0.5 turbidity criteria (Zeraatkar et al., 2022). The suitable medium was added to 96-well microplates during the experiment. Microdilution was conducted by adding different amounts of SeNPs, $NaSeO_3$ solution, and specific antibiotics for each strain (colistin for gram-negative and vancomycin for gram-positive bacteria) to the microplates. Microorganisms grown at Macfarland 0.5 turbidity were transferred to microplate wells and cultured at 37 °C for 24 hours to study antimicrobial interaction.

RESULTS AND DISCUSSION

FTIR Spectra of SeNPs and Cp extract

FTIR spectra were utilised to identify the functional groups of the bioactive components in the Cp extract and their role in the bioreduction, coating, and stability of SeNPs. The FTIR spectra of the final synthesis liquid arising from the reaction were analyzed to assess it. The shifts in frequency seen at 3320, 1636, 1425, 1261, 1081, and 695 cm^{-1} in Figure 1's spectra indicate that the groups associated with these locations are linked to bioreduction, stability, and coating. The events include stretching of -OH and -COOH groups at 3320 cm^{-1} due to alcohol and carboxyl groups, as well as N-H stretching and vibration at 1636-1425 cm^{-1} related to amide groups. The peak at 1425 cm^{-1} corresponds to C—O—H stretching, while the peaks at 1261 cm^{-1} and 1081 cm^{-1} are attributed to —S and vinyl group stretching, respectively. The shift at 695 cm-1 is due to —S stretching (Ezhuthupurakkal et al., 2017; Hatzikioseyian et al., 2022; Ramamurthy et al., 2013)while the morphology and the properties of the adsorption beads are studied by SEM, FTIR and rheological analysis. Langmuir isotherm describes the adsorption equilibrium with maximum loading Q0 29.411 mg Ni/g SeNPs and 0.651 mg Ni/g alginate-SeNPs. The adsorption of nickel is fast and equilibrium is attained within one hour. Continuous flow experiments in packed beds reveal early elution patterns and non-symmetric sigmoidal profiles due to the reduced adsorption capacity of the immobilized SeNPs, the short depth of the beds and the apparent non-uniform flow pattern. The Bohart-Adams equation fits adequately the main sigmoidal part of the breakthrough curves, however fails to predict the early breakthrough data. By using the advection–dispersion-reaction equation (ADR.

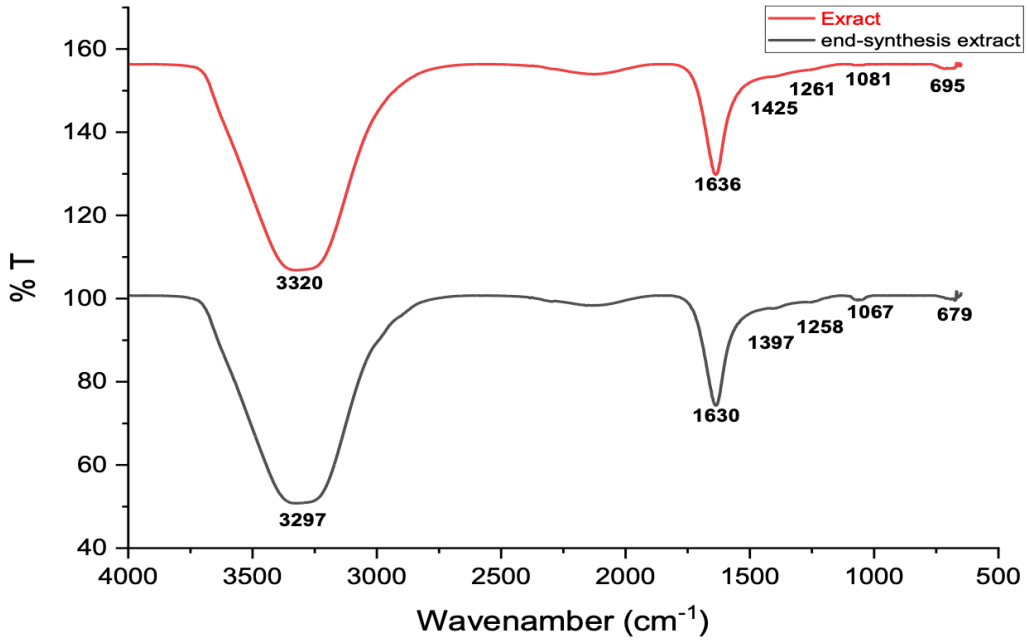

Figure 1. FTIR spectra of Cp extract and liquids obtained as a result of synthesis.

