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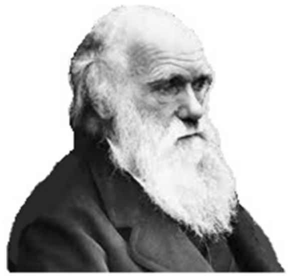

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Green chemistry for the preparation of silver nanoparticles using mint leaf leaves extracts and evaluation of their antimicrobial potential

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ABSTRACT

The aim of this study was to develop a green synthesis of silver nanoparticles using mint plant leaves extracts. The fresh suspension of plant extracts was yellowish-green in color. However, after the addition of $AgNO_3$ within 15min, the suspension showed a change in color to dark brown after 5 hours of incubation at room temperature. The formation of silver nanoparticles was confirmed using UV-Vis spectral analysis and revealed silver surface plasmon resonance band to be in a range around 200-500 nm. The XRD pattern showed the characteristic bragg peaks of (111), (200) and (220) planes of the face center cubic (FCC) silver nanoparticles $AgNP_S$. Under scanning electron microscope (SEM), silver nanoparticles with a mean particle size of 26 nm and a spherical shape were most frequently observed. In testing, the synthesized AgNPs colloidal solution showed better antibacterial activity against both Gram-positive and Gram-negative bacterial strains. The diameter of the inhibition zones of AgNPs at 50 µg/ml concentration against bacterial strains such as *Bacillus subtilis* and *Escherichia coli* were 25 mm and 20 mm, respectively.

***Keywords*:** Green synthesis, Silver Nanoparticles, Mentha (Mint)

1. INTRODUCTION

Nanoparticles are those particles which have less than 100 nm in diameter and exhibits as new or which accelerate size dependent properties if compared with larger particles of the same material. Nanoparticles present widely in the nature for so many of year because of

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productive form of photochemical and volcanic activity, created by plants and algae, or as the product of combustion and food cooking. Recently it has found from vehicle exhausts. Nanoparticles are of interest because of their two main properties such as chemical reactivity and optical behavior.

Silver is one of the most widely used metals in nanotechnology and it has many applications in medicine, electronics, optics, and space industries (1, 2). Antibacterial, antifungal, antiseptic, antiviral, and anti-angiogenic properties of this metal have made it as a useful agent in medicine (3, 4). Today, special interest has been directed to the green synthesis of nanoparticles and a variety of biologic systems have been used for this purpose (5, 6). For example, using plants in the synthesis of nanoparticles, due to avoiding toxic and polluting substances, is an eco-friendly method (7). Reduction of metal ions in solution and actually their neutralization is the base of nanoparticles synthesis (8). Plants are the main economic and effective sources for the synthesis of nanoparticles. Studies show that some medicinal herbs and natural products, generally used by people, have biological and pharmaceutical effects (9). Since medicinal herbs do not have chemical harmful effects and have beneficial effects against any pathogens and bacteria, they can be used as antiseptic, antiviral, antioxidant, and antimicrobial agents (10). Many studies have been performed on synthesis of gold and silver nanoparticles using plant extracts (11). According to the studies on green synthesis using bio-compatible agents, the main advantage of using plants for nanoparticles synthesis is that this method is bio-secure, easy to use, and includes a wide variety of metabolites that are involved in ion reduction process (12). Green synthesis of nanoparticles using plants such as Mentha Leaf is easily available and its extract has a good potential to reduce ions, it was used in this study. High levels of phenol and flavonoid in this plant have also been reported (18).

Since, nanotechnology is very expensive due to the high costs of synthesis and identification of the material's structure, through using herbal agents in the synthesis of nanoparticles, the costs can be greatly reduced. Synthesis of nanoparticles requires three materials, including metal ions, reducing agents, and protective agents that all of them are chemicals. The main aim of this study was to synthesize silver nanoparticles using Mentha Leaf extract as the reducing agent of Silver Nitrate ($AgNO_3$). Green synthesis of nanoparticles has been an exploring research topic in recent days due to their advanced use in biomedical, chemical and related fields. We herein report the synthesis of silver nanoparticles by the reduction of aqueous Ag^+ and with the extract of Mint (Mentha) leaves. Mint is known for its medicinal values such as ginger has been used to treat skin diseases, colorectal cancer, arthritis, heart condition and also have been reported for its antibacterial properties (19-23).

