

GREEN SYNTHESIS OF SeNPS USING *Sonchus maritimus* BASED NANOSIZED METAL OXIDES FOR *in vitro* BIOLOGICAL APPLICATIONS AND *in vivo* ACUTE TOXICITY EVALUATION

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ABSTRACT. This investigation aimed to characterize the green synthesized selenium nanoparticles using *Sonchus maritimus* L. extract and evaluate their antioxidant and antibacterial properties. Moreover, acute toxicity of nanoparticles was performed in Wistar rats. The synthesis of SeNPs was confirmed by Ultraviolet-visible, Fourier Transform Infrared Spectroscopy, Scanning Electron Microscopy and Energy Dispersive X-ray analysis. Antioxidant activities of *S. maritimus* and SmE-SeNPs were determined by DPPH and FRAP assays. Antibacterial activities were tested against Gram positive and negative pathogen bacteria. The SEM results showed that SeNPs had a spherule-like structure reaching up to 26.48 nm. In addition, *S. maritimus* and SmE-SeNPs had DPPH scavenging activity and reducing power. SeNPs exhibited activities against *Escherichia coli* and *Staphylococcus aureus*. The intraperitoneal toxicity test of SeNPs showed no mortality and minor behavioral variations. In conclusion, *S. maritimus* can be considered as biocatalyst stabilizers for the biosynthesis of SeNPs which might be used in several applications due to their biological efficiency.

Keywords: SeNPs, *Sonchus maritimus*, antioxidant, antibacterial, toxicity test.

INTRODUCTION

One of the most exciting technologies of the XXI century is nanotechnology. It is the skill of observing, measuring, manipulating, assembling, and producing materials at the nano-scale level, often between 1 and 100 nm (BAYDA *et al.*, 2020). It enables the conversion or self-

assembly of individual atoms, molecules, or molecular clusters into specific structures to produce materials with novel and significantly distinct properties (KHODADE *et al.*, 2017). The range of applications for nanoparticles in fields including medicine, electronics, chemistry, catalysis, and energy, there has been an increase in the commercial demand for them in recent years (SINGH and DHALI WAL, 2015). In biology, the nano-metric field still requires additional research to create new materials (CHETEHOUNA *et al.*, 2020). Different techniques, including conventional chemical synthesis and environmentally friendly synthesis, have been used to create metal nanoparticles (EL-SHA FEY, 2020). Due to their bioactivity, plants are widely used in medicine, food, and pharmaceutical industries (STANKOVIĆ *et al.*, 2022). The green synthesis of nanoparticles employing biogenic materials such as plant extracts by their bioactive constituents which can act as important biocatalysts in the formulation of nanoparticles, also as natural nanoparticle stabilizers (FRITEA *et al.*, 2017). Selenium is a trace element, vital for human health (FARSADEGH and JAFARIZADEH-MALMIRI, 2019), which the human body needs from 40 to 300 micrograms per day (RAJESHKUMAR *et al.*, 2018). It possesses an important role in the control of human metabolism (DJALALINIA *et al.*, 2021). Recent studies have found that nanoparticles of elemental selenium have a variety of unusual biological properties, including immunomodulation anticancer and bone growth stimulation (XIA *et al.*, 2022).

Sonchus maritimus L. belongs to the Asteraceae family, which is one of the largest plant families in the world, characterized by antioxidant and anticancer activity and proved effect against infections and pathogenic bacteria (HAMEED *et al.*, 2021). The *Sonchus* genus is distinguished by its abundance of known secondary metabolites, including terpenes, sterols, flavonoids, and coumarins (FOUAD *et al.*, 2020). Phenolic compounds are well known for their diverse activities, from antioxidant, antimicrobial, and anti-inflammatory (KATANIĆ STANKOVIĆ *et al.*, 2022). These compounds possess a significant role in the green synthesis of nanoparticles and serve as natural reductants of Se salt and stabilizers of SeNPs (IKRAM *et al.*, 2021). It is well known that plant leaf extracts have already been used in the synthesis of diverse nanomaterials (FAN *et al.*, 2020).

This study aimed to characterize and evaluate the antioxidant, antimicrobial activities, and *in vivo* acute toxicity of eco-friendly synthesized selenium nanoparticles by *S. maritimus* aqueous extract.

