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Research Article

Implementation of *Eruca sativa* Extract for the Preparation of Nano-Selenium Particles

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Abstract

Background: Nanoparticles can act as a carrier platform to ferry drugs to their target site of action to improve their activity and reduce the toxicity of some compounds, such as selenium, which acts as a cofactor for a variety of enzymes involved in oxido-reductive activities. ***Objective***: This study suggested the implementation of *Eruca sativa* as a reducing agent to formulate selenium nanoparticles (SeNPs). ***Method***: First, a 1% *Eruca sativa* extract solution will be dropped on 10 mM sodium selenite to produce a nanoselenium solution and characterize its physicochemical properties. ***Results***: The prepared nano-selenium is monodispersed with a small particle size (39.4 nm), as confirmed by different characterization techniques. ***Conclusion***: A 1% *Eruca sativa* extract solution has a powerful reducing effect that can be used in nanoselenium creation.

Keywords: *Eruca Sativa*, Nano-Selenium, UV-Vis spectra, FT-IR, Dynamic light scattering, Scanning electron microscopy.

استخدام مستخلص *Eruca sativa* لإعداد جزيئات السيلينيوم النانوية

الخلاصة

الخلفية: يمكن أن تعمل الجسيمات النانوية كمنصة حاملة لنقل الأدوية إلى موقع عملها المستهدف لتحسين نشاطها وتقليل سمية بعض المركبات، مثل السيلينيوم، الذي يعمل كعامل مساعد لمجموعة متنوعة من الإنزيمات المشاركة في الأنشطة المؤكسدة و الاختزالية. **الهدف**: اقترحت هذه الدراسة تنفيذ *Eruca sativa* كعامل اختزال لصياغة جسيمات السيلينيوم النانوية (SeNPs). **الطريقة**: تم أضافة محلول مستخلص *Eruca sativa* بنسبة 1٪ على سيلينيت الصوديوم 10 mM لإنتاج محلول نانوسيلينيوم وبعدها تم توصيف خصائصه الفيزيائية والكيميائية. **النتائج**: النانو السيلينيوم المحضر أحادي التشتت بحجم جسيم صغير (39.4 نانومتر)، كما تؤكده تقنيات التوصيف المختلفة. **الأستنتاج**: محلول مستخلص *Eruca sativa* بنسبة 1٪ له تأثير اختزال قوي يمكن استخدامه في إنشاء النانوسيلينيوم.

