RESEARCH ARTICLE

Biosynthesis of silver nanoparticles by Achillea *eriophora* DC. extract

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ABSTRACT

Nanoparticles have a wide range of applications due to their unique biological and physicochemical properties. Among metal nanoparticles, the silver nanoparticles are of special importance due to their wide application. Although there are several chemical and physical methods for the synthesis of silver nanoparticles, biological methods are more suitable due to their time and low energy, non-use of toxic solvents and biocompatibility. In this study, aqueous extract of dried flowers of *Achillea eriophora* (Shirazi yarrow) was used as a reducing agent for the synthesis of silver nanoparticles. The reaction was performed at room temperature and showed a change in color from pale yellow to dark brown to form silver nanoparticles. UV-Vis spectroscopy, scanning electron microscopy (SEM), Fourier transforms infrared spectroscopy (FTIR) and particle sizing (PSA) were used to evaluate the physicochemical properties of the formed nanoparticles. The presence of an absorption peak at a wavelength of about 450 nm confirmed the formation of silver nanoparticles. SEM results showed that, the shape of particles was spherical and their particle size ranges were from 38 to 144 nm. FTIR results also showed the role of reducing groups on the surface of nanoparticles. The results of PSA showed that the particles have low polydispersity and the extract is desirable for the synthesis of silver nanoparticles. Based on these results, it can be said that the synthesis of silver nanoparticles by Shirazi yarrow extract is a simple, fast, non-toxic and biocompatible method and can be used in food, medicine and agriculture.

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INTRODUCTION

Today, nanotechnology is one of the most exciting and emerging disciplines in science and research, which is growing rapidly due to its widespread use in science and industry. One of the important issues in nanotechnology research is the synthesis of different nanoparticles with particle sizes less than 100 nm. The unique optical and catalytic properties of these nanoparticles have made it possible to have a wide range of applications in the chemical, medical, pharmaceutical, electronic, and agricultural industries. One of the most popular metallic nanoparticles is silver nanoparticles (AgNPs), which is widely used in various fields due to their unique physical and chemical properties [1-4]. These nanoparticles are harmless to humans, but at low concentrations have beneficial effects on bacteria, viruses, other microorganisms and can use for degradation of chemical pollutants [5-7]. Because of their widespread use of silver nanoparticles, various methods for its synthesis have been reported. Particle size is one of the factors considered in these methods. Therefore, the smaller the particle size and the smaller their accumulation, the designed method will be the more desirable [6]. Today, researchers are developing new methods for synthesizing high amounts of silver nanoparticles. So far, various types of chemical and physical

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methods have been used to synthesize silver nanoparticles, but because of the high amounts of toxic substances, high temperatures, high costs, time-consuming and potential environmental hazards are not suitable [8-10]. Hence, the need for high-efficiency, low-cost, biocompatible and non-toxic methods are increasing. One of these methods is the production of nanoparticles in a biological way [11,12]. Biological methods, such as the use of bacteria, fungi, proteins, nucleic acids and plants are used to make silver nanoparticles because they are simple, non-toxic, cost-effective and biocompatible [13]. In the meantime, the use of plants or their extracts is more beneficial for the biological synthesis of nanoparticles extracellular. Phytochemical compounds in plants can play an important role in the regeneration and stabilization of nanoparticles. The synthesis of metal nanoparticles using plant extracts can be used as an economical and valuable method for the production of large-scale metal nanoparticles [14].

In recent years, several plants have been successfully used for the rapid and efficient extracellular synthesis of gold, silver, copper and other nanoparticles. Extract of pine, persimmon, magnolia, geranium and alfalfa plants are used to successfully synthesize silver nanoparticles. Results show that, the high stability of the silver nanoparticles synthesized by this method, and the biomolecules in the extract, which include alkaloids, alcoholic compounds and polysaccharides, were considered as agents for the reduction factor of silver ions [15].

