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Green Synthesis of Spherical Silver Nanoparticles Using *Ducrosia Anethifolia* Aqueous Extract and Its Antibacterial Activity

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Abstract

The present study introduces a simple, cost-efficient, and eco-friendly method for the synthesis of silver nanoparticles (AgNP) using aqueous extract *Ducrosia anethifolia* and its antibacterial activity. *Ducrosia anethifolia* aqueous extract was used both as a reducing and capping agent. Synthesized silver nanoparticles were characterized using several techniques including UV-Vis spectroscopy, powder X-ray diffraction (XRD), Transmission electron microscopy (TEM), dynamic light scattering method (DLS), and FT-IR analysis. The results demonstrated that the size range of the synthesized nanoparticles was 4 to 42.13 nm, with the average size of 11.4 nm and in a spherical shape. Moreover, X-ray diffraction analysis indicated that the silver nanoparticles were highly crystalline in nature. Examination of antibacterial activity of synthesized on Gram-positive and Gram-negative bacterial revealed that they had a substantial antibacterial effect.

Keywords: *Ducrosia Anethifolia*; silver nanoparticle; Green synthesis; antimicrobial performance

1 Introduction

Given their unique properties, metal nanoparticles have a broad array of applications, each of which is associated with a certain characteristic. (1.4) Moreover, of such nanoparticles, silver nanoparticles are utilized in a vast variety of fields such as catalyst in chemical reactions, solar cells, photography, electronics, food industry, dentistry, and most importantly, antibacterial effects on Gram-positive and Gram-negative bacteria (5). This bioactivity is due to the particle and nanometer size (1-100) of silver nanoparticles, giving them special physical, chemical, magnetic, and optical properties which are different from their bulk structure (8-6). Therefore, silver nanoparticles are considered as a potent antibacterial material, comparable to, and even in some cases, stronger than common antibiotics (9). Currently, there are various physical and chemical methods of producing metal nanoparticles (10, 13) which include chemical reduction (14), electrochemistry (15), mold-based synthesis (16), hydrothermal synthesis (17), and vacuum deposition or vaporization (18), and Sol-Gel technique (19).

However, it should be noted that chemical methods have a poorer performance and that chemical methods are toxic due to the use of toxic reducing chemicals like citrate, borohydride, and other organic, toxic compounds (20). In addition, other disadvantages of these techniques include costliness, high energy consumption, and eco-toxic nature. Thus, the need for a biocompatible and cost-efficient method, devoid of the abovementioned drawbacks, is strongly felt. Biosynthesis methods can benefit from microorganism cells or plant extracts to produce nanoparticles. Recently, green synthesis techniques have turned into an important branch of nanotechnology (21-29).

This is the reason for the wide application of medical plants in the synthesis of silver nanoparticles (30-33). In fact, medical plants have bioactive molecules which make possible the green synthesis of silver nanoparticles. Such

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active molecules work as reducing and capping agents for nanoparticle synthesis, making nanoparticles useable for biomedical applications (34-35). Synthesis of nanoparticles, an indicator of the relationship between biotechnology and nanotechnology, has increased due to the rising demands for eco-friendly technologies (36).

Various studies have shown that different plant compositions abound in high levels of strong antioxidants such as polyphenols, sugar, nitrogen reserves, and amino acids (37). These compounds function as reducing (38) and coating agents for NPS-synthesis (39-40). The plant extract used to synthesize NPS can be useful due to their lack of need for processes such surface coating, and lower reaction time (41). Additionally, they do not produce any detrimental waste (42).

An aromatic and medicinal plant belonging to the *Apiaceae* family, *Ducrosia anethifolia* grows wild in areas of Iran-Turan as well as the Gulf of Oman. In Persian language, this plant is called *Moshgak*, *Roshgak*, and *Moshkbu* (*literally* meaning Musk-smelling) and has anti-inflammatory and pain-relieving effects (43). The main chemical compositions of this herb include: n-decanal, n-dodecanal, chrysanthenyl acetate, myrcene-limonene, and α-pinene. Also, the aliphatic compounds of this plant are believed to act as an antibacterial agent against Gram-positive and Gram-negative bacteria (44). The present study introduced a quick way of producing

silver nanoparticles using *Ducrosia anethifolia* plant extracts and examined their properties and inhibitory effects on Gram-positive and Gram-negative bacteria.