2. MATERIALS AND METHODS

2. 1. Preparation of the plant leaf extracts

Ten grams of fresh leaves of mint were taken and washed with distilled water separately. The Leaves were cut into fine pieces and crushed with 50 ml sterile distilled water using motor and pastel. The contents were boiled with constant stirring for 15 minutes. After cooling contents were filtered with Whatmann No. 1 filter paper (pore size 25 μm). Dark yellow colored extracts were obtained, which were used as reducing agent and stabilizer.

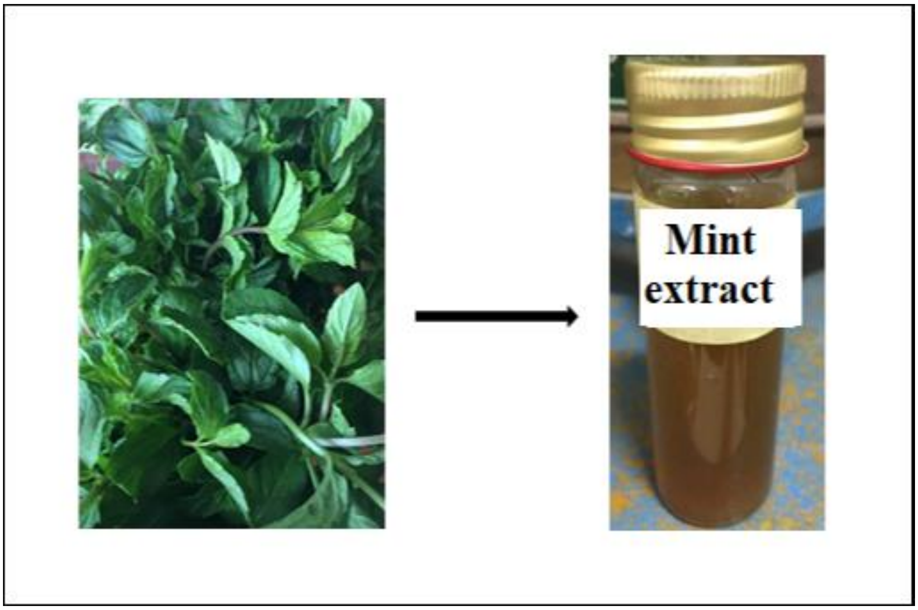

Fig. 1. The conversion of Mentha leaf into a Mentha(Mint) extract

2. 2. Synthesis of silver nanoparticles

In this study, silver nitrate $AgNO_3$, (Reagent World, USA, purity 99.99 %). 15 ml of 0.1 M Silver nitrate solution was prepared, 15 ml of Mint extract was added to 10 ml of silver nitrate solution and put the mixture on the magnetic stirrer at 60-70 °C for 20 minutes. To ensure the formation of AgNPs, Within 10-15 min, the color solution changes to dark brown color due to the formation of AgNPs. A gray precipitate was collected by filtration, washed with ethanol and distilled water several times, finally dried in air at 30 °C for 24 hours

3. CHARACTERIZATION

The optical properties were examined via (CARY, 100 CONC plus UV-Vis-NIR, Split-beam Optics, Dual detectors) spectrophotometer equipped with a xenon lamp at a wavelength range at (300-900 nm). All films were deposited on glass substrates. The size and shape of the nanoparticles were determined by X-ray system (Shimadzu - XRD6000, Shimadzu Company /Japan). The X-Ray source was Cu-K_α radiation with 0.15406 nm wavelength. The system operates at 40 KV and 30 mA emission current. The scanning electron microscope, a sample of silver nanoparticles was loaded onto the sample holder. This was allowed to be analyzed in a fully automated scanning electron microscope SEM (Make – JEOL, Model – 6390).