Heidari et al. was prepared the green synthesis of silver nanoparticles by the aqueous extract of rosemary and extracted the spherical nanoparticles with a particle size range between 20-40 nm [16]. Silver nanoparticles using an aqueous extract of *Camellia sinensis* were synthesized by Etemadi et al. [17] and Kavoosi and Yaghoubi also reported the preparation of silver nanoparticles by using extracts *of Origanum majorana* [18]. Shokri reported degradation of 4-Nitrophenol from industrial wastewater by nano catalytic ozonation [19]. Cadmium oxide nanoparticle was extracted by using *Achillea wilhelmsii* flower which described by Karimi et al. Regarding to their studied, some compounds such as tannins, flavonoids, alkaloids, and carotenoids existing in the extract were responsible for the reduction of cadmium oxide ions and the stability of nanoparticles [20]. In addition, the flowers of this species have also been used to produce successful gold nanoparticles [21]. Kazemivash et al. studied green synthesis of silver nanoparticles by using of *Bunium persicum* seed extract. The results showed that the extract of *Bunium* seed for preparation of AgNPs that were almost spherical in shape with an average diameter of 51nm [22].

Achillea is a plant from *Asteraceae* that has many medicinal and therapeutic properties which contains of flavonoids, alkaloids and sesquiterpen lactones, phenolic and polyphenolic compounds and for treating of hypoglycemic, nerve tonic, anti-hemorrhoid, anti-diarrhea, antacid, carminative, appetizer, anthelmintic and anti-bacterial remedies is used [23,24]. In this study, aqueous extract of dried flowers of *Achillea eriophora* was used as a reducing agent for the synthesis of Ag nanoparticles for the first time. *Achillea eriophora* is one of the most important species of *Achillea* genus that is endemic of Iran [25] and has higher essential oil content than other species of this genus [26]. Considering that the extract of this genus is rich in antioxidant and reducing agents, therefore, its effect as a safe biological source in reduction of silver ions to nanoparticles can be interesting and important. The aim of this study was to investigate and introduce *Achillea eriophora* as a useful and available bioactive source for the synthesis of silver nanoparticles.

MATERIALS AND METHODS

Preparation of Herbal Extract

Flower shoots of the *Achillea eriophora* were collected in May 2018 from the Bagh e Shadi forests of Khatam city (Yazd province). The plant was identified in agricultural college of Maybod Branch, Islamic Azad University, Yazd. The flower branches were dried in shade. For extract preparation, 2.5 g of flower branches was washed with deionized water and then grinded and extracted with 100 ml of distilled water with 5 min boiling. The extract was filtered after cooling.

Synthesis of Silver Nanoparticles

In order to synthesis of silver nanoparticles, $AgNO_3$ solution of 20 mM concentration was used. Therefore, 10 ml of prepared extract was added to 90 ml of $AgNO_3$ solution at room temperature and caused to change the color of the extract from pale yellow to brown which indicated the production of AgNPs [20].

Characterization of Silver Nanoparticles

UV-Vis Spectroscopy

In UV-Vis Spectroscopy method, 200 μL of obtaining solution was diluted to 1mL with deionized water. A Philips spectrophotometer (Model: PU8625u/vis) was used to measure the optical density of solutions in a range of 400-600 nm.

Scanning Electron Microscopy

Morphology and particle size of AgNPs was determined by Scanning Electron Microscope (Model: Phenom Pro X). To do this, the resulting solution was centrifuged three times at a speed of 12,000 rpm and the resulting sediment was photographed.

Infrared Spectroscopy (FT-IR)

FT-IR technique was used to study the functional groups involved in the synthesis and reduction of nanoparticles. For FT-IR, the obtained solution was centrifuged at 12,000 rpm for 10 minutes, and after dispersing the top solution, the resulting sediment was washed with deionized water and dried in an oven at 60 ° C for 1 hr. The resulting powder was analyzed with FT-IR (Shimadzu, Model: IR Prestige-21) in the mid IR region of 500-4000 cm^{-1}.

Particle Size Analyzer

Typical applications of dynamic light scattering analyzer are the characterization of particles, emulsions or molecules, which have been dispersed or dissolved in a liquid. Determination of the sizes of metallic nanoparticles or quantum dots and analysis of estimate population of aggregates large and small, for small molecules are two applications of this product.

To prepare the sample, 1 mL of AgNPs solution was diluted in 10 ml with distilled water and analyzed by the Particle Size Analyzer (PSA) (Model: 90Plus/BI-MAS) based on the Dynamic Light Scattering (DLS) assay.

