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GREEN BIOSYNTHESIS OF SELENIUM NANOPARTICLES USING PARSLEY (*PETROSELINUM CRISPUM*) LEAVES EXTRACT

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Abstract: Selenium (Se) is an essential trace mineral with various biological effects. Different physico-chemical and biological methods have been reported in literature for selenium nanoparticles (SeNPs) synthesis. The aim of this study was to use the parsley (*Petroselinum crispum*) leaves extract in order to prepare SeNPs by an eco-friendly method. SeNPs were characterized from structural and morphological point of view by using analytical techniques such as: dynamic light scattering (DLS), UV-Vis spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR) and Atomic Force Microscopy (AFM). DLS method revealed particles with maximum distribution being registered around 400 nm and an apparent zeta potential value of -14.2 mV. On the other hand, the parsley leaves extract was characterized concerning the total phenols content, vitamin C and antioxidant capacity, in order to evidence potential properties as biocatalyst or natural stabilizers for SeNPs synthesis. This study reports for the first time a green approach for SeNPs biosynthesis by using parsley leaves extracts.

Keywords: selenium, nanoparticles, *Petroselinum crispum*, antioxidant capacity, AFM

INTRODUCTION:

In the last years, obtaining nanoparticles became an interest domain due to their large applicability in various areas such as medicine, biology (Salata, 2004) agriculture (El-Ramady *et al.*, 2014) and electronics (Chaudhary and Mehta, 2014). Different methods (chemical, physical or biological ones) for nanoparticles synthesis are used. One of eco-friendly and economic method is to start the biosynthesis of nanoparticles using biogenic material that contains bio-macromolecules or bioactive compounds which can act as potential biocatalyst in the synthesis of nanoparticles. In addition, these bioreducing agents can act also as natural stabilizers for nanoparticles (Sharma *et al.*, 2014).

Biosynthesis of SeNPs by different plant extracts such as: *Allium sativum* (Babu *et al.*, 2017), dried vitis vinifera (Raisin) extract (Sharma *et al.*, 2014), fenugreek seed extract (Ramamurthy *et al.*, 2012), flower of *Bougainvillea spectabilis* Willd (Deepa and Ganesan, 2015) have been previously reported.

Phenolic compounds, flavonoids and minerals present in plant materials may facilitate the synthesis of nanoparticles (Sharma *et al.*, 2014). From the phenols class, flavonoids are predominant compounds in parsley. In the aqueous extract of parsley leaves, apigenin (4', 5, 7,-trihydroxyflavone), cosmosiin (apigenin-7-O-glucoside), oxypeucedanin hydrate (coumarin 2",3"-dihydroxyfuranocoumarin) and apiin (apigenin-7-O-apiosyl-(1 --> 2)-O-glucoside) were detected (Chaves *et al.*, 2011; Farzaei *et al.*, 2013). A recent study (Rajendran *et al.*, 2015) demonstrated that apigenin is able to form apigenin linked gold nanoparticles.

The aim of this study was to use the parsley (*Petroselinum crispum*) leaves aqueous extract in order to prepare SeNPs by an eco-friendly method. SeNPs were characterized by using various analytical techniques such as: DLS, UV-Vis spectroscopy, FTIR and AFM. dynamic light scattering (DLS), UV-Vis spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR) and Atomic Force Microscopy (AFM).

MATERIALS AND METHODS:

Total phenols, vitamin C content and antioxidant capacity of parsley leaves extract

The parsley leaves extract was obtained by homogenization of fresh leaves with distilled water in the ratio of 1:10 (w/v). The mixture was then centrifuged at 5000 rpm, for 15 minutes. The bioactive compounds and antioxidant capacity was determined from supernatant. Total phenols content (TPh) was determined by using the modified Folin-Ciocalteu method (Singleton *et al.*, 1999). Briefly, 100 µl of parsley extract was mixed with 1750 µl distilled water, 200 µl Folin-Ciocalteu reagent (dilution 1:10, v/v) and 1000 µl of 7.5 % Na_2CO_3 solution. The mixture was incubated at room temperature, in dark, for two hours. The absorbance was measured at 765 nm using Shimadzu mini UV-Vis spectrophotometer. Total phenols content was expressed as mg Gallic Acid Equivalents (GAE)/g of fresh weight (fw).