Characterization of SeNPs

UV-vis Data

After 30 minutes of stirring a solution containing Cp and sodium selenite, a red color shift was seen as a result of the reaction. The color change persisted until the 50-minute mark. The UV-vis analysis (Figure 1) determined the maximum absorbance values of 327.8 nm within the wavelength range of 290-400 nm, indicating the synthesis of SeNPs through bioreduction with Cp. The color change occurred because of the creation of SeNPs by reduction and the vibrations on the plasma surface, known as surface plasma resonance (SPR). This was demonstrated by the peak absorbances recorded in UV-vis spectroscopy (Ramamurthy et al., 2013; Perumal et al., 2021; Fadl et al., 2022; Puri & Patil, 2022; Shin et al., 2022; Vundela et al., 2022)respectively, which suggested that C. papaya fruit extract could be a competitive reducing and stabilizing agent during phytofabrication of nanoparticles. UV–Vis and FTIR spectroscopy showed the formation of SeNPs from sodium selenite, which could be related to the reducing and stabilizing activities of C. papaya fruit extract. The SeNPs were found to be stable with a Zeta potential of −32 mV. The average hydrodynamic size of SeNPs was found as 159 nm by dynamic light scattering. The SeNPs showed a broader XRD pattern with no sharp Bragg's peaks and found to be amorphous. SEM showed that SeNPs were spherical in shape and EDX pattern showed that SeNPs were made up of Se (71.81%.

