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RESEARCH PAPER

Green Synthesis and Antibacterial Activity of Silver Nanoparticles Using *Dracocephalum Moldavica* Leaves Extract

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ABSTRACT

In the presence work we firstly synthesize silver nanoparticles by a green procedure using D. *moldavica* leaves extract. Nanoparticles were prepared by a simple precipitation method via aid of sun light irradiation in a short time about 15 min. morphology of the nanostructures were characterized by scanning electron microscopy and for better estimation of grain size transmission electron microscopy was also applied. X-ray diffraction pattern approved crystallinity and purity of the silver nano products. Dynamic light scattering was used for measuring of the size distribution. Ultra violet-visible absorption illustrate the band gap of the prepared nanoparticles. The antibacterial activity of silver nanostructures was evaluated against clinical isolates of Escherichia coli bacteria. The Kirby-bauer method was accomplished for determination of zone of inhibition.

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INTRODUCTION

Nano particles are basically rated in a size range of 100 nm. Nano particles properties are obviously different than particles with larger dimensions. Based on changes in specific characteristics such as size, shape and distribution, some new features have been observed [6].

The key role of Nano particles in pharmaceutical, industrial and biotechnological applications has been proven [10]. According to physiochemical properties, silver Nano particles play an important role in biology and medicine [6]. Antibacterial, antifungal, anti plasmodial and larvicidalfeatures of silver Nano particles are proved specifically [26].

Biological approaches have been considered widely since they are eco-friendly, cost effective and without any toxic chemical effects during the synthesis of Nano particles [25–20]. The synthesis of Nano particles by biological methods is more justified since its higher energy and time efficiency. This method does not require poisonous solvents or any environmentally dangerous material. Green synthesis of nanoparticles is an eco-friendly method based on using natural solvent [8]. More stability and faster rate of synthesis, is an advantage of plants-based production of Nano particles compared to the other methods. In addition, the Nano particles obtained from this method, are more different in shape and size [17].

Recently, the green synthesis of AgNPs has been reported, where plant extracts such as fenugreek leaf extract, Daucuscarota extract,

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Dioscoreabulbifera tuber extract, and Citrus lemon extract are the reducing agents [11, 12, 21, 23,5 and16]. In addition, studies have proven that extracts of neem [21], Hibiscus cannabinus [3], tamarind [2], Murrayakoenigii [15], Parthenium leaf extract [16], Rosa rugose [4], Hibiscus rosasinensis [15], Nelumbonucifera [27], Hedera helix [1]and oak fruit bark extract [25,24] are effective extracts in the biosynthesis of Ag NPs.

Dracocephalummoldavica L. is a perennial, herbaceous plant belonging to the Lamiaceae family [24,9]. some pharmacological studies have recentlyproven antioxidant, antiseptic, antibacterial, and carminative specifications of the plant's essential oil [24], and the areal parts of D. moldavica are used in traditional West Azerbaijani (Iranian) medicine for general diuretic, digestive, sedative, and antiemetic applications [9].

It is believed that the reduction of Ag ions and the stabilization of following NPs is under Terpenoids participation [18]; As a result of exploring medicinal properties, D. moldavica leaf extract used as a reducing and stabilizing agent during the synthesis of AgNPs.

As there appears to be no report concerning the synthesis of NPs using D. moldavica seed extract to date, this study designed to photosynthesize AgNPs using D. moldavica leaf extract, and to subsequently examine the antimicrobial properties of synthesized NPs.

MATERIALS AND METHODS

Reagent and Materials

All materials used in this experimental study including Nutrient broth media, Meuller- Hinton agar media, and silver nitrate for the synthesis of nanoparticles were purchased from Merck company in Germany. Standard strain of the *Escherichia coli cells* (AATCC 11229) was purchased from the Center of Scientific and Industrial Research of Iran.

