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Green synthesis of silver nanoparticles and antioxidant activities in *Bunium persicum* (Boiss.) B. Fedtsch. seeds extract

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ABSTRACT

In this study, the antioxidant activity and green synthesis of silver nanoparticles of ethanolic extracted from the seeds of *Bunium persicum* (Boiss.) B.Fedtsch was investigated for the first time. B.persicum is a local plant of Iran and the antioxidant activity of this plant was studied using the DPPH method and shows notable results in scavenging the free radicals. Antioxidant activity of extract checked in comparison to BHT.IC50 of *B.persicum* respectively was 1000 mg/mL; revealing a notable antioxidant effect. Another experiment was performed on green synthesis of silver nanoparticles in this plant extract. Green synthesis of silver nanoparticles in *B. persicum* was confirmed by the Fourier transforms infrared spectroscopy (FTIR), scanning electron microscopy (SEM), UV-Vis spectroscopy, Energy dispersive X-ray diffraction analysis (EDX) and the X-ray diffraction (XRD). Ag NPs were almost spherical in shape, with an average diameter of 51 nm. Results showed significant antioxidant effects that cause the formation of spherical silver nanoparticles with nearly homological size in this plant and can be used as the basis of medical studies in the future.

Keywords: *Antioxidant Activity, Bunium Persicum, DPPH, Green Synthesis, Silver Nanoparticles*

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INTRODUCTION

Bunium persicum (Boiss.) B.Fedtsch. members of the Apiaceae family, carry economical and medicinal value, growing wild in arid regions of Iran and with tolerance and compatibility to circumstances of drought [1]. The Apiaceae family contains 423 genera and 3000 species including herbs, shrubs, trees and aromatics plants, that are found throughout the world, particularly in northern hemisphere [2]. Two notable characteristics of the Apiaceae family are their "umbel" inflorescence and "Shizocarp" fruit. "Umbel" inflorescence that means "sunshade" (a flat or convex flower in which all pedicles spread from a single point of the apex). Shizocarp fruit contains two mericarps that are often joined to each other from extended oil canals (vittae); with ethereal oils and resins existing within these canals [3]. Nowadays, with increasing awareness of the abnormal maintainer risks and chemical drugs, the use of this plant's seeds as a natural additive has expanded [4]. In previous studies on the essential oil of *B. persicum*, compounds of cuminaldehyde, gamma-terpinene, trans-3-Caren-2-ol, acetic acid, Caryophyllene and 1,3,8 -p- menthatriene were recognized [5, 6]. Seeds of *B. persicum* have also been used in traditional medicine. Multiple remedial effects have been qualified for seeds of this plant in ancient Iranian medical books, including antihistaminic properties and remedies for digestive disturbance, diuretic disorders, convulsions, asthma and dyspnoea. In addition, the seeds are also