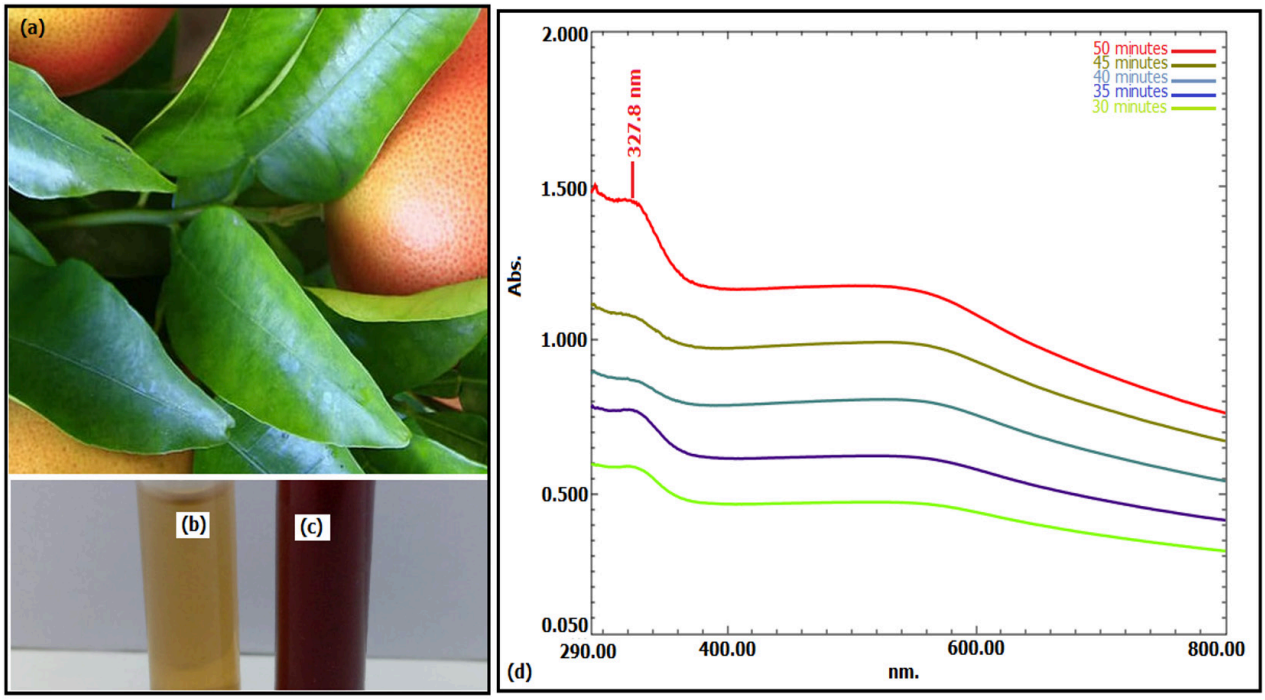

Figure 1. *Citrus paradisi* leaves (a), leaf extract (b), liquid containing colloidal SeNPs at the end of synthesis **(c)**, and UV-vis analysis data showing the synthesis of SeNPs and their presence along their maximum wavelength absorbance bands (327.8) (d)

XRD Data of SeNPs

Selenium nanoparticles were dropped onto a powdered carbon adhesive tape. Then, it was fed into the device and phase analysis was performed.The XRD data was used to analyze the crystal structures of particles synthesized using Cp (20-80 theta). The crystal nanosizes were determined by applying the Debye-Sherrer equation to the FWHM values in the data. Expansions were observed in the Bragg angles of the data obtained at 2-theta on the following planes: (102), (133), (191), (202), (210), (244), (264), (293), and (310). The full width at half maximum (FWHM) values for these angles are 23.50, 29.70, 41.32, 43.56, 45.58, 51.07, 55.62, 61.06, and 65.06, as shown in Figure 2. The results indicated that the crystal structures of the produced SeNPs matched the values specified in the Joint Committee on Powder Diffraction Standards (JCPDS file no 06–0362). Additional modest signals detected in the data were associated with different biomolecules found in the extract. (Fouda et al., 2022). The crystal size was determined to be 42.0 nanometers using the Debye-Scherrer formula. The Debye-Sherrer equation was used to determine that the average size of SeNPs in a study including *Ficus benghalensis* leaf extract ranged from 45 to 95 nm. (Tripathi et al., 2020). SeNPs were determined to have a size of 41 nm in a different green synthesis work (Zeraatkar et al., 2022).

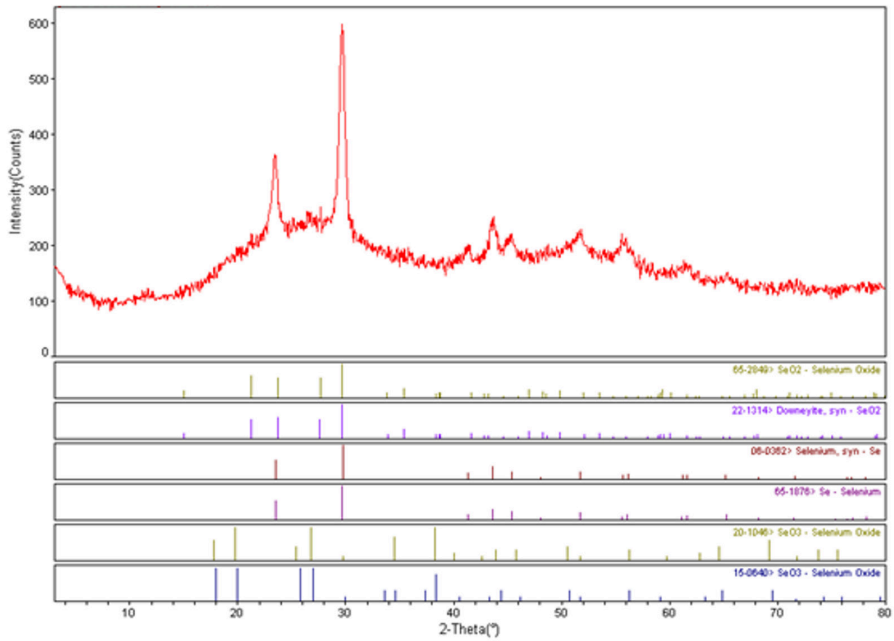

Figure 2. Crystallographic data of SeNPs produced using a certain method was analyzed using X-ray diffraction at a certain angle and reflected on a specific plane.