Preparation of Dracocephalummoldavica Extract

D. moldavica leaves were collected from areas that had grown wild. The leaves were washed several times with distilled water to remove the dust particles. The leaves were cut into small pieces and 150 g were boiled in a 500-mL glass beaker along with 1000 mL of sterile distilled water for 20 minutes and allowed to stand for 6 hrs at room temperature. The color of the aqueous solution changed from watery to yellow color. The aqueous extract was separated by filtration with Whatman No. 40 filter paper. The leaf extract used for biosynthesis of silver nanoparticles from silver nitrate.

Synthesis of Silver Nanoparticles

The source of silver was silver nitrate (1 mM) in distilled water. Silver nitrate solution was prepared

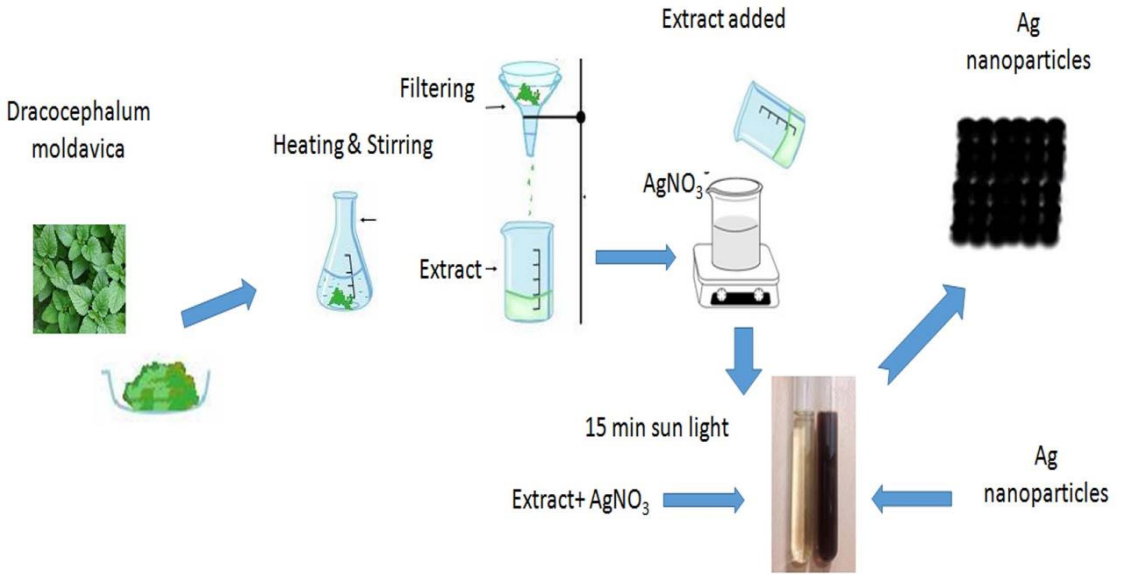

Fig. 1. Schematic of nano silver preparation

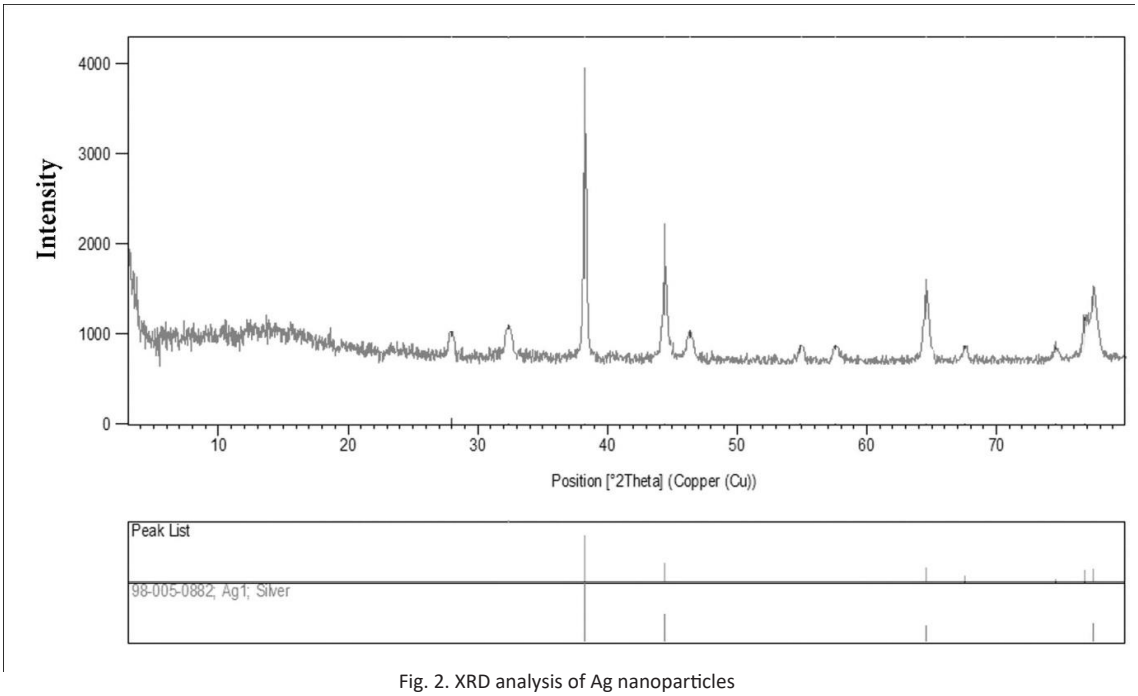

Fig. 2. XRD analysis of Ag nanoparticles

and was reduced using *Dracocephalummoldavica* extract at room temperature. The system was stirred and reduction took placerapidly at room temperature under sun light and completed in 15 minutes. 150 ml of collected filtrate was mixed with 600 ml of 3 mM silver nitrate solution. The color of the solution gets changed from yellow to dark brown which indicates the formation of silver nanoparticles. The reduced solution was centrifuged at 7000 rpm for 15 minutes. The centrifugation process was repeated for three times to remove any impurities adsorbed on the surface of silver nanoparticles. The dried powder was used for the experimental work (Fig. 1). The formation of AgNPs was further more confirmed by spectroscopic analysis, TEM, DLS, XRD and SEM techniques.

RESULTS AND DISCUSSION

Characterization of Silver Nanoparticles