The titrimetric method using 2.6-dichlorophenolindophenol reagent was applied for determination of vitamin C, according to Contreras-Calderón *et al.*, 2011. Three grams of parsley leaves were homogenized with 30 ml of 2 % oxalic acid solution, and then diluted to 100 ml with 2 % oxalic acid solution and filtered. 10 ml of filtered solution was titrated with 0.01 % blue 2,6-dicloro-phenol-indophenol solution and the final point was considered when the solution reached pink color for 30 seconds. The calibration curve was performed with vitamin C solution. Results were expressed as mg ascorbic acid

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equivalents/100 g fresh parsley leaves. The antioxidant capacity of parsley extract was evaluated by two methods. FRAP (Ferric Reducing Antioxidant Power) is a simple spectrophotometric method based on the reduction of ferric tripyridyltriazine complex [Fe(III)-TPTZ] by a reductant, in acid medium (Benzie and Strain, 1996). Trolox was used for the calibration curve and the result is expressed as µmol Trolox equivalents (TE)/g fw. CUPRAC (CUpric Reducing Antioxidant Capacity) method is another simple method used for evaluation of antioxidant capacity of a wide variety of polyphenols and plant extracts (Özyürek *et al.*,2011). For the evaluation of antioxidant capacity of parsley extract, 1 ml of $CuCl_2$ (1.0 x 10^{-2} M), 1 ml of Neocuproine solution (7.5 x 10^{-3} M) and 1 ml of ammonium acetate buffer (1.0 M) at pH=7 were mixed with 500 µl parsley extract and 600 µl distilled water and allow to stay at room temperature for 30 minutes. The absorbance at 450 nm was recorded against a blank solution. The calibration curve was performed with the Trolox solution. Results were expressed as µmol Trolox equivalents (TE)/g fw.

Preparation, physico-chemical and morphological characterization of selenium nanoparticles

The leaves of *Petroselium crispum* (Plain Leaved 2 cultivar) were obtained in the laboratory from seeds provided by a company specializing in production and marketing of seeds (Agrosel SRL). Fresh parsley leaves were washed and freeze-dried (Freeze dryer Alpha 1–2 Christ - Martin Christ, Germany). Distilled water was added to dried leaves in the ratio 1:10 (w/v) and homogenized. After filtration, sodium hydrogen selenite ($NaHSeO_3$) solution was added (ratio 1:10 and 1:1 v/v) in concentration of 10000 ppm and allowed to stay overnight, at room temperature. When the solution color turns red, it was centrifuged at 5000 rpm for 10 minutes. The supernatant was removed and the red SeNPs were washed with distilled water, followed by repeated centrifugations (three times), filtrations and drying. The characterization of SeNPs was performed by UV-Vis, FTIR spectroscopy and DLS, while morphological details were observed by AFM. UV-Vis spectrum of selenium nano-colloidal solution was recorded in the range of 200-800 nm using Shimadzu UV-VIS 1700 PharmaSpec, Shimadzu Corp. Kyoto, Japan spectrophotometer. DLS measurements were performed by using ZEN 3690 (Malvern Instruments) in order to determine the average particle size, size distribution and zeta potential of nanoSe. AFM microscopy (SPM/AFM 5500 Keysight Technologies) was applied in order to observe the morphology and surface topography of the drop-coated film of nanoSe, using tapping mode with RTESP tip. The resonance frequency of the tip was 281.335 kHz and the scanning range was 2.11 µm.

Statistical analysis

All the experiments for the determination of total phenols, vitamin C and antioxidant capacity were conducted in triplicates. The values are presented as the mean ± standard deviation (SD).

RESULTS AND DISCUSSION:

Assessment of total phenols, vitamin C and antioxidant capacity of parsley leaves extract

Parsley is a popular fresh herb well known for aromatic and biochemical compounds. The parsley leaves extract was characterized from point of view of bioactive compounds such as total phenols and vitamin C content, and for antioxidant capacity using two different methods (FRAP and CUPRAC). The results are presented in Table 1.

Table 1.
Assessment of total phenols, vitamin C and antioxidant capacity of parsley leaves extract

	Total phenols (mg GAE/g fw)	**Vitamin C (mg vit C/100 g fw)**	**FRAP value (µmol TE/g fw)**	**CUPRAC value (µmol TE/g fw)**
Parsley leaves extract	10.39 ± 5.50	160.32 ± 14.55	4.42 ± 0.70	0.86 ± 0.12

The values are presented as the mean ± standard deviation (SD).

The parsley leaves are a very rich source of vitamin C. The amount of vitamin C of parsley cultivar in our experiment was 160.32 mg/100 g. The result is similar with that obtained by Karklelienė *et al.*, 2014 who investigated the productivity and biochemical composition of different dill and leafy parsley cultivars. The highest amount of vitamin C was recorded in Moss Curled parsley cultivar (162.8 mg/100 g).

