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Original Article

Total Phenolic and Flavonoids Contents, Radical Scavenging Activity and Green Synthesis of Silver Nanoparticles by *Laurus nobilis* L. Leaves Aqueous Extract

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Abstract

In this project, total phenolic and flavonoid contents of the aqueous extract of *Laurus nobilis* L. leaves were evaluated by colorimetric methods. These natural products which are found in plants extracts, can be considered as reducing and stabilizing agents in the synthesis of metal nanoparticles. Regarding to high amounts of these compounds in the extract, silver nanoparticles were synthesized by aqueous extract of *Laurus nobilis* L. leaves through a simple and eco-friendly route. Characterizations of nanoparticles were evaluated by using Ultra Violet-Visible spectroscopy (UV-Vis), Fourier Transform Infra-Red spectroscopy (FT-IR), X-Ray Diffraction analysis (XRD), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). XRD analysis confirmed the crystalline nature of nanoparticles and the average size of synthesized silver nanoparticles were found 19.65±13.49 (nm) by TEM analysis. Radical scavenging activity of the extract and silver nanoparticles were also evaluated by DPPH (1,1-Diphenyl-2-picrylhedrazyl) assay. The extract showed the best results in comparison with silver nanoparticles and BHT (Butylated Hydroxyl Toluene) as a reference antioxidant.

Keywords: *Laurus nobilis* L., Phenolic and flavonoid contents, Silver nanoparticles, Radical scavenging activity

Introduction

Laurus nobilis L. (Family *Lauraceae*) commonly known as Bay, being a native plant from the Mediterranean region which is traditionally used in folk medicine and as a spice. The extracts of *L. nobilis* have been subjected for evaluating many biological activities. The leaves extracts are used to suppress the high blood sugar; fungal and antimicrobial infections; to treat eructation, flatulence and gastrointestinal problems. Anti-inflammatory, anticonvulsive, antiepileptic and antioxidant properties have also been evaluated for the plant extracts [1-6].

In the recent decades, the use of biological systems like plants extracts for reducing metal salts to metal nanoparticles has attracted considerable attention. Plant extracts can act as both reducing agent and stabilizer in the synthesis of nanoparticles [7]. The bio reduction process is actually complex, but it is believed that the plants extracts include natural compounds involving the reducing agents which can be responsible for the bio reduction procedure [8-10]. The compounds like proteins, sugars, flavonoids, etc. have been reported as the molecules which may contribute in biochemical pathways leading to the biosynthesis of noble metal nanoparticles [11-13].

Among the metallic nanoparticles, silver has been enormously utilized for its high antimicrobial, antioxidant, cytotoxic, and catalytic properties [14-19]. Many techniques of synthesizing Silver nanoparticles (Ag NPs) have been reported in the literature [20-23], but they are always expensive and involve the use of toxic and hazardous chemicals. Therefore, the use of biological systems

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like plants extracts can be considered as environmentally friendly processes for nanoparticle synthesis without using toxic chemicals. The advantage of using plants which make it suitable for large-scale nanoparticles synthesis is that they are easily available, safe to handle, and possess a broad variability of metabolites that may aid in reduction.

This study investigates a simple, rapid, efficient and sustainable route of Ag NPs preparation by aqueous extract of *L. nobilis* leaves and evaluates total phenolic and flavonoid contents of the extract as the potent compounds which may be responsible for the reduction of silver ion to Ag NPs. Radical scavenging activity of the extract and synthesized Ag NPs were also evaluated in this project. The green synthesis of Ag NPs by *L. nobilis* extract has not been reported before.

Material and Methods

Chemicals**:** All the chemicals and solvents used in this study were purchased from Merck Company (Germany).

