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Green Synthesis, Characterization and Antimicrobial Activity of Ag Nanoparticles Using Mint Extract

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Abstract: In this work, we describe the biological method are used for the production of silver nanoparticles using plant extract. Silver (Ag) nanostructures were successfully prepared by a simple, highly efficient, and low-cost using the hydrothermal method by using the mint extract and evaluate their antimicrobial activity. The resulting nanostructures were characterized by XRD, FESEM, and UV-VIS spectroscopy. The nanoparticles structural properties were studied using X-Ray diffraction (XRD) and showed all the diffraction peaks are indexed to (F.C.C) structure. The crystallite size of Ag NPs was calculated and equal to 25 nm. FE-SEM images of silver showed nanoparticles that assembled in flower-like shape with a diameter of 10 nm-20 nm. The optical absorption explained by UV-Visible spectroscopy, the Ag NPs has sharp absorbance with the highest peak at 400 nm. The optical transmittance of the Ag film deposit time was around 40% at wavelength 400 nm then increases sharply at wavelength 400-900 nm. The energy gap increase to 3.4 eV. The antimicrobial activity was evaluated by agar well disc diffusion method against various microorganisms. The zone of inhibition against (Escherichia coli) was 20 mm, and fungus (Bacillus subtilis) was 25 mm. The use of silver nanoparticles in drug delivery systems might be the future thrust in the field of medicine.

Keywords: Green Synthesis, Silver Nanoparticles, Mint Extract, Hydrothermal Method, Antimicrobial Activity

1. Introduction

Nanotechnology is a broad-based science involving manipulation of atoms, electrons, protons, and neutrons in a variety of ways to generate new understanding of how materials can be developed to solve many problems in medicine, engineering, agriculture, surface science, marine science, and geology. It involves three dimensions at a minuscule size ranging up to 100nm [1]. The nanoparticle has potential applications in various fields such as healthcare, food and feed, cosmetics, environmental health, biomedical science, chemical industries, drug and gene delivery, energy science, electronics, mechanics, and space industries, However, Ag NPs have been established to be simple because they have sensed antimicrobial activity against various microorganisms. Almost 5000 years ago, Romans, Greeks, Egyptians, and Indians used silver in several forms to preserve the food products [2]. In the recent decade, Ag NPs have been received the huge attention of the scientists due to their remarkable defense against various pathogenic microorganisms. The exceptional characteristics of Ag NPs have made them applicable in various fields like biomedical, drug delivery, water treatment, agricultural [3]. It also has been achieved extensively in the drug delivery system for the treatments of cancer [4], diabetes [5], allergy [6], infection [7] and inflammation [8]. Nanoparticles are grouped into organic, inorganic, metal and semiconductor nanoparticles due to their superior material properties. There are many ways to synthesize nanoparticles such as solid reaction, co-precipitation, chemical reaction, and a sol-gel method, microwave irradiation, etc. The various nanoparticles like gold, silver, copper, iron, palladium, and zinc. Among these Silver nanoparticles, are known as excellent antimicrobial agents, and therefore they could be used as alternative disinfectant agents. On the other hand, released silver nanoparticles could pose a threat to naturally occurring microorganisms. In recent years green synthesis provides an advancement over chemical and physical method as it is cost-effective, environment-friendly, easily scaled up for large-scale synthesis. This technique eliminates the use of energy, high pressure, temperature and toxic chemicals [9, 10]. As plant medicated nanoparticles preparation is easy to handle, safe and economical [11]. It finds more advantages over chemical and physical method. In biological method plants have been used for the synthesis of nanoparticles were coated by the plant extract which has medical benefits and can be used as a drug and cosmetic applications [12]. All the parts of the plant like leaf, stem, flower, seed, and skin of the fruits were used earlier for the synthesis of Ag NPs. In this report, mint leaf has been used as a traditional medicine to cure various diseases. The plant mainly confirms the presence of phytochemicals such as steroids flavonoids, alkaloids, terpenoids, proteins, phenols and anthraquinones, which are already, reported [13]. The current investigation focuses on the extract of mint used to synthesize Ag NPs using a hydrothermal method enhancing the importance of plant sources and implementing green chemistry for the future research.

2. Experimental Section

2.1. Materials

Twenty gram of fresh leaves of mint were taken and washed with distilled water separately. Leaves were cut into fine pieces and crushed with 100 ml sterile distilled water using motor and pastel. Contents were boiled with constant stirring for ten minutes. After cooling contents were filtered with Whatman No.1 filter paper (pore size 25 µm). Dark yellow colored extracts were obtained, which were used as reducing agent and stabilizer.