2 Materials and method

2.1 Instruments

The powder X-ray diffraction (XRD) pattern measurements of the samples were recorded on a Holland-Philips X-ray powder diffractometer using Cu K□ radiation (λ= 0.1542 nm) with scattering angles (2□) of 5-80◦, operating at 40 kV and a cathode current of 20 mA. Additionally, some specimens of synthesized silver nanoparticles AgNPs for TEM studies were prepared by ultrasonic dispersion of the NPs in ethanol, and the suspensions were dropped onto a carbon-coated copper grid. TEM was carried out using a (CM30 3000Kv). FT-IR spectra were recorded to investigate the functional group on samples which carried out on a Bruker VERTEX 80 v model using the KBr disk method. The size distribution of Ag NPs was characterized by the DLS approach, using a computerized inspection system (MALVERN Zen3600) with DTS® (nano) software. UV-Vis spectroscopy (UV-Vis) analyses were taken using a Varian Cary 50 UV–vis spectrophotometer. Spectra were recorded in a range of 350-800 nm.

2.2 Materials and methods

Silver nitrate ($AgNO_3$) was purchased from Merck Company for this study and was used without any renewed purification. Fresh samples of Ducrosia anethifolia parts consisting of leaves and stems were identified and collected. To remove pollution, all of glassware were cleaned with dilute HNO_3 and rinsed with distilled water as well as dried in an oven under air atmosphere.

Fresh Ducrosia anethifolia extract was preferably used to reduce aqueous Ag+ solution to Ag NPs. So, the collected Ducrosia anethifolia was washed with distillated water to remove any previous contamination, and then dried in the shade at the room temperature. Afterwards, the dried plant was grinded so as to yield powders with mesh 20. In the next step, 5g powder was added to 50 ml distillated water while being completely stirred for 2h. The solution was cooled at room temperature and filtered through Grade1 Whatman filter paper. The obtained fresh extract was used to prepare AgNPs.

In this research, several concentration ranges from 20 ml of 5% to 20% of plant extract was used to synthesis Ag NPs.Typically, 20 ml fresh filtered extract was interacted with 5 ml aqueous 0.01 mM $AgNO_3$ under reflux by magnetic stirred. After interaction for 30 min, color of suspension changed from light-yellow to brown which this change indicates formation of Ag NPs. The brown Ag suspension was cooled at room temperature and kept for characterization by several techniques.

3 Result and discussion

3.1 UV-Vis Assessment

After silver nitrate (AgNO3) was added to extracts of Ducrosia anethifolia, the color of the suspension changed within some minutes. The extract was yellow by itself, but it turned brown during the process of silver nanoparticles synthesis which was due to the surface plasmon resonance (SPR) of silver nanocrystals as they are dependent on the size and shape of nanoparticles. As shown by Fig. 1, there is a sharp peak almost 465 nm which supports silver nanoparticles synthesis.According to the previous literature, the observed bond corresponds to absorption by colloidal silver nanospheres in 450-500 nm(33, 45-47).

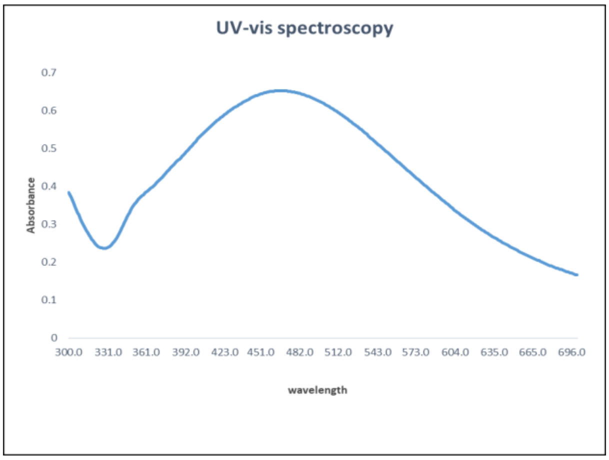

Figure1: UV-vis spectroscopy of Ag NPs.

3.2 TEM and DLS investigation

Morphology and size distribution of synthesized nanoparticles was investigated by TEM images (Fig. 2). Fig. 2 shows high-quality crystalline structure and spherical morphology of AgNPs which is matched with observed peak for nanospheres in UV-Vis spectroscopy.

The size distribution of synthesized silver nanoparticles was in the ranges from 4 to 42.13 nm with average size of 11.4 nm. Fig. 3 depicts the particle size distribution histogram for nanoparticles obtained from DLS technique. According to the analyses, the silver nanoparticles had an average size of 117 nm. These findings are not in agreement with those achieved from TEM technique. The reason for this difference is that DLS measures hydrodynamic diameter of nanoparticles as well as their surrounding cover. Moreover, in an aqueous environment, it is likely that some nanoparticles connect to each other and create an aggregation of nanoparticles. Subsequently, DLS measures the overall diameter of the set of nanoparticles. Depending the size of nanoparticles as well as the magnitude of biological components connected to various plant extract nanoparticles, the particle size distribution of nanoparticles accounts for a highly narrow to a highly broad range in DLS. Similar findings were reported by G. Singhaj et al who showed that silver nanoparticles formed from extracts of Ocimum sanctum differed on average in terms of their particle size nanoparticles in DLS and TEM techniques, suggesting that the latter showed the average particle size of 14.3 nm, while the former the average particle size of 22.3 nm(48).

