Green Synthesis of Silver Nanoparticles Using Parsley Stem: Characterization and Evaluation of Their Antioxidant Properties

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***Abstract* –** In the study, silver nanoparticles (AgNPs) were successfully synthesized by an environmentally friendly synthesis method using parsley stem extract. The synthesized silver nanoparticles were characterized by ultraviolet/visible light absorption spectrophotometer (UV-Vis), X-ray diffraction (XRD), and scanning electron microscopy (SEM) analysis. As a result of the characterization, it was determined that 31 nm spherical nanoparticles were formed, showing a spectrum at ~460 nm wavelength. DPPH and CUPRAC methods were used to examine the antioxidant activities of plant extracts and AgNPs. In DPPH and CUPRAC removal activity, AgNPs obtained by green synthesis provided a high rate of inhibition removal compared to the extract. The characterization of silver nanoparticles synthesized by the green synthesis method and their antioxidant activity were investigated, and the obtained values indicate the presence of an antioxidant capacity.

Keywords –Antioxidant, Green Synthesis, Nanoparticle, Parsley Stem, Petroselinum Crispum

I. Introduction

Nanoparticles (NPs) are defined as particles with an average particle size between 0 and 100 nm. NPs show completely new or improved properties compared to larger particles of the material, and these new properties are achieved due to differences in certain properties such as distribution, particle structure, and size [1]. Silver nanoparticles (AgNPs) find wide application in various fields such as integrated circuits, sensors, biolabeling, filters, antimicrobial deodorant fibers, cell electrodes, low-cost paper batteries (silver nanowires), and antimicrobials. The antimicrobial properties of silver nanoparticles are used in different medical fields, various industries, livestock, packaging, accessories, cosmetics, health, and military applications [2]. AgNPs provide many benefits to pharmaceutical and biomedical applications due to many advantages such as using environmentally friendly materials, avoiding toxic chemicals in synthesis protocols, and being environmentally friendly. In recent years, nanoparticles have been formed mostly from microorganisms [3] and plants [4]. Microbial-derived synthesis procedures have some disadvantages such as strong strain identification, maintenance of aseptic conditions for abundant growth of bacteria, and possibilities of contamination and infection [5]. Also, the synthesis of nanoparticles from microorganisms is relatively expensive and time-consuming for plants [6].

Synthesis conditions are very important as the shape and size of nanoparticles vary according to environmental variables. Therefore, the application areas can be changed according to the shape and size of the nanoparticles. Particle size control during silver nanoparticle synthesis is a very important criterion in the field of biosynthesis. In the last fifteen years, many methods have been developed to control the form and size of particles synthesized in solution media. Scientists have reported the effects of reaction requirements such as temperature, solvent, and silver nitrate concentration on the synthesis rate and particle size of AgNPs [7]. Various studies have been conducted on the synthesis of silver nanoparticles using various plant extracts [8]. Parsley (*Petroselinum crispum*) is widely used as a green vegetable and garnish and is used for medicinal purposes in many countries, especially in cough, gastrointestinal discomfort, dermatitis, exanthema, macula, alphosis, and hemorrhoids. In previous studies, silver [9], zinc [10], and selenium [11]. were used as NPs in parsley. Parsley is a rich source of iron, vitamins (riboflavin, beta-carotene, thiamine, and vitamins E and C), essential oils, and fatty acids. These bioactive compounds function as both reducing and stabilizing agents [12]. It has been determined that the aqueous extract of parsley leaves contains apigenin (4′,5,7,-trihydroxyflavone), cosmosiin (apigenin-7-O-glucoside), oxypeucedanine hydrate (coumarin 2″,3″-dihydroxyfuranocoumarin) [13]. The focus is on the green synthesis of AgNPs, which are easily synthesized, made using renewable and non-toxic reductants, and used for pharmaceutical purposes. Although high amounts are spent each year for the synthesis of substances used as biological agents, green synthesis nanoparticles are an important alternative to powder technology synthesized for pharmaceutical purposes. Since the leaves of parsley are generally used, the stems of this plant are waste. This study aimed to synthesize silver nanoparticles and to determine their characterization and biological activities by using parsley stem extract as a reducing agent for silver ions. Physicochemical characterization of biosynthesized nanoparticles was carried out using UV-Vis, XRD, and SEM technology. The antioxidant activity was investigated using the DPPH free radical capture method and copper ion-reducing antioxidant capacity (CUPRAC).