Preparation of the extract: The leaves of *Laurus nobilis* L. were collected from seaside area of Roudsar in north of Iran on July 2015. The leaves were well washed and dried in shade. Then 20 g of dried leaves were powdered and soaked in de-ionized water (200 mL) and boiled for 10 min. After filtration, the extract was centrifuged (EBA 20 Hettich, Germany) at 4000 rpm for 15 minutes to remove the plant residue and impurities and kept in a dark bottle at 4°C for further uses.

Determination of Total phenolic content: The total phenolic content in the aqueous extract of *L. nobilis* was determined using Folin-Ciocaletu reagent which was explained by Oliveira *et al*. previously [24]. The amount of total phenolic compounds was expressed in terms of Gallic acid equivalent (mg/L of the extract, and or mg/g of dried plant material), using a regression equation that was obtained from Gallic acid calibration curve ($Y = 0.0105X + 0.0138, R^2 = 0.9955$). Experiment was performed in triplicate and expressed as mean±Standard Deviation (SD).

Determination of Total flavonoids content: Total flavonoid content was determined by aluminum chloride colorimetric method which is based on the formation of a complex flavonoid-aluminum having the maximum absorption at 415 nm. The procedure is explained by Chang *et al*. previously [25]. The calibration curve was plotted for quercetin and a regression equation was obtained ($Y = 0.0673X + 0.0051, R^2 = 0.9961$). Flavonoids content of the extract was expressed in terms of quercetin equivalent (mg/L of the extract, and or mg/g of dried plant material). Experiment was performed in triplicate and expressed as mean±Standard Deviation (SD).

Synthesis of silver nanoparticles: A 50 mL solution of (0.01 M) $AgNO_3$ was gradually added to 10 mL of the aqueous extract of *L. nobilis* leaves, while the mixture was kept in an ultrasonic apparatus (S15H, Germany). Then the mixture was stirred in a magnetic stirrer (500 rpm) at room temperature. The solution turned light yellow after 20 min and gradually a dark brown color appeared, which confirmed the formation of Ag NPs. The mixture was filtered after 4 hours and was washed several times by de-ionized water. Finally, synthesized silver nanoparticles were dried at room temperature for 24 hours and kept for further evaluations.

Characterization of Silver Nanoparticles: The structure and size of the synthesized Ag NPs was studied by different methods. UV-Visible spectroscope (Bio-Tek, Shimabzu, UV 1800) was used for monitoring the synthesis of nanoparticles. 1 mL of the suspension was collected from the purified sample at the end of reaction, and then was sonicated at 4000 rpm for 15 min. The UV-Vis spectra was recorded over the 200-800 nm range. FT-IR analysis of the dried Ag NPs was carried out by KBr pellet method using FT-IR spectroscope (Perkin-Elmer, Spectrum100, Germany); The phase structure and material identification of Ag NPs was studied using the X-ray diffractometer (PANalytical X'Pert Pro MPD). The SEM and TEM techniques were used to visualize the size and morphology of the Ag NPs. Scanning electron microscopic (SEM) analysis was carried out using (ZEISS, SIGMA VP-500, Germany). Thin film of the sample was prepared on a carbon coated copper grid by dropping the sample on it and then the film was dried under mercury lamp. Transmission electron microscope (EM10C-100 KV, Zeiss, Germany) was used for TEM analysis. TEM grid was prepared by placing 5µL of the sample on carbon-coated copper grid and drying under mercury lamp.

Radical scavenging activity: A solution of DPPH (1,1-Diphenyl-2-picrylhedrazyl) in methanol

(40µg/mL) was prepared freshly. Then, 2.5 µL of the solution was added to 10µL of the samples (the extract, suspension of silver nanoparticles and BHT) separately. The mixtures were incubated in dark for 30 min. Finally, the absorbance of the samples, was taken by the spectrophotometer at 517 nm [26]. For calculating the percentage of radical scavenging activity, the following equation: $\%I = 100[\frac{A_C - A_S}{A_C}]$ was used. In the equation, A_s is the absorbance of sample and A_c is the absorbance of control (containing all of the reagents without sample). The average of two independent experiments was expressed as the result.