4. RESULTS AND DISCUSSION

According to the UV-Vis spectrum that medium containing silver nanoparticles had the maximum absorption at 305 nm due to the interaction of light with the surface of silver nanoparticles. This interaction indicates the existence of surface plasmon resonance (SPR) (Figure 2).

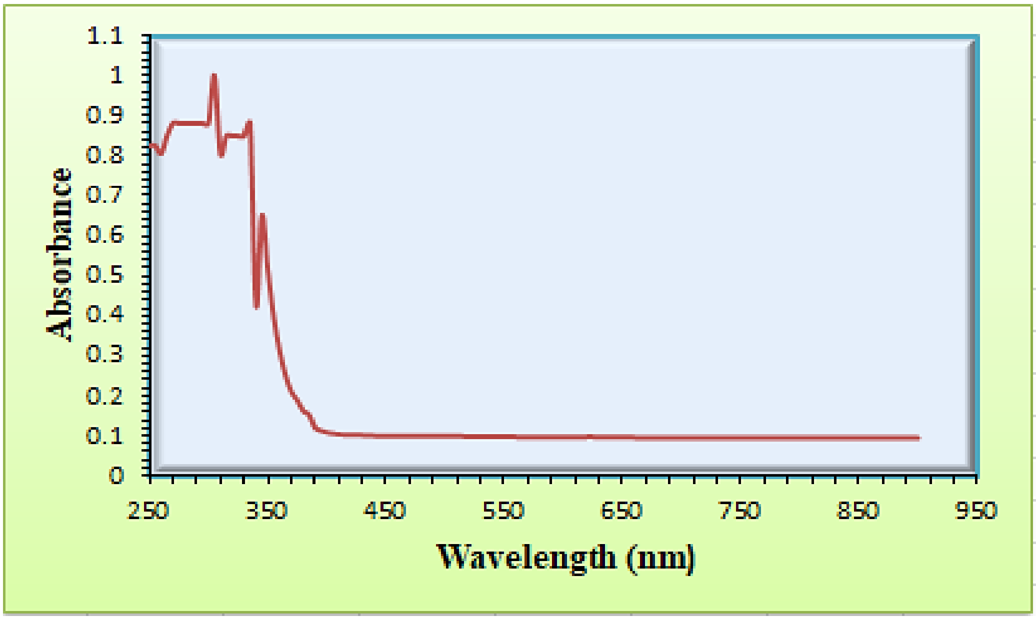

Fig. 2. UV-Visible absorption spectrum of silver Nanoparticles (AgNPs) synthesized by reducing silver ion with Mentha extract