II. Materials and Method

A. *Preparation of plant extract*

The parsley needed to prepare the extract will be purchased from the grocery store and only the stems are used. To remove the residues and contaminated organic compounds on the stems, they were washed first with running tap water and then twice with distilled water and left to dry at room temperature. The pieces cut to a total of 5 g were taken into a beaker containing 100 ml of distilled water and infused for 30 minutes. The extract was then filtered with No. 1 Whatman No:1 filter paper (0.44 µm) and stored at 4 °C out of sunlight for later use.

B. *Synthesis of silver nanoparticles*

Silver nitrate ($AgNO_3$) solution (5 mM), to be used for the preparation of silver nanoparticles, was obtained by adding 50 mL of distilled water and stirring at room temperature on a magnetic stirrer. For the preparation of silver nanoparticles, which are aimed to be examined, 3 mL of the extract cooled to room temperature was taken and added to 50 mL of 5 mM $AgNO_3$ solution and mixed in a water bath at 85 °C for 45 minutes. In this process, the color change of the transparent colored solution towards dark brown, which is a well-known phenomenon in the literature and is accepted as an indicator of the formation of silver nanoparticles, was observed. The resulting dark solution was centrifuged at 14.000 rpm for 20 minutes to remove the upper liquid phase and the solid part at the bottom was washed 3 times with distilled water. The solid part (silver nanoparticles) obtained will be left to dry in an oven at 65 °C for 24 hours and stored for use in subsequent characterization processes [14].

C. *Characterization of silver nanoparticle*

To determine the time point of the maximum production of silver nanoparticles, absorption spectra of the samples were taken at 300-800 nm using a UV-vis spectrophotometer. The mean particle diameter of silver nanoparticles was calculated from the XRD model. The samples will be air dried and characterized by SEM electron microscopy for their detailed morphology and size. The analyzes were made by the Eastern Anatolia High Technology Center (DAYTAM), which operates within Ataturk University.

D. *DPPH Method*

Free radical scavenging activity of silver nanoparticles and extract synthesized from parsley stem were determined according to the method determined by [15]. using 2,2-diphenyl-1-picrylhydrazil (DPPH) free radical. According to this; The sample solution (1 mL) was added to the DPPH solution (0.267 mM, 4 mL, 0.004% methanol solution). The absorbance of the sample was read at 517 nm after 30 minutes of incubation at room temperature and in the dark. Milligram Trolox equivalent per gram extract (mg TE/g) was used as the unit of measurement.

E. *CUPRAC Test (Copper Ion Reducing Antioxidant Capacity)*

The reducing antioxidant capacity of silver nanoparticles and extract synthesized from parsley stem was determined according to the method specified by [16]. According to this; 0.5 mL of sample, the solution was premixed and added to the reaction mixture containing $CuCl_2$ (1 mL, 10 mL), neocuproin (Sigma-Aldrich) (1 mL, 7.5 mL), and ammonium acetate buffer (1 mL, 1M, pH 7.0). Similarly, a control sample was prepared by adding $CuCl_2$-free sample solution (0.5 mL) to the premixed reaction mixture (3 mL). The absorbance of the control sample was then subtracted from the absorbance of the sample and measured at 450 nm after a 30-minute incubation of the sample and control sample at room temperature. CUPRAC activity is expressed as Trolox equivalent (mg TE/g).

III. RESULTS

SEM Analysis

SEM images of AgNPs obtained from parsley stem by the green synthesis method are given in Figure 1. From the SEM images, it is seen that the particles have different diameters and sizes. The morphology of silver nanoparticles was found to be spherical and their average diameter was 31 nm.