Figure 1. *The conversion of Mentha leaf in (Mint) extract.*

2.2. Biosynthesis of Ag NPs Using Mint Extract

0.1 M of Silver nitrate was dissolved in 50 ml of distilled water and it was added to 25 of mint extract and stirring at room temperature for 30 min. The brown color change was observed after 15mins. The mixtures were added to sealed Teflon-lined of 100 ml capacity, which was heated and maintained in the oven for 3 h at 190 gray precipitate was collected by filtration, washed with ethanol and distilled water several times, finally dried in air at 30°C for 24 hours.

2.3. Antibacterial Assay

The antibacterial activity of Ag-NPs that prepared by mint aqueous extracts were evaluated against two types of bacteria. The antibacterial assessment was performed using agar well diffusion method against different pathogenic microorganisms Escherichia coli (gram negative) and Baccillus subtilis (gram positive) and measuring the inhibition zones (mm). The pure cultures of bacteria were sub cultured on Mueller-Hinton agar (MHA). Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. Wells of 5 mm diameter were made on nutrient agar plates using gel puncture. Using a micropipette, 25 µL of nanoparticle solution was poured onto each well on all plates. After incubation at 37°C for 24 hrs, the diameter of zone of inhibition was measured in mm.

3. Characterization

The UV-Visible spectral measurements were used to confirm the formation of silver nanoparticles by using Shimadzu – UV1800 spectrophotometer instrument in the range between 200-900 nm. The sample was centrifuged at 4000 rpm for15min, then dried and ground with KBr to form a pellet and analyzed on thermo nicolet iS5 model. The SEM analysis was carried out to determine the morphology and the mean particle size of nanoparticles. The sample was prepared on a carbon-coated grid by just dipping a very small amount of the prepared Ag NPs on the grid, by using blotting paper the extra solution was isolated, then the sample was allowed to dry for SEM analysis using JEOL-JSM-5610LV with a resolution of 1µm at 15kV with 27mm. X-ray diffraction pattern can be used to determine the crystalline structure. X-ray system (Shimadzu - XRD6000, Shimadzu Company /Japan). The X-ray source was Cu-Kα radiation with 0.15406 nm wavelength. The system operates at 40 KV and 30 mA emission current. The sample is scanned from (20 - 90 degree).

4. Results and Discussion

4.1. XRD Diffraction Patterns

The XRD diffraction patterns of synthesized Ag nanoparticles. The XRD patterns of Ag contain 3 main peaks at diffraction angles 2θ˚: 38.2˚, 44.4˚, 64.6˚ corresponding to (111), (200), and (220) planes respectively as shown in figure 2 also, this result agrees well with that presented in references. All the diffraction peaks are indexed to F.C.C structure and there is no trace of the hexagonal face, which matched well the standard peaks (JCPDS 04-0783). The average crystallite size was calculated by using Scherrer formula, the peak widths of a strong diffraction plane were calculated and listed in Table (1), the strong and narrow peaks may be ascribed to the preferential growth along (111) plane of Ag crystallites. Table 1 shows the crystallite sizes of Ag-NPs. The average crystallite size from XRD has been estimated using Scherer formula: [14].

$$D = k\lambda/\beta\cos\theta \quad (1)$$

Where k = constant (0.89<k<1), λ = wavelength of the X-ray, β = full width at half maximum (FWHM) of the diffraction peak, and θ = diffraction angle. The crystal size of Ag of the prevailing peak nanoparticles of (111) about 25 nm.

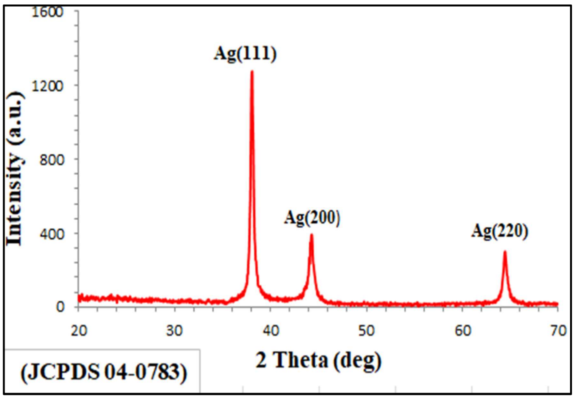

Figure 2. *XRD pattern of synthesized Ag nanoparticles using Mint extract.*

Table 1. *The crystallite sizes of Ag NPs.*

Material	h k l	2 Theta (deg)	Crystallite size (nm)
	111	38.2	25
Ag	200	44.4	20
	220	64.6	24

4.2. FE-SEM Analysis

The morphological features of synthesized Ag nanoparticles from mint extract using hydrothermal was studied by (Scanning Electron Microscope). The figure showed the high density of Ag NPs synthesized. The electrostatic interactions such as hydrogen bond, bio-organic bond, and capping molecules are the reason for biosynthesis of siver nanoparticles. The Synthesized of Ag nanoparticles was flower-like in shape with a diameter of 10 nm-20 nm as shows in figure 3.