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used for seasoning of the foods and drinks as well as in perfumery [7-9]. Other studies confirm that essential oil of *B.persicum* has a strong antimicrobial [10-12], antifungal [13], antioxidant activity [14]. Also, investigation of *B. Persicum* Hydroalcoholic extract showed the inhibitory effect on Glucose-Induced [15]. Nowadays, nanotechnology, or the fabrication of nanomaterials with functionalities that have positive applications in health, environment, industries or any other field of human life, have been considered. The main techniques of making nanoparticles are summarized in two top-down and bottom-up methods. The top-down method was first introduced by Feynman, where bulk materials reduce into nanoparticles by the physical, chemical or mechanical operations. This method includes the use of laser ablation, atomization, annealing, radio frequency (RF) sputtering, arc discharge, focused ion beam lithography and electron beam evaporation. Drexler first proposed bottom-up production, where nanoparticles are created by combining the constructive units and putting them together. The systems used in this method be composed of chemical vapor and atomic layer deposition (belong to the gas-phase methods), sol-gel processes, reduction of metal salts, electrodeposition and templated synthesis (belong to the liquid-phase methods) [16]. Reduction of the corresponding metal cation is a straightway reaction to gain metal nanoparticles, which leads to the production of colloidal properties [17]. By considering risk of physical and chemical nanoparticles productions, new studies have focused on low-risk and environmentally friendly methods of metallic nanoparticles. Natural ingredients such as vitamins, plant extracts and microorganisms could be notable for nanotechnology [18, 19]. Green synthesis has been further developed due to the simplicity of the method, low-costs, reproducibility and sustainability of products compared to other methods. Green synthesis methods include ultrasound, microwave, hydrothermal, magnetic and biological methods, among others [20]. The green synthesized iron nanoparticles are used for disinfection of water and soil remediation of heavy metals [21, 22]. Studies show that the shape and size of the nanoparticles of gold can be controlled by the alteration of reaction temperature. The resulting nanoparticles are used in applications such as hyperthermia and optical fiber architectures [23]. Silver nanoparticles synthesized using plant extracts exhibited highly antibacterial [24, 25], anticancer [26, 27] and wound-healing activity [28]. In another study, silver nanoparticles had an inhibitory effect on the activity of larvae and resulted in mortality of the tested specimens [29, 30]. Silver nanoparticles (NPs) have been subjected to Medical research because of their size, shape and antimicrobial activity [31, 32]. Related studies have shown that temperature variations with stirring could affect the shape and size of the silver nanoparticles [33-35]. Sunlight can also enhance the speed of the synthesis of silver nanoparticles, with the size of the silver nanoparticles produced under sunlight between 10-80 nm [36]. Due to the importance of B.persicum in the pharmaceutical and food industries, and in order to complete previous researches, antioxidant activities and green synthesis of silver nanoparticles of their seed extract were evaluated for the first time in this paper.

MATERIALS AND METHODS

Collection and identification the plant

Seeds of *B.persicum* were collected in May. 2016 from 15 km of Bardeskan, an eastern city of Iran. They were identified in the Herbarium of Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS), Tehran under code number 1560-AUPF (*Bunium persicum* (Boiss.) B.Fedtsch.).

Preparation of seed plants extract

Firstly, 100g of seeds were grinded and extracted with 500 mL of 99% ethanol solution by maceration for 72 h. Finally, solutions were evaporated at room temperature to gain dry mass used for the experiments.

Synthesis and Characterization of silver nanoparticles

In the next step, 100mL stock solution (0.001M) of silver nitrate ($AgNO_3$) and seed extract of *B.persicum* was taken separately. For the reduction of Ag^+ ions, 10 mL of the aqueous extract was added to 90 mL of aqueous $AgNO_3$ solution and was stirred for 5 minutes. The final mixture was placed for 5 minutes in the light and color changes from light green to brown was observed. Part of the solution was centrifuged (at 12000 rpm for 1 hr) to separate the nanoparticles.UV-Vis Spectroscopy (SHIMADZU, Model:UV_1650PC, Kyoto, Japan), SEM (Philips, Model: XL30), EDX (Philips, Model:D6792) , FTIR(SHIMADZU,Model:8400S, Kyoto, Japan) and XRD (D8 ADVANCE, Bruker) methods were used to ensure the synthesis and characterization of silver nanoparticles. In UV-Vis Spectroscopy method, the silver solutions were diluted

by 0.2 mL aliquot solutions with 2 mL deionized water. In this study, a Shimadzu spectrophotometer was used to measure the optical density of solutions/ suspensions in a range of 200 nm to 800 nm, and deionized water was used as the blank. Morphology and size of nanoparticles were investigated by SEM. In this method, the sample was fixed, cleaned and dried. Then, the surface of samples was covered with a thin layer of gold foil. For elemental analysis of the synthesis of silver nanoparticles, the purity of the approximate value of each of the elements was verified using EDX. Using the FT-IR spectroscopy on the extract, the biomolecules were identified, which are agents the reduction of silver ions to metallic silver. For FT-IR evaluation, the bio-reduced solution was centrifuged at 12,000 rpm for 1 hr. The fouling layers were located on the potassium bromide crystals and the FTIR spectrum was obtained in the mid IR region of 400-4000 cm^{-1}. In addition, the crystalline structure of biosynthesized AgNPs confirmed by XRD analysis. In this method , Colloidal sample was placed on glass slide as a thin film and carried out using a PANalytical, the X'Pert-Pro MPD x-ray diffractometer by Cu-Kα radiation of wavelength 0.15418nm in the scattering angle range of 30°–80°.