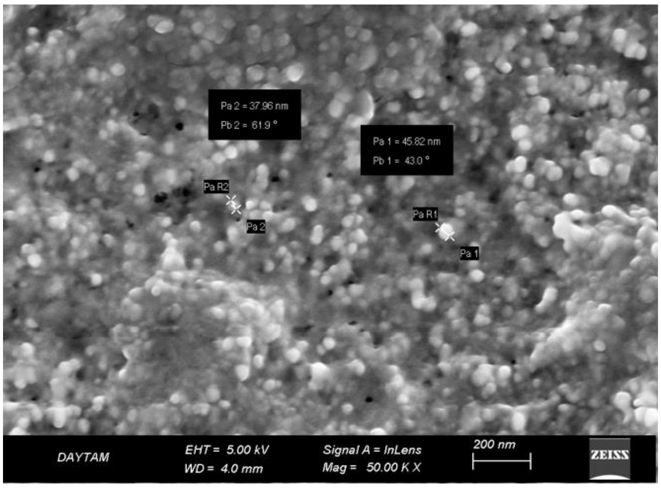

Figure 1. Images of SEM analyzes of synthesized silver nanoparticles

UV-Visible Spectral Analysis

Biosynthesis of AgNPs using parsley stem extract showed yellow to black color change in an aqueous solution. UV-vis spectroscopy analysis revealed that AgNPs peaked at the specific absorbance value of 460 nm.

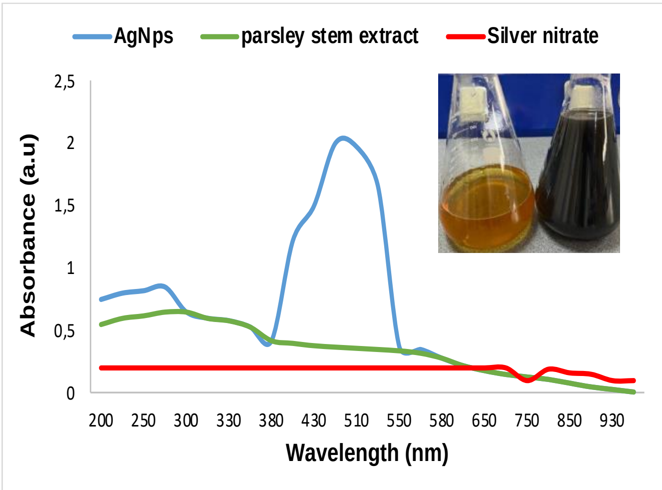

Figure 2. UV-Vis spectrum of synthesized AgNPs and parsley stem

XRD Analysis

In XRD analysis results, peaks of 63°, 81°, 99°, 70°, and 40° overlapping with 27.8, 28.37, 32.16, 46.21, and 54.77, respectively, in 2θ are sharp peaks representing the spherical crystal structure of silver (Figure 3).

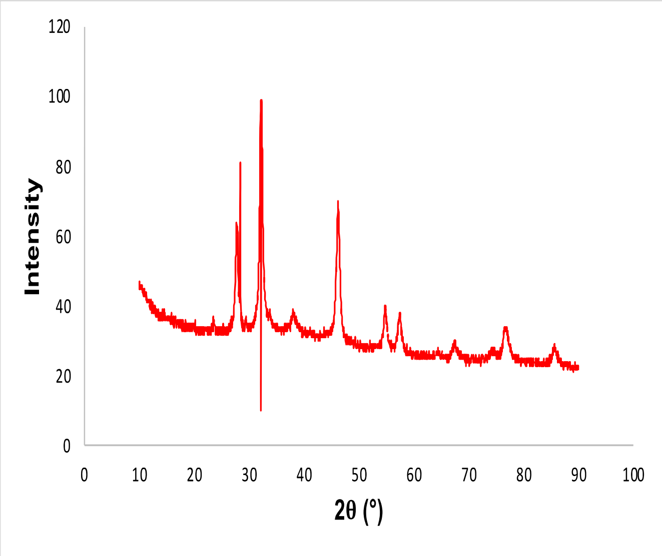

Figure 3. XRD results of synthesized AgNPs

Antioxidant Activity Using DPPH Method

The antioxidant activity of parsley stem aqueous extract and AgNps were compared with the positive control, ascorbic acid (Figure 5). At the highest concentration of 400 µg/mL, DPPH radical scavenging activities were 72% for ascorbic acid, 32% for parsley stem extract, and 48% for AgNps. AgNps synthesized with the green synthesis method provided a high rate of radical removal.

