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Selenium nanoparticles from *Euphorbia retusa* extract and its biological applications: antioxidant, and antimicrobial activities

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Abstract

The biosynthesis selenium nanoparticles (SeNPs) were prepared from aqueous extracts of *Euphorbia retusa*, and the active components of the plant extract were identified. Additionally, an examination was conducted to determine the antioxidant and antimicrobial properties of metal nanoparticles derived from this plant. The SeNPs were characterized using a variety of techniques, such as UV-visible, transmission electron microscopy (TEM), and zeta potential spectroscopy. As a result, the levels of phenolics, flavonoids, and tannins that were present in the nanoparticle samples that were created were lower than the levels that were present in the aqueous extract to begin with. The antioxidant activity of metal nanoparticles (IC_{50} = 0.247mg/ml) and *E. retusa* extract (IC_{50} = 0.054 mg/ml) was assessed making use of the DPPH assay. The findings for the plant extract showed much higher efficacy than those for the metal nanoparticle solutions, which showed significantly lower effectiveness. To determine whether the samples have antibacterial properties, they were tested against several bacterial and fungus species. The activity of the selenium nanoparticles against *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus epidermidis*, *and Candida albicans* was shown to be the most powerful. In conclusion, the creation of selenium nanoparticles is an essential step in the process of enhancing the biological properties of an organism.

Keywords: Euphorbia retusa, Nanoparticles, SeNPs, Phytochemical, Aantioxidant, Antimicrobial.

1. Introduction

The chemically green production of nano-sized particles made of several elements (including selenium) by living microbes is common in nature. This phenomenon has piqued the interest of scientists over the last years, not only because of the wide range of uses of these nanoparticles (NPs) in Nano medicines, as well as the uniqueness of techniques and processes of NPs production associated with "green synthesis" [1]. Biological synthesis also uses phytonutrients to replace harmful compounds employed as reducing and stabilizing agents [2]. fast developments in nanotechnology toward the synthesis of nanomedicine agents hold enormous promise for improving cancer therapy techniques. Nanoparticles (NPs) have several uses in various fields of research currently. NPs have been repeatedly claimed to play a crucial role in modern medicine in recently. They've been studied for a variety of clinical uses, including medication transporters, genetic transfer to malignancies, and adjuvants in radiology [3].

Inorganic nanoparticles have various positives and special properties for improved sensing and medication administration. Nonetheless, just a small number of inorganic NPs have been converted into medical practical's [4]. These nanoparticles offer unique physical features like conductivity, stability, and visual qualities, making them an excellent choice for biological and manufacturing research. (https://www.nature.com/subjects/nanoparticles).

Targeted therapies are the most effective cancer drugs since they are more effective than most other

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treatment options (frequent targeted therapy) and fewer possible adverse impacts, such as decreased applicability, decreased necessity larger concentrations, decreased adverse side effects, limited therapeutic measurements, resistance to different medications, and nebulous targets [5]. Trace amounts of selenium (Se) can be found in the diets of both humans and animals. Selenocysteine is one of at least 25 selenoproteins and enzymes in the human body that rely on dietary selenium [6].

Selenium nanoparticles (SeNPs) are gaining popularity in the medical profession due to their antibacterial and antitumor capabilities, as well as being biodegradable and non-toxic when compared to their analogues. Selenite (SeO_3^{-2}) and selenite (SeO_4^{-2}), biogenic Se factor A (SefA) and metalloid reeducates Rar A, which are naturally found on the of SeNPs, give the nanoparticles stability and keep them from aggregating. SeNPs are effective chemotherapeutic and chemo preventive drugs. Antibiotics work better against cancers when combined with them. Additionally, they are used in nano-biosensors and environmental remediation [7]. SeNPs are effective antitumor and targeted therapies with less cytotoxicity as contrasted to selenium (Se) molecules, which makes them suitable for use in medicine [8-12].

The genus Euphorbiaceae (spurge family) contains approximately 8100 species, 2000 of which are *Euphorbia* species. This family was recently discovered to have roughly 300 genera and 10,000 species that are used in folk medicine to cure venomous bites and trichiasis, as well as wart removers [13]. *Euphorbia retusa* is an arid yearly or brief plant with a raised stem 20 to 60 cm tall, sessile leaves, but a few yellow green flowers that can exists in North Africa, Pakistan, and Palestine, as well as Egypt's deserts sandy deserts and Sinai [14,15].