Evaluation of antioxidant activity of the extract by DPPH

The capability of the extracts to scavenge DPPH (1, 1-Diphenyl-2-picrylhydrazyl) radical was specified according to the method illustrated by Blois *et al.* [37]. Sample stock solutions (1.0 mg/mL) were diluted in ethanol to ultimate concentrations of 750, 500, 250, 100 and 50 mg/mL. BHT (Butylated hydroxytoluene) was used as a standard in 4 to 250 mg/L solution. 0.1 mM of DPPH was prepared in ethanol and 3 mL of this solution was mixed with 1 mL of different concentrations of sample and standard solution separately and allowed to react at room temperature and in darkness. Ethanol (1 mL) with DPPH solution was used as controlling. After 30 min the absorbance values were measured at 517 nm and converted into the percentage inhibition of DPPH activity using the following formula of Bors *et al* [38].

$$\text{Inhibition of DPPH activity (\%)} = (A_{control} - A_{sample}) / A_{control} \times 100 \quad (1)$$

IC_{50} extract was obtained from the percent inhibition curve against the different concentrations of the extract.

Statistical analysis

Microsoft Excel 2013 was used for analyzing data and results were reported in the form of Mean ± Standard Deviation.

RESULTS

Biosynthesis of silver nanoparticles

In this experiment the green synthesis of silver nanoparticles from silver salt using *B.persicum* seed extract was a reducing factor. Silver nanoparticles were synthesized by *B.persicum* seed extract at 10 minutes of incubation. The solution of the extract and silver nitrate salt did not change color alone. Upon addition of the extract to $AgNO_3$, the biosynthesis reaction was initiated and color of solution changed from green to brown (Fig. 1) and

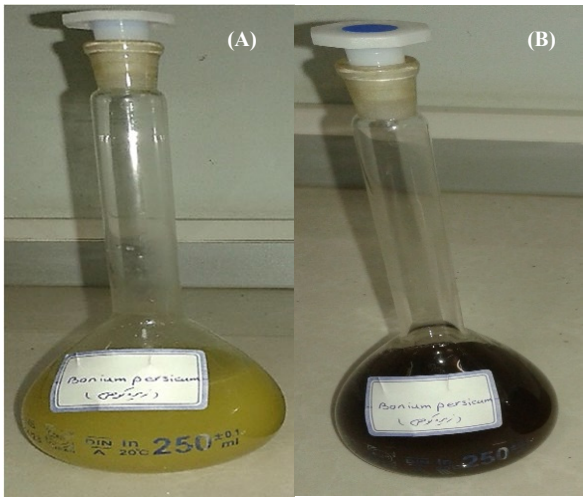

Fig. 1. Optical photograph of colloidal solution of (A) *B.persicum* seeds extract, (B) $AgNO_3$ solution reduced with 5 mL of *B.persicum* seeds extract.

the intensity of the brown color increased during the incubation period. Thereafter, the absorption spectrum of nanoparticles absorbed by UV-vis spectrophotometer Scan Drop of Germany results showed the wavelength shift in ~420 in Fig. 2 and confirmed the formation of colloidal silver nanoparticles. Reduction factors of silver ions in the extract were identified by spectra of FT-IR that are shown in Fig. 3. Fourier transform infrared spectroscopy analysis of the silver nanoparticles *B.persicum* seed extract showed a wide absorption peak at 3100–3500 cm^{-1} that is related to OH factor stretching vibration. Two peaks appeared at 2856.38 cm^{-1} and 2925.81 cm^{-1} due to C-H stretching from alkanes. The control extract absorption peak in 1747.39 cm^{-1} shifted to 1741.60 cm^{-1} and illustrated the presence of C=O group from esters. A single absorption peak located at 1585.38 cm^{-1} was attributed to the stretch vibration of C=C. Additionally, scanning electron micrographs (Fig. 4) showed the silver nanoparticles of *B.persicum* seed extract with crystalline and spherical structures with sizes around ~50 nm. Results showed the formation of nanoparticles with almost identical dimensions and homogeneous structure. Finally, application of the EDX illustrated a large peak of silver around 3.10 keV that confirmed its formation in the suspension (Fig. 5). According to the figure, in this scan range, the impurity peak was not found. Therefore, Fig. 6 shows the XRD pattern of synthesized AgNPs, using the aqueous seeds extracts of B.persicum. The AgNPs production demonstrated by the four distinct refraction peaks at 38.1°, 44.4°, 64.6° and 77.3° can be defined

