



Eco-Friendly Ultrasonication-Assisted Synthesis of Silver Nanoparticles

Using *Foeniculum Vulgare*: A Potent Antimicrobial Agent

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Abstract

This research delves into the green synthesis of silver nanoparticles (Ag NPs) utilizing the aqueous extract of *Foeniculum vulgare* (fennel). The synthesis process involved mixing the fennel extract with an aqueous silver nitrate solution at two different temperatures, 25 °C and 60 °C, under ultrasonication. The synthesized Ag NPs were subjected to a comprehensive characterization using various analytical techniques. The UV-visible spectroscopy revealed a distinct absorption peak at 450 nm, indicative of the successful formation of Ag NPs. Fourier-transform infrared (FTIR) spectroscopy identified the presence of functional groups such as amides, amines, and flavonoids, which played a role in the reduction and stabilization of the nanoparticles. Powder X-ray diffraction (p-XRD) analysis confirmed the crystalline nature of the Ag NPs. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) analyses showed that the nanoparticles were predominantly spherical, with diameters ranging from 10 nm to 30 nm, irrespective of the synthesis temperature. The antimicrobial efficacy of the Ag NPs was evaluated, revealing inhibition zones of 26 mm and 17 mm against *Escherichia coli* at 25 °C and 60 °C, respectively. The Ag NPs demonstrated moderate antibacterial activity against Gram-negative bacteria and limited antifungal activity. These findings highlight *Foeniculum vulgare* as a viable and eco-friendly source for the synthesis of Ag NPs, with promising applications in antimicrobial treatments.

Keywords: *Foeniculum vulgare*, Ag Nanoparticles, SEM, TEM, antimicrobial activity.

1. Introduction

The green synthesis of nanoparticles has garnered significant interest in material and nanoscience research owing to its inherent benefits. These advantages encompass a non-toxic synthesis process, the absence of hazardous chemicals, high safety standards, and a minimal environmental impact. The metal nanoparticles have witnessed a tremendous development of the area within the realms of nanotechnology due to unique properties, like a smaller size that can be arranged from 1 to 100 nm in diameter and a larger surface area to volume ratio. Nanosized particles of metals have been attracting interest recently owing to their distinctive chemical, catalytic, optical, physical, and electrical properties, as well as their vital antimicrobial activity against various types of bacteria [1–7]. Amongst numerous metal nanoparticles, Ag NPs have been intensively studied, due to their exceptional physicochemical properties [8]. Also, it has been determined that the valuable Ag metal can be considered safe due to its displaying insignificant toxicological effects compared to other metals [1, 9]. According to the reports, Ag NPs appear promising for use against multidrug-resistant bacteria, as well as in many critical applications, such as anticancer, catalytic electronics, optics, imaging, and biomedical fields. This is because they have unique properties, such as reactivity and physical properties [9–12]. Various methods are reported for the synthesis of nanomaterials, however, most of these methods require the use of potentially hazardous and toxic reactants/reagents and surfactants that are not compatible with biological applications.[13–15] Developing eco-friendly and non-toxic routes is of great interest and necessary specifically for biological applications. In this regard, plant extracts are considered suitable and safe alternatives to toxic reagents/surfactants for the synthesis of nanomaterials. The aqueous extracts of various medicinal plants have been used successfully for the preparation of metal nanoparticles with controllable dimensions [11, 16, 17]. The plant extracts act as mild reducing agents as well as surfactants i.e. the functional groups help in controlling size and shape. The mild nature of the plant extracts results in better controlling the kinetics whereas the functional groups help stabilize the nanoparticle's surface [11, 18–22]. In addition, this green route is easily scalable and cost-effective.

Ag nanoparticles with uniform size distribution have also been prepared by using plant extracts [23]. Ag is easily reducible and the phytochemicals present in plant extracts can help to reduce Ag⁺ ions into Ag nanoparticles without the addition of a capping agent [24, 25]. Extracts from different parts of the plant, including the leaves, seeds, roots, flowers and stems, have been used to synthesize Ag NPs. Each part of the plant contains different chemically effective compounds that exert a physiological impact on humans [16, 26, 27]. *Foeniculum vulgare* (fennel) is a medicinal and aromatic plant that belongs to the *Foeniculum* genus and Apiaceae family. Fennel is a herb with a strong flavor, light yellow flowers and the potential to be

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used in the medicinal, nutritional and pharmaceutical fields. In different parts of the