MATERIALS AND METHODS

Collecting of plant samples

The plants of *S. maritimus* were collected in November from Djamaa village in El-Oued state, Algeria, and were taxonomically verified by Pr. Halis Youcef, a botanist in CRSTRA Touggourt, Algeria. The voucher plant is stored in the plant bank of the Department of Biology, El Oued University (voucher specimen no: FSNV/DB/consult/2021/88-14-05). The leaves were rinsed with distilled water and let to completely dry at room temperature, then were ground to powder and stored until use at room temperature.

Preparation of leaves extract

The method of extraction is in detail described by DEROUICHE *et al.* (2022). For preparation of the aqueous extract, 5 g of *Sonchus maritimus* dry leaf powder was added to 50 mL of distilled water. The mixture was filtered through Whatman filter paper No. 1 and dried in a stove at 50°C, after being macerated at room temperature for 24 h.

Green synthesis of selenium nanoparticles

In the SeNPs preparation process 100 ml of 0.1 M sodium selenite was mixed with 20 mL of the aqueous leaf extract of *Sonchus maritimus* L. and 80 mM of ascorbic acid solution was added dropwise until a slightly yellow color was achieved. After color changing the reaction mixture was incubated with constant stirring in the dark at 130 rpm for 72°C to avoid photo-catalysis. When the solution color turns red, the samples were obtained by centrifugation, washed twice with distilled water and ethanol, dried, and stored in an amber-colored sample vial until use (KHANDSUREN and PROKISCH, 2021).

Physical characterization of SeNPs

Various analytical approaches were employed to characterize the nanoparticles. Using an ultraviolet-visible spectrophotometer (Jenway) the absorbance peaks in the UV-Visible spectrum, which was recorded between 200 and 800 nm, were used to identify the synthesized SeNPs. The Fourier Transform Infrared Spectrophotometer (Thermo Scientific iS5) was used to determine the functional groups found in the biomaterial and confirm the presence of Se-O peak, through using KBr between 4000-400 cm⁻¹. Scanning Electron Microscopy and Energy Dispersive X-ray were used to examine the surface morphology, identify the elements present in the samples, and determine the average particle size.

DPPH radical scavenging ability test

The relative antioxidant capacity of plants and nanoparticles was assessed using a DPPH (2,2- Diphenyl-1-picrylhydrazyl) radical scavenging test, by a spectroscopic method, Plant extract and SeNPs have their anti-radical activity which measured using the method of NWIDU *et al.* (2017). Different concentrations of extracts solution and colloidal solution of SeNPs were prepared (200, 175, 150, 125, 100, 75, 50, 25, 10 and 5 µg/mL) in distilled water. 20 µL of the extracts, SeNPs, or standard were mixed with 160 µL of a 0.1 mM DPPH in ethanol solution before being mixed with 20 µL of distilled water. Under similar conditions, ascorbic acid (as a standard) over the concentration range of 200, 175, 150, 125, 100, 75, 50, 25, 10 and 5 µg/mL. For 40 min in the dark at 37°C, the mixtures were incubated. At 517 nm, the sample absorbance was measured. The negative control was made the absorbance of a blank sample which made up of an equal volume of distilled water and DPPH solution (A₀). The IC₅₀ was calculated though plotting the percentage inhibition:

$$D\% = [(A_0 - A_1/A_0) * 100] \quad (1)$$

where A₀ and A₁ correspond to absorbance of the control and absorbance of the sample respectively. IC₅₀ was calculated by utilizing the regression line equation against various concentration of SeO and plant extract.

Ferric reducing ability “FRAP”

According to the study by OYAIZU (1986), 1 mL of the extract or the SeNPs at various concentrations (200, 175, 150, 125, 100, 75, 50, 25, 10 and 5 µg/mL) in addition to 2.5 mL of phosphate buffer solution (0.2 M, pH 6.6) were added to 2.5 mL of 1% potassium ferricyanide (K₃Fe(CN)₆) solution. After 20 minutes of incubation at 50°C in a water bath, the reaction is stopped by adding 2.5 mL of 10% trichloroacetic acid. The mixtures are centrifuged at 3000 rpm for 10 min, and then 2.5 mL of the supernatant, 2.5 mL of distilled water, and 0.5 mL of an 0.1 % solution of aqueous ferric chloride (FeCl₃) are mixed. Absorbances of samples (plant extract or the SeNPs) are read at 700 nm. The absorbance of ascorbic acid, which serves

as a standard for antioxidants, was measured under identical conditions as the samples. As described in YAZDANI *et al.* (2019), FRAP value was calculate as follows:

$$D\% = 100 - [(Ac / As) \times 100] \quad (2)$$

where *Ac* and *As* represent the absorbance of the control and absorbance of the sample, respectively. IC₅₀ was calculated by utilizing the regression line equation against various concentrations of SeO and plant extract.