EDX Profiles of Synthesized Particles

Figure 3 displays prominent peaks in the element profile of the particles collected using Cp, showing a significant concentration of the Se element, attributed to SeNPs (Chitti Kondal Rao et al., 2022). The low signals of oxygen and carbon in the graph were caused by the bioactive components present in the structures of the phytochemicals in the extract. Bioactive compounds are structures involved in coating and stabilising SeNPs (Chitti Kondal Rao et al., 2022; Fouda et al., 2022; Hashem et al., 2022).

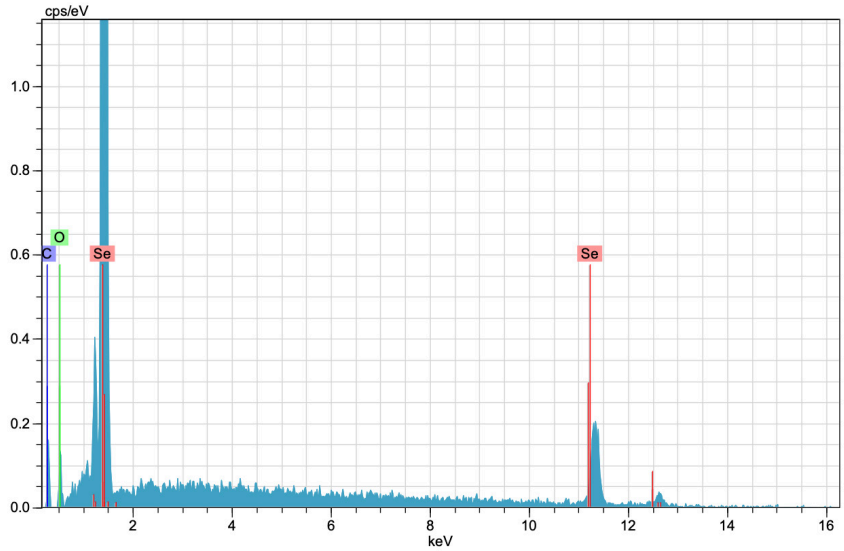

Figure 3. EDX profiles displaying the elemental makeup of particles produced with Cp extract

Morphologies of Synthesized SeNPs

The morphological appearance of SeNPs synthesized using Cp extract source was determined using TEM, AFM, and DLS data provided in Figure 4. The images showed that the SeNPs had a spherical shape, no clustering, and an average size of 45 nm, with some being smaller than 50 nm (Saranya et al., 2022). The green synthesis investigation reported that the synthesized SeNPs exhibited a spherical shape and had an average size of 156.93 nm according to DLS results (Saravanakumar et al., 2022)the SeNPs were synthesized using Trichoderma extracts (TE. SeNPs with a semi-spherical

shape were observed in a green synthesis investigation, with a size distribution of 37.5 nm (Alizadeh et al., 2023). The study reported that SeNPs produced using the extract had a spherical shape and were evenly distributed in the AFM image (Kazemi et al., 2021).