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INTRODUCTION

Nanotechnology is a novel and growing field with important applications in science and technology. In the pharmaceutical industry, nanoparticles act as carrier platforms to ferry drugs to their target sites of action. It is based on the creation of small particles at the submicroscopic level with a particle size less than 100 nm. Such formulations enhance biological activity, reduce toxicity, and provide a means for the controlled release of drugs, especially in encapsulated products [1]. For instance, nanoparticles of biologically important trace metals like selenium, copper, chromium, iron, or silver [2,3]. Selenium is one of the most medicinally and biologically important trace elements. It constitutes what are called seleno-proteins, which are enriched with seleno-cystien. Seleno proteins act as cofactors for a variety of enzymes involved in oxido-reductive activities, such as glutathione peroxidase and thioredoxin reductase [4]. These enzymes possess a cytoprotective effect, and their activation helps boost fertility, reduce inflammatory reactions, and provide a chemo-preventive effect against different types of cancer [5]. The formulation of selenium as nanoparticles (SeNPs) is one of the most challenging strategies [6]. Many methods have been suggested, which can be chemical, physical, or biological. The chemical methods are widely used, although they are toxic, costly, and non-ecofriendly [7]. Nowadays, there is a trend to implement herbal products based on biological methods (green biosynthesis), which have gained a great deal of popularity as they are more eco-friendly, inexpensive, and biocompatible [8]. Herbal products with prominent antioxidant power are highly recommended to achieve that target [9]. Such formulations may kill two birds with one stone, as the nanoparticle formulation will enhance permeation of both selenium and the plant extract into that target site, which may help achieve a synergistic effect [10]. *Eruca sativa* is an annual herb that is widespread in the Mediterranean area. It is known traditionally as jarjeer, or rocket salad. It is one of the members of the Brassicaceae family, which includes vegetables enriched with different types of glucosiniolates and used in our daily lives, such as broccoli, Brussels sprouts, cabbage, and collards. It is known for its therapeutic properties as an astringent, cytoprotective, hepatoprotective, anticancer, diuretic, digestive, emollient, tonic, depurative, laxative, rubefacient, and stimulant [11]. Glucosinolates are glycosides present abundantly in the cruciferous plants that belong to the family Brassicaceae. They are made up of an S-β-D-glucopyrano unit anomerically connected to an O-sulfated and alkyl-substituted (Z)-thiohydroximate moiety [12]. *Eruca sativa* is endowed with different types of phytochemicals, such as phenolic acids, flavonoids, terpenes, carotenoids, tannins, glycosides, saponins, sterols, alkaloids, and other secondary metabolites [13]. Furthermore, it is enriched with some fatty acids like erucic acid (major content), oleic acid, linoleic acid, and saturated fatty acids. The main phytochemicals are kaempferol (a flavonoid derivative), glucosativin (4-mercaptobutyl glucosinolate), glucoerucin (glucosinolate derivatives), and eruric acid, which is an unsaturated fatty acid [14]. Hydrolysis of glucosatives yields 4-mercaptobutyl-isothiocyanate (sativin), to which the bitter taste of Jareer can be ascribed [15]. Accordingly, this study suggested the implementation of *Eruca sativa* as a reducing agent to formulate SeNPs, which were later recommended to have therapeutic applications in different medical abnormalities.

METHODS

Materials

The following materials were used: n-hexane (BDH/England), sodium selenite (Sigma, USA), De-ionized water (Hayat, Iraq), ethanol 80% (Riedel-de Haen, Germany), Ethyl acetate (Reagents, USA), Sodium disulphate (Reagents, USA) and Sodium acetate (The Chemical Co., USA).

Extraction of Eruca Sativa

E. Sativa species (*Brassica napus*) was collected from a local farm in Al-Adhmayia region in February 2022. The collected crop was cleaned, washed thoroughly, dried in a dark, well-ventilated place, and finally ground by a grinder machine to a fine powder to be used for extract preparation. One hundred grams of dried powder of *E. sativa* was macerated with 500 ml of n-hexane (99.8%) for 24 hours, then another 500 ml of n-hexane was added and left for another 24 hours. The last step was repeated twice until reaching a final volume of n-hexane of two liters. The prepared extract was filtered, and the defatted plant materials were left to dry at room temperature for approximately 6 hours. Then 500 ml of 80% ethanol was added. This step was performed five times until the color of the ethanolic extract disappeared. Then the extract was filtered and evaporated to 30 ml using a rotary evaporator (4 rpm at 4 °C) [16]. Partitioning of the ethanolic extract was achieved by adding 20 ml of distilled water to the ethanolic extract and 50 ml of ethyl acetate to the ethanol/water extract in a separatory funnel. This step was repeated three times, then sodium disulfide was added to the ethyl acetate fraction, which was then filtered and left to dry [16]. The yield of about 40 g of extract was stored in a dark, sterile screw bottle at 4° until used.

Characterization of E. sativa extract

The extract was characterized through measuring the total flavonoid content and its antioxidant power using reducing power assay.

Total Flavonoid Content

The total flavonoid content was determined according to the previously reported method [17]. Briefly, 250 µl of the extract (0.75 mg/ml) was mixed with 1.25 ml of D.W. and 750 µl of a 5% sodium nitrite solution, then left at room temperature for 6 min. After that, 150 µl of 10% aluminum chloride solution was added, and the mixture was again left for 5 minutes. Then 0.5 ml of 1M sodium hydroxide was added, and the volume was made up to 2.5 ml with D.W. and mixed well. Finally, the absorbance was determined versus blank at 510 nm. A calibration curve was prepared using Gallic acid as a standard. Results were expressed as mg of Gallic acid equivalents per gram of extracts.