RESULTS AND DISCUSSION

Test of changing the color of the solution

Fig.1 shows the color of solution from pale yellow to dark brown after adding the silver nitrate solution to the extract solution at room temperature, over a period of 60 minutes. As it is evident in the Fig.2, at first, the change in color

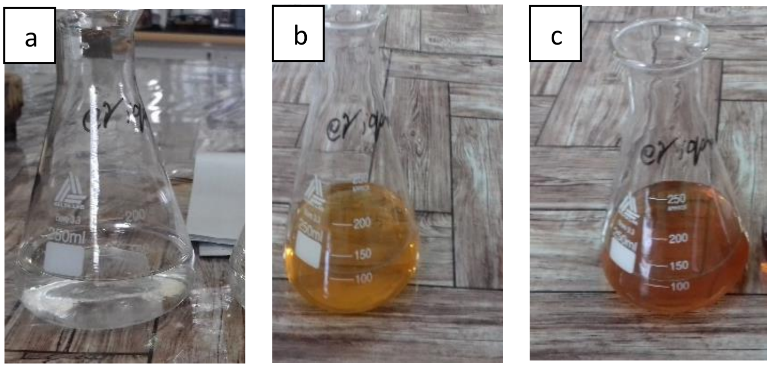


Fig. 1. The color change of the solution obtained from the interaction of the extract and the silver nitrate: (a) $AgNO_3$ solution before adding to the extract; (b) changing the color of the solution obtained by adding silver nitrate to the herbal extract after 30 minutes (c) Change the color of the solution after 60 minutes

Fig. 2. The color change of the solution obtained by the interaction of the extract and the silver nitrate after 2 hours.

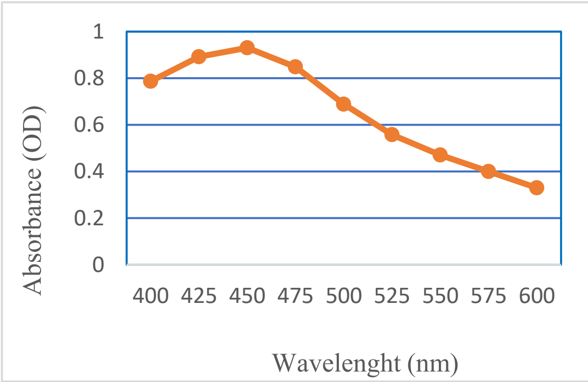


Fig. 3. Spectrophotometric analysis of Ag nanoparticles produced by *Achillea eriophora*

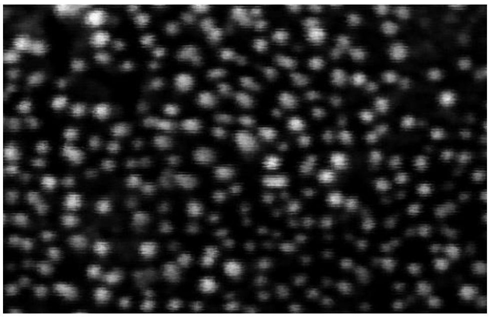


Fig. 4. SEM image of synthesized nanoparticles by the *A. eriophora* extract with a spherical shape and particle sizes between 38-144 nm

was slowly and after two hours the color of extract completely changed. This color changing reflected the biosynthesis of silver nanoparticles. This was similar to the results of Ramteke et al. [27] and Sivaraman et al. [28]. According to research on yarrow, it could be said that there might be diversity in the compounds in yarrow extract, including proteins, flavonoids [21], terpenes, water-soluble heterocyclic compounds such as alkaloids [24] Which are strong antioxidants and have a high potential for reducing metal ions [29], which made the extract of this plant could act as a good reducing agent and in a relatively short time which reduced silver ions and the production of silver nanoparticles. The color change observed in the solution is a clear sign and the first sign of the formation of silver nanoparticles in the reaction [30] and this color change was owing to surface Plasmon vibrations in the nanoparticles [31].

Physical and chemical properties

Results of spectrophotometric analysis

After observing the color change of the solution, spectrophotometric method was used to confirm the synthesis of silver nanoparticles by the extract of *Achillea eriophora*. Silver nanoparticles, depending on the method of preparation, the size and shape of the produced particles have an absorption strip of 380 to 450 nm. The findings from the Uv-Vis spectrum showed that, λ_{max} was about 450 nm and confirmed the formation of Ag nanoparticles (Fig. 3). Other experiments have shown that absorption at wavelengths between 400-450 nm indicates the production of silver nanoparticles [32].