Two analytical methods, FRAP and CUPRAC, have been used to determine *in vitro* the antioxidant capacity of aqueous leaves extracts of parsley. FRAP assay was originally developed by Benzie and Strain in 1996 to measure reducing power of plasma. Subsequently, this assay has been adapted to be used for measuring the antioxidant capacity of plant extracts. The principle of this method consists in the reduction of ferric 2,4,6-tripyridyl-s-triazine (TPTZ) to a blue colored product, in the presence of an antioxidant agent (Prior *et al.*, 2005). The degree of hydroxylation and extent of conjugation of polyphenols is direct correlated with the reduction power (Ou *et al.*, 2002). CUPRAC assay is a version of the FRAP assay where Cu^{2+} is used instead of Fe^{2+}. The assay is based on the reduction of Cu (II) to Cu (I) in the presence of reducing agents from samples (Prior *et al.*, 2005).

Spectroscopy Analysis, Morphology and Size Analysis of nanoSe

Biogenic synthesis of SeNPs by aqueous parsley extract was confirmed by the color conversion: light brown which is characteristic for the extract has turned into orange-red specific for SeNPs (Fig.1).

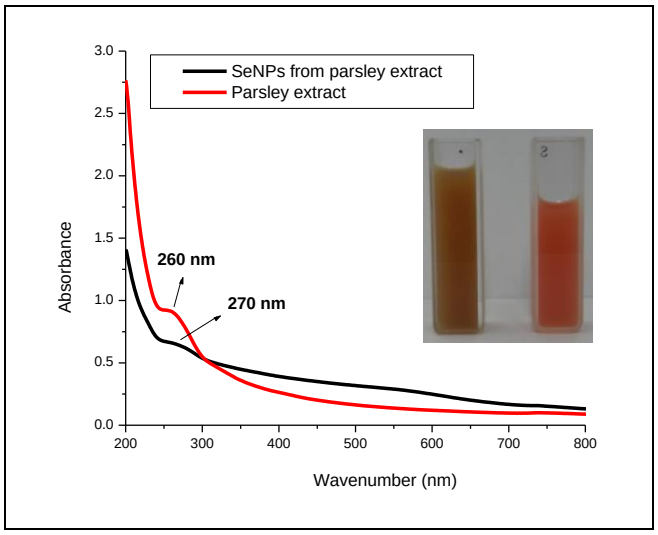

Fig.1. UV-Vis spectra of parsley extract and SeNPs biosynthesized by parsley extract. Inset: Photograph of parsley extract (left) and biosynthesized SeNPs (right)

The maximum absorbance at about 270 nm is characteristic for colloidal nano-selenium, as previously reported by Shah *et al.*2010. This result is in agreement with other studies in literature, demonstrating that the reducing agent was strong enough to ensure complete conversion of the precursor molecules into nano sized selenium particles. SeNPs are known to exhibit a regular maximum absorption in the wavelength region of about 300 cm^{-1} when spherical particles with the size of 50-100 nm are formed, depending on the experimental condition. Furthermore, the morphology and surface topography of the SeNPs was confirmed by AFM imaging (Fig.2a). The AFM images and results showed that the SeNPs were spherical in shape having the diameter ranging from tens nm to maximum 300 nm.