Result and Discussion

Total phenolic and flavonoid contents of the aqueous extract of *L. nobilis* leaves was evaluated. Results showed that the plant is rich in flavonoids (21.576±0.0763mg/L; 2.049±0.0031mg/g) and phenolic compounds (23.964±0.0698mg/L; 2.272±0.0028mg/g). It is proposed that various biomolecules existing in aqueous plant extracts such as polyphenols, polysaccharides, proteins, etc. may take role in Ag NPs formation [27-28]. *Laurus nobilis* (Bay) has previously been screened for chemical ingredients and biological activities by researchers. Phenolic compounds were isolated from *L. nobilis* extracts and their application as natural antioxidant compounds were evaluated [29]. Gold nanoparticles were synthesized by *L. nobilis* aqueous extract at room temperature and its antimicrobial activity was also evaluated by Khalil *et al*. They reported that 2 h of extraction by reflux-heat using aqueous ethanol at 35% concentration achieved high yields of polyphenols from *L. nobilis* in comparison with two other species which were analyzed in the study. HPLC analysis revealed the presence of four phenolic compounds in *L. nobilis* extract, and the plant was richer in phenolic compounds in comparison with two other species [30].

There are a few reports in the literature which shows the relation between phenolic content, and potent in green synthesis of Ag NPs. Subramanian *et al*. demonstrated that the stem bark extract of *Shorea roxburghii* contain high level of total phenolic compounds and the plant extract could be used as a green reducing agent for synthesis of Ag NPs [31]. Goodarzi *et al*. revealed that the order of the plants reducing capacity was similar to that of their phenolic content and antioxidant potential [18]. Begum *et al*. through kinetic studies using FTIR and Cyclic Voltammetry proposed that polyphenols or flavonoids present in tea leaves were responsible for the formation of Ag and Au nanoparticles [32]. Ahmad *et al*. reported that the phenolic compounds in pineapple, exhibit excellent antioxidant activity and these natural antioxidants seems to be a good choice for synthesis of Ag NPs [33].

In the next part of our study, green synthesis of Ag NPs through *L. nobilis* leaves aqueous extract was carried out. The appearance of pale yellow to dark brown color of the reaction mixture indicated the biosynthesis of silver nanoparticles (Fig. 1). It is well known that silver nanoparticles exhibit striking color change (light yellow to brown) due to the excitation of surface Plasmon vibrations in the particles [34]. Characterization of synthesized nanoparticles was revealed by different methods.

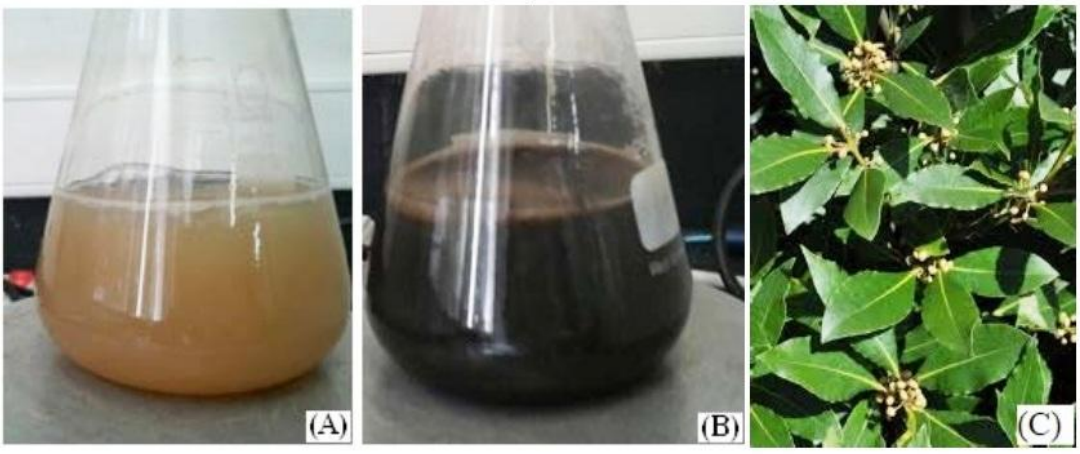

Fig. 1 Color change before (A) and after (B) biosynthesis of Ag NPs by *Laurus nobilis* L. (C) aqueous extract.