Morphological analysis by scanning electron microscopy (SEM)

Scanning electron micrographs indicated a high concentration of synthesized silver nanoparticles by the plant extract, and the shape of the nanoparticles was almost spherical without uniform dispersion (Fig.4). According to the results of SEM images, silver nanoparticles had a particle size ranges from 38 to 144 nm, which is similar with the results of other studies. Kim and Song obtained different results in their research on the synthesis of nanoparticles by five plants; they obtained nanoparticles with dimensions of 15 to 500 nanometers [33]. It should

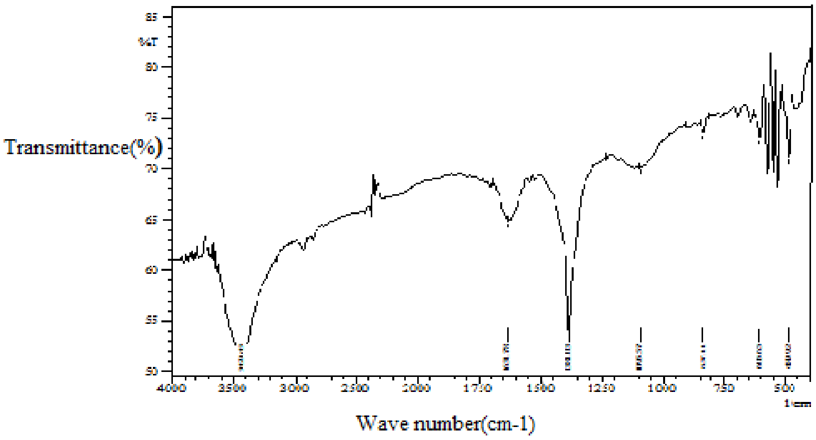


Fig. 5. The FTIR absorption spectra of Ag nanoparticlesby *Achillea eriophora* extract

be noted, that parameters such as pH, temperature, time, and various concentrations of silver salt as well as the extract could affect the size variation of nanoparticles produced [34]. Increasing the concentration of the extract can cause the formation of anisotropic particles [35]. In general, it can be said that the shape and size of the nanoparticles formed depend on the interaction of biomolecules in the extract with metal ions.

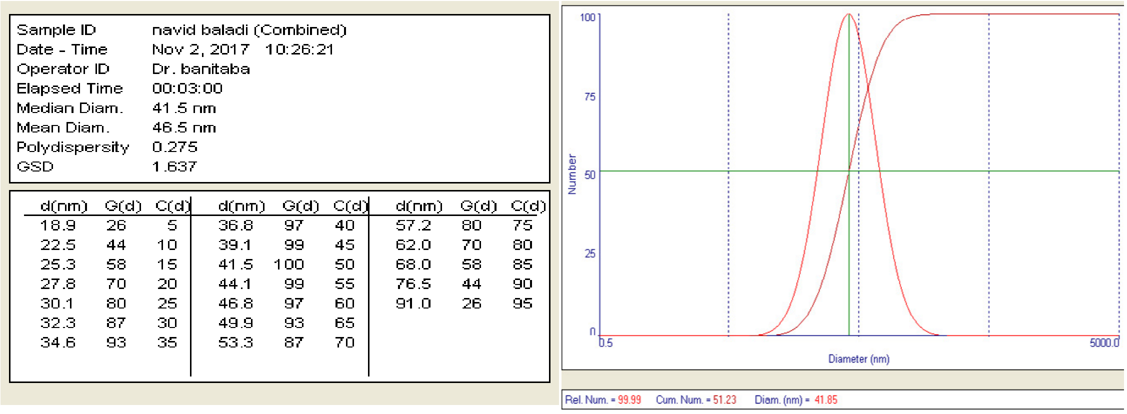
Infrared Spectroscopy (FT-IR)

FT-IR spectra provided the information on the groups involved in the reaction of silver ion reduction and stability of nanoparticles. Fig.4 shows the FT-IR spectra of synthesized nanoparticles in the wave number of 500 to 4000 cm^{-1}. The FT-IR spectra showed that, the clear absorption peaks at 3429.43, 1631.78, 1381.03, 1095.57, 837.11 and 605.65 cm^{-1}, respectively. The absorption band at wave number of 3429.43 cm^{-1} is related to N-H groups which are due to the peptide bands of the proteins presented in the extract. A specific absorption band in the wave number of 1631.78 cm^{-1} could be attributed to the C=C alkenes. The observed spectrum in 1381.03 cm^{-1} is related to the C-H alkanes groups. The detected band in 1095 cm^{-1} is attributed to the stretching of the C-O in the ether joints. The observed peaks at 837 and 605 cm^{-1} are associated to the C=C alkenes group and C-H bending in the aromatic compounds, respectively. All of these compounds are involved in the reduction of silver ions and converted them into silver nanoparticles.