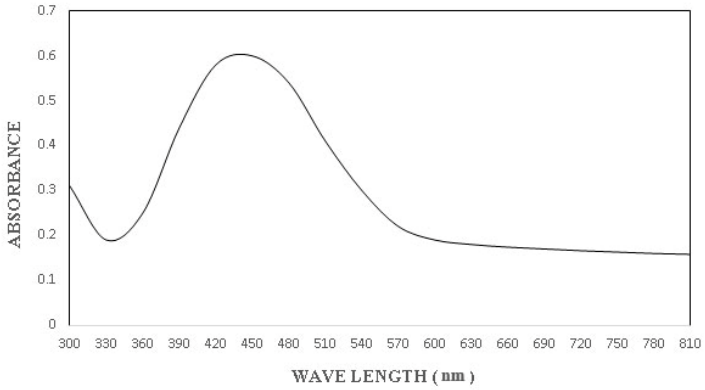

Fig. 2. The absorption spectrum of silver nanoparticles at 30 °with concentrations of silver nitrate of *B.persicum* seed extract

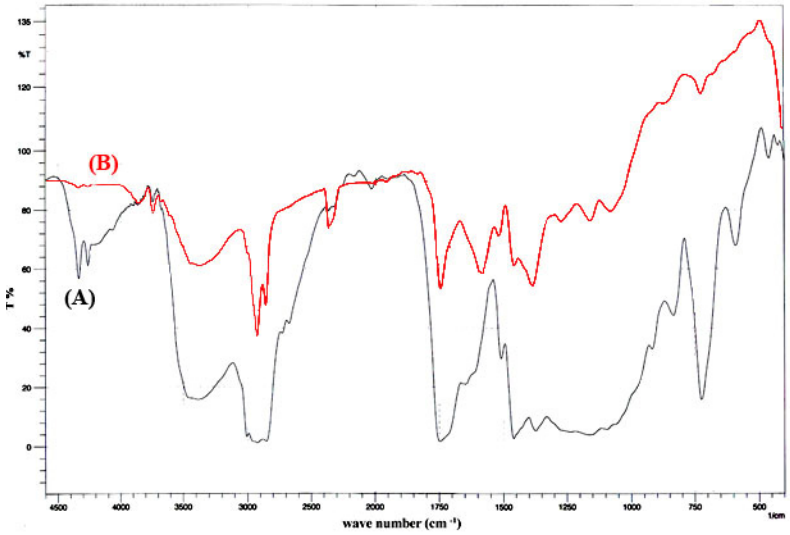

Fig. 3. FTIR absorption spectra of (A) control *B.persicum* seed extract and (B) stabilized Ag nanoparticles.

to (111),(200), (220) and (311) crystallographic planes of metallic Ag and was matched with JCPDS file no. 04–0783.

Antioxidant activity of total extract

In this research, Method DPPH analysis was used to study the antioxidant activity of the extract.

B.persicum seeds extract revealed a very high scavenging of DPPH and showed an antioxidant activity comparable with BHT. The *B.persicum* extract demonstrated a notable result in inhibiting DPPH, reaching up to 83.90% at concentration 18.75 µg/mL and its IC_{50} was 253.6247 µg/mL compared with the IC_{50} of BHT of 87.45 µg/mL. IC_{50} = 253.6247± 1.4921 (Mean ± Standard Deviation).