Figure 2 shows UV-Vis spectra exposed from the reduction of silver ions which have poly dispersed nanoparticles with broadening peak in the absorbance band at the wavelength of 460 nm. This, confirms the Nano crystalline character of the particles [35]. Nobel metal particles are ideal candidates for study with UV-Vis spectroscopy, since they exhibit strong surface Plasmon resonance absorption in the visible region and are highly sensitive to the surface modification [36].

FT-IR spectrum of plant extract before and after synthesis of Ag NPs was carried out to identify the water soluble organic compounds from the leaves extract which may take part in the reduction procedure of silver ions (Fig. 3). A strong peak at 3400 cm^{-1} indicates the presence of O-H groups in phenolic compounds, and N-H groups in proteins. The peak observed at 2936 cm^{-1} is due to aliphatic C-H stretching. The peaks at (1400 and 1600) cm^{-1}indicates C=C stretching in alkenes and aromatic compounds and also C-H bending in aliphatic compounds. The peaks at (1000-1300) cm^{-1} indicate C-C and C-O stretching in the compounds like alcohols and phenols. FT-IR confirmed the presence of bio-reducing organic compounds on the surface of Ag NPs which are likely to be responsible for nanoparticle synthesis and stability.

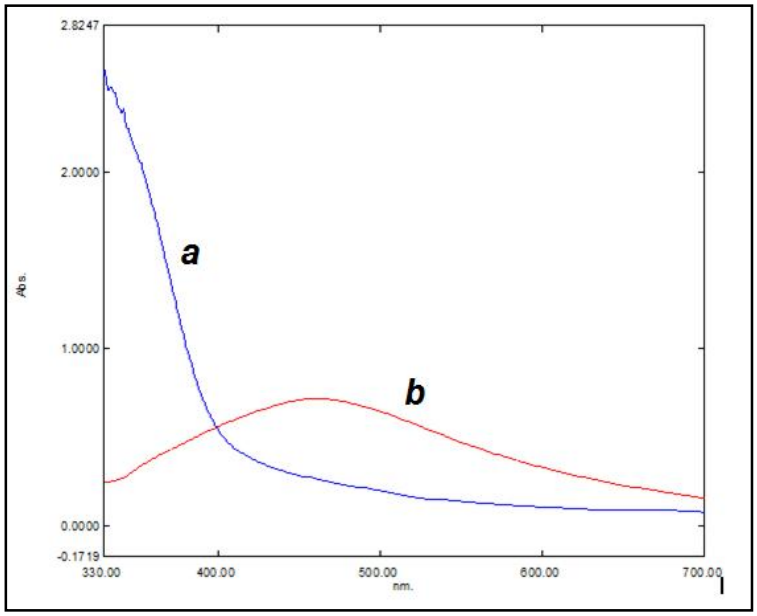

Fig. 2 UV-Vis spectrum of (a) *Laurus nobilis* L. leaves aqueous extract and (b) Ag NPs/*L. nobilis* extract suspension.