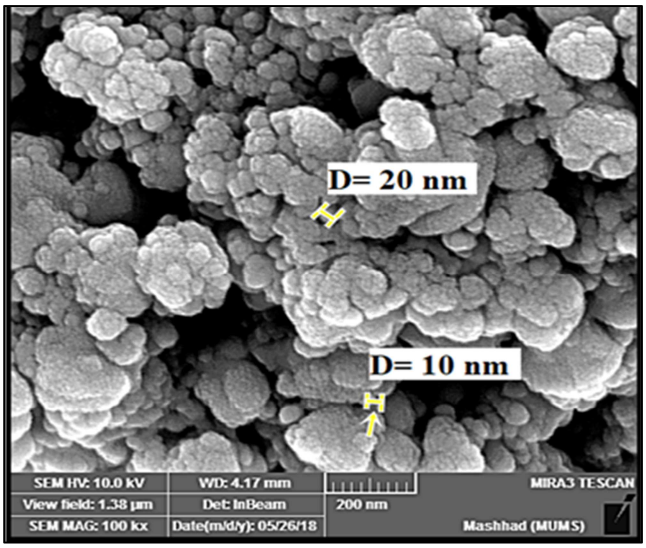

Figure 3. *SEM images of Ag NPs that prepared using mint extract.*

4.3. UV-Vis Analysis

The reduction of silver ions into silver nanoparticles during exposure to plant extracts was observed as a result of the color change. The color change is due to the Surface Plasmon Resonance phenomenon. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with a light wave. The optical property of Ag NPs was determined by UV-Visible spectrophotometer. After the addition of silver nitrate to the plant extract. The Ag NPs has sharp absorbance with the highest peak at 400 nm and progressively decreased while nanometer increased due to quantum size effect as shown in figure 4.

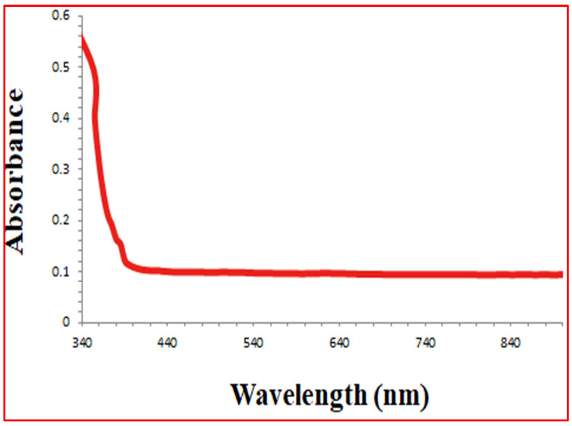

Figure 4. *UV-visible absorption spectrum of (Ag-NPs) synthesized by mint extract.*

Figure 5 shows the optical transmittance for Ag nanoparticles as a function the wavelength at the range (300-1000) nm. The UV-Vis spectra are very important because it provides the details related to the optical band. The optical transmittance of the Ag film deposit time was around 40% at wavelength 400 nm then increases sharply at wavelength 400-900 nm. Also it is observed that the optical transmittance spectra shift towards shorter wavelength as a particle size decrease.

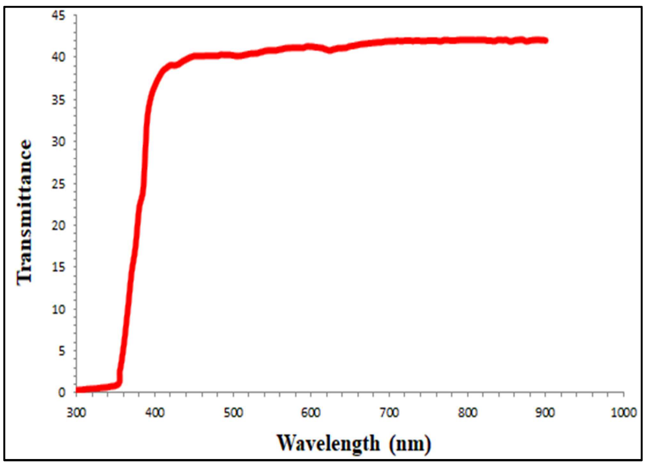

Figure 5. *Transmittance Spectra of Ag film.*

Figure 6 shows that the graph between (αhv)2 versus photon energy (hv) gives the value of the direct band gap. The extrapolation of the straight line to (αhv)2 = 0, gives the value of the band gap. From the UV-spectra shows the absorbance decreases with increasing wavelength and the energy gap increase to 3.4 eV.

