Research Article

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Effectiveness of six different methods in green synthesis of selenium nanoparticles using propolis extract: Screening and characterization

<https://doi.org/10.1515/gps-2020-0065>

received September 04, 2020; accepted October 29, 2020

Abstract: Selenium nanoparticles (Se NPs) were fabricated with propolis hydro-alcoholic extract and six different methods, namely, hydrothermal, microwave irradiation, ultrasonication, UV radiation, self-assembling, and conventional heating. Results indicated that antioxidant activity, turbidity, pH, and brix values of the provided hydroalcoholic propolis extract were 85.8%, 2.235% a.u., 4.1, and 3.2°Bx, respectively. Gas chromatography analysis revealed that approximately 38 bioactive compounds were detected in the provided extract within 40 min of retention time, including chalcone. Results also revealed that each method had advantage in fabrication of Se NPs compared to others, but spherical Se NPs with overall appropriate physicochemical attributes of particle size (50–60 nm), polydispersity index (0.362), zeta potential (−41.8 mV), maximum broad absorption peak (321 nm), and antioxidant activity (12.4%) were synthesized using the ultrasonication method with a frequency of 20 kHz and a power of 300 W for 10 min.

Keywords: accelerated method, antioxidant activity, green synthesis, physicochemical properties, selenium nanoparticles

1 Introduction

Selenium, a trace element, is known as essential poison for the human body, and its high amounts in the diet can be lethal and its low amount can be resulted in a chronic and decreasing body deficiency system [1]. Selenium nanoparticles (Se NPs) have high surface-to-volume ratio with more specific biological, chemical, physical, and pharmaceutical attributes and have attained much interests [2]. Furthermore, numerous researches shown that Se NPs have unique antioxidant, antimicrobial, and anticancer activities [3,4].

Biological fabrication of inorganic NPs based on natural sources including plants and their derivatives, microorganisms, and enzymes is known as the green synthetic method and considered as an eco-friendly, one step and cost-effective technique that can be used as an alternative of the common physical and chemical metal NP synthesis methods [5–7]. In this method, natural bioreductants (e.g., flavonoids, phenolic compounds, organic acids, and enzymes) and biostabilizers (e.g., proteins and carbohydrates), which are existed in the plant and microorganisms, are studied [8–10]. However, compared to traditional physicochemical synthesis techniques, such as microwave heating, ultraviolet (UV) irradiation, ultrasonication, hydrothermal, and common heating, green synthesis of the metal NPs is slow [1,11]. Therefore, to overcome this problem, which is too important for fabrication of metal NPs in commercial scale, the green synthesis method of the NPs is generally accelerated using physical and chemical heating methods as mentioned earlier [3,4].

Propolis, a bee glue, is collected by honey bees from numerous plant flowers, and due to its unique biological attributes such as antibacterial, antifungal, and antiviral activities against several microorganisms, anti-inflammatory, antiulcer, antitumor, and immune-stimulating activities, it received great attention by the researchers and scientists [12,13]. Several studies indicated that propolis is composed of various valuable bioactive compounds

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such as alcohols, aldehydes, aromatic acids and esters, flavanones, flavones, hydrocarbon esters, ethers, hydroxyl and keto waxes, ketones, terpenoids, steroids, sugar, several vitamins (e.g., B, C, and E), resins, and silicon [14,15]. These bioactive compounds have the ability of metal ion reduction to their elements and to synthesis their NPs [16]. Shubharani *et al.* synthesized Se NPs using hydroalcoholic extract of bee propolis and evaluated their antioxidant and bactericidal activities against numerous bacteria strains. They demonstrated that propolis extract had high bioreduction and stabilizing properties to green synthesis of Se NPs [17].

Thus, the main purposes of the current study are to (i) provide hydroalcoholic propolis extract and determine its specification, (ii) produce Se NPs by propolis extract and six different heating methods, namely, microwave, ultrasonication, UV irradiation, hydrothermal, self-assembling at room temperature, and mild heating using heater and stirrer, and (iii) evaluate particle size, polydispersity index, zeta potential, and antioxidant activities of the fabricated Se NPs.

2 Materials and methods

2.1 Materials

Propolis and selenite salt (Na_2SeO_3), with purity of 99%, were obtained from a local market (Tabriz, Iran) and Merck Company (Merck Co., Darmstadt, Germany), respectively.

2.2 Methods

2.2.1 Preparation of propolis extract

To prepare propolis extract, propolis was cleaned manually by hand to remove its debris and insect residuals and put into a freezer for 24 h at -20°C . Then, the frozen and solidified propolis was grounded using a laboratory miller. Thereafter, 10 g of the provided powder was added into the 100 mL of ethanol solution (20% V/V) and vigorously shaken by hand for 60 min and filtrated through Whatman No. 40 filter paper. Finally, alcohol was evaporated from the extract solution using a rotary evaporator (Buchi Rotavapor R-100 System).

2.2.2 Synthesis of Se NPs

According to the previous studies, selenium salt solution with a concentration of 10 mM was provided [1–4]. Then,

200 μL the provided propolis extract was added into 20 mL of the prepared selenium salt solutions, and for the synthesis of Se NPs, the samples were used in six different synthetic methods, namely, hydrothermal, microwave irradiation, ultrasonication, UV radiation, self-assembling, and conventional heating. In the hydrothermal method, colloidal solution was placed in a laboratory autoclave at 121°C and 1.5 atm for 15 min. In microwave radiation technique, the mixture solution was heated by an 800 W microwave device (MG2312w, LG Co., Seoul, South Korea) for 30 s. In the ultrasonication manner, the probe of equipment, with a tip diameter of 3 mm, was immersed in the provided solution, and the sample was treated for 10 min using a laboratory ultrasound device (Hiesche, UP400S, Berlin, Germany) with a frequency and a power of 20 kHz and 300 W, respectively. Another mixture solution was exposed to a 365 nm UV light from a 6 W UV lamp up to a maximum time of 1 h at room temperature. In the self-assembling technique, colloidal solution was kept at room temperature (33°C) overnight and in conventional synthesis process, colloidal solution was placed on a magnetic heater-stirrer set at 300 rpm and 60°C for 30 min.

2.3 Analysis

2.3.1 Propolis extract

To recognize the number and the type of the existed components in the propolis extract, gas chromatography mass spectrometry (Agilent GC-MS 5973, Santa Clara, CA) under electron impact ionization (70 eV) was utilized [18,19]. pH and brix of the propolis extract were measured using a pH meter (Delta 320, Shanghai, China) and refractometer (Atago, ABBE, NAR-1T, Tokyo, Japan), respectively.

2.3.2 Se NPs

Fabrication of Se NPs in the colloids were easily confirmed using the UV-Vis spectrophotometry analysis by a Jenway UV-Vis spectrophotometer (6705, Staffordshire, UK), via their surface plasmon resonance (SPR) signal detection [3,4]. Dynamic light scattering apparatus (Nanotracer Wave, Microtrac, USA) was utilized to evaluate the formed Se NP particle size and zeta potential, their distributions, and polydispersity index (PDI) [20,21]. Transmission electron microscopy (TEM, CM120, Philips, Amsterdam, Netherlands) was utilized for morphological assessment of the resulted Se

NPs. Antioxidant activity of the resulted Se NPs using propolis extract was assessed using the described method by Sayyar and Jafarizadeh-Malmiri and Eq. 1 [22]:

$$I\% = (A_{\text{Control}} - A_{\text{Sample}})/A_{\text{Control}} \times 100 \tag{1}$$

where $I\%$, A_{control} , and A_{sample} are percentage of inhibition, absorbance of control, and samples, respectively, at the wavelength of 517 nm.

2.3.3 Statistical analysis

All synthesis and analysis were carried out three times, and the results were presented according to the obtained mean of data. Data were analyzed using the analysis of variance using Minitab v.16 statistical package (Minitab Inc., PA, USA). Tukey’s compression test at 5% level of significance was used to compare the mean values of the obtained data.

3 Results and discussion

3.1 Specifications of propolis extract

Results show that antioxidant activity, turbidity, pH, and brix values of the prepared hydroalcoholic propolis extract were 85.8%, 2.235% a.u., 4.1, and 3.2°Bx, respectively. GC-MS chromatogram of the propolis extract is shown in Figure 1. Results indicated that there were approximately 38 bioactive components in the provided extract, within 40 min of retention time. Chalcone, as a main bioactive compound of propolis, was highlighted with peak centered at retention time of 35.01 min (Figure 1) and its anticancer, antimicrobial, anti-inflammatory, and antidiabetic activities have been demonstrated [23–26]. The most important chemical compounds in the propolis extract include chalcone, flavone, phosphoric acid, acetic acid, butanol, butanoic acid, and *n*-butyl acetate and butyl ester. Some of the main chemical compounds of the propolis extract are presented in Table 1. As clearly presented in Table 1, *n*-butyl acetate had highest amount (area%) compared to other presented compounds in the provided propolis extract. Due to the presence of carboxyl group (COOH) in its chemical structure, *n*-butyl acetate can easily reduce selenium ions and convert those into their elements, Se NPs can be formed based on the provided selenium element [13,14,17].

Figure 1: GC-MS chromatogram of the provided propolis hydroalcoholic extract.

Table 1: Most important chemical compounds of the propolis hydroalcoholic extract

Compounds	Peak number	Retention times (min)	Area (%)
Acetic acid	2	1.43	5.40
Butyl alcohol	3	1.50	14.69
<i>n</i> -Butyl acetate	6	1.90	40.39
Butyric acid	9	4.37	1.28
Chalcone	23	33.68	2.43
Phosphoric acid	26	35.01	8.03
Phthalic acid	27	36.24	3.08
Flavone	31	37.39	0.37
Methyl ester	33	39.19	0.21

3.2 Effectiveness of the synthesis methods on characteristics of the formed Se NPs

Reduction of selenium ions into Se NPs using natural existed reductants in propolis extract was observed by change in the color of mixture solution containing sodium selenite and propolis extract from light brown (at the beginning of synthesis process) to red-dish orange after accomplishing the NPs fabrication procedure due to SPR of the formed Se NPs [1–4]. Figure 2 shows

Figure 2: Appearance and color of the mixture solutions containing synthesized Se NPs using six different fabrication methods.

the appearance and color of the colloids having synthesized Se NPs using six different fabrication methods.

Characteristics of the resulted Se NPs with six different NP fabrication methods, as well as particle size, PDI, zeta potential, and antioxidant activity are presented in Table 2. As shown in this table, synthesized Se NPs using ultrasonication and self-assembling at room temperature had a minimum particle size of 94.89 and 98.68 nm, respectively. The statistical analysis shows insignificant differences between particle sizes of the formed Se NPs via these two methods. However, the particle size of the formed Se NPs using microwave heating (158.6 nm) was significantly higher than that of those synthesized using other methods. It seems that in the microwave accelerated technique; NPs are mainly produced via heat effect. While, ultrasounication, heat, and high pressure affect the synthesis of inorganic NPs [27,28]. In fact, by passing ultrasonic wave through a solution, a lot of bubbles are generated, grown, and finally collapsed, which cause acoustic cavitation. High local temperature (5,000 K) and pressure (1,000 bar) are generated in the mixture solution by collapsing of the formed bubbles, which can easily

integrate molecular chains [29,30]. Fardsadegh *et al.* could successfully synthesize Se NPs using *Pelargonium zonale* leaf extract and microwave heating, with a particle size of 136 nm [4]. Obtained results revealed that fabricated Se NPs using microwave heating and hydrothermally methods had minimum PDI of 0.222 and 0.226 nm, respectively. The statistical analysis show insignificant differences between PDI of the produced Se NPs using these two techniques. However, PDI of the fabricated Se NPs using self-assembling (0.446) and UV radiation (0.437) was significantly higher than that of those formed using other techniques. Several studies indicated that microwave heating has some advantages over other metal NPs synthesized techniques, and formation of the uniform and monodispersed NPs is one of those [9,10]. It has been demonstrated that the rate of particle growth is controllable through microwave heating based on controlling of the dielectric loss factor (microwave energy absorption by substance) combined with the dielectric constant. Controlling of the heating rate decreases the growth of NPs and avoids their agglomeration [31]. Figure 3 shows the particle size distribution of the resulted Se NPs through six different methods. Achieved narrow and sharp picks for the most fabricated Se NPs using synthetic methods indicated the high homogeneity and uniformity of the prepared Se NPs. Sheikhlou *et al.* had already provided Se NPs using walnut leaf extract and microwave heating, with PDI of 0.206 [32].

Zeta potential is defined as surface electric charge of the formed NPs, whose value has a direct relationship with the stability of the resulted NPs in colloids [3,4]. According to Table 2, the synthesized Se NPs with microwave heating had a maximum value of -55.6 mV. While minimum zeta potential value (-34.4 mV) was obtained for the made Se NPs via UV radiation. Numerous researches revealed that high values of zeta potential ($>+30$ and <-30) for the mixture solution containing made inorganic NPs show their high stability [19,21]. Sheikhlou *et al.* made Se NPs using the walnut leaf extract and microwave heating, with a zeta potential value of -24.7 mV [32].

Table 2: Physicochemical attributes of the formed Se NPs using propolis extract and six different synthetic methods

Method	Particle size (nm)	PDI	Zeta potential (mV)	Antioxidant activity (%)
Microwave heating	158.6	0.222	-55.6	11.0
Self-assembling	98.68	0.446	-47.4	12.1
Hydrothermal	110	0.226	-44.9	11.1
UV irradiation	132.9	0.437	-34.4	11.1
Ultrasonication	94.89	0.362	-41.8	12.4
Conventional heating	116.5	0.251	-46.1	11.1

Figure 3: Particle size distribution of the fabricated Se NPs using propolis extract and microwave heating (a), self-assembling (b), hydrothermal (c), UV irradiation (d), ultrasonication (e), and conventional heating (f) methods.

Fardsadegh et al. fabricated Se NPs using *Pelargonium zonale* leaf extract and microwave irradiation, with a zeta potential value of -24.6 mV [4]. It seems that because of the presence of natural stabilizing materials in the propolis extract such as waxes, ketones, terpenoids, steroids, and sugars, made Se NPs had high zeta potential and stability in the colloidal solutions [14,33]. Zeta potential distribution of the resulted Se NPs utilizing propolis extract and six synthetic techniques is shown in Figure 4.

Antioxidant activity of the resulted Se NPs utilizing propolis extract using six different methods is presented in Table 2. Attained results shown that while antioxidant of the provided propolis extract was 85.8%, the synthesized Se NPs using ultrasonication (12.4%) and self-assembling (12.1%) methods had highest antioxidant

activity compared to that of those synthesized using microwave irradiation, UV radiation, hydrothermally, and conventional heating methods. Antioxidants are capable to decrease oxidative stress via interaction with free radicals and terminate the adverse chain reactions, which in turn convert those into harmless products [34]. Selenium has high antioxidant activity, and the obtained results indicated that by changing selenium from its bulk form to its NPs state, its surface-to-volume ratio increases and in turn results in a drastic change in its properties [1,2]. High antioxidant activity of other synthesized metal and metal oxide NPs, such as silver chloride and zinc oxide NPs using different plant extracts, namely, *Beta vulgaris* peel and *Mangifera indica* leaves extract, was also reported [35,36].

Figure 4: Zeta potential distribution of the fabricated Se NPs using propolis extract and microwave heating (a), self-assembling (b), hydrothermal (c), UV irradiation (d), ultrasonication (e), and conventional heating (f) methods.

3.3 Screening of the method for Se NPs synthesis with more desirable attributes

In the metal NPs synthesis, at a colloidal form, the main objectives are to synthesize NPs with small particle size and high stability [4,20,37]. Generally, by formation of NPs, their surface-to-volume ratio increases, which in turn leads to drastic increase in their activity and reactivity [1,3]. For example, antibacterial, fungicidal, and antioxidant activities of the metal and metal oxide NPs such as ZnO NPs drastically increase [19,22]. In other words, most of physicochemical and biological attributes of the synthesized NPs are influenced by their particle size. Obtained results indicated that, by using ultrasonication and self-assembling methods, Se NPs with a small particle size and moderately high antioxidant activity

were synthesized. Conversely, simplified manipulation and short operation time are two main parameters in fabrication of metal NPs in a large scale [38,39]. Therefore, obtained results revealed that the synthesis of Se NPs using the propolis extract and ultrasonication was rapid, cost-effective, and eco-friendly manner, which could result Se NPs with desirable physicochemical and biological characteristics. UV-Vis spectra and TEM image of the resulted Se NPs, and utilizing propolis extract and ultrasonication accelerated synthetic method are presented in Figures 5 and 6, respectively. Results show that the spherical formed Se NPs had a particle size ranging from 50–70 nm and maximum broad absorption peak ($\lambda_{\max}$) in the wavelength of 321 nm. Previous studies indicated that Se NPs have $\lambda_{\max}$ in the wavelength ranging from 270 to 350 nm [3,4].

Figure 5: UV-Vis spectra of the mixture solution containing fabricated Se NPs using propolis extract and ultrasonication method.

Figure 6: TEM image of the fabricated Se NPs using propolis extract and ultrasonication method.

4 Conclusion

Selenium, with unique pharmaceutical and medicinal attributes, was converted into its nanoparticles using propolis extract and six different synthetic methods. Results revealed that propolis extract, due to the presence of natural reducing and stabilizing biocompounds in its composition, such as charcones, flavones, phosphoric acid, acetic acid, butanol, butanoic acid, butyl ester, hydroxyl and keto waxes, ketones, terpenoids, steroids, and sugars, had potential application in green synthesis of Se NPs.

In addition, attained results show that physicochemical properties of the resulted Se NPs using propolis extract drastically influence the applied synthetic methods. In this study, ultrasonication and self-assembling methods could fabricate Se NPs with small particle size and high antioxidant activity. While fabricated Se NPs using microwave heating had minimum PDI and maximum zeta potential values. Hence, the type and the concentration of bioreductant and biostabilizing agents of the natural sources, and type of the inorganic NP synthetic method, are main parameters to fabricate metal and metal oxide NPs with desirable characteristics.

Acknowledgments: The authors appreciate the support of Islamic Azad University – Mamaghan branch to accomplish this research.

Conflict of interest: The authors declare that they have no conflict of interest.

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