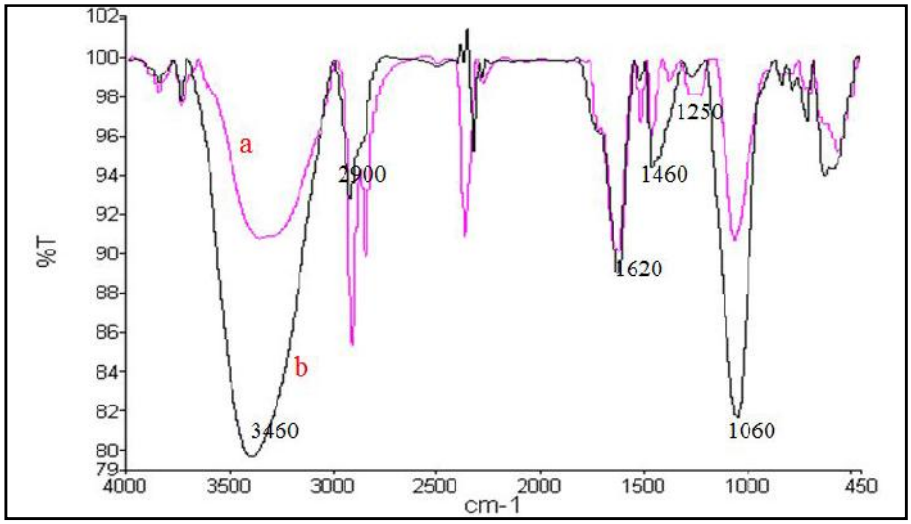

Fig. 3 FT-IR analysis of (a) synthesized Ag NPs by *Laurus nobilis* L. leaves aqueous extract and (b) *Laurus nobilis* L. leaves aqueous extract.

X-ray diffraction pattern recorded for the silver nanoparticles are shown in Fig. 4. The high intense peaks at 2θ degrees of 38.18, 44.54, 64.86, 77.55 can be attributed to the (111), (200), (220), (311) Bragg reflections respectively. This indicates that the synthesized Ag NPs by aqueous extract of *L. nobilis* leaves had crystalline nature and confirms the face centered cubic (FCC) structure. The average size of Ag NPs were calculated 18.77 nm, according to Debye-Scherer equation (1): ($D = K\omega/\beta\cos\theta$). Where ω is the X-ray wavelength (1.540560 Å), β is the width of XRD peak at half height, θ is the Bragg angle, and K is the shape factor with value 0.9.

SEM image of silver nanoparticles synthesized by aqueous extract of *L. nobilis* leaves is shown in Fig. 5. SEM image shows size, shape and distribution of nanoparticles. It confirms that particles are small and spherical in shape. As it is seen, the extract of *L. nobilis* has an important role in stabilization and prevention of agglomeration of Ag nanoparticles. A typical transmission electron microscope (TEM) image of the nanoparticles and their size distribution are presented in Fig. 6. The mean diameter and standard deviation of silver nanoparticles was found 19.65±13.49 (nm).

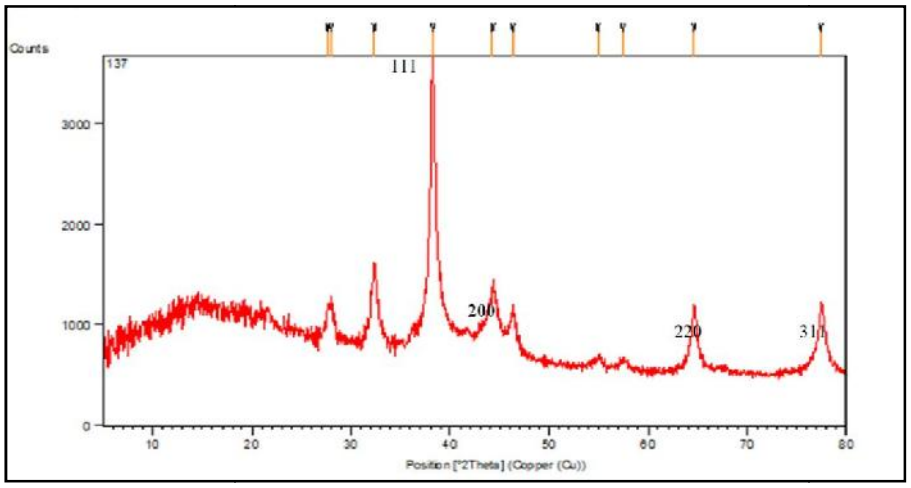

Fig. 4 XRD patterns of Ag NPs synthesized by aqueous extract of *Laurus nobilis* L. leaves.