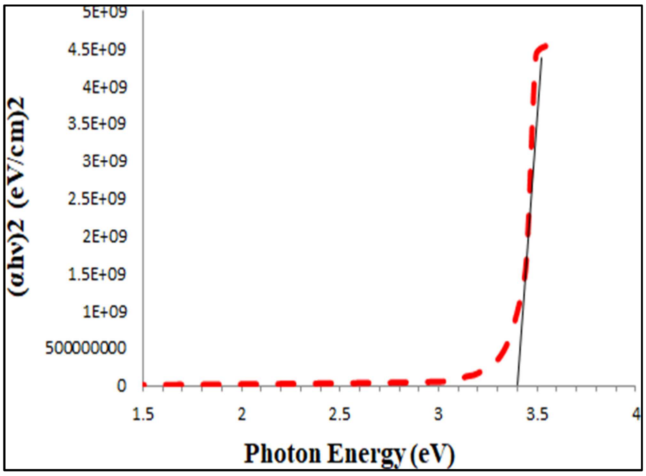

Figure 6. *(αhv)2 versus photon energy gap of Ag film.*

5. 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 50µl/well. AgNO3 was used as a control. The silver nanoparticles biosynthesized from plant extracts showed inhibition zone against microorganisms Escherichia coli, Bacillus 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 be nanoparticle adhere 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 for nanoparticlesto 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 Ag-NPs 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 7. The diameter of the inhibition zones of Ag-NPs against the bacterial strains such as, Bacillus subtilis (25mm) and Escherichia coli (20mm) at 50 µg/ml concentration.

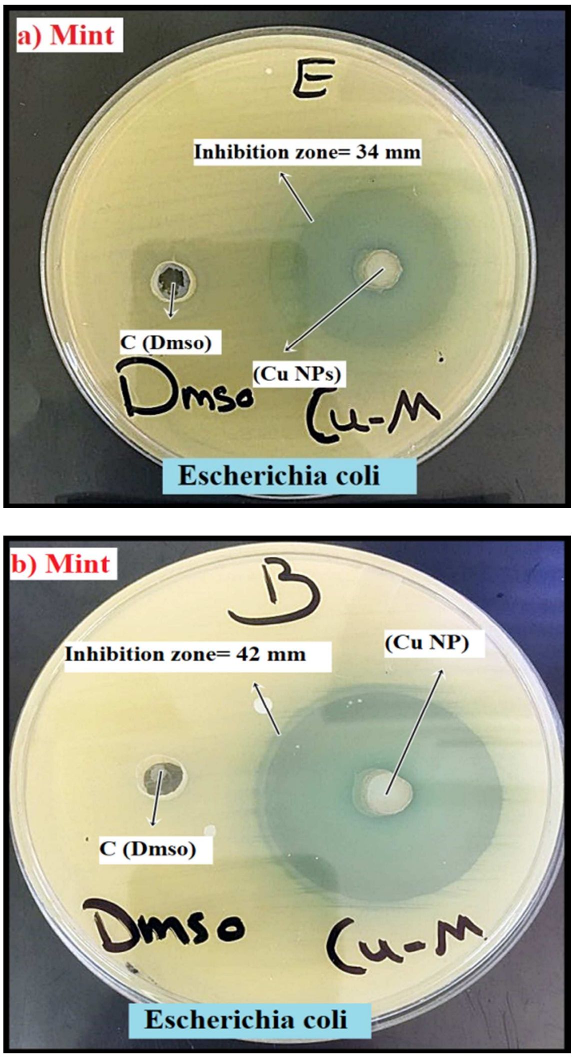

Figure 7. *Antimicrobial activity of synthesized silver nanoparticles from Mint (a) E. coli, (b) B. subtilis.*

Table 2. *Zone of inhibition of antimicrobial activity of silver nanoparticles (mint).*

S.No	Test pathogens	Zone of inhibition (mm)	Control
1	E. coli	20	
2	B. subtilis	25	

6. Conclusion

In summary, silver nanostructure was the successfully prepared by a green synthesis method using mint extract with a hydrothermal. The present study proves the use of the medicinal plant for biosynthesis of silver nanoparticles which is rapid, cost-effective and environmentally safe with potential use against microbes. The results showed the silver nanoparticles in the size range of 10-30 nm. The produced Ag nanoparticles were polycrystalline in nature with the F.C.C phase with flower like in shape. The optical properties data revealed that the direct optical band gap of silver was 3.4 eV. The synthesized stable aqueous dispersions of Ag nanoparticles using plant extract are ideal for diverse industrial and biomedical applications.

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