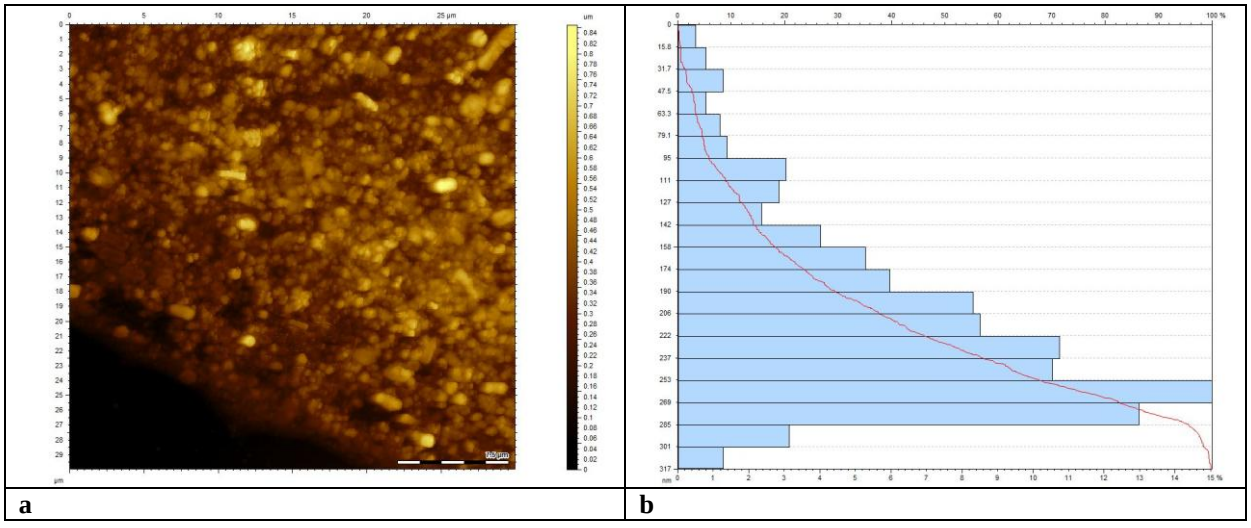

Fig.2. a. AFM image of SeNPs biosynthesized by parsley extract; **b**. Size (diameter) distribution histograms of corresponding structures

The size distribution and the zeta potential of colloidal SeNPs were assessed by using the DLS method (Fig.3).

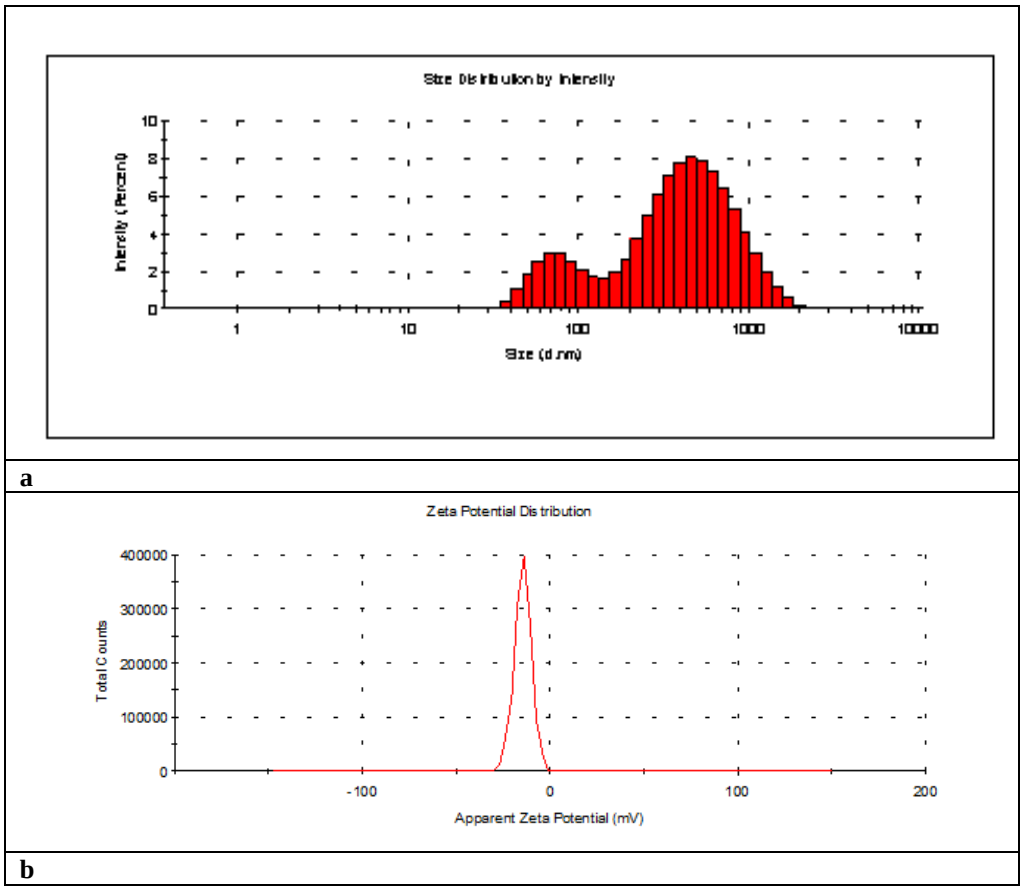

Fig.3. a. Size distribution of SeNPs recorded by DLS measurement. **b**. Zeta potential measurement of SeNPS

Fig. 3 shows the DLS measurement of the biosynthesized SeNPs by parsley extract indicating two maximum distribution: particles with the size under 100 nm (25 %) and above 100 nm (75 %), the maximum distribution being registered around 400 nm, which is in agreement with AFM histogram. Of course, some differences between these two techniques arise because AFM images were recorded from a limited area (30µm x 30µm) of a dried film of SeNPs, meanwhile the DLS measurements were performed in solution. Furthermore, the apparent zeta potential was recorded at a maximum value of -14.2 mV, which indicates that these nanoparticles do not form aggregates in solution leading to a stable dispersion.