Reducing power assay

The reducing power of the extract was measured according to a previously reported method [17]. Briefly, different concentrations of the extract were prepared by a fold serial dilution of 1mg/ml aqueous solution of the extract, but in this study, a serial dilution of 0.75 mg/ml of the extract was used to prepare concentrations ranging from 5.85 mg/ml to 0.75 mg/ml, then a mixture of 2.5/ml of 0.2M phosphate buffer (pH = 6.6) and 2.5/ml of aqueous solution of 1% potassium ferocyanide [K3Fe(CN)6] was mixed with each of these concentrations, the mixture was incubated at 50 C for 20 min. and 2.5 ml of 10% trichloroacetic acid (TCA) was added to the mixture and centrifuged for 10 min. at 3000 RPM. The supernatant was mixed with 2.5/ml distilled water and ferric chloride (FeCl3). Finally, the absorbance of different concentrations of the extract was measured at 700 nm and compared with the control, which was prepared by adding 1 ml of distilled water instead of the extract. The absorbance, or percent of absorption, increased of the extract versus concentration was plotted and compared with that of different concentrations of Vitamin C as standard [18]. The absorbance of the reaction mixture with different concentrations of the extract was compared with the control, which was prepared by adding 1 ml of distilled water instead of the extract. Absorbance propagation percentage was calculated for each concentration, and a plot of extract concentrations versus their absorbance was drawn. The percentage of absorption increase was sketched and compared with that of different concentrations of vitamin C [18].

Eruca Sativa Based- Nanoselenium Biosynthesis and Characterizations

Biosynthesis of Eruca Sativa Based- Nanoselenium

The biosynthesis process was based on the implementation of E. sativa extract as a biological reducing agent. Briefly, a solution of 1% w/v of the extract was loaded into a solution of 10 mM sodium selenite at the following proportions (1:2, 1:4, 1:10, and 1:20 sodium selenite/extract). The former was prepared by dissolving 0.172 g of sodium selenite (Na2SeO3) in 100 ml of deionized water. Then, the final mixture was mixed using a hot plate magnetic stirrer at 60 °C and pH around 9 in the dark for 12 hours, and then autoclaved at 121 °C and gas pressures of 1.5 bar for 15 minutes [19]. Next, the autoclaved mixture was exposed to filter sterilization using a Millipore filter membrane (0.22 µm) and to ultrasonic vibration (20 KHz by a Q700 sonicator) to ensure an even dispersion of the nanoparticles in the liquid and to get micron-sized colloidal particles. Finally, the prepared colloid was sent for the characterization process, as seen in the following sections.

Identification and characterization of nano selenium

UV-Vis spectra analysis

UV-Vis spectrum was performed to measure the reduction of metallic selenium ions after 10 to 15 min of color change (consider time zero), then after 24 and 48 hr. A small aliquot was drawn from the solution and a wavelength from 220nm to 1000nm on UV-Vis spectrophotometer was applied [20].

Dynamic Light Scattering (DLS)

A method to characterize the particle size and poly dispersity index (PDI) values of different Nano solutions together with zeta potential of the fabricated SeNPs [21]; The samples were analyzed in the Nanotechnology Department, Technology University using particle size and zeta potential analyzer Brookhaven®.

Fourier transform infrared spectroscopy (FTIR)

FTIR was used to confirm the involvement of O–H, N–H, C=O and C–O functional groups during the formation of SeNPs, which were associated with bioactive molecules capping their surface [22]. Absorptions of IR radiation by different functional groups within the sample are plotted as a function of wavelength, typically presented in units of wavenumbers or cm^{-1} [23].