DISCUSSION

Nowadays, using a safe biological procedure to synthesize nanoparticles is desirable. Plant extracts, due to their phytochemicals compounds, such as flavonoids, phenolic and terpenoid, are able to reduce metal ions to metal nanoparticles. In this study, the formation of silver nanoparticles in *B.persicum* seed extracts was investigated by four methods. Initially,during synthesis, a change in color from green to brown was observed. During the time, this change in color became more severe. This gradual color change can be caused by the stimulation of superficial plasmons resonance and the reduction of silver salts. In similar studies, this color change indicates the first formation process

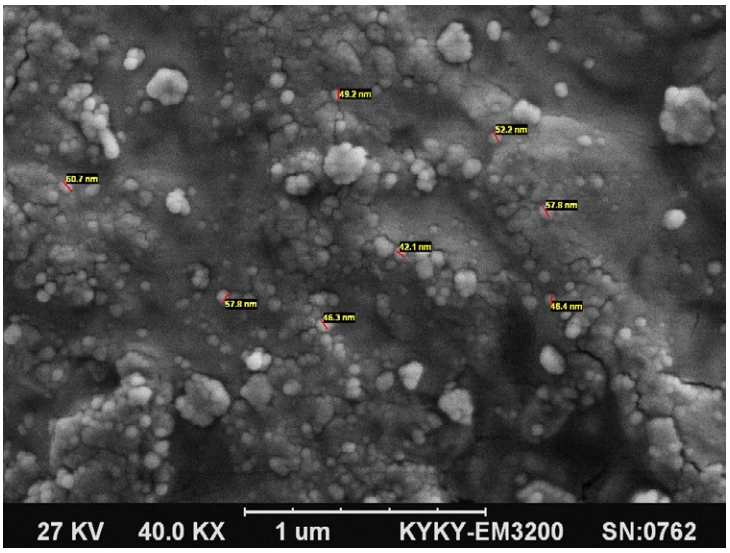

Fig. 4. A typical SEM image of silver nanoparticles constructed in reaction of 1 mM silver nitrate and aqueous seed extract of *B.persicum*

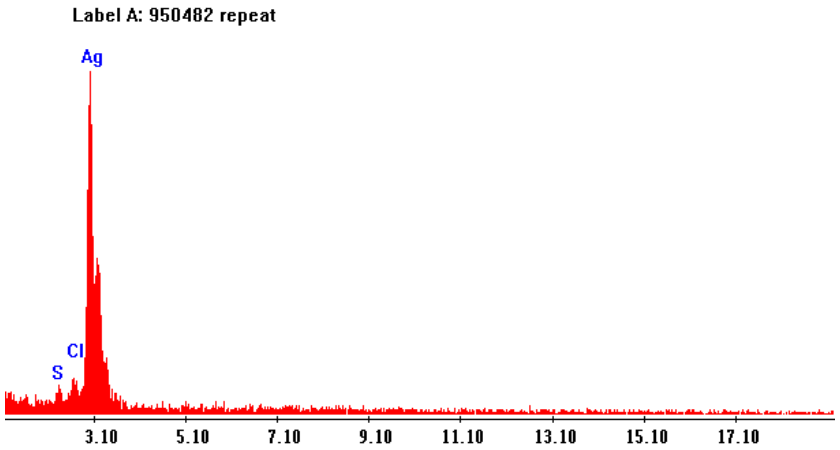

Fig. 5. EDX spectra of silver nanoparticles green synthesized using aqueous seed extracts of *B.persicum*