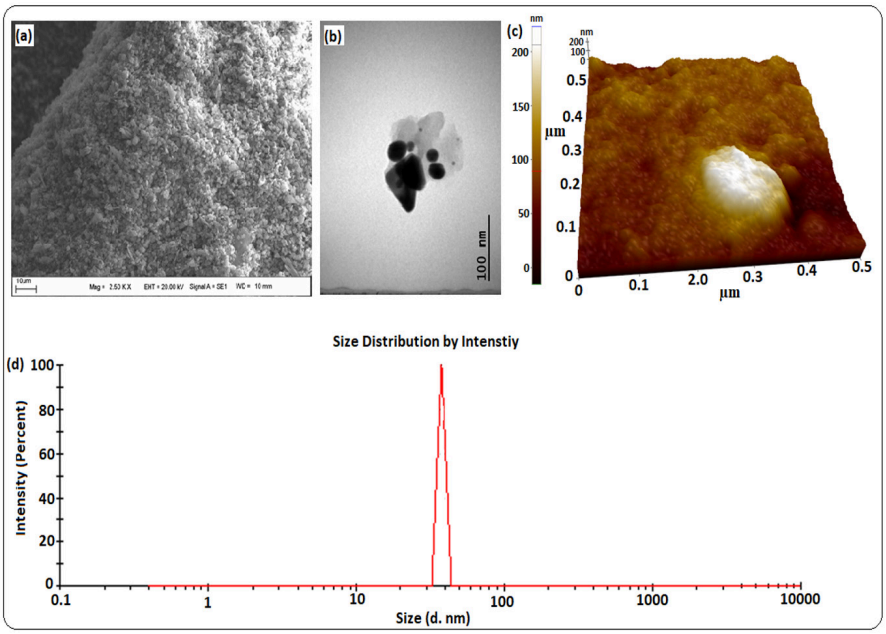

Figure 4. Morphological features of SeNPs produced using Cp extract were analyzed by SEM (a), TEM (b), AFM (c), and DLS (d) techniques

Surface Structures of SeNPs and Mass Loss Points Due to Temperature Change

The density-dependent charge distribution of SeNPs synthesized using Cp was observed to be -20.54 mV, as depicted in Figure 5. The EDX profile in Figure 3 confirmed the existence of bioactive components including carbon and oxygen, aligning with the surface charge distribution findings in Figure 5. The nanoparticles negative surface charge is a crucial characteristic that enhances stability and biocompatibility, which is beneficial for biomedical uses. Having two distinct charges of nanoparticles in the same setting leads to outcomes like aggregation and fluctuation, which have a detrimental impact on stability. SeNPs, having positive qualities in the physiological environment and a negative surface charge distribution, readily interact with biological structures that have these properties in the same environment (Chitti Kondal Rao et al., 2022; Gharbavi et al., 2022; Hatami et al., 2020; Mariadoss et al., 2022; Saravanakumar et al., 2022; Chen et al., 2023;)Bacillus cereus, Salmonella enterica, and Escherichia coli. Zeta potential data from a green synthesis investigation revealed that SeNPs produced using fruit extract had a surface charge of -12.44 mV (Gharbavi et al., 2022).

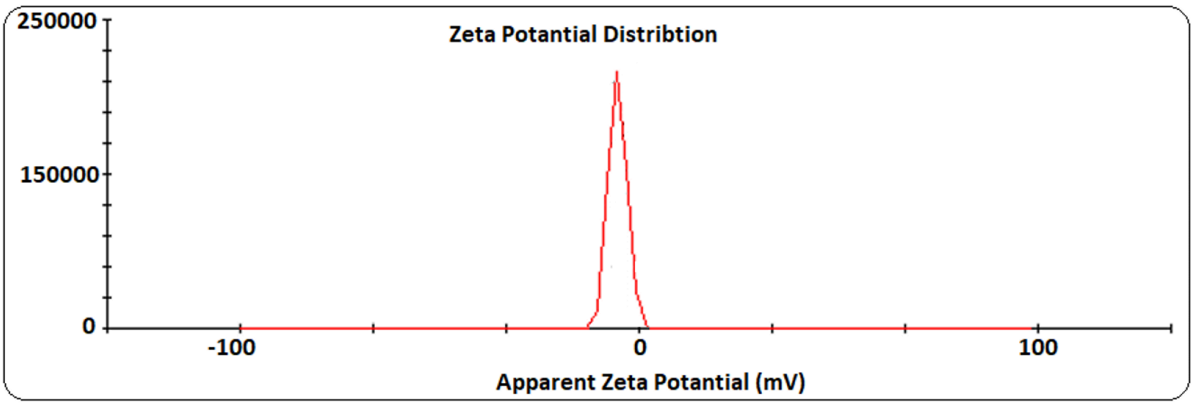

Figure 5. Surface charge distributions of SeNPs synthesized using Cp were graphed based on zeta potential analysis results.

The TGA-DT data presented in Figure 6 was utilised to assess the mass losses of the SeNPs due to temperature variations during the synthesis process. Mass losses of 22.64% and 49.37% were recorded at two distinct temperature ranges. The bulk of the losses occurred due to the degradation of SeNPs. The significant mass reductions were mostly caused by the bioactive elements responsible for the stability of the SeNPs (Baran., 2019; Padalia & Chanda, 2021; Alizadeh et al., 2023; Younas et al., 2023). Furthermore, the data obtained from XRD, EDX, and Zeta potential analyzes in Figure 2, Figure 3, and Figure 5 showed the existence of bioactive components.