The XRD pattern of silver nanoparticles is shown in Fig. 2. XRD pattern of magnesium hydroxide is indexed as a pure hexagonal structure with suitable agreement to literature value (JCPDS card no. 98-005-0882, Space group: F m -3 m, cell constants: a, b, c: 4.0860 angstrom). The crystallite size evaluation was also performed using the Scherrer equation:

$$Dc=0.9\lambda/\beta\cos\theta$$

Where β is the width of the observed diffraction peak at its half maximum intensity (FWHM) and λ is the X-ray wavelength (CuKα radiation, equals to 0.154 nm). The calculated crystallite size is about 24 nm.

The SEM images of silver nanoparticles are shown in Fig. 3. It seems by applying leave extract mono-disperse nanoparticles were prepared; SEM images confirm nanoparticles with average diameter of 51 nm have been obtained.

Transmission electron microscopy (TEM) technique was used to visualize the morphology of the Ag NPs. The 80 kV ultra-high-resolution transmission electron microscope (Zeiss- EM10C). The TEM images show the spherical morphology of prepared nanomaterials with 38 nm diameter (Fig. 4).

DLS analysis was applied for size distribution of nanoparticles. As well as shown in Fig. 5, the particles size were distributed in 30-50 nm. It can be concluded that the uniform particles size was formed via applied synthesis route.

The optical properties of prepared silver nanoparticles was characterized by UV-visible spectroscopy using a Double beam

spectrophotometer (Perkin Elmer lambda 15). The bio reduction of silver ions in aqueous solution was monitored by UV-VIS spectra of the solution via wavelength range from 360-600 nm (Fig. 6). The broad strong absorbance peak was observed in the UV-Vis spectrum of prepared silver nanoparticles.