Antibacterial activity

The Antibacterial activity was carried out using disc diffusion method (MWITARI *et al.*, 2013). The disc diffusion assay on Mueller Hinton agar plates against common Gram- negative (*Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922) and Gram-positive (*Staphylococcus aureus* ATCC 25923) bacteria was performed to evaluate the antibacterial properties of the aqueous extract of *Sonchus maritimus* and the synthesized SeNPs. Bacteria were conserved on nutrient agar plates at 4°C. Activation of bacteria was performed at 37°C in the incubator, overnight. The 0.5 McFarland standard bacterial suspension was prepared in sterile physiological water. The suspension was spread uniformly on the dried surface of Muller Hinton agar by streaking swab three times. After that, sterile paper discs (Whatman No. 3) of 6 mm were impregnated with 20 µL (10, 20 and 30 mg/mL) of Se-NPs and leaves aqueous extract solutions, and discs were than dried in a clean bench before were placed on the inoculated agar surface. Discs impregnated with preparation solvents (sterile distilled water) were used as control. The all plate was incubated at 37°C for 24 h. Gentamicin, amoxicillin, ciprofloxacin, cotrimoxazol, and ceftazidime were used as standards against all pathogens. After incubation, the zone of inhibition around each disc were measured in millimeters unit. Experiments were carried out in triplicate.

Acute toxicity testing

An acute toxicity test of the SmE-SeNPs was carried out according to the method of LORKE (1983). Fifteen (15) male albino Wistar rats 8 weeks old, weighing 141.38 ± 3.85 g, were obtained from the Institute Pasteur of Algeria, and were kept in plastic cages at the Animal room of the Department of Cellular and Molecular Biology, Faculty of Natural and Life Sciences, El-Oued University, Algeria. Rats were divided into three groups of five rats in each ($n = 5$). All rats were maintained fasting for 12 h; each group of rats was injected intraperitoneally with a single dose of SmE-SeNPs (Control, 2.5 and 5 mg/kg body weight). The rats were observed for 24 h to monitor their behavior as well as mortality. All experimental procedures employed, also rat care and handling, were in accordance with international guidelines provided by the local ethics committee (41EC/DCMB/FNSL/EU2022) of the Department of Cellular and Molecular Biology, Faculty of Natural and Life Sciences, El-Oued University, Algeria.

RESULTS AND DISCUSSION

Green Synthesis and Characterization of SeNPs

UV-visible spectroscopy

As shown in Fig. 1, the SeNPs were successfully synthesized using an aqueous extract of *S. maritimus* and the transitions from yellow to ruby red was a clear indication that SeNPs had been synthesized (CITTRARASU *et al.*, 2021). The ability of plant extract in the synthesis

of nanoparticles was confirmed firstly by the visual identification of color change (RAJESHKUMAR *et al.*, 2020). Extraction is an essential step in the isolation and identification of phenolic compounds (BOUKADA *et al.*, 2022). The phytochemicals such as flavonoids and alkaloids found in plant extract may be responsible for the broad shoulder at 360 nm (ATHISA *et al.*, 2022). According to the UV-visible spectrum of different time phases in the synthesis of SeNPs, observation of a significant plasmon resonance peak from 300 nm indicated the presence of SeNPs in the samples. This supported by different previous studies suggest that Se-NPs were detected between 250 and 400 nm (GHADERI *et al.*, 2022). ANANTH *et al.* (2019) showed a strong absorbance peak at 370 nm of SeNPs synthesized from sodium selenite using the reduction power of ascorbic acid. Also, in the study by MALHOTRA *et al.* (2014), the maximal absorption of SeNPs was found and produced by wet chemical approach at 390 nm.