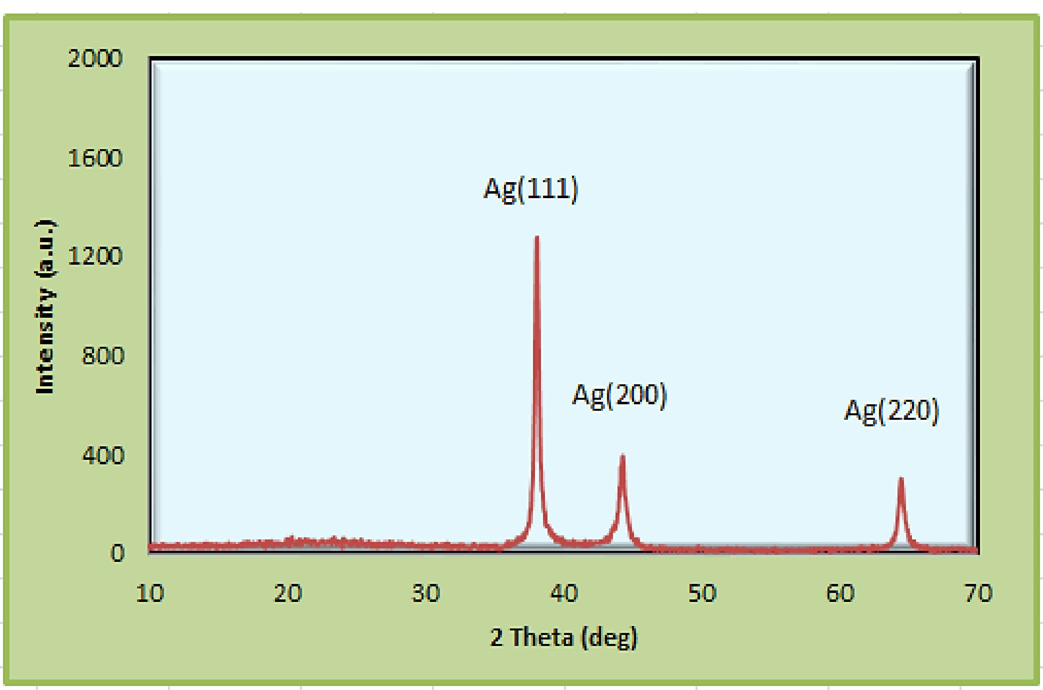

Fig. 3. XRD pattern of synthesized Ag nanoparticles using Mint extract

And the crystalline structures of AgNPs were confirmed by the XRD analysis as shown in Fig. 3. The peaks located at 2θ° = 38.2°, 44.4°, 64.6° index to the (100), (200), (220). Are characteristic diffractions of face-centered-cubic (FCC) structured metal Ag (JCPDS Card no. 04-0783).

According to the scanning electron microscope (SEM) silver nanoparticles with mean particle size of 26 nm and spherical shape were the most frequent particles (Figure 4).

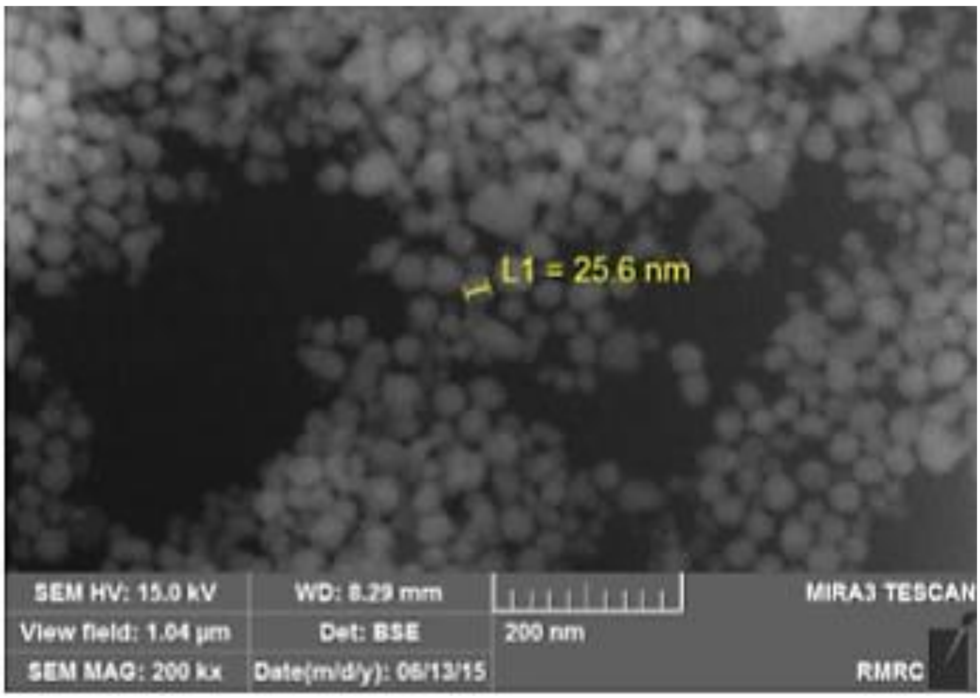

Fig. 4. SEM images of Ag nanoparticles

4. 1. Antimicrobial assay

Biosynthesized silver nanoparticles by this method were studied for antimicrobial activity against pathogenic bacteria by well diffusion method; it was observed that silver nanoparticles have antibacterial activities at concentration of 50 μl/well. $AgNO_3$ was used as a control. The silver nanoparticles biosynthesized from plant extracts showed inhibition zone against microorganisms *Escherichia coli*, *Baccillus subtilis* (Gram negative) and *E. coli* (Gram positive). A maximum zone of inhibition (MZI) is listed in Table 1. From the table, it is evident that the nanoparticles synthesized are good candidates for their usage as an antibacterial agent. The mechanism of inhibitory action of silver nanoparticles on microorganisms, still not very clearly understood. Several possibilities could benanoparticle adhesion to the cell membrane and further penetration inside or by their interaction with phosphorus containing compounds like DNA and hampering the normal replication process, loss of cell viability and eventually resulting in cell death. It is also preferable fornanoparticlesto attack on the respiratory chain. It has also been suggested that a strong reaction takes place between the silver ions and thiol groups of vital enzymes ultimately inactivate them.