The aerial section of the plants, which includes saliva, alkaloids, flavonoids, tannins, triterpenes, sterols, and thirteen deoxy phorbol esters, is essential for several biological processes [16-18].

We created selenium nanoparticles and tested their antioxidant and antibacterial activities in vitro using *Euphorbia retusa* shoot extract. Various spectroscopic techniques, including UV, Zeta potential, and SEM, were used to analyze these selenium nanoparticles.

2. Materials and Methods

2.1. Plant material and extraction process

The *Euphorbia retusa* plant was taken in Wadi Hagoul, which is in the northern section of Egypt's Eastern Desert. As a result, the plant was washed, air-dried, and cut into little pieces. Ten grams of the plant were saved in a 250-milliliter conical flask, and one hundred fifty milliliters of methanol were added. After being shaken for two hours at 25 ℃ in a horizontal water bath shaker, the mixture was filtered through Whatman filter paper no. 1. (125 mm, Cat No 1031 127, Germany). The final extract was placed in a clean bottle and kept at 4 ℃ [19].

2.2. Synthesis of metal nanoparticles

Utilizing the method of Devasenan *et al*. [20] the green protocol was attempted for the manufacture of metal nanoparticle solutions using *E. retusa* extract. In 20 ml deionized water, 1 mmol of selenium sulphate was thoroughly dissolved. The salt solution was progressively added to a stirred plant extract solution at 25 °C. The mixture was stirred for an additional two hours until there was a noticeable change in the color of the solution. In addition to the color intensity of the plant extract and the metal salt solution, the absorbance of the solution was determined. The solution of metal nanoparticles was kept in a dark bottle and refrigerated at 4 °C.

2.3. Characterization of metals nanoparticles

The generated nanoparticles' physical characteristics and chemical make-up, including their size, form, nature of the surface, crystal structure, and morphology information, were determined using a TEM at the Electron Microscope Unit, Mansoura University in Egypt (JEOL TEM-2100, Tokyo, Japan). The study was performed at a 200 nm range. UV-VIS (Shimadzu UV-VIS 2450) spectral analysis was used to investigate the optical characteristics of Selenium nanoparticles.

According to Bhattacharjee [21], in the Electron Microscope Unit, Mansoura University, Egypt, using Malvern Instruments Ltd. Zeta Potential Ver. 2.3, the surface energy of the produced selenium nanoparticles in solution was measured using the zeta

potential method (Kassel, Germany). Nanoparticle surface properties can be studied using this method, and the particles' control is likely to last for a very long period [22].

2.4. Phytochemical Analysis

2.4.1. Total Tannin Contents

The tannin concentration was determined using the vanillin-hydrochloride method [23], which involved measuring the absorbance of the sample after treatment with newly generated vanillin-hydrochloride. The tannin contents of the extracted plant sample were expressed as grams tannic acid equivalents / 100-gram dry plant. The tannin capacity of the tested samples was estimated using the tannic acid standard curve ($y = 0.0009x$; $r^2 = 0.955$).

2.4.2. Total phenolic contents

Quantitative analysis of phenolic components in the plant extract was performed. Issa *et al*. [24] detailed an approach to using the Folin-Ciocalteu (F-C) assay, and we followed their lead, calculating the characteristics as milligrams gallic acid equivalents of dry plant using the gallic acid standard graph. To implement this, we used a Gallic acid standard graph ($y = 0.0062x$, $r^2 = 0.987$).

2.4.3. Total flavonoid contents

The amount of flavonoids is reported as the milligrams of catechin equivalent per gramme of dry plant material. The aluminum chloride colorimetric assay published by Zhishen *et al*. [25], utilizing the Catechin "secondary metabolite" standard curve, was used to analyses the extracted plant material. Flavonoids in general were determined by fitting a standard curve to the data ($y = 0.0028$ x, $r^2 = 0.988$).

2.5. Antioxidant Activity

Kitts *et al.* [26] used the DPPH• assay with ascorbic acid as a reference to examine the antioxidant capacity of the analyses plant extract and its metallic nanoparticles formulations. Every sample was serially diluted in an equivalent share of methanol. Each sample was serially diluted with an equal quantity of 0.135 mM DPPH• solution. The samples were then kept at 25 ℃ in the dark for 30 mins. The absorption of colors intensity in the samples was determined at λ = 517 nm. The IC_{50} values were plotted on a graph, and the antioxidant capacity was stated as follows:

$$\% \text{ Radical scavenging activity} = [1 - Asample/Acontrol\,] * 100$$

The IC_{50} values showed how much antioxidant was needed to lower the beginning concentrations of DPPH• solutions by 50%. The IC_{50} values are inversely related to the antioxidant activities of the materials studied [27].