Scanning Electron Microscopy (SEM)

The most versatile instruments available for the examination and analysis of the nanostructure morphology, topography and size characterizations through 3D image capture of the powder sample at an accelerated voltages 10kV [24]. Energy Dispersive X-

ray Spectroscopy (EDAX). It gives qualitative status as well as quantitative measurement of elements that may be involved in the formation of nanoparticles [25].

Inclusion criteria for the nano selenium solution

The target nano particle should include the following criteria: mean particle size < 50 nm with PDI value (< 0.7) to indicate mono disperse solution and zeta potential range (> +30 and < -30) which expresses high stable phenomena [26].

Statistical analysis

Statistical analysis was performed using the statistical package for social science (SPSS) (IBM SPSS statistics for windows, Version 23). Data were expressed as mean±SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) The level of significance was set at <0.05, 0.01 and 0.001.

RESULTS

In this study, the total polyphenol content and reducing power of *Eruca sativa* extract were tested to see if this plant extract could change selenium ion (Se^{+2}) to elemental selenium (Se^{0}) in an experiment to make nanoselenium. According to the experiment that was performed to estimate TPC, 0.9 g of the extract is equivalent to 1 mg of gallic acid. A plot of log concentrations versus absorbance at 700 nm showed the right and downward shifting curves of E. sativa extract compared to the vitamin C curve as a standard, which indicated that the reducing power of extract was less than that of vitamin C within these selected concentrations (Figure 1).

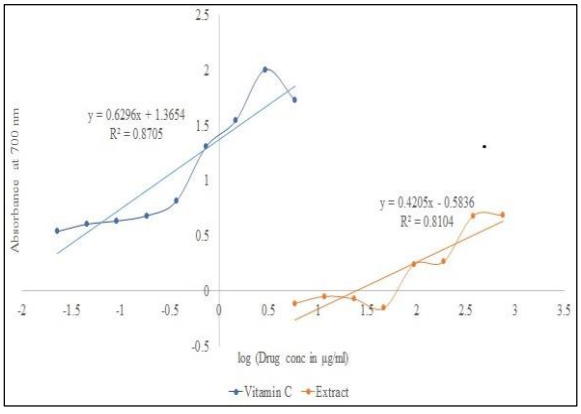

Figure 1: Reducing power assay of E. sativa compared to vitamin C as standard.

The color change upon the addition of sodium selenite to the *Eruca sativa* extract was considered an indication of SeNP synthesis (Figure 2). The UV-vis spectrum of SeNPs biosynthesized from extracts of *Eruca sativa* is shown in Figure 3. The absorbance spectrum was recorded in the range of 220-1000 nm. A strong absorption peak was observed between 268 and 964 nm, with maxima at 268 nm, confirming the presence of nanoselenium in the samples.

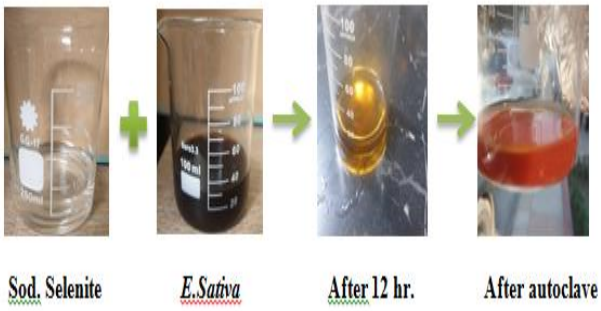

Figure 1: Visual observation for nano-selenium formation.