The synthesized AgNPs colloidal solution has shown better antibacterial activity against both Gram-positive and Gram-negative bacterial strains and they showed the inhibition zone on the petri plates using the agar diffusion method in Figure 5. The diameter of the inhibition zones of AgNPs against the bacterial strains such as, *Bacillus subtilis* (25 mm) and *Escherichia coli* (20 mm) at 50 μg/ml concentration as shown on Table 1.

Table 1. Zone of inhibition of antimicrobial activity of silver nanoparticles (Mint)

S. No	Test pathogens	Zone of inhibition (mm) at 50 μg/ml concentration	Control
1	*E. coli*	20	-
2	*B. subtilis*	25	-

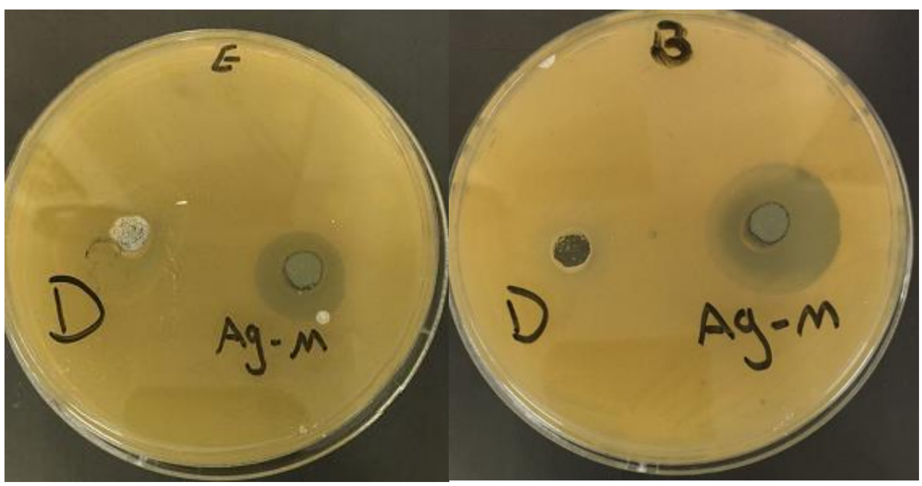

Fig. 5. Antimicrobial activity of synthesized silver nanoparticles from Mint (a) *E. coli*, (b) *B. subtilis*.

5. CONCLUSIONS

According to the results of the present study and since Mentha Leaf is a cost-effective, available and useful plant in medicine, it can be used as the best choice for the biosynthesis of nanoparticles. Among the methods of nanoparticles synthesis, biosynthesis is considered to be the cost effective, safe, and bio-compatible one. Since in this method, Silver nanoparticles are synthesized without using hazardous chemicals, they can be used in the health-related industries such as healthcare and the environment. This study showed the successful synthesis of silver nanoparticles using the extract of Mentha L. This method is a cost-effective and rapid method that can be applied at room temperature. The result of the studied showed that the green synthesized silver nanoparticles possessed to have significant antimicrobial properties. Thus, the green synthesized silver nanoparticles from Mint can be used as a curative agent for targeted drug delivery to cure diseases. This may be due to the mode of action of silver ions against the bacteria. These silver ions can cause a destruction of peptidoglycan cell wall and lysis of cell membrane. The silver ions bind to DNA bases, causes and condense the DNA to lose its ability to replicate, thereby prevents replication via binary fission. Also, it leads to induction of ROS (Reactive Oxygen Species) synthesis, thereby forming highly reactive radicals that destroy the cells.

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