2.6. Antimicrobial Activity

The extraction process MeOH of *E. retusa* was test counteract four Gram-negative bacterial strains (*Escherichia coli* AYCC-10566, *Pseudomonas aeruginosa* AYCC-9427, *Salmonella typhimurium* AYCC-25566, and *Klebsiella pneumoniae* AYCC-10331), three Gram-negative bacterial strains (*Bacillus cereus* EMCC-1080, *Staphylococcus aureus* AYCC-6578, *Staphylococcus epidermidis* AYCC 12298, and *Bacillus subtilis* AYCC-10247), and one fungus (*Candida albicans* AYCC10721). The bacterial isolates were obtained from the Cairo Microbiological Resources Centre (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University. *C. albicans* was collected from the Laboratory of Mycology at Mansoura University's Faculty of Medicine in Egypt.

The antibacterial activity of microorganisms was determined to use the agar diffusion procedure [28]. For fungus, though, the tests were carried out with the spore's suspension technique [29]. In a nutshell, 10 mg/disc of the MeOH extract was placed onto filter paper discs having a diameter of 5 mm. at 1×10^8 colony forming units (CFU)/mL of the bacteria were put onto petri plates that were coated with nutritional agar medium. Fungi were cultured in Petri dishes using a dextrose agar potato medium with a spore concentration of 1×10^6 spores/mL. The plates were immediately sealed with Parafilm® tape once the MeOH extract discs were positioned in the middle (Sigma, St. Louis, MO, USA). We incubated the plates at 37 ℃ for 24 hours to grow bacteria and at 28 ℃ for 72 hours to grow mold. At three different points, we determined the inhibitory zone widths in millimeters. ampicillin, ceftazidime, and amphotericin were used as positive controls.

3. Results and Discussion

3.1. Characterization of the metal/metal oxide nanoparticles

3.1.1. Transmission Electron Microscope (TEM)

TEM analyses were used to define the nature and crystallography of the nanoparticles prepared using *Euphorbia retusa* plant extract, for instance, particle size, shape, and aggregation. Greater spatial resolution analysis was done on the samples (100 nm). The produced selenium nanoparticles' Microstructures and sizes are shown in Figure 1. The creation of the selenium nanoparticles' sphere and tetrahedral forms is depicted in (Figure 1).

3.1.2. UV-Visible spectrophotometer

The synthesized Se-NPs solutions were analyzed for their optical properties using a UV–Vis spectrophotometer with a scan range from 226 to 1100 nm. The results specified that the maximum absorbance reads of Se-NPs were recorded at 245 nm, this indicated the development of the respective nanoparticles in the liquid, and it was verified as a sign as shown in (Figure 2a). The maximum absorption peak was recorded for the *E. retusa* plant extract at a wavelength of 245 nm with absorbance at 0.860.

3.1.3. Zeta potential analysis

Zeta potential analyses (Figure 2b) were run for the prepared metal nanoparticles using *E. retusa* plant extract to investigate the surfaces charges in suspensions to use the Zeta Potential Ver. 2.3 from Malvern Measurements Ltd. Zeta potential performance (Figure 2b) was useful to identify the surfaces charges. A moving double ion layer covers nanoparticles, as it disperses in the solution, the electric potential at the border of the two layers is documented as the Zeta potential of the particles and has generally has values in the range of +100 mV to -100 mV. The synthesized selenium nanoparticles using *E. retusa* extract have Zeta Potential values of -19.0 mV.