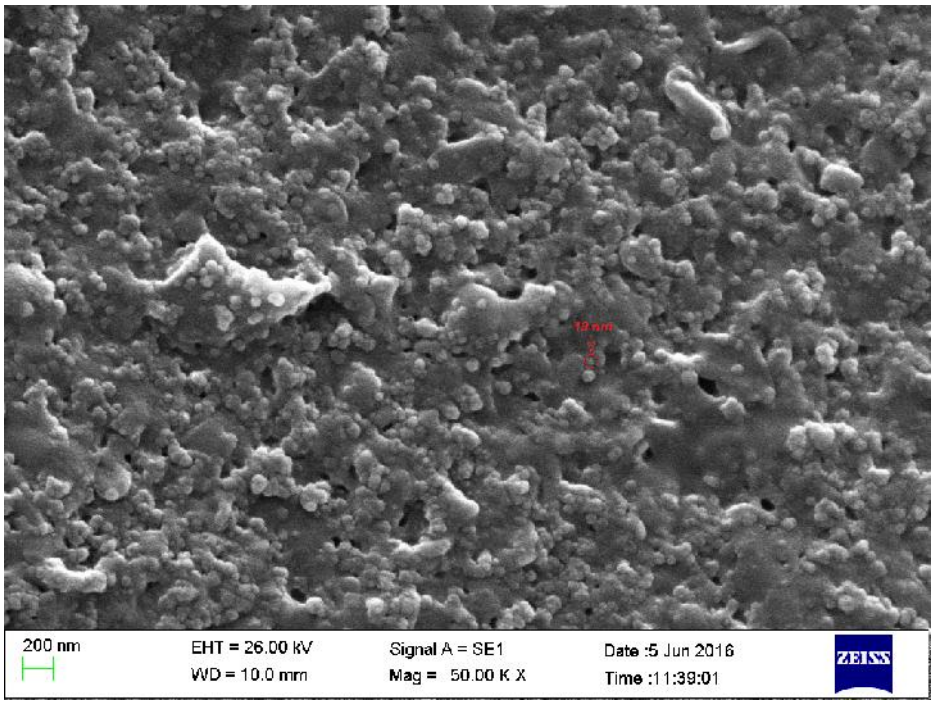

Fig. 5 SEM image of synthesized Ag NPs by aqueous extract of *Laurus nobilis* L. leaves.

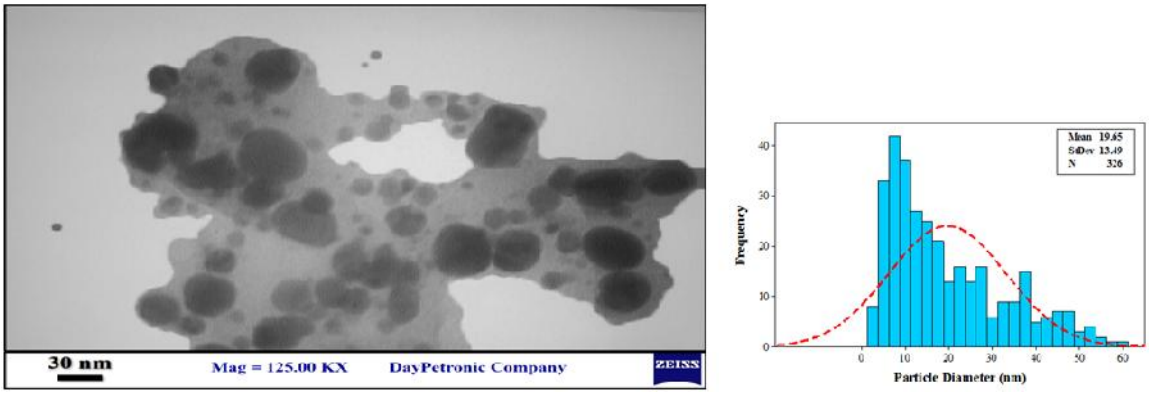

Fig. 6 TEM image and corresponding particle size distribution histogram of synthesized Ag NPs by aqueous extract of *Laurus nobilis* L. leaves.