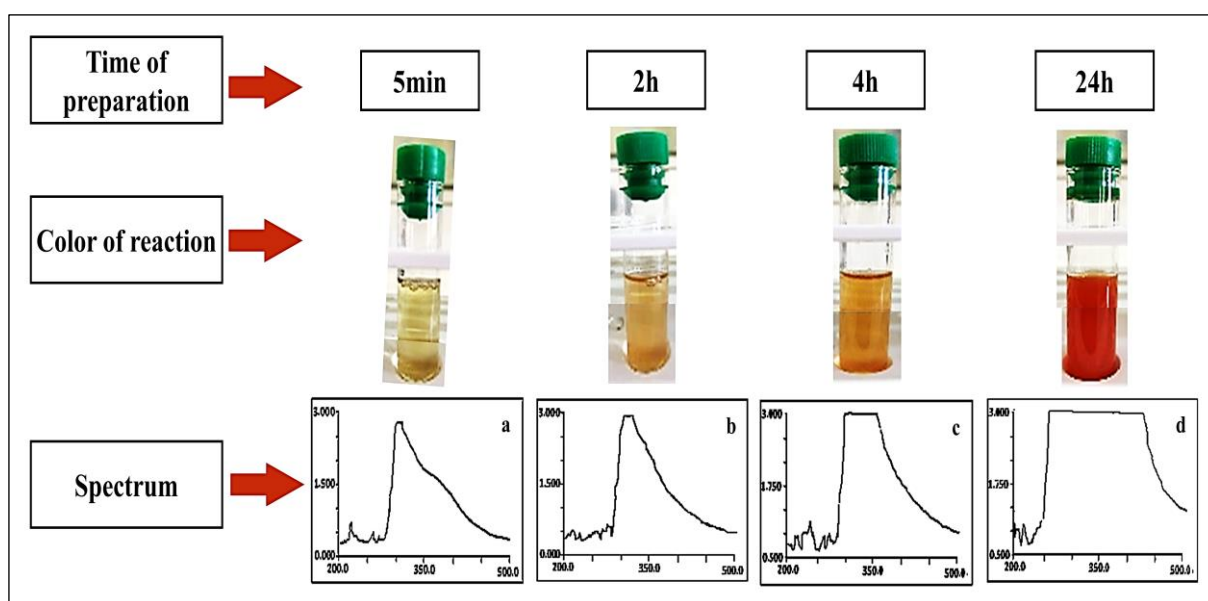


Figure 1. UV-visible spectrum and colored reaction of biosynthesized SmE-SeNPs.

FT-IR spectroscopy

FT-IR technique allows to detect the presence of various stabilizing and reducing functional groups of metabolites to confirm their participation in the synthesis of SeNPs; the functional groups present on the surface of SeNP can be identified using the FT-IR technique though measure the vibrational frequencies of chemical bonds (ALIPOUR *et al.*, 2021). The FT-IR spectrum of the *S. maritimus* aqueous extract and SeO-NPs was shown in Fig. 2, respectively. The appearance of identified peaks in both spectrums; a large absorption band appeared at about 3400 cm^{-1} representing the extending hydrogen-bonded O-H alcohol and phenols that exist in the plant extract. In addition, other peaks were depicted in both spectra of each of the aqueous extracts and SeNPs such as peaks about 1600 cm^{-1} indicating the presence of the -N-H group (bending vibrations). The intense peak of about 1400 cm^{-1} designates the -C-H group, and the secondary -OH bending vibrations are of about 1200 cm^{-1} . The functional groups C-O and C=O stretching vibration are represented by peaks of about 1100 cm^{-1} and 1050 cm^{-1} , respectively. Strong intensity -C-H bending vibrations were related to the peaks seen around 824 cm^{-1} . These determinations of different wave numbers are in accordance with RAJAGOPAL *et al.* (2021) and NAGALINGAM *et al.* (2022). Based on the obtained results and in accordance with MULLA *et al.* (2020), polyphenols, flavonoids, and proteins present in the leaf's aqueous extract were used to cap the synthesized SeNPs. This last confirmed its

formulation through the distinguishable peak at 588 cm^{-1} which indicates Se-O interaction (MULLA *et al.*, 2020).