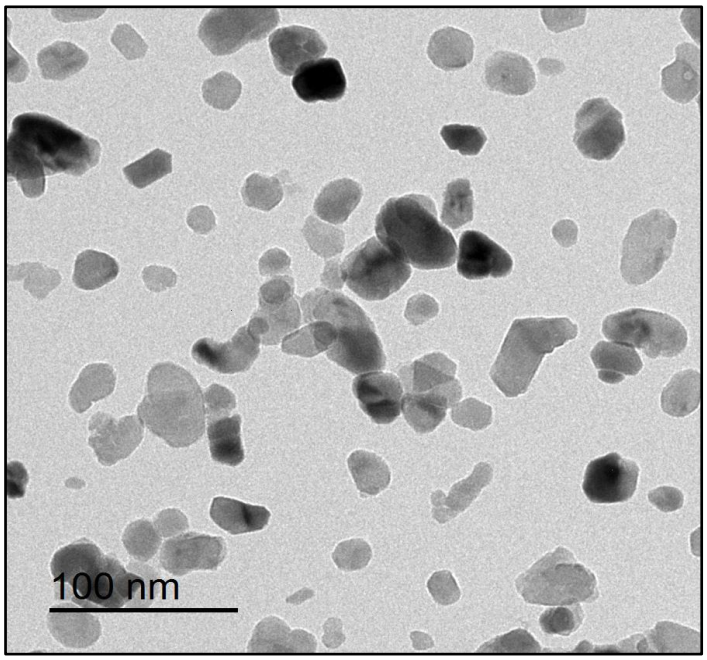

Fig. 1. TEM configurations of SeNPs

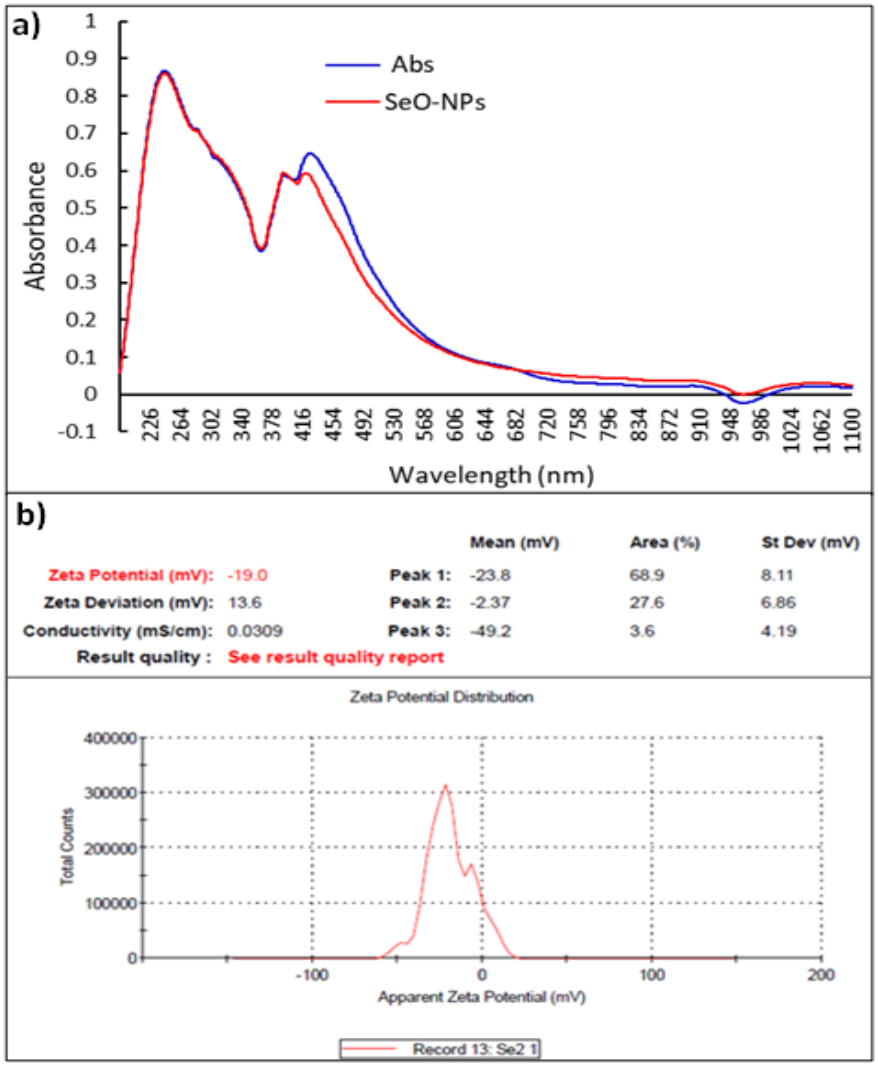

Fig. 2. Characterization of *Euphorbia retusa*-Se-NPs. a) The UV-visible spectroscopy graphs of the prepared zinc nanoparticles, b) Zeta potential analysis of SeNPs.