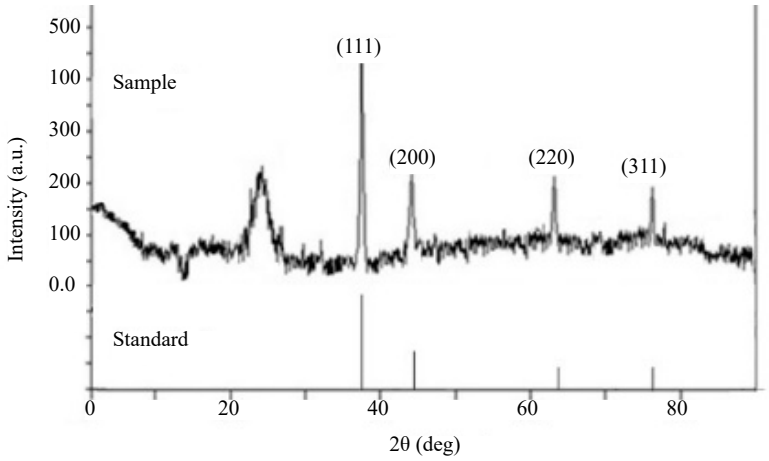

Fig. 6. XRD spectrum of silver nanoparticles synthesized from seeds extract of *B.persicum*.

of silver nanoparticles [39, 40]. In previous studies [41, 42], inspection of silver Solution by UV-vis spectrophotometry show the absorbance in the range of 400–450 nm, therefore, the shift of wavelength to ~420 nm indicates a reduction of Ag^+ to metallic Ag in our experiment. Presence of polyphenolics, particularly flavonoids groups in the *B.persicum* seed extract is responsible for Ag^+ reduction.

Investigating of Fourier transform infrared spectroscopy results showed that presence of capping or reducing groups such as OH factor is responsible for the conversion of silver ions to silver particles in total extract.

Also, the shifted absorption peak (caused by C=O) and appearance of a new peak (caused by C=C) confirmed the formation of silver nanoparticles in the silver solution. FTIR analysis indicated the presence of terpenoids, flavonoids, carboxylic acid, phenols and esters groups that confirmed the function of plant extract material in the reduction of Ag^+ ions to silver nanoparticles (43,44). Besides the other methods, scanning electron micrographs showed the formation of silver nanoparticles from this plant extract with sizes about ~ 50 nm. Previous observations illustrated that reaction time and metal ion concentration effect on size and optical properties of nanostructures. Also, increasing the concentration of the extract can lead to the formation of anisotropic particles (45,46). In general, it can be stated that the size and shape of the nanoparticles formed due to interactions between the biomolecules (phenols and phenolic acids) of the extract with metal ions. The EDX (47) proved the synthesis of silver with a single large peak. Finally, the result of XRD pattern confirmed the presence and crystalline structure of AgNPs, in comparison with standard authenticated [48]. On the other hand, plants are rich in natural antioxidants. Due to the presence of groups such as flavonoids, the plants can inhibit free radical action and play important roles in the pharmaceutical and food industries. Nowadays, usage of these natural antioxidants, especially as a preservative of oils, has been considered. In the pharmaceutical industry, these natural antioxidants are also used to produce anticancer drugs. In laboratories, several methods have been reported to detect the antioxidant activity of plants [49]. In this study, DPPH analysis was used to illustrate the ability of the components of *B.persicum* extract to act as donors of hydrogen atoms [50]. As a result, the radical scavenging activity of the seed plant extract increased with increased concentration which proves the high antioxidant activity of the plant. Microwave-assisted hydrodistillation was carried out on the essential oil of *B.persicum*, and this confirmed the same result [51]. This antioxidant activity is due to the presence of phenolic groups that have the ability to donate electrons and recover metal particles and form nanoparticles [52].

CONCLUSION

This study revealed the seed extract of *B.persicum* has a high antioxidant effect and can be used as a preservative for oils in the food industry. Additionally, this study showed that antioxidant activity of seed plant rescued silver ions to silver nanoparticles. Green synthesis of silver nanoparticles as an eco-friendly method could be a suitable alternative to current physical and chemical methods, especially in the medical sphere. In this study, spherical silver nanoparticles of a homogeneous were produced.

Due to the healing properties and the anti-infectious of *B.persicum* seeds, silver nanoparticles have the potential utilized in the pharmaceutical industry. *B.persicum* is a local plant and grows in many areas of Iran and its use as a powerful antioxidant is recommended.

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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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