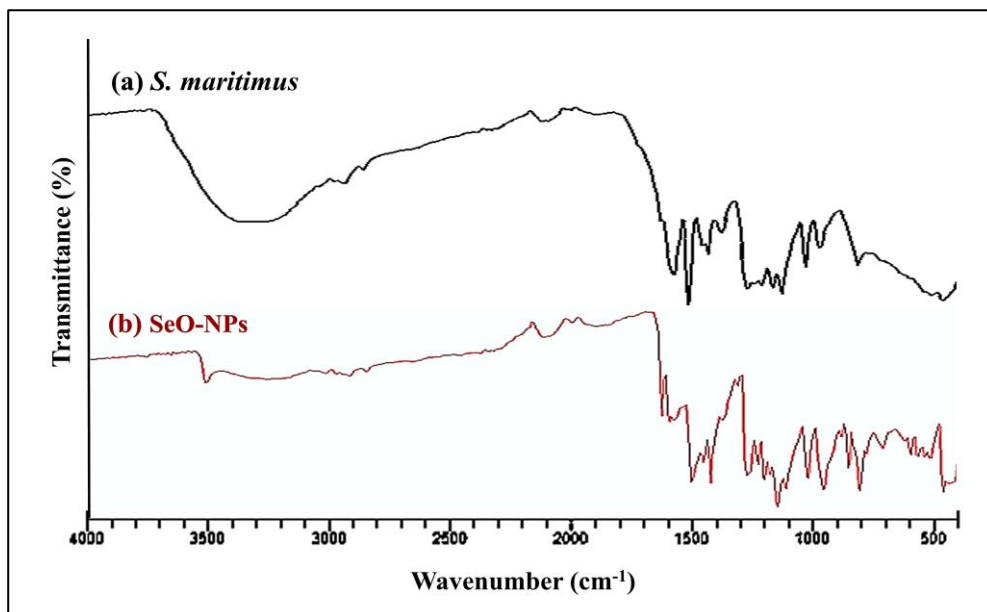


Figure 2. FT-IR analysis of functional groups present in *S. maritimus* and SeO-NPs.

SEM with EDX analysis

SEM was used to determine the surface morphology and average size distribution of the synthesized nanoparticles (ABU-ELGHAIT *et al.*, 2021). Fig. 3a shows the histogram that represents the average particle size of SeO-NP, which was found at around 26.48 nm, while the SEM image demonstrates the surface morphology when it showed a spherule-like shape. PANDEY *et al.* (2021) revealed that the biogenic synthesis of selenium gave a spherical shape with a diameter between 5 nm and 50 nm. EDX microanalysis equipment was used to investigate the elemental content of specific regions inside SEM slices, and employed to determine the quantitative and qualitative status of elements that might be involved in the formulation of nanoparticles (SHAHBAZ *et al.*, 2022). In Fig. 3b, an EDX spectrum of SeO-NPs shows Se and O as major elemental compositions, which indicates the purity of prepared samples.