3.2. Phytochemical analysis

The initial phytochemical investigation may be helpful in identifying the bioactive components, which may then facilitate the creation of new drugs [30,31]. Significant sources of natural components are the main classes of secondary metabolites and bioactive molecules called phenolics and flavonoids. The most prevalent and significant natural phenolics are assumed to be flavonoids because they are one of the most diversified and common groups of natural chemicals [32,33]. As a result, *Euphoria retusa* shoot extract contains phenolics (142.01 mg GA equivalent/g dry extract), flavonoids (42.94 catechin equivalent/g dry extract), and tannins (16.51 mg GA equivalent/g dry extract) in our current study. According to a previous study, *E. retusa* includes triterpenes, alkaloids, flavonoids, and tannins [34,35]. Saponins, tannins, triterpenes, and flavonoids were found in the leaves of *E. heterophylla* [36], as well as tannins, flavonoids, and alkaloids in the leaves of *E. hirta*, are examples of phytochemical findings from this study that are consistent with other members of the Euphorbiaceae family [37]. The family Euphorbiaceae has generally been found to include a wide range of phytochemical substances, including tannins, diterpenes, triterpenes, alkaloids, flavonoids, lectins, millamines, and esters of sterol [18].

Polyphenol compounds produce an electron resonant hybridization action by biologically lowering salt ions and turning them into nanoparticles while also stabilizing those particles in a stable, pro state [38,39]. Since phenolics, which include flavonoids, are hard to break, they are utilized in the bio-reduction of selenium ions and their creation into nanoparticles. The flavonoids connect to the surface of the nanoparticles and accumulate there, neutralizing the charges of the selenium ions to create zero-valent molecules in the nanometer range. As a result, new substances are created that are very small in size, growing their surface area and being active, effective, and distinctive chemically [40,39]. Concerning the phenolics (82.71 mg GA equivalent/g dry extract), flavonoids (15.64 mg catechin equivalent/g dry extract), and tannins (4.73 mg GA equivalent/g dry extract) in the environmentally friendly selenium composite nanoparticles made with *Euphoria retusa* extract, there were significant reductions (Table 1).

Table 1. The phytochemical analysis of the investigated extracted samples.

Samples	Phytochemical Analysis		
	Phenolics' Contents	Flavonoids' Contents	Tannins' Contents
Euphorbia retusa	142.01	42.94	16.51
E. retusa-SeNPs	82.71	15.64	4.73

Phenolics Content "mg gallic acid/1 gm dry extract", Flavonoids Content "mg catechin/1 gm dry extract", Tannins Content "mg tannic acid/1 gm dry extract"

3.3. DPPH antioxidant activity

The DPPH• free radical test was used to evaluate the potential antioxidant scavenging activity of the *E. retusa* extract and its metal nanoparticles. The potential of the sample to snare DPPH free radicals in the solution via a free radical pathway is known as its antioxidant capability (Table 2). The comparison of the results of the tested samples with that of ascorbic acid verified that the plant extract has a better activity for trapping the free radicals of DPPH in the solution than the metal nanoparticle solutions. The results, in general, agree with phytochemical results as the phenolic contents enable better efficiency of the sample to trap the free radicals in the solution.

The extracts of *E. retusa* demonstrated antioxidant activity in a dose-dependent manner, as determined by the findings ($P \leq 0.05$), which was equivalent to ascorbic acid as a reference standard (Table 2). At 50 mg/ml, the scavenging activities of 87.69% and 87.73% for *E. retusa* and SeNPs, respectively. However, the lowest concentration (5 mg/ml) shows the lowest antioxidant activity in the studied samples. Based on IC_{50} values, the most potent antioxidant capacity was recorded for the extracted *E. retusa* with IC_{50} value at 0.054 mg/mL relative to the result of ascorbic acid (IC_{50}= 12.78 mg/ml). With an IC_{50} of 0.247 mg/ml, the synthesis of SeNPs caused by the action of the *E. retusa* extract clearly reduced the antioxidant scavenging activity. The *E. retusa* extract in this study contained high concentrations of phenolics, flavonoids, and tannins; these compounds are essential for green synthesis as reducing agents because they can transform ions into nanoparticles and are widely recognized for their antioxidant potential based on their structure, in particular the number and behaviour of the hydroxyl groups. The antioxidant activity of these substances is mild to moderate [41]. During the process of biosynthesis, the groups that were responsible for the extract's antioxidant action were used, which resulted in a reduction in that activity.

3.4. Assessment of the antibacterial activity.

The antibacterial effectiveness of the *E. retusa* extracts was assessed using the disc diffusion method and its metal nanoparticle solutions against several Gram-positive and negative bacterial species, as well as pathogenic yeast, *C. albicans* fungal species. The results (Table 3) specified no activity of the extracted plant against all the tested microbial species. The results of the present research contradict those of previously reported Euphorbiaceae species with antibacterial activity [42-44]. The antibacterial activity of methanol extracts indicated some degree of antimicrobial activity against a variety of

microorganisms, according to Philip et al. [45] and Abdallah [35].