Based on other studies in the field of biological synthesis of nanoparticles by plants, the plant can play a role in restoring metal ions and stabilizing these nanoparticles [36]. In addition, the studies have shown that, biological molecules such as phenol and flavonoids, and proteins existing in plant extracts play an important role in reducing ions and turning them into metal nanoparticles [37]. Khan and Ahmad prepared gold nanoparticles with purified protein, which confirmed the effect of proteins and amino acids present in the plant extract in the synthesis of nanoparticles [38]. Previously, Bankar et al reported the effects of proteins on the synthesis of nanoparticles and demonstrated the role of proteins in the process of synthesizing nanoparticles [39]. Filippo et al. reported the effect of carbohydrates such as glucose and maltose on ion reduction [40] and Nadagouda et al. reported the effect of flavonoids on the synthesis of nanoparticles [41]. Based on the above studies, it could be stated that the various compounds existing in the extract play a major role in this field. As previously mentioned, Yarrow's plant contained many natural compounds such as antioxidant compounds, phenols, flavonoids and terpenes. In the present study, the results of IR spectra showed specific peaks of amides related to proteins. Peaks with aromatic rings and ether connections were also visible. On this basis, it could be stated that flavonoids, terpenes and aromatic compounds present in the extract were responsible for the reduction of silver ions and the stability of silver nanoparticles.

Investigating the distribution of nanoparticle diameter and size

Dynamic light scattering is a physical method



Sample ID	navid baladi (Combined)
Date - Time	Nov 2, 2017 10:26:21
Operator ID	Dr. banitaba
Elapsed Time	00:03:00
Median Diam.	41.5 nm
Mean Diam.	46.5 nm
Polydispersity	0.275
GSD	1.637

d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)	d(nm)	G(d)	C(d)
18.9	26	5	36.8	97	40	57.2	80	75
22.5	44	10	39.1	99	45	62.0	70	80
25.3	58	15	41.5	100	50	68.0	58	85
27.8	70	20	44.1	99	55	76.5	44	90
30.1	80	25	46.8	97	60	91.0	26	95
32.3	87	30	49.9	93	65			
34.6	93	35	53.3	87	70			

Fig. 6. Particle size distribution and particle diameter diagram for synthesized silver nanoparticles by *Achillea eriophoria* extract in terms of number of particles

used to determine the distribution of particles in solutions and suspensions. The graphs obtained from the particle size analysis are shown in Fig. 5. As it is shown in the Fig.5, the average particle diameter was equal to 41.5 nm, which was equivalent to the results of the SEM microscope images with nanoparticle size in the range of 38-144 nm. The polydispersity of particles was about 0.275 which identified the homogeneity of the synthesized nanoparticles and indicated the extract was desirable for the synthesis of silver nanoparticles.

CONCLUSION

The present study is conducted an easy, cost-effective, safe and single-phase bioassay of silver nanoparticles using the aqueous extract of Shirazi Yarrow's flowers. In this study, silver nanoparticles were synthesized extracellularly by yarrow extract at room temperature. Analysis and evaluation of the results showed that, the compounds in the extract, such as terpenes, flavonoids and aromatic compounds contributed to the reduction of silver metal ions and eventually stabilized the synthesized nanoparticles. Therefore, it can be concluded that the amount of these compounds along with the reductive and antioxidant properties of plant extract for the synthesis of desirable nanoparticles, is important and necessary. The results of spectrophotometric analysis and physicochemical properties showed that, the Yarrow's plant had a high potential for the reduction of free radicals and metal ions. In addition, considering the safety and environmental aspects, produced nanoparticles by this method had the potential to be used in industries related to human health such as nutrition and healthcare as a result of the non-use of hazardous chemicals. The results of this study show that the uses of *Achillea eriophora* extract as a simple, fast, low cost and without the need to use environmentally harmful substances, is a good option for the production of silver nanoparticles.

CONFLICT OF INTEREST

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

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