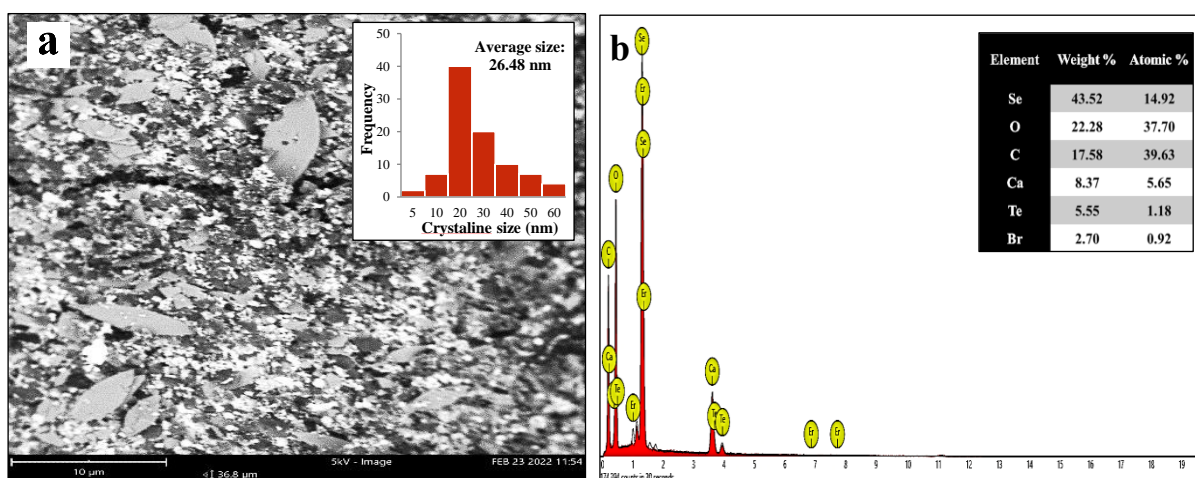


Figure 3. Morphological, size and chemical composition of SeO-NPs based on Scanning Electron Microscope (SEM) micrographs and EDX spectra.

Biological activities of SeNPs

Antioxidant activity

In our study, the antioxidant activities of *S. maritimus* aqueous extract and SeNPs green synthesized evaluated by two methods. DPPH scavenging activity while DPPH was a stable substance takes hydrogen or electrons from the antioxidant agents (VYAS and RANA, 2018). FRAP assay, where the antioxidant activity of the agents present in the sample is expressed by their reducing power to convert ferric iron to ferrous iron (PERERA *et al.*, 2016). The results presented in Figure 4 showed that selenium nanoparticles and aqueous extract of *S. maritimus* possess a moderate antioxidant activity compared to standard (ascorbic acid) in each of DPPH and FRAP methods which expressed by inhibition concentration 50 (IC₅₀); 1511.31 $\mu\text{g/mL}$ and 823.91 $\mu\text{g/mL}$ for SeNPs, 1131.01 $\mu\text{g/mL}$ and 440.04 $\mu\text{g/mL}$ for *S. maritimus* extract, respectively. The low antioxidant activity of SeNPs and *S. maritimus* extract may be related to difference of their scavenging and reducing mechanisms of free radical compared to ascorbic acid mechanisms in these protocols. Another study revealed that selenium nanoparticles green synthesized by *Geobacillus* cell-free extract had an antioxidant activity through DPPH and FRAP tests (KUMAR *et al.*, 2020). HERNÁNDEZ-DÍAZ *et al.* (2021) reported that antioxidant compounds exert their effects using different mechanisms depending on the nature of the substance and its ability to react with the substrate in which it is dissolved and confirmed that SeNPs had the ability to scavenge the free radicals and modulate its generation, which allow to its exploit in the biomedical application. PRIYANKA *et al.* (2020) reported that antioxidant activity of a plant extract depends on the amount of phenols present in it.

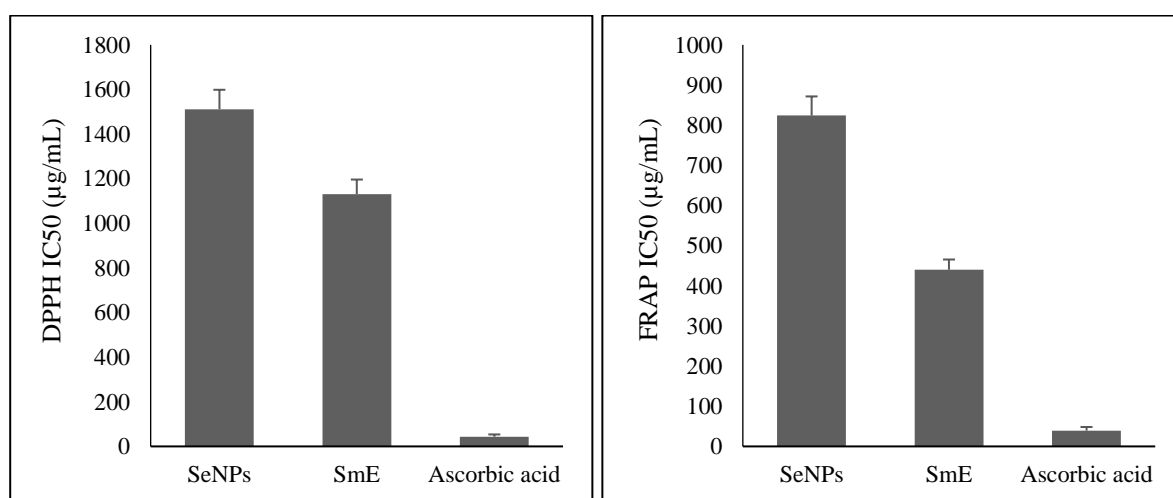


Figure 4. DPPH scavenging and FRAP activities of *S. maritimus* aqueous extract, SeO-NPs and ascorbic acid.