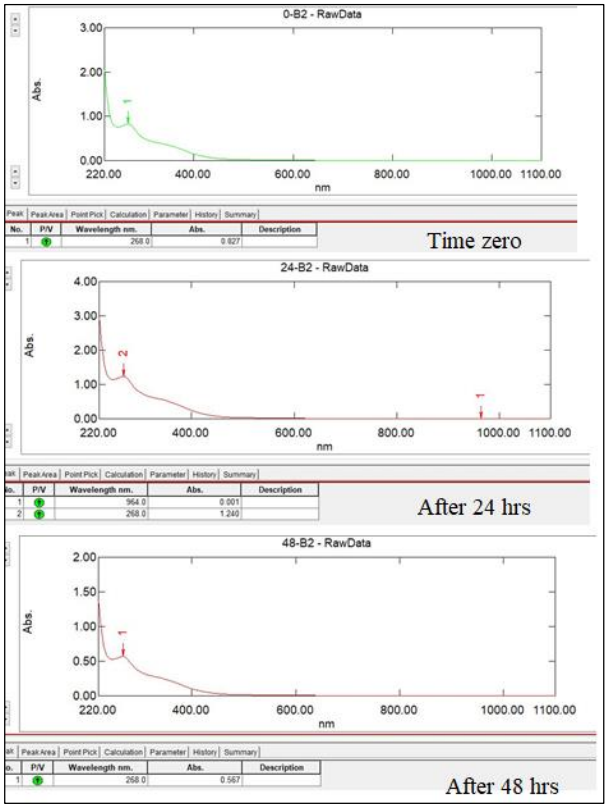

Figure 2: UV-visible spectrum of selenium nanoparticles.

As can be seen in Table 1, the mean particle size values of the produced SeNPs vary from 39.4 to 124.6 nm, measured by a particle size analyzer at -90 plus (Brookhaven). The minimum particle size for the produced SeNPs (39.4 nm) was achieved using 1:2 Na2SeO3:extract solutions. At the obtained optimum synthesis conditions (1:2), the zeta potential value of the fabricated SeNPs was -56.57 mV, while the PDI value of the fabricated SeNPs was 0.242.

Table 1: Mean particle size and poly disparity of nano particles

Samples Na Selenite: *E. sativa*	Mean particle size (nm)	Poly dispersity
1:2	39.4 (minimum)	0.242
1:4	48.2	0.198
1:10	57.9	0.005
1:20	124.6 (maximum)	0.304

The zeta potential of the synthesized SeNPs is shown in Figure 4. SEM images of nanoparticles observed from the micrograph showed that the majority are spherical, with a small percentage of elongated particles, and range in size from 64.12 to 66.53 nm. A uniform distribution of SeNPs on the surface of the pellets is observed (Figure 5).

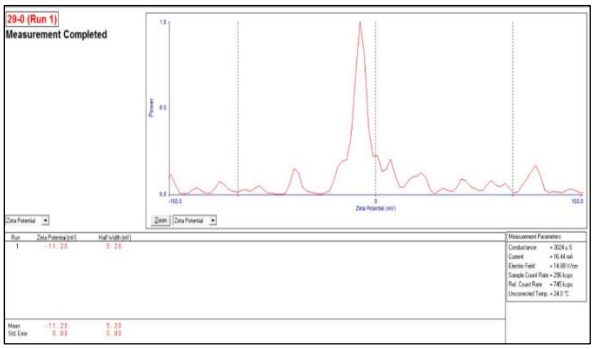

Figure 3: Zeta potential of the synthesized Se NPs made from sodium selenite with *Eruca Sativa* extract in percentage 1:2 (v/v).

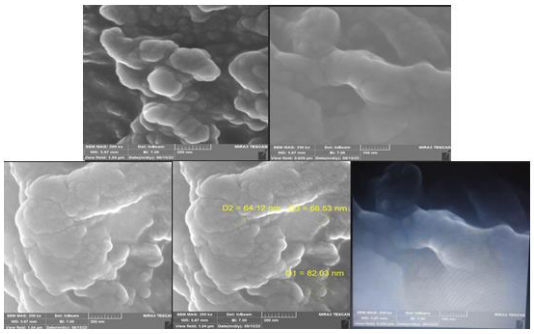

Figure 4: SEM image of Selenium nanoparticles made from sodium selenite with *Eruca Sativa* extract in percentage 1:2 (v/v).