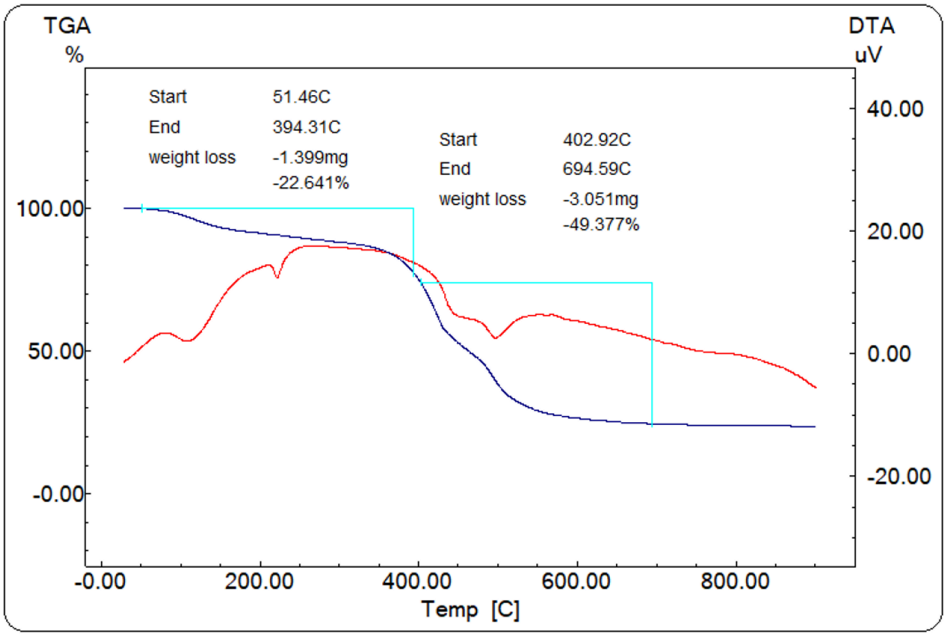

Figure 6. Temperature of mass loss points were determined using TGA-DT for SeNPs synthesized using the Cp extract

Antibacterial Effects of SeNPs

Several characteristics of nanoparticles, including shape, concentration, size, surface charge, and contact duration, play a crucial role in hazardous effects. Nanoparticles interact with cells in physiological contexts due to features such as electrostatic attraction force and hydrophobic contact (Doan et al., 2020; Mehravani et al., 2021; Remya et al., 2015; Webster, 2020). Pores of big circulatory arteries are particularly present in tumour tissues. These regions facilitate the easy passage of things like nutrients and oxygen (Chen et al., 2021; Hosny et al., 2021). Certain biomolecules exhibit an affinity for interacting with nanoparticles. Substances negatively impact the activities of biomolecules involved in metabolic processes as DNA, proteins, and enzymes (Rolim et al., 2019; Barabadi et al., 2020; Donga et al., 2020; Hosny et al., 2022; Webster, 2020).

The emergence of antibiotic resistance in microorganisms hinders the effectiveness of combating infections and leads to more severe issues. Research efforts to find alternative antimicrobial drugs to address this issue continue to be crucial and relevant (Silva et al., 2021; Wongpreecha et al., 2018; Das et al., 2022). SeNPs have the potential to address antibiotic resistance in addition to their effectiveness in other biological processes. Stable SeNPs were quickly synthesized using the Cp extract to examine their antibacterial effects on pathogenic strains by the microdilution method, in order to aid in the hunt for antibiotic agents. The synthesized SeNPs were found to be effective against microorganisms with MICs of 6.00 and 12.00 µg mL^{-1}, as shown in Figure 8 and Table 2. SeNPs exhibited the most favorable impact on Gram-negative Pseudomonas aeruginosa. The impact occurred at a concentration slightly below the Minimum Inhibitory Concentration (MIC) of the Na2SeO3 solution, which was almost equivalent to the concentration of antibiotics utilised. Properties like form, surface charge, size, and contact duration significantly influence the antibacterial capabilities of nanoparticles. Microorganisms and nanoparticles interact in the physiological environment through processes such electrostatic attraction, adsorption, and hydrophobic contact (Ahmed et al., 2016; Ferreyra Maillard et al., 2018; Babu et al., 2020; Mariadoss et al., 2022). Due to this interaction, the morphology of the cell wall and membrane is adversely altered. Furthermore, metabolic activities including energy metabolism in the membrane structure are disturbed, affecting their shape and function. Important biological molecules like DNA and RNA, which have an attraction to these substances, experience dysfunction. Due to these adverse impacts, bacteria are unable to perform their essential functions. Consequently, the mortality of microorganisms occurs due to these consequences (Cui et al.,