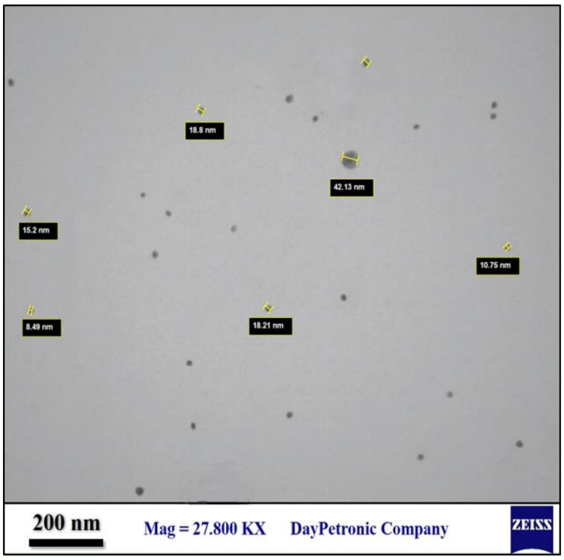

Figure 2: TEM image of synthesized AgNPs using Ducrosia Anethifolia extract

3.3 FTIR analysis

To investigate the functional groups of extracts on the surface of AgNPs, FTIR analysis was carried out. Several peaks were observed in FTIR plot (Figure 4) that were indicate of various functional groups on the surface of AgNPs. According to Fig. 4, the peaks witnessed at 3500 cm-1 range was associated with the amide group N-H stretching, whereas the band seen at 3232 cm-1 accounted for the O-H stretching of the carboxylic acid group. Furthermore, the peak witnessed at 3500-3200 cm-1 was corresponded to alcohol/phenol O-H stretching which is capable of overlapping with carboxylic acid and amine groups. Also, the weak peak seen at 2918 cm-1 accounted for the alkane C–H stretching vibrations of methyl, methylene, and methoxy groups. Two strong peaks at 1622 and 1616 cm-1 represented the C=O stretching vibrations due to carbonyl stretching groups in carboxylic acid, and moreover, the peaks at 1383, 1100 and 616 cm-1 were assigned to C–H bending, C–OH bond stretching and aromatic ring, respectively (49). All of the mentioned bands were considered as principal functional groups in molecular structures in Ducrosia anethifolia extract which play a major role in formation of silver nanoparticles

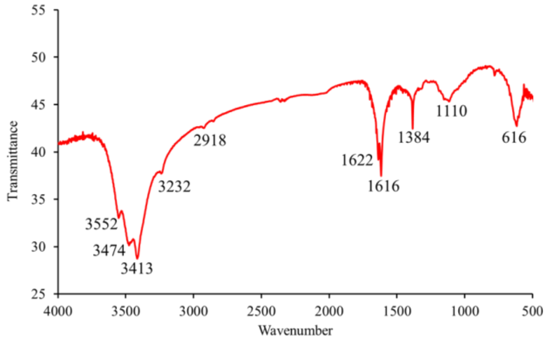

Figure 4: FTIR spectroscopy of prepared silver nanoparticles by green synthesis

3.4 XRD spectroscopy

The peaks caused by X-ray diffraction at 2θ=3.2°, 44.2°, 64.5°, and 77.8°, all shown by Fig. 5. These angles can be indexed to the 111, 200, 220 and 311 planes of reflections of silver, respectively. This diffraction pattern is indicative of natural crystalline structure of silver nanoparticles and those produced with a face-centered cubic structure (FCC). These findings are in line with those obtained from silver nanoparticles produced from plant extracts. Likewise, there was a series of peaks witnessed at ranges 10 to 20 which corresponded to the bioorganic crystalline phase of plant extracts. For instance, in the previous study conducted on silver nanoparticles synthesis using Wheat grass extracts, we showed that peaks caused by X-ray diffraction at 2θ=38.22°, 44.37°, 64.54°, and 77.47°, suggesting that the results of the present study are supported (43, 49-53).