FT-IR analysis was carried out to identify the possible biomolecules and plant extract-metal ion interactions responsible for the formation and stabilization of selenium nanoparticles [27]. The result of the FT-IR analysis of *Eruca sativa* extract is presented in Figures 6-A, -B, and -C. Figure 6-A shows the spectrum of the *Eruca sativa* extract that did not contain metal selenium; Figure 6-B shows the sodium selenite sample spectrum; and Figure 6-C shows the spectrum of the nano-selenium sample, which shows the peaks of both the control and test samples; similarly, B and A show the transmission peaks of the control samples. EDAX analysis gives the qualitative as well as quantitative status of elements that may be involved in the formation of nanoparticles.

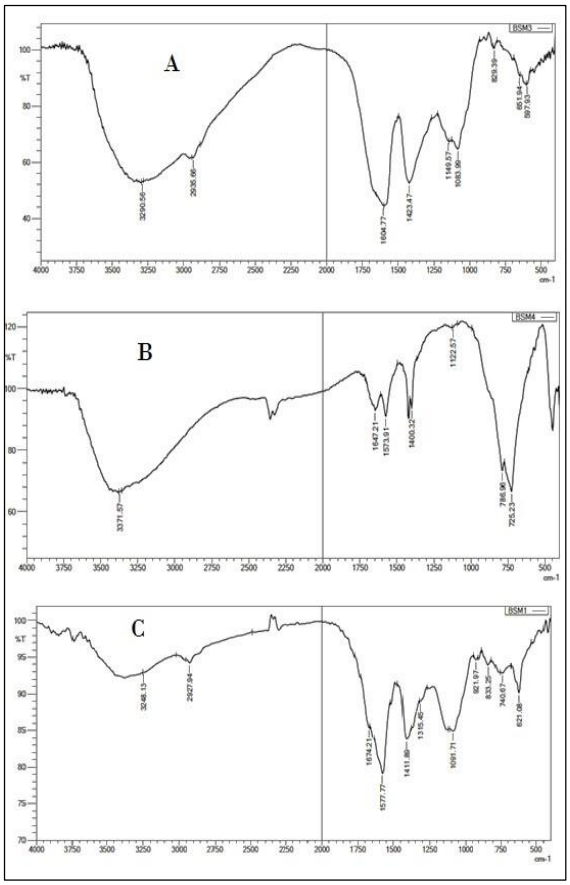

Figure 5: FTIR spectrum of (A) *Eruca sativa* extract, (B) sod. selenite sol. and (C) selenium nanoparticles made from sodium selenite with *Eruca sativa* extract in percentage 1:2 (v/v).

Figure 7 shows the elemental profile of synthesized nanoparticles using *Eruca sativa* extract.

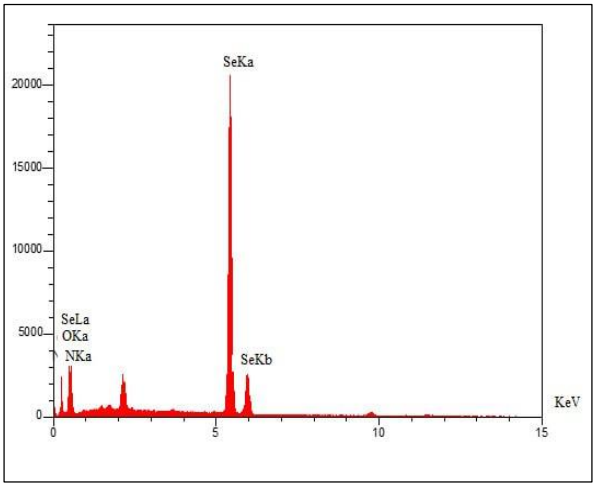

Figure 6: EDAX spectrum of selenium nanoparticles made from sodium selenite with *Eruca sativa* extract in a percentage 1:2 (v/v).