Fourier Transform Infrared Spectroscopy

The biosynthesis of SeNPs was characterized by FTIR analysis (Fig.4) in order to investigate the functional groups responsible for the synthesis and stability of nanoparticles.

The marker bands of SeNPs (powder) show in the high wavelength range (Fig. 4a) an intense absorption peak at 3280 cm^{-1} due to -OH stretching of the aromatic rings and a sharp peak at 2918 cm^{-1} representing ether-methoxy-OCH_3 groups. These peaks indicate the presence of a biopolymer associated with the SeNPs, probably resulted from the cell walls. In the low wavelength range, there appear amide I band at 1636 cm^{-1} (C=O stretch of the ester group), amide II at 1530 cm^{-1} (N-H bending), 1394 cm^{-1} (C-H asymmetric bending in CH_2 and CH_3 groups), 1220 cm^{-1} (secondary -OH bending). In the low wavenumber region (Fig. 4b), the large and intense band at 1050 cm^{-1} represents the superposition of in plane C-H bending, but also the characteristic Se-O stretching vibration, according to literature (Kannan, 2014). Additional bending vibrations of Se-O bond are emphasized at 668 and 465 cm^{-1}.

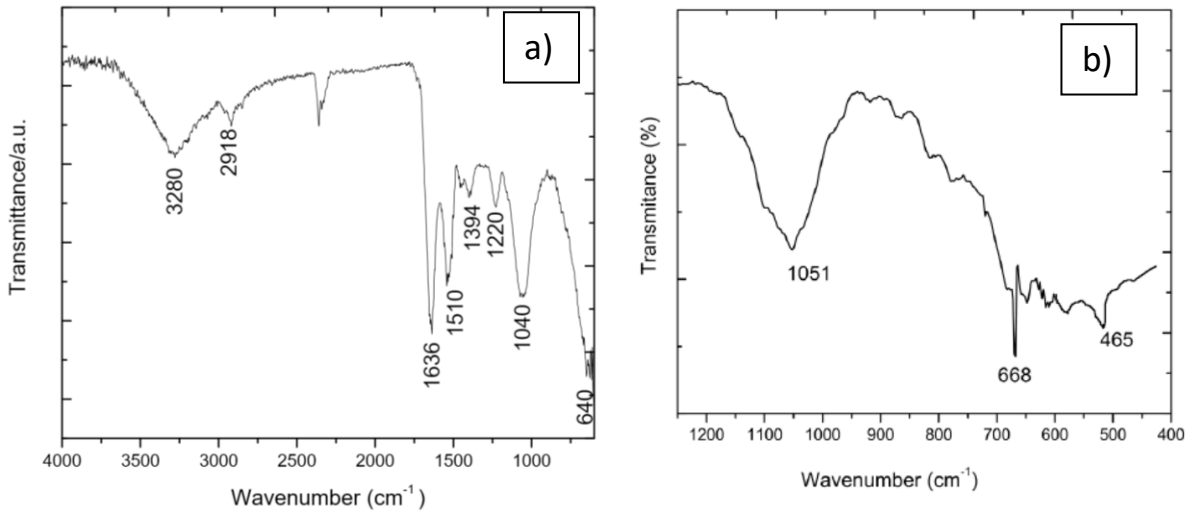

Fig. 4 FTIR spectrum of powder SeNPs obtained from parsley extract: a) general spectrum in the range 4000-400 cm^{-1}; b) detailed spectrum of powder SeNPs in the range 400-1200 cm^{-1}.

CONCLUSIONS:

In this study, for the first time in literature, the green synthesis of SeNPs by aqueous parsley leaves extract, at room temperature, was reported. SeNPs were characterized by various techniques: UV-Vis, FTIR, AFM, DLS. The FTIR analysis demonstrated the presence of alcoholic groups and aromatic ring in the parsley extract being responsible for the biosynthesis of SeNPs. According to AFM and DLS data, the spherical shape and the maximum distribution around 400 nm was confirmed. Further studies are necessary in order to demonstrate possible medical and biological applications such as food supplements, anticancer agent, or crop bio-fortification agent.

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