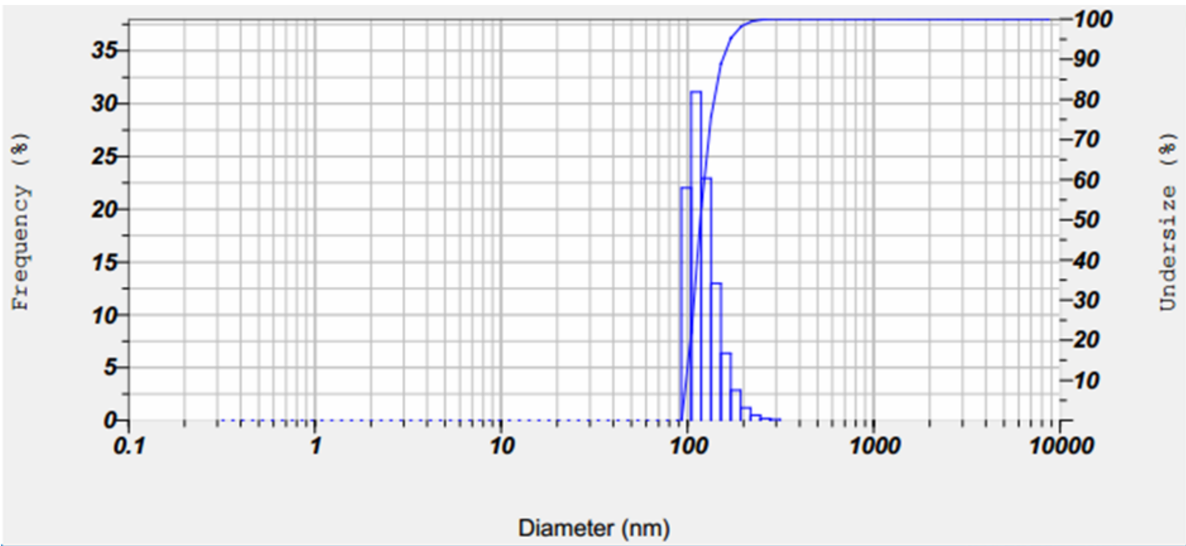

Figure 3: DLS of green synthesized Ag NPs by Ducrosia Anethifolia extract

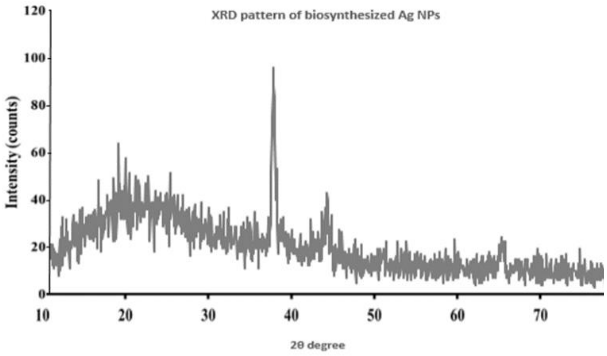

Figure 5: XRD pattern of biosynthesized Ag NPs using Ducrosia Anethifolia extract

Nonoparticles were effective against all strains of tested Gram-positive and Gram-negative bacteria at ranges 32-128 μg/ml. Both Enterococcus faecalis and Pseudomonas aeruginosa showed similar values for MICs and MBCs.Nanoparticles had no bactericidal activity in concentration up to 128μg/ml.Mechanism of AgNPs bacterial inhibition was performed in four ways as follows: i) Ag+ could form a stable complex with thiols and phosphate in amino acids and nucleic acids, ii) AgNPs led to the disruption ATP formation and DNA replication by producing Ag+, iii) AgNPs was capable of generating reactive oxygen species (ROS) or oxygen radical species (they could damage the DNA by means of oxidative stress), and iv) AgNPs disrupted the respiration functions of the cell and permeability through attaching and penetrating into the cell membrane of bacterial. By addressing this reasons, the green synthesized AgNPs demonstrated the best antibacterial activity(54-55-56).

4 Antibacterial activity of green Ag NPs

The MICs and MBCs obtained from synthetic nanostructures against various strains of bacteria are shown in Table 1.

5 Conclusion

Given the wide application of metal nanoparticles in industry as well as the desperate need for a low-risk and safe treatment without any adverse effects on both humans and environment, it is believed that green synthesis methods are less costly, highly safer and eco-friendly. In this study, Ducrosia anethifolia aqueous extracts were used as the reducing agent for reduction of Ag+ cation to the AgNO3 to Ag0. Therefore, AgNO3 reaction at the presence of Ducrosia anethifolia aqueous extracts resulted in the synthesis silver nanoparticles (AgNPs). Various methods of identifying the characterization of nanoparticles such as XRD, TEM, FTIR, DLS, and UV-Vis have supported successful synthesis of AgNPs. Likewise, the synthesized nanoparticles were shown to have substantial anti-bacterial properties, especially against Gram-positive and Gram-negative bacteria.

Table 1: The MICs and MBCs obtained from green Ag NPs

Gram positive bacteria				Gram negative bacteria			
Staphylococcus aureus (ATCC 25923)		*Enterococcus faecalis* (ATCC51299)		*Escherichia coli* (ATCC 25922)		*Pseudomonas aeruginosa* (ATCC27853)	
MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
32	ND	128	ND	64	ND	128	ND

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