Table 2. The antioxidant results (% scavenging activity, and IC_{50} (mg/ml)) of the *Euphorbia retusa* extract**.**

Treatment	Concentrations (mg/ml)	% Scavenging activity	
		Euphorbia retusa	*E. retusa*-SeNPs
Plant extract-NPs	50	87.69±1.75	87.73±1.99
	40	76.32±1.66	79.06±1.69
	30	60.54±1.45	62.92±1.18
	20	33.11±0.84	35.77±0.98
	10	17.82±0.09	20.15±0.15
	5	10.61±0.03	9.79±0.02
	IC_{50} (mg/ml)	0.054	0.247
Ascorbic acid	Concentrations (mg/ml)	% Scavenging activity	
	20	67.91±1.27	
	15	57.96±0.89	
	10	46.71±0.71	
	5	39.88±0.56	
	2.5	8.27±0.06	
	1	2.64±0.01	
	IC_{50} (mg/ml)	12.78	

Table 3. Antimicrobial activity of the greenly synthesized nanoparticles using *Euphorbia retusa* shoot extract on various pathogenic microbial strains.

Tested organism	Treatments		Standared antibiotics		
	E. retusa	*E. retusa* SeNPs	Ceftazidime	Ampicillin	Amphotericin
Gram-negative bacteria					
Escherichia coli	0	38.47±0.13	17.42±0.05	26.17±0.92	-
Pseudomonas aeruginosa	0	14.66±0.06	R	21.52±0.11	-
Klebsiella pneumoniae	0	25.73±0.08	17.28±0.09	33.36± 0.69	-
Salmonella typhi		38.69±0.15	17.37±0.07	24.42±0.49	-
Gram-positive bacteria					
Bacillus cereus	0	16.44±0.06	14.51±0.06	9.75±0.03	-
Bacillus subtilis	0	26.88±0.09	13.69±0.07	10.79±0.04	-
Staphylococcus aureus	0	12.66±0.04	21.00±0.80	30.80±1.10	-
Staphylococcus epidermidis	0	24.77±0.07	28.54±1.00	29.66±0.71	-
Fungi					
Candida albicans	0	18.78±0.09	-	-	13.62±0.61

In addition, the solutions of the synthesized nanoparticles revealed a broad spectrum of antimicrobial activity against the diverse microbial species. In particular, the selenium nanoparticles revealed the most potent activity against *E. coli* (inhibition zone diameter = 38.3 mm), *K. Pneumonia* (21 mm), *B. cereus* (23 mm), *B. subtilis* (22 mm), *S. epidermidis* (24 mm) bacterial strains, and *C. albicans* (21 mm) fungal strains. These outcomes are comparable to those that were just revealed by Vinu *et al*. [46] against *Vibrio parahaemolyticus* and El-Zayat et al. [47] for *Ephedra aphylla* extracts against a range of microbial species. The SeNPs solution, on the other hand, exhibited the highest antibacterial efficiency against *E. coli*. The aqueous extract of *E. retusa* contains powerful antibacterial selenium nanoparticles, and their derivative elements, like selenium sulfide, are used to treat infectious disorders

including Malassezia and Tinea versicolor. However, excess selenium caused toxicity and selenosis. Thus, the present study concentrated on minimizing cell toxicity and developing selenium's bio-functional properties. Nanotechnology has made selenium safer and more functional than biosynthesis [48].

Investigating the sensitivity and tolerance of several bacterial species was important, as well as their tolerance, persistence, sample concentration, and host response [49], so that we could talk about the mechanism of action behind these behaviours. Significant changes were seen in the antibacterial potency as a function of the nanoparticle's size, shape, and aggregation characteristics [50].

4. Conclusions

E. retusa extract produced selenium nanoparticles in a green synthesis (SeNPs). The active components in the plant extract oxidize metal ions or convert them to zero states by forming nano-sized metal oxides in solution. This section estimated phytochemical content from selenium nanoparticles. TEM, zeta potential, and UV-Visible spectra intended metal nanoparticles. These solutions of the isolated plant and its metal nanoparticles showed reduced antioxidant and increased antibacterial properties. Thus, ecofriendly selenium nano-solutions may be used in large-scale pharmaceutical investigations as effective antibacterial agents.

5. Conflicts of interest

"There are no conflicts to declare".

6. Acknowledgments

"None

7. References

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