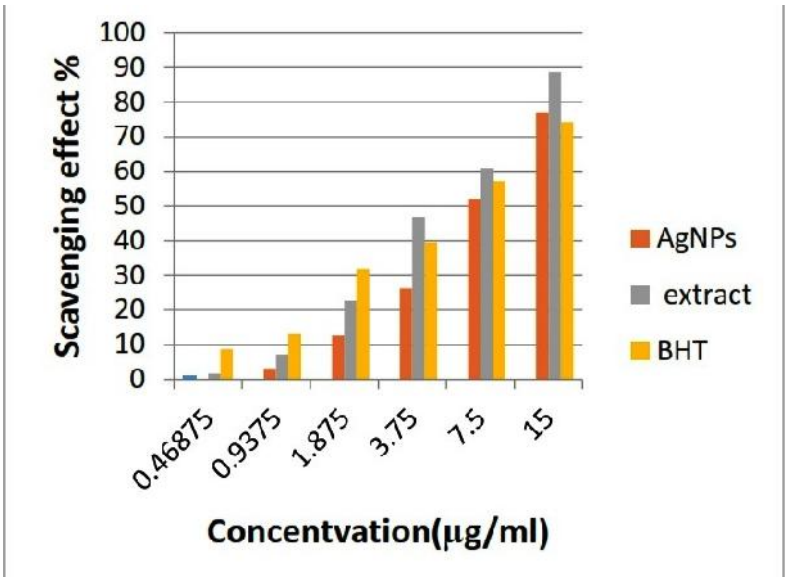

Fig. 7 DPPH radical scavenging effect of *Laurus nobilis* L. aqueous extract and Ag NPs/extract suspension in comparison with BHT.

Radical scavenging effect of the aqueous extract of *L. nobilis* and synthesized Ag NPs were evaluated by DPPH scavenging assay. As shown in Fig. 7 the aqueous extract of *L. nobilis* exhibited higher scavenging activity (61-89)% in concentrations 7.5-15 (µg/mL) compared to Ag/*L.nobilis* suspension and DPPH (as a standard). In concentrations 3.75-7.5 (µg/mL), extract was better than BHT and AgNPs respectively and, when the concentration reduces, the BHT shows better results than the other samples. Previous study in antioxidant activity on *L. nobilis* leaves ethanol extract indicated that *L. nobilis* has a high antioxidant potential [29]. Our result is in agree with the previous study with the advantage of using distilled water as solvent. In contrast to synthetic antioxidants which have adverse effects on human health, natural antioxidants are proposed because of their health promoting properties.

In conclusion we developed a simple, rapid and efficient green method to synthesis stable Ag NPs by aqueous extract of *Laurus nobilis* L. leaves. These nanoparticles were synthesized with an average size of 19.65±13.49 (nm) and spherical in shape and were characterized by UV-Visible and FT-IR spectroscopy; XRD crystallography; SEM and TEM microscopy. Total flavonoids and phenolic contents of the aqueous extract of *L. nobilis* leaves were also evaluated as the biomolecules which may have important role in the formation and stabilization of Ag NPs. Radical scavenging assay for the extract and synthesized Ag NPs showed good results in comparison with BHT as a chemical antioxidant. As *L. nobilis* is a

common garden plant in temperate regions, it can be considered as a valuable source for bio reduction of metal ions to metal nanoparticles and also as a natural antioxidant.

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