DISCUSSION

Plant extract or other biological mass, such as bacteria, fungi, yeast, or plant biomass, could be an alternative method for the synthesis of nanoparticles in an eco-friendly manner that is less time-consuming, safer, and lower-cost [28]. In the reducing power assay test, Perlis Prussian blue showed up when ferric ion in the form of ferric ferrocynide was changed into ferrous ion. This test gives an idea about the potential of *Eruca sativa* extract to donate electrons to free radicals [29]. According to the experiment that was performed to estimate flavonoids content, 0.9 g of the extract is equivalent to 1 mg of gallic acid, which was compatible with the results reported by Abdul-Jabbar, in which alcoholic extract and fresh rocket leaf contained nearly equal amounts of total flavonoids content (0.81mg and 0.80 mg QE/g, respectively) [30], owing to the presence of phenolic compounds [31]. In accordance, 1% of the extract solution (prepared by dissolving 1g of *Eruca sativa* extract in 100 ml of deionized water) was used to prepare nanoselenium in the current study. The reduction of selenium ions into SeNPs induced by *Eruca sativa* extracts was confirmed by the gradual conversion of color from dark brown to light yellow, then to reddish orange, after the addition of an acidic sodium selenite solution and reacting for 12 h. The final reddish-orange color is the most significant property of nanoparticles. This result has been previously obtained by many researchers using different types of green plants [32]. The sample peak optical density, or absorbance, correlates linearly and directly to the concentration of nanoparticles in solution [32]. According to research, this SeNPs peak was created by the Surface Plasmon Resonance (SPR), which is a resonance [33] phenomenon induced by the interaction of the electrons of metal nanoparticles with incoming photons. This result is compatible with many previous studies associated with various ranges of UV-visible maximum absorption peaks. Ramamurthy *et al*. found an absorbance peak ranging from 200–400 nm, with the maximum peak at (390) nm, when using fenugreek extract for SeNPs synthesis [6]. Another SeNP was synthesized by (34) using garlic cloves, which reported an absorption peak at 260 nm. *Withania somnifera* was used to synthesize spherical-shaped SeNPs with a maximum absorbance of 310 nm by dynamic light scattering (DLS) [35]. From the current study, it can be recognized that there formulas were uniformly dispersed particles, mostly as nano-spheres, and no other peak was observed in the whole spectrum, which means Se0 has successfully formed [36]. The polydispersity index (PDI) is a dimensionless and scaled index in which values bigger than 0.7 indicate that the sample has a very broad particle size distribution and is probably not suitable to be analyzed by the dynamic light scattering (DLS) technique, while values smaller than 0.05 are mainly seen with highly monodisperse standards [37]. DLS was performed to assess the particle size of different nanosolutions, and it can be seen that when increasing extract concentration in relation to sod, in selenite, there was an increase in particle size; in the current study, the largest size (124.6 nm) was related to the largest ratio of extract sample. This can be explained by the fact that there was twice as much extract solution available to turn Na2SeO3 into Se0. This made the nanoparticles start and grow more quickly, and in some cases, they also clumped together. The least concentrated nanosample has a much smaller mean size of approximately 39.4 nm; however, it is characterized by a narrow size scatter in comparison with the largest extract ratio. The obtained results were in line with the findings of Skandalis *et al*. in relation to the synthesis of nanosilver from *Arbutus unedo* [38]. The zeta potential value of the fabricated Se NPs was -56.57 mV, which indicated that the formed NPs were surrounded by negatively charged groups and had high stability at the same time. The polydispersity index (PDI) value of the fabricated Se NPs was 0.242; this small PDI value indicated that the formed Se NPs were monodispersed [39]. However, previous studies on green synthesis of SeNPs using Pelargonium zonale indicated that SeNPs with mean particle size, PDI, and zeta potential values of 136 nm, 0.321, and -24.6 mV, respectively, were fabricated at optimum synthesis conditions [40]. The obtained results indicated that *Eruca sativa* extract could be effectively synthesized into SeNPs with smaller particle sizes and higher stability due to the low value of the zeta potential (< -30 mV) as compared to Pelargonium zonale leaf extract, which could be related to the strong reluctant and stabilizing agents in *Eruca sativa* extract [41]. The SEM image of SeNPs synthesized from *Eruca sativa* extracts shows that they were assembled on the surface due to interactions such as hydrogen bonds and electrostatic interactions between the bio-organic capping molecules bound to the SeNPs. SEM analysis of the synthesized SeNPs was clearly distinguishable owing to their size differences. SEM analysis of the prepared nanosolutions indicated the formation of selenium nanoparticles. Figure 5 shows the nanoparticle size range of 64.12–66.53 nm. The formation of nearly uniform particles indicates that *E. sativa* extract could form monodisperse nanoparticles, as has been shown previously by PDI results. These results are compatible with previous results obtained from the biosynthesis of nanoselenium with Allium Sativum extract [32]; also, SEM results agreed with others who reported a 74.25 nm average size of SeNPs. Spherical SeNPs were produced from *Diospyros montana* with calculated SeNP sizes of 80 nm [42]. Researchers prepared SeNPs from leaf extracts of *Capsicum annuum* and *Allium sativum* with reported spherical particle sizes of 50–150 and 40–100 nm, respectively [43,34], which support the current findings. FT-IR can measure the vibrational rates of chemical bonds to figure out what kinds of functional groups are