Antibacterial activity

As presented in Figures 5 and 6, SeNPs had an important antibacterial activity compared to *S. maritimus* aqueous extract, which did not give a considerable activity against Gram-positive and Gram-negative bacteria. The inhibition zone against the Gram-positive bacteria was observed as concentrations discs of SeNP that reach up to 24.8 mm for *Escherichia coli* and 11 mm against *Staphylococcus aureus* (MILOVANOVIĆ *et al.*, 2021). However, SeNPs showed no activity against *Pseudomonas aeruginosa*. These results are in accordance to the other studies, which indicate that antibacterial action of SeNPs is still obscure and their action

mechanism are not clear due to difference in synthesis parameters of SeNPs itself (SHOEIBI and MASHREGHI, 2017; SOUZA *et al*, 2022).

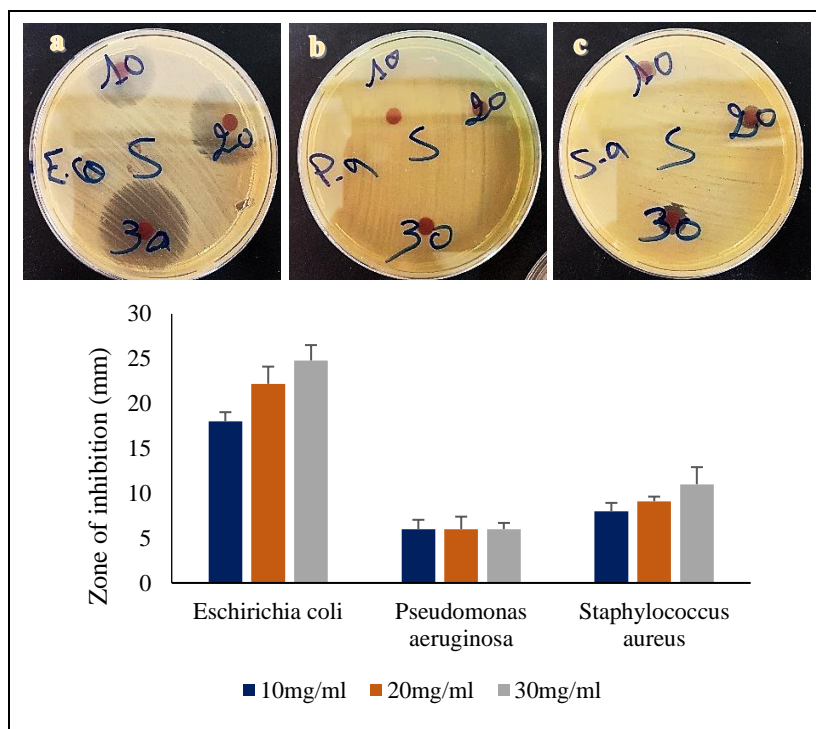


Figure 5. Antibacterial activity of SeO-NPs against *Escherichia coli* (a), *Pseudomonas aeruginosa* (b) and *Staphylococcus aureus* (c).