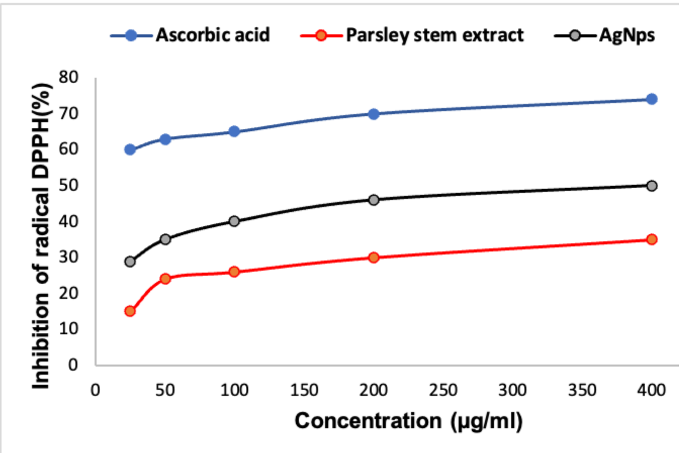

Figure 5. Percentage of inhibition of DPPH free radicals with different concentrations of parsley stem extract, AgNPs, and ascorbic acid

Reduction Potential Using CUPRAC Method

The antioxidant activity of parsley stem aqueous extract and AgNps were compared with the positive control, ascorbic acid. At the highest concentration of 400 µg/mL, reduction potential using the CUPRAC method was 96% for ascorbic acid, 48% for parsley stem extract, and 53% for AgNps. AgNPs had a higher total antioxidant capacity than parsley stem extract (Figure 6). The silver nanoparticle synthesized with the parsley stem extract showed a stronger antioxidant capacity than the extract.

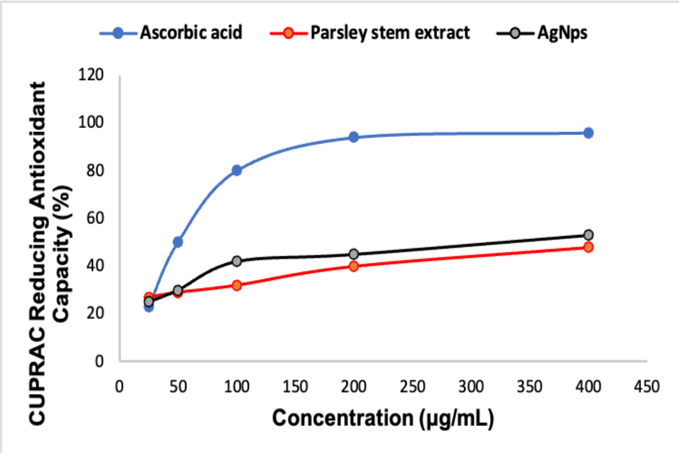

Figure 6. Percentage of inhibition of CUPRAC reducing antioxidant with different concentrations of parsley stem extract, AgNPs, and ascorbic acid

IV. DISCUSSION

AgNPs in spherical morphology have been reported by many different researchers [17]. Similarly, some researchers reported that the absorption spectrum of AgNPs is between 425-461 nm [18]. It shows that the silver ion is reduced to silver nanoparticles when exposed to the bioactive components of the plant extract [19]. Agarwal et al. [20] synthesized AgNPs from *Cymbopogon citratus* and reported that the aforementioned peaks belong to silver. The silver nanoparticle synthesized with the parsley stem extract showed a stronger antioxidant capacity than the extract, and this difference may be due to the chemical structure of the tested sample [19]. Previous studies have shown that the level of antioxidant capacity depends on the plant species and botanical family [21]. The greater surface area-to-volume ratio, which increases the reactivity of AgNPs towards radicals, can be attributed to the increased antioxidant activity of AgNPs [22].

V. CONCLUSION

In this study, AgNPs were synthesized using an environmentally friendly green approach. AgNPs have good antioxidant properties. The green synthesized AgNPs approach is fast, cost-effective, environmentally friendly, non-toxic and suitable for large-scale production. However, more research is needed to demonstrate various biological properties (such as antifungal, antidiabetic, anti-inflammatory) and their mechanisms of action.

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