on the surface of the nanoparticles [44]. Figure 6 shows two absorption peaks located around 3248.13 and 3290.56 cm-1 can be assigned as the absorption bands of O-H carboxylic acid and one peak at 3371.57 cm-1 assigned as the absorption peak of the N-H group. The presence of these functional groups (carboxylic acid and amid group) renders the SeNPs' stable and also serves as reducing agents in the conversion of sodium selenite to elemental selenium (45). Also, FTIR spectra depicted that IR bands in the regions 2935.66 and 2927.94 cm-1 for (C-H) asymmetric bending, in addition to three peaks at 1674.21, 1647.21, and 1604.77 cm-1 may be due to the (C=C) group. Similarly, two peaks at 1577.77 and 1573.91 cm-1 confirmed the presence of the (C=N) group; the absorption peaks at 1423.47, 1411.89, and 1400.32 cm-1 revealed the presence of the (O-H) group; two peaks at 1149.57 and 1122.57 cm-1 reveled the (C-O-C) group; two absorption peaks at 1083.99 and 1091.71 cm-1 related to the (C-N) group; and one peak at 829.35 cm-1 revealed the presence of the (C-H) group. Usually, the fingerprint region for each compound has a unique pattern characterized by a large number of peaks, which are almost never discussed [46,47]. The different functional groups obtained through FT-IR analysis of SeNPs synthesized by *Eruca sativa* help in the reduction of biosynthesized SeNPs. The EDAX detector was used to confirm the presence of metallic selenium ions. This analysis showed that selenium made up the largest percentage of the sample solution (80.27%), which suggests that the sample solution was very pure, along with C (1.41%), N (1.41%), and O (7.79%), respectively [22]. It has been reported that Se was the major element (61.60%), followed by C (29.96%) and O (4.41%) in a nanosolution that was synthesized by adding 2 mL of aqueous fruit extract of *E. officinalis* dropwise into 10 mM sodium selenite.

Conclusion

The present study was carried out to synthesis of Selenium nanoparticles using extract of *Eruca Sativa*. The biomolecules of the extract acted as reducing, stabilizing as well as capping agents leading to the formation of stable spherical Selenium nanoparticles as confirmed by the characterization tests used.

Conflicts of interest

There are no conflicts of interest.

Funding source

The authors did not receive any source of fund.

Data sharing statement

All data are available upon reasonable request to the corresponding author.

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