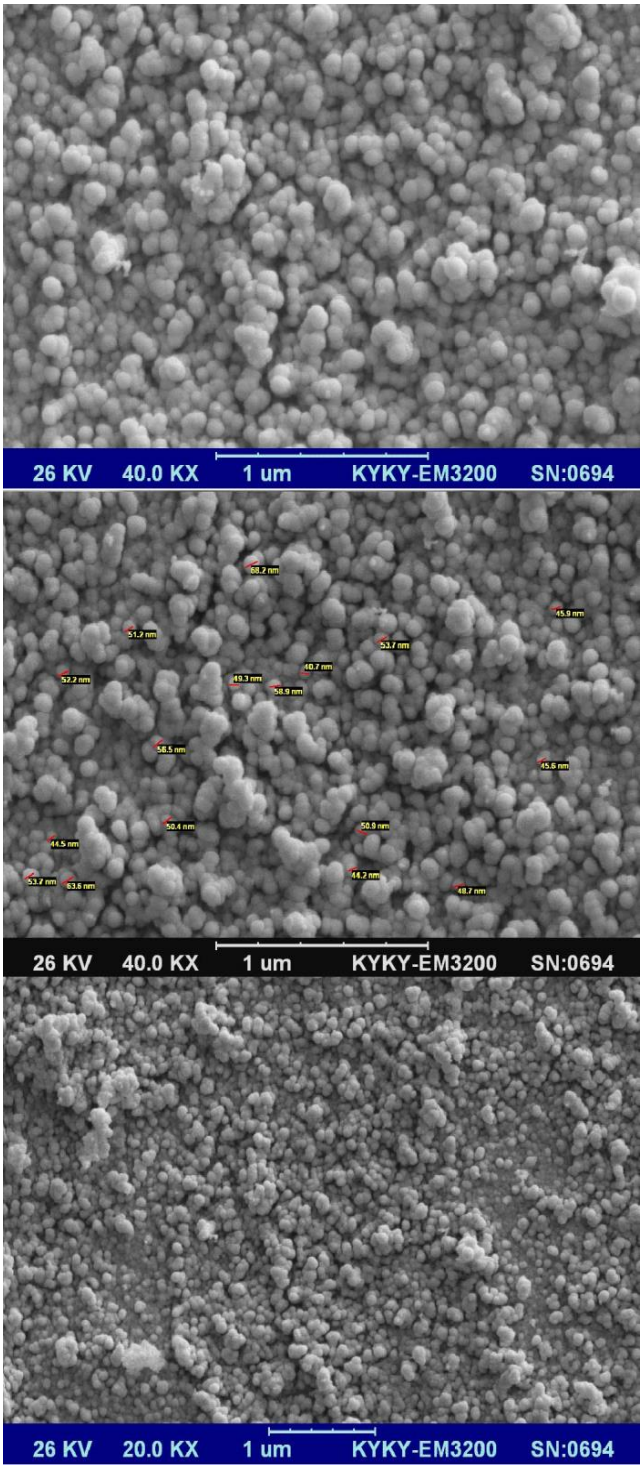

Fig. 3. SEM images of the silver nanostructures

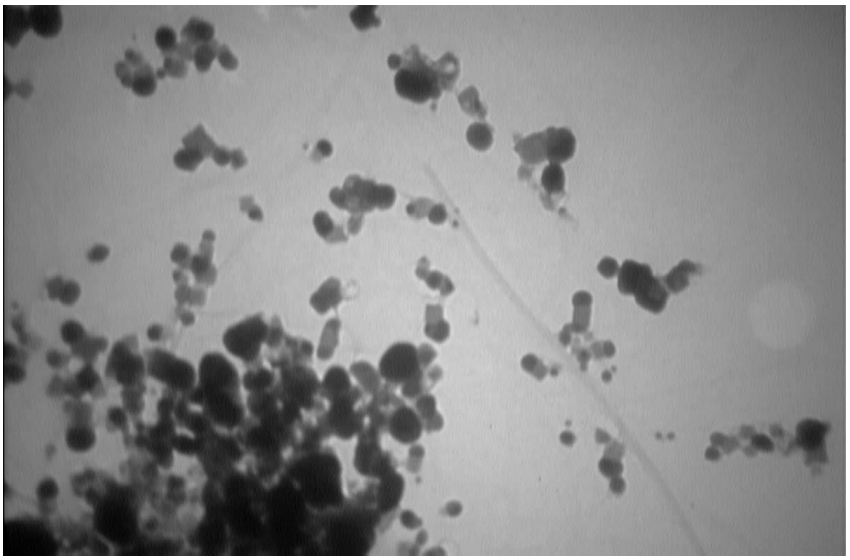

Fig. 4. TEM images of the Ag nanoparticles

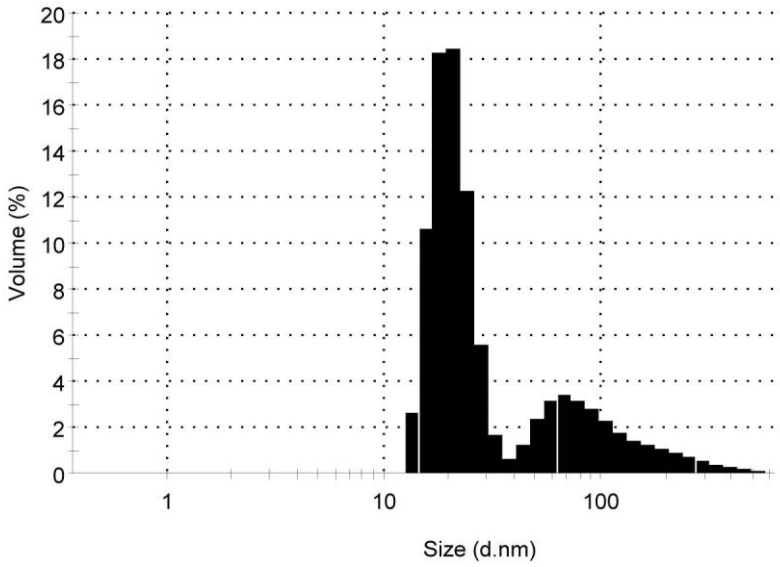

Fig. 5. Particle size analyzer of the prepared product

Anti-Bacterial Assay of Silver Nanoparticles

The produced Ag nanoparticles was evaluated for antibacterial activity. Agar well diffusion method [16] was used and the pH was adjusted at 7.3. Clinical isolates of Escherichia coli bacteria were selected for the investigating. Briefly, sterile molten Mueller Hinton agar (20 ml) was poured into sterile Petri dishes and allowed to solidify at room temperature. Pyre cultures of pathogenic bacteria as 0.5 macfarland (108 Cfu/ml) was swabbed on the Muller-Hinton agar plates. Plate containing media as well as culture were dividedin to four equal parts and previously prepared discs were placed on each part of the plate. The discs were placed in the following order: disc soaked with double distilled water as negative control, disc soaked with *D. moldavica* extract, disc soaked