2012; Ahmed et al., 2016; Ezhuthupurakkal et al., 2017; Jha et al., 2017; Donga et al., 2020).

The study on green synthesis found that the MIC of SeNPs against Pseudomonas aeruginosa and Staphylococcus aureus bacteria was 100 µg mL^{-1} (Srivastava & Mukhopadhyay, 2015). It was shown that the effective MIC value of SeNPs synthetized through Nepeta extract on P. Aeruginosa was 4 µg mL-1 (Zeraatkar et al., 2022).

Table 2. Effective MIC values of SeNPs synthesized by Cp extract, Na_2SeO_3 solution, and antibiotics on the suppression of bacterial growth.

Organism		Antibiotic* µg $m^{L-}1$	Na_2SeO_3 Solution µg mL^{-1}	SeNPs µg mL^{-1}
Gr (-)	*P. aeruginosa*	4.00	8.00	6.00
Gr (+)	*B. subtilis*	8.00	16.00	12.00

* Colistin for *P. Aeruginosa*, vancomycin for *B. Subtilis.*

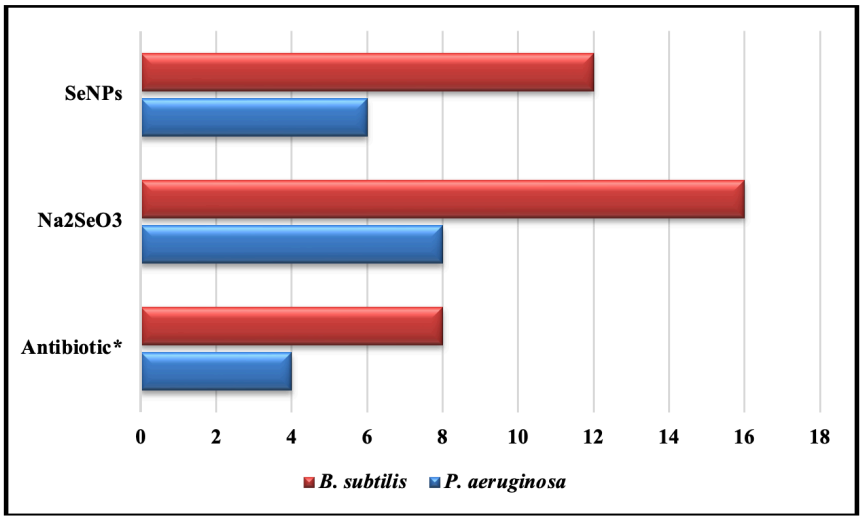

Figure 8. MIC values of SeNPs synthesized by Cp, antibiotics and sodium selenite solution suppressing the growth of bacteria.

CONCLUSION

SeNPs are highly precious materials. Acquiring these elements using green synthesis methods is crucial for ensuring biocompatibility. SeNPs were synthesized using green synthesis using Cp extract in an environmentally friendly, inexpensive, and rapid process. The SeNPs were analyzed using TEM, AFM, DLS, UV-vis, XRD, and EDX data to determine their characteristics. The SeNPs were found to be spherical and uniform, with an average size of 45 nm, a surface charge of -20.54 mV, and a maximum absorbance wavelength of 327.8 nm. SeNPs' suppressive effects on pathogenic strains were investigated via the microdilution method. Concentrations ranging from 4 to 16 µg ml^{-1} were effective in inhibiting bacterial growth.

SeNPs produced with Cp will greatly enhance medical applications as antibacterial agents by streamlining the synthesis process, given their significance in several utilisation areas.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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Consent to participate
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