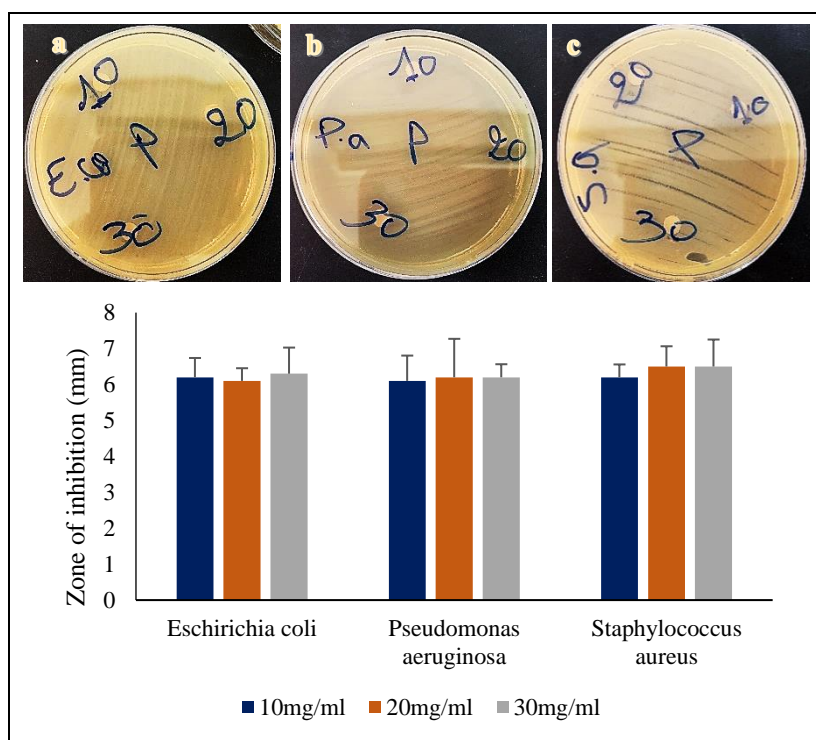


Figure 6. Antibacterial activity of *S. maritimus* aqueous extract against *Escherichia coli* (a), *Pseudomonas aeruginosa* (b) and *Staphylococcus aureus* (c).

Just now, numerous studies have been carried out to demonstrate the anti-microbial capabilities of nanoparticles against Gram-positive and Gram-negative bacteria, while the concept's underlying molecular insights have not been fully investigated (VINU *et al.*, 2021). However, there are some theories about their antibacterial activity. Menon *et al.* suggested that SeNPs can induce oxidative stress due to the production of reactive oxygen species that lead to protein denaturation by interacting with thiol and sulfhydryl groups of transmembrane proteins or interacting with intracellular proteins, thus inhibiting the food and respiratory metabolic pathway, leading to DNA damage by deteriorating DNA replication and leading to cell death (MENON *et al.*, 2020). DEV *et al.* (2022) informed that bacterial cells were probably killed by the SeNPs with bioactive molecules that coat their surface, which increases the importance of SeNPs for use in the biomedical field.

Acute toxicity study

Depending on some factors and behaviors in toxicological control, such as the case of the eyes, movement, sleep, and diarrhea (DEROUICHE *et al.*, 2020). The results shown in Table 1 demonstrate that these physiological parameters were normal (N) in albino Wistar rats treated with different doses of SmE-SeNPs (2.5 and 5 mg/kg) compared to control rats which were injected with normal saline solution (0.9% NaCl). Additionally, no unusual symptoms or side effects appeared, and no mortality cases (0) were reported before 14 days. These results are in accordance with the study by RANJITHA and RAI (2021), which reported the safe and therapeutic applications of these green-synthesized Se nanoparticles.

Table 1. Intraperitoneally acute toxicity test of SmE-SeNPs on physiological parameters of Wistar albino rats (0 = none, N = normal).

Parameters	0 h		3 h		24 h		Day 7		Day 14	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
Dead rats	0	0	0	0	0	0	0	0	0	0
Eyes	N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	N	N	N	N
Movement	N	N	N	N	N	N	N	N	N	N
Diarrhea	N	N	N	N	N	N	N	N	N	N

CONCLUSION

The current work illustrates the subtleties and effective biosynthesis of selenium nanoparticles using aqueous leaves extract of *Sonchus maritimus*. The green-formulated nanoparticles were identified by UV-Vis, FTIR and SEM with EDX. Due to synergistic action, ascorbic acid and plant extract together greatly affected the surface shape and size of SeNPs. The average size was found to be about 26.48 nm. Antibacterial tests were carried out against *Escherichia coli* and *Staphylococcus aureus*. It has been observed that green synthesis of selenium nanoparticles by *S. maritimus* are characterized as antioxidants with no toxicity recorded in the studied doses, which raises the advantage of biocompatibility when used in the treatment of diseases in humans. An in-depth understanding of the nanoparticles' nature would emerge from a study of the biological proprieties *in vivo*, which might be used for future research and be helpful for deliver drugs to humans suffering from harmful diseases.

Acknowledgments

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