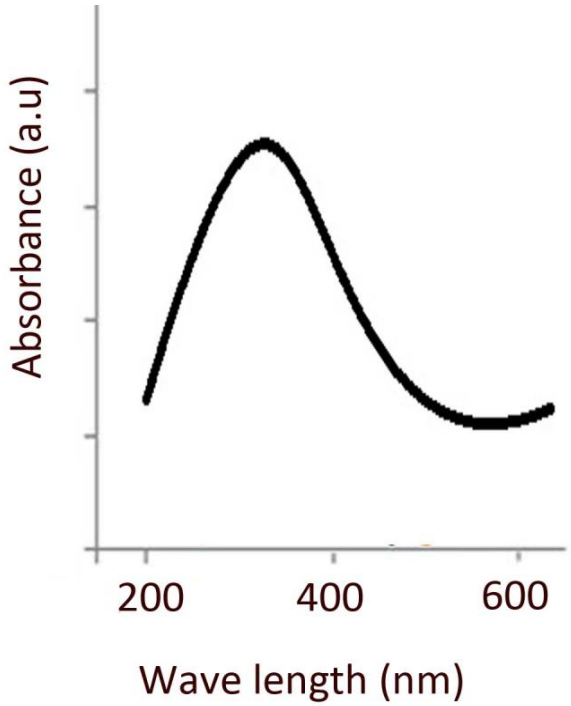

Fig. 6. UV-Vis absorption of the silver nano products

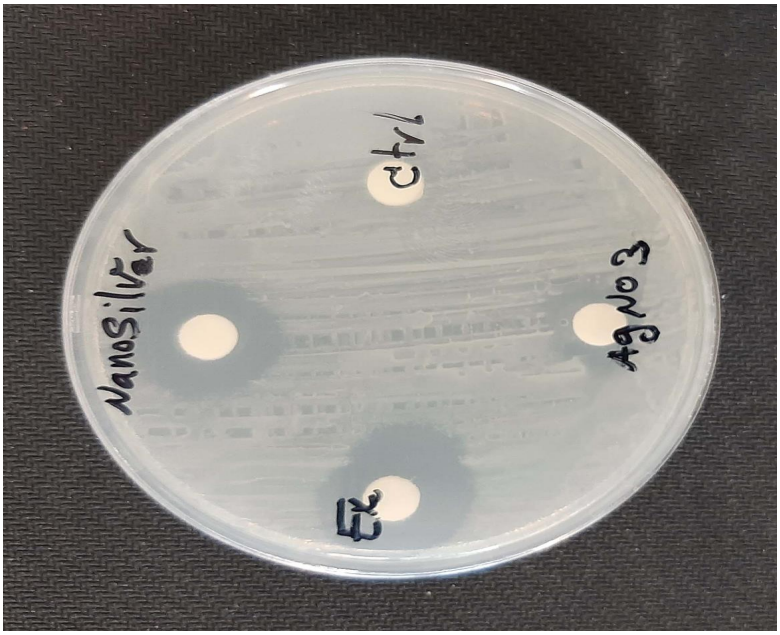

Fig. 7. Antibacterial activity of the Ag on the E-Coli bacteria

with 1mM silver nitrate solution and disc soaked with solution containing *D. moldavica* extract mediated synthesized silver nanoparticles. The plates were incubatedat 37°C for 24 hours. Then, the maximum zone of inhibition were observed and measured for analysisagainst each type of test

microorganism.

Antibacterial property analysis in this study, the antimicrobial property of AgNPs was investigated by growing Escherichia coli colonies on Muller-Hinton agar plates supplemented with AgNPs. Results obtained in previous studies also support the antibacterial potential of AgNPs. In the Muller-Hinton agar plate the zone of inhibition was observed around both the disc ($AgNO_3$ andAgNPs) as shown in Fig. 7.

In comparison to $AgNO_3$ and AgNPs, The zone of bacterial inhibition by AgNPs was more than AgNO3. No zone of inhibition was obtained in case of control and *D. moldavica* extract.

CONCLUSION

Green synthesis of nanoparticles has been an emerging research area now a day. The advancement of green synthesis has an advantage over chemical and physical methods. The plant extract synthesized silver nanoparticles are environment friendly, cost effective and easily scaled up for large scale synthesis of nanoparticles; furthermore there is no need to use high temperature, pressure, energy and toxic chemicals [7]. Chemical antimicrobial agents are increasingly becoming resistant to a wide spectrum of antibiotics. An alternative way to overcome the drug resistance of various micro organisms is therefore urgently needed. Ag ions and silver salts have been used for decades as antimicrobial agents in various fields due to their growth inhibitory abilities against microorganisms [22]. This study concludes that *D. moldavica*leaves has the capability to synthesize AgNPs and its medicinal activities. The confirmatory report of our studies indicates that herbal medicine can be used in fish health management and curing of diseases.

CONFLICT OF INTEREST

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

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