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ORIGINAL ARTICLE

Green Synthesis of Silver Nanoparticles Using *Pimpinella anisum* L. Seed Aqueous Extract and Its Antioxidant Activity

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KEYWORDS

Pimpinella anisum L.;
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ABSTRACT: An aqueous extract of *Pimpinella anisum* was used for green synthesis of silver nanoparticles by bio reduction of an aqueous solution of silver nitrate. Silver nanoparticles were characterized by UV–Vis spectrometry, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) analysis, scanning electron microscopy (SEM) and energy-dispersive X-ray analysis (EDAX). The increase in absorption at 420 nm was used for recording the formation of a colloidal suspension of silver nanoparticles. The binding properties of the capped Ag nanoparticles synthesized from aqueous extract of *P. anisum* were analyzed by FTIR. XRD studies revealed that most of the nanoparticles were cubic and face centered cubic in shape. SEM analysis showed the size and shape of silver nanoparticles and EDAX confirmed the presence of silver. The synthesized silver nanoparticles showed DPPH free radical scavenging activity.

INTRODUCTION

Pimpinella anisum L. is an annual medicinal plant belonging to the Apiaceae family. This species is widely distributed and is found in Europe, Turkey, Iran, the Caucasus, Central Asia, Syria and Egypt. The essential oil and seeds of *P. anisum* have been in Iranian traditional medicine for many purposes [1, 2].

In recent years many researchers have investigated the use of plant extracts for the green synthesis of metal nanoparticles. The synthesis of silver nanoparticles using plant extracts has been the subject of several recent studies because of the relationship to the biological activities of the plants [3-10]. Among many methods for synthesis of silver nanoparticles the green approach has been considered because of use of environmentally safe materials [11, 12]. Living plants due to antioxidant properties of their natural compounds

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are preferred for biosynthesis of silver nanoparticles [13, 14].

This study reports a simple, eco-friendly and economical method to synthesize silver nanoparticles using aqueous extracts obtained from seeds of *P. anisum*. The prepared nanoparticles were characterized by instrumental methods (UV-VIS absorption, FTIR, XRD, SEM, EDAX, and TEM). The antioxidant properties of these nanoparticles were evaluated using DPPH assay.

MATERIALS AND METHODS

All analytical reagents used in the study were of analytical grade and were purchased from Merck, Germany.

Collection of plant material

Seeds of *P. anisum* L. were obtained from a local market in Sabzevar, Iran.

Preparation of plant extract

The dried seeds of *P. anisum* were thoroughly ground to a powder. Then 10 g of dried powder was mixed with 100 mL of distilled water and the mixture was extracted with ultrasound probe (Hielscher ultrasonic processor, Model UP 50H, Germany) for30 minutes with pulsed sonication at 0.5 duty cycles, 100% of the available amplitude, room temperature and constant frequency 30 kHz. Then the extract was filtered with Whatman filter paper (No.1) and the filtrate was collected and stored at 4°C for further study. Ultrasound-assisted extraction is a proven method for the extraction of intracellular material, functional, and bioactive components. Ultrasound increases the extraction yield significantly and reduces the processing time considerably [15].

Preparation of silver nanoparticles

P. anisum aqueous extract (10 mL) was mixed with 90 mL of 1 mM$AgNO_3$ aqueous solution in an Erlenmeyer flask. The reaction mixture was heated at 80 °C for one hour. The yellow-brown colored solution changed into dark brown, indicative of the reduction of Ag^+ ions to metallic Ag.

Characterization of silver nanoparticles by instruments

FTIR spectra were obtained using a Perkin-Elmer Model 10.02.00 instrument. XRD measurements were made on a BrukerD8 Advance instrument using Cu K(α) radiation(λ = 0.15406 nm) at an operating voltage of 45 kV and a current of 40 mA on a glass slide drop coated with the nanoparticle solution. Nanoparticles on specimen stubs were visualized by scanning electron microscopy (SEM; VEGA3 TESCAN, Czech Republic), and elemental analysis (EDAX) was accomplished using a Sirius SD EDS attachment. Transmission electron microscopy (TEM; CM120 Philips, Netherlands) shows morphology and the size range of nanoparticles.

Antioxidant assay

The antioxidant activity of the silver nanoparticles was measured using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) [16]. Briefly, 1 mL of of DPPH_solution (0.1 mM in methanol) was added to 1.5 mL of nanoparticle solutions of different concentrations (20, 40, 60 and 80 µg/mL). The same process was repeated for synthetic antioxidant butylatedhydroxytoluene (BHT) aqueous solution as standard and positive control. All test tubes were shaken and allowed to stand for 30 min in the dark at room temperature and then the absorbance was measured at 517 nm against a blank (without nanoparticle solution). The assays were carried out in triplicate.

RESULTS AND DISCUSSIONS

Formation of silver nanoparticles

The reduction of silver ions by an aqueous extract of *P. anisum* to silver nanoparticles was followed by recording the absorption spectra of the synthesized silver nanoparticles against a deionized water blank as a function of time by periodic sampling of 0.2-mL aliquots diluted with 2 mL deionized water (Figure 1).

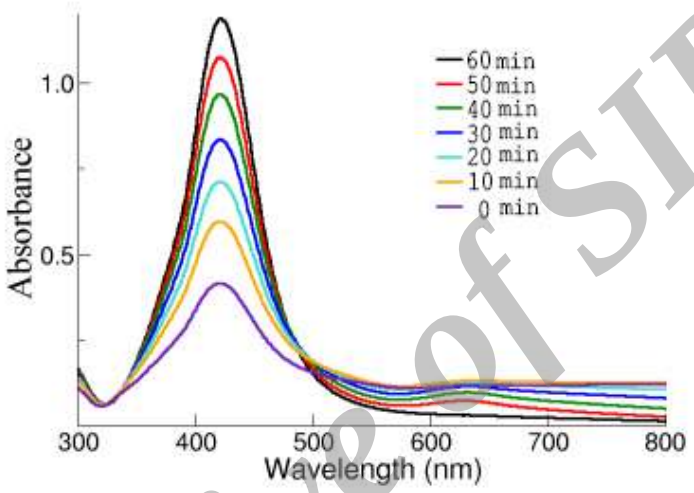

Figure 1. UV–Vis absorption spectrum of silver nanoparticles formed by reduction of 1 mM aqueous silver nitrate at 10-min intervals

FTIR analysis of silver nanoparticles

The binding properties of Ag nanoparticles synthesized from aqueous extract of *P. anisum* seeds were investigated by Perkin Elmer Spectrum (version 10.02.00) with wavenumber range of 400-4000 cm^{-1}. Dried and powdered Ag nanoparticles were mixed with potassium bromide to produce pellets and the result was FTIR spectra (Figure 2) of silver NPS synthesized from *P. anisum* seed aqueous extract contain a broad peak at 3402.32 due to the hydrogen bonded hydroxyl group. The peak at 1642.82 cm-1 represents the C=O group. FTIR spectra confirm that the silver ions to silver nano particles were due to the bioreduction of capping materials of *P. anisum* seed extract.

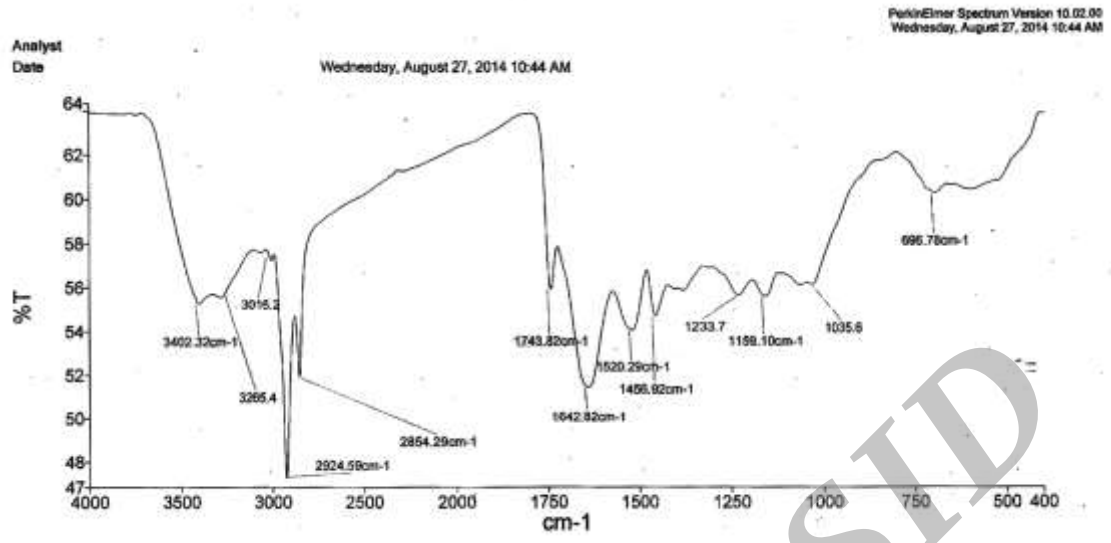

Figure 2. FTIR spectrum of the silver nanoparticles

XRD

The silver nano particles synthesized by *P. anisum* seed extract were analyzed by X-ray diffraction (Figure 3).Full width at half maximum (FWHM) data was used according to the formula of Scherer to determine mean particle size. The equation is as follows: $d = 0.9 \lambda / \beta \cos\theta$ Where d is crystallite size of AgNPs particles, λ is wavelength of X-ray radiation source, β is the angular FWHM of the XRD peak at the diffraction Bragg angle θ.

The XRD Spectrum (Figure 3) showed four diffraction peaks at 38.155, 46.304, 64.585 and 77.182, which are indexed as (111), (200), (220) and (311) crystalline planes of nano silver. The XRD pattern clearly showed that the Ag NPs are crystalline in nature.

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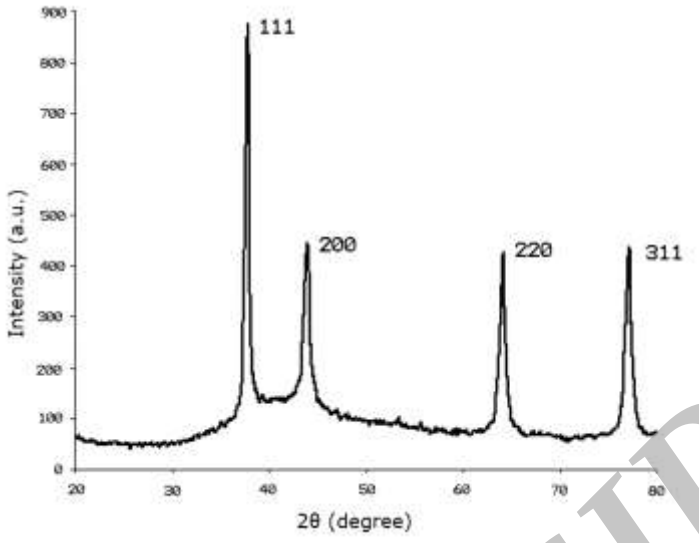

Figure 3. XRD pattern of the silver nanoparticles

SEM and EDAX

The silver nano particles produced by reduction of $AgNO_3$ by a *Pimpinella anisum* seed aqeous extract were examined using scanning electron microscopy (Figure 4). The images showed relatively uniformshaped nanoparticles. Analysis of the specimen by EDAX showed a peak at 3 keV, which is confirmatory for the presence of silver.

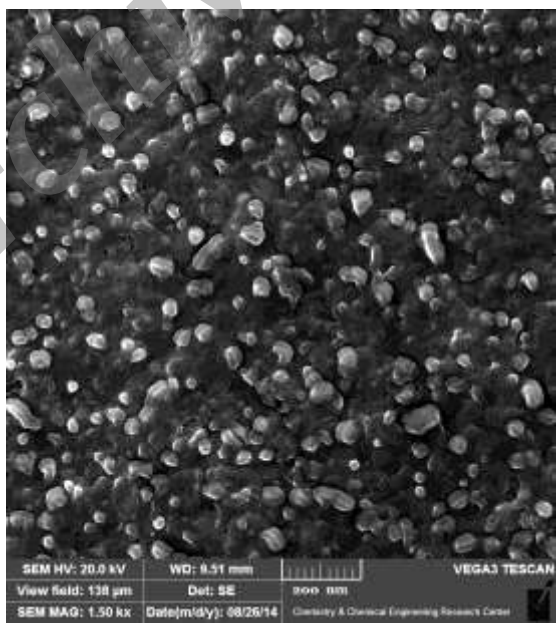

Figure 4. SEM images of silver nanoparticles formed by the reaction of 1 mM silver nitrate and aqueous extract of *P. anisum* seeds

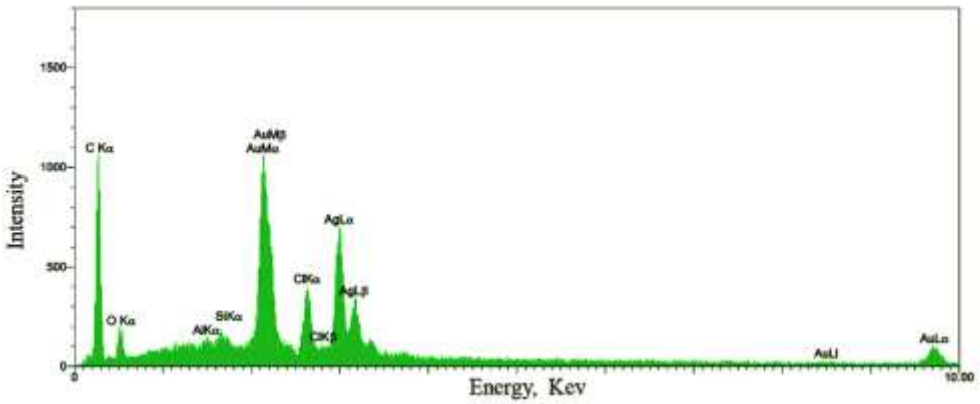

Figure 5. EDAX spectrum of synthesized silver nanoparticles, showing presence of silver

TEM

Size distribution and morphology of silver nanoparticles has been widely studied by transmission electron microscopy (TEM) as a powerful instrument. It shows the major parts of nanoparticles have spherical morphology, in which few nanoparticles were agglomerated(Figure 6, Figure 7). By comparing the size of the scale bar silver nanoparticles were in the range of 20 to 200 nm.

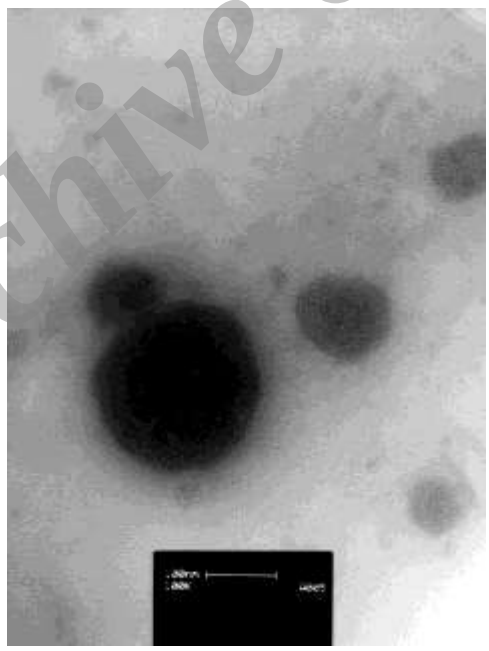

Figure 6. Transmission electron microscope image of silver nanoparticle synthesized by aqueous extract of *Pimpinella anisum* seeds.(Scale bar 100 nm)

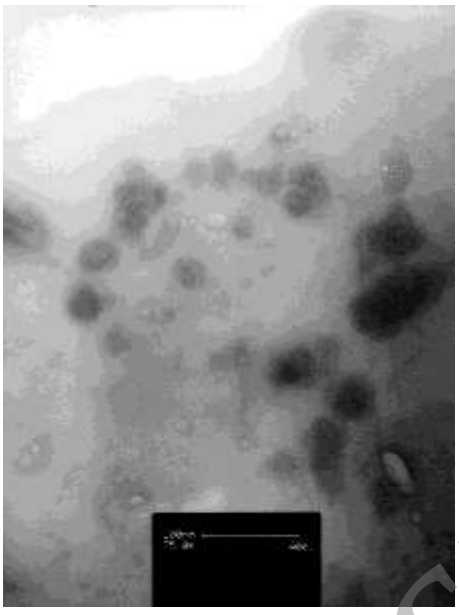

Figure 7. Transmission electron microscope image of silver nanoparticle synthesized byaqueous extract of *Pimpinellaanisum* seeds.(Scale bar 200 nm)

Antioxidant activity

Antiradical activity assay is based on the reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH). Due to the presence of an odd electron in DPPH, solutions of DPPH give a strong absorption maximum at 517 nm. As this electron becomes paired off in the presence of a hydrogen donor, i.e. a free radical scavenging antioxidant, the absorption strength is decreased. The resulting decolorization is directly proportional to the number of electrons captured [17]. In the present work, the antioxidant properties of synthesized nanoparticles were determined using the DPPH free radical scavenging assay method. The percentage inhibition of DPPH Activity is calculated by the following relationship:

Percentage inhibition of DPPH activity = (Abs Blank - Abs Sample/ Abs Blank) x 100

The blank was 1 mL DPPH solution and 1.5 mL methanol.

The absorbance also decreases with increase in concentration of the silver nanoparticles. The results are tabulated (Table 1). In any case, the ability of synthetic antioxidant is higher than silver nanoparticles because of lower IC_{50} values of BHT. Higher IC_{50} means higher consume of extracts for scavenging of 50% of DPPH free radical.

Table 1. Comparison IC_{50} values of silver nanoparticles and BHT

	20 ppm[a]	**40 ppm[a]**	**60 ppm[a]**	**80 ppm[a]**	
Reductant	**Percentage inhibition of DPPH activity**				**IC_{50}**
Silver nanoparticles	23.5±1.2	30.6±1.2	35.7±0.8	48.9±1.6	87.7±1.1
BHT	76.0±4.8	93.8±0.8	94.6±1.1	96.7±0.9	14.9±0.9

[a]Concentration of silver nanoparticles and BHT

CONCLUSIONS

Green synthesis of silver nanoparticles using *P.anisum* seed aqueous extract and ultrasound as extraction method has been reported. Extraction by ultrasound reduced the processing time. Crystalline and spherical shape synthesized silver nanoparticles were prepared at ambient conditions without using any chemicals or surfactants as reducing or capping agents. This green approach is environmentally friendly, low cost and easier than conventional chemical synthesis methods. The silver nanoparticles were characterized by UV-Vis, FTIR, XRD, SEM, EDAX and TEM. Also Ag nanoparticles showed antioxidant activity. This fast and convenient synthesis can be used for large-scale production of other metal nanoparticles and can be valuable in environmental, biotechnological, pharmaceutical and medical applications.

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