ORIGINAL ARTICLE

Green synthesis of Silver nanoparticles using the aqueous extract of Prangos ferulaceae leaves

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INTRODUCTION
The field of nanotechnology is one of the most promising areas in modern material science and technology which has grown very rapidly all over the world for the past decades. It is defined as the science in the design, synthesis, characterization and application of nanoscale materials [1, 2]. Because of thermal conductivity, chemical stability, catalytic and biological activities, the synthesis of silver nanoparticles is very important [2-16]. Size, shape, distribution and surface area to volume ratio of the silver nanoparticles act as affecting factors on their biological activities such as antibacterial, antifungal and antioxidant [10-21]. Up to now, large number of preparation and synthesis procedures including physical, chemical, electrochemical, photochemical and etc methods have been studied and reported for the silver nanoparticles [5-9, 10-16, 22-26]. Although these methods are very successful to produce well-defined silver nanoparticles, but each of them has some disadvantages such as high cost and time of synthesis, the use of hazardous and harmful toxic chemicals, release of hazardous by-products in environment, difficulty in purification, low rate of material conversions and high energy consumption [27, 28]. So, using of the nontoxic and environmentally sustainable synthesis processes is attractive especially if they are intended for invasive applications in medicine [29]. The use of plant extracts in synthesize of the metal nanoparticles has more advantageous over other methods due to the elimination of harmful reagents, less reaction time, cost effectiveness, ease of scale up, economic viability and most importantly ecofriendly [3-5, 8, 10-16, 17-20, 30, 31]. In the synthesis of nanoparticles by the plant extracts, the extract could acts both as reducing agents and stabilizing agents [8, 10-20, 31]. The major natural products responsible for the spontaneous reduction of metal ions are flavonoids, terpenoids, carboxylic acids,
quinones, aldehydes, ketones and amides [10- 20, 32-34]. A number of plants that were studied for the synthesis of silver nanoparticles in different sizes and shapes are Cardiospermum halicacabum L. [4], Olea europaea [5, 12], Zizipora tenuior L [8, 11], Justicia adhatoda [9], Nasturtium Officinale [14], Syzygium cumini (L.) [18], lemon [19], Garlic [20], Tribulus terrestris [28], Pimpinella anisum L. [29], Eucalyptus hybridra [32], Chenopodium album [33-34], Petroselinum crispum [35], Aloe Vera [36], Securinega leucopyrus [37], Matricaria chamomilla [38], Dracaena mahatma [39] and etc. Prangos is a perennial genus of the Apiaceae and distinguished by the presence of winged fruits. The Prangos genus is represented by 15 species in Iran, of which four are endemic. They are widely used in folk medicine as tonic, antiflatulent, antihaemorrhoids, for the treatment of intestinal worms and treatment of leukoplakia. Prangos ferulaceae is the most widespread species of the genus in Iran. It is a long and permanent herb that is mainly used as a rich herb in animal feeding [40]. In the present study, for the first time, we have reported a suitable green route for the synthesis of silver nanoparticles using aqueous extract of Prangos ferulaceae leaves as reducing and stabilizing agent.

EXPERIMENTAL

Preparation of plant extract

Prangos ferulaceae leaves were collected from Sardab, Zanjan, Iran and washed several times with double distilled water and then dried to remove the residual moisture. The clean and dry leaves were cut into fine pieces and then were powdered. About 8.0 g of obtained powder was dipped into a beaker containing 125 ml of Millipore water. The content of beaker was mixed at room temperature (T = 25°C) and the extraction was allowed to proceed during 30 min. The aqueous extract was filtered using Whatmann filter paper and then centrifuged at 8,000 rpm for 30 min to remove heavy biomaterials. The extract was stored at 4 °C until used.

Synthesis of silver nanoparticles

For the synthesis of silver nanoparticles, 1, 2 and 3 ml of aqueous extract of Prangos ferulaceae leaves were slowly added to 30 ml of 1 mM aqueous silver nitrate solution for the reduction of Ag¹⁺ to Ag⁰. The solution was allowed to react at room temperature (25°C), 50°C and 80°C and kept for 72 h. The color change was noted by visual observation indicating the formation of silver nanoparticles. The completion of the reaction was monitored by UV-Visible spectroscopy.

Characterization of silver nanoparticles

The synthesized silver nanoparticles were confirmed and characterized by the following methods; UV-Vis spectroscopy, scanning electron microscopy (SEM), dynamic light scattering (DLS), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FT-IR). UV-Vis spectra of synthesized silver nanoparticles were monitored as a function of reaction time on a spectrophotometer (PG, UK) in 400-700 nm range operated at a resolution of 10 nm. The DLS and zeta potential was measured by using a Zetasizer Nano ZS 3600 (Malvern Instrument ltd, UK). Measurements were made by means of DLS in the range of 0.6-6000 µm. 750 µl of sample was transferred in the clear disposable zeta cell for the measurement of zeta potential. The zeta potential was calculated using Henry’s equation. SEM analysis was done using Hitachi S-4500. The crystalline nature of silver nanoparticles was studied by XRD analysis using an X-Ray diffractometer (Bruker AXS D8 ADVANCE). FT-IR spectroscopy was done using a Perkin Elmer infrared spectrometer (model FT-PC-160) in the range 400-2000 cm⁻¹.

RESULTS AND DISCUSSION

The green synthesis of silver nanoparticles was done using aqueous extract of Prangos ferulaceae leaves and aqueous silver nitrate solution. Visual observation, UV-Vis, XRD, SEM, EDAX, DLS and FT-IR studies confirmed the bio-reduction of aqueous silver ions to silver nanoparticles.

Characterization of silver nanoparticles by UV-Vis Spectroscopy

It is obvious that silver nanoparticles in aqueous solution show yellowish brown color due to excitation of surface plasmon vibrations of silver nanoparticles [41]. When the extract of Prangos ferulaceae leaves was mixed with the aqueous solution of the silver ion, it started to change the color from colorless to yellowish brown during the time, which may be the indicates the formation of silver nanoparticles and the intensity of color was directly proportional to the
formation of nanoparticles (Fig. 1; A; 1 after 30 min, 2 after 120 min and 3 after 24 h). In contrast, the color of control solution (without silver nitrate ions) remains unchanged during this period of the experiment (Fig. 1; B; 1 after 30 min, 2 after 120 min and 3 after 24 h). The obtained results showed that when the extract concentration was increased, the color of the reaction solution changed to reddish brown and finally to colloidal brown, which would lead to the formation of well-defined and stable silver nanoparticles (Fig. 1; C; 1 only 1 mL of extract, 2 only 2 mL of extract and 3 only 3 mL of extract after 24 h). In other words, when the concentration of the biological material mediating nanoparticle synthesis is increased, higher contents of the biomolecules involved in the metal reductive.

The effect of temperature was according to extract concentration and the color of the reaction solution was increased with increasing temperature. These results are in consistent with other studies such as *Malus domestica* and *Artemisia Absinthium* in the synthesis of silver nanoparticles [42, 43]. On the other hand, UV–Vis spectroscopy is generally recognized as an important technique to examine the metal nanoparticles in aqueous suspensions [44]. The UV-Vis spectrum of silver nanoparticles was recorded after of 15, 30, 45, 60, 90, 105, 120 min, and 24 h from the initiation of reaction between the extract of *Prangos ferulaceae* leaves and aqueous silver nitrate solution.

As shown in Fig. 2, the UV-Visible spectra show an absorption band at about 440 nm.
which corresponds to the absorbance of silver nanoparticles and steadily increases in intensity as a function of time of reaction without any shift in the peak wavelength [10-22].

The spicy brown color in the reaction solution results from absorption of the colloidal silver nanoparticles in the visible region of the electromagnetic spectrum (380-500 nm) due to the excitation of their surface plasmon vibrations [16-22]. The UV-Visible absorption spectra of silver nanoparticles exhibits characteristic surface plasmon resonance bands centered at 425 nm. The location of the surface plasmon resonance peak on the lower end of the absorption range (380-500 nm) indicates that the colloidal dispersion was primarily composed of small spherical silver nanoparticles which are in agreement with SEM and DLS results.

**DLS analyses of silver nanoparticles**

The particle size of the synthesized silver nanoparticles was determined using DLS measurements. Fig. 3 shows the DLS pattern of the suspension of silver nanoparticles synthesized using *Prangos ferulaceae* leaves extract. The DLS analysis shows a wider hydrodynamic size range for silver nanoparticles; approximately 5 to 100 nm. On the other hand, the results show that the sizes of silver nanoparticles are influenced by a number of factors including reaction temperature and extract concentration. To find out the effect of temperature and optimum condition, synthesis of silver nanoparticles was carried out in different temperatures including: 25°C (Fig. 3A), 50°C (Fig. 3B) and 80°C (Fig. 3C). It was found that the sizes of silver nanoparticles decreased with increasing the temperature reaction. As can be seen, the optimal temperature for the formation of silver nanoparticles is 80°C, because the smallest silver particles were obtained in this condition. In order to find out the effect of extract concentration for the formation of silver nanoparticles, three experimental with different ratio of the *Prangos ferulaceae* leaves extract to silver nitrate solution i.e. (1 to 30, green curves in Fig. 3), (2 to 30, blue curves in Fig. 3), and (3 to 30, red curves in Fig. 3) were tested. It was found that the silver nanoparticles which are synthesized at 80°C with ratio of the *Prangos ferulaceae* leaves extract to silver nitrate solution of 2 to 30 have the smallest particle size (less than 10 nm) compare to other ratio (Fig. 3a and b). In general, the average hydrodynamic sizes of nanoparticles decreased with increasing reaction temperature and plant extract concentration. The zeta potential of the synthesized silver nanoparticles was found to be less than -21.0 mV at all experimental conditions. This high value confirms the repulsion among the silver nanoparticles and thereby increases in stability of the nanoparticles formulation [45]. The negative potential value could be due to the possible capping of the bio-organic components present in the *Prangos ferulaceae* leaves extract [46, 47].

![Fig. 2: UV-Vis spectra of colloidal silver nanoparticles synthesized using *Prangos ferulaceae* leaves extract and aqueous silver nitrate solution recorded at different reaction times.](image)
SEM and EDAX analyses of silver nanoparticles

SEM has provided further insight into the morphology and size details of the synthesized nanoparticles. The micrographs of the synthesized silver nanoparticles were synthesized using the aqueous extract of *Prangos ferulaceae* leaves in optimum conditions are shown in Fig. 4a. The result shows that the synthesized silver nanoparticles have spherical shape and well distributed without aggregation. The particle sizes were found in the ranges of 10-20 nm. EDAX analysis confirmed the presence of elemental silver in the synthesized nanoparticles. The EDAX spectrum exhibits an optical absorption band peaking at 3.0 KeV, indicates the binding energy of silver (Fig. 4b). This result is consistent with other studies on the EDAX analysis of silver nanoparticles synthesized by using extracts of other plants such as *Eichhornia crassipes*, *Eruca sativa* and *Spinacia oleracea* [48, 49]. The EDAX spectrum also shows the presence of weak signals from C, O, N, and K elements, which might have appeared due to the X-ray emissions from the metabolites present in the extract of the *Prangos ferulaceae* leaves [50]. In other words, chemical analysis of the produced silver nanoparticles by EDAX, confirmed both the existence of silver and the organic component that covers the Ag aggregates; the latter is implied by the presence of the C, O and N peaks in the EDAX spectrum. The EDAX analysis also proved that the Ag nanoparticles are in metallic form, with no formation of Ag$_2$O in them and free from any other impurities [51].

Fig. 3: The effect of extract concentration (1 to 30, green curves in A, B and C), (2 to 30, blue curves in A, B and C), and (3 to 30, red curves in A, B and C) and temperature reaction (A: 25°C, B: 50°C and C: 80°C) on the particle size of the synthesized silver particles.
XRD pattern of silver nanoparticles

The crystalline nature of silver nanoparticles was confirmed by the analysis of XRD studies. The XRD pattern of the silver nanoparticles that were synthesized in optimum conditions is shown in Fig. 5. The peaks in the X-ray diffraction pattern are due to reflections from the (111), (200), (220), and (311) planes and confirm the crystalline structure of the synthesized silver nanoparticles. Also, two peaks were also observed Fig. 5 at 27.89 and 32.24°. These peaks may be due to the metabolites which are present the extract and responsible for silver ions reduction and stabilization of resultant nanoparticles [48-51].

FT-IR analyses

FT-IR measurements were done to identify the major functional groups on the Prangos ferulaceae extract and their possible involvement in the synthesis and stabilization of silver nanoparticles. The spectra of Prangos ferulaceae leaves before and after reaction with silver nitrate are shown in Fig. 6. The FT-IR spectrum of the Prangos ferulaceae leaves extract shows several characteristic peaks; absorption band of O-H and N-H at 3405 cm⁻¹, C-H band at around 2926 cm⁻¹, characteristic absorption band at about 1623 cm⁻¹ for acetyl -C=O, -C-N asymmetric stretching at 1354 cm⁻¹ and C-O stretching at 1130 cm⁻¹.
Fig. 5: XRD pattern of the synthesized silver nanoparticles.

Fig. 6: FT-IR spectrums of (A) *Prangos ferulaceae* leaves extract and (B) silver nanoparticles.
The FT-IR spectrum of silver nanoparticles (reaction product) shows some of the Prangos ferulacea leaves extract peaks such as -C=O, C-O -C-N that have been decreased and some peaks have been lost, indicate that these groups are effective in the reduction of the silver ions to silver nanoparticles. The peaks observed at 3446 cm⁻¹ (-OH), 2924 cm⁻¹ (germinal methyl), 1118 cm⁻¹ (C-O-C) and 599 cm⁻¹ (C=C) suggest the presence of flavonones or terpenoids which adsorbed on the surface of silver nanoparticles. Such interactions between metal nanoparticles and plant constituents have been reported in previous study [51]. It seems that the oxidation of aldehydic groups of terpenoids to carboxylic acids causes the simultaneous reduction of silver ions to silver nanoparticles followed by capping on the surface of synthesized silver nanoparticles [52].

Chemistry involved in silver nanoparticles formation

Previous studies indicated that Prangos ferulacea leaves contain natural products including phenolic, flavonoids, terpenoids, and coumarins [53-56]. These natural products have hydroxyl group as a functional group in the structure and play a key role for the reduction of silver ions and producing and stabilization of silver nanoparticles. In other words, in the suggestion mechanism [9, 12-16, 21-25, 50-52], silver ions in aqueous solution dehydrated the hydroxyl group of the Prangos ferulacea leaves extract to carbonyl group and convert to silver nanoparticles.

CONCLUSION

Green synthesis of silver nanoparticles by using plant extract had many advantages such as safe, non-toxic, ecofriendly route of synthesis which can be manufactured at a large scale. The results in the present study show that the aqueous extract of Prangos ferulacea leaves can act as reducing as well as capping agent and can be used for the synthesis of silver nanoparticles. Hence, water soluble metabolites containing hydroxyl functional group (such as flavonoids, proteins and coumarins) are suggested to be responsible for the reduction and stabilization of silver nanoparticles. The XRD, SEM, EDAX and DLS techniques confirmed that the synthesized nanoparticles have spherical shape with an average size was about 10 nm. Also, the synthesized silver nanoparticles were found to be stable at room temperature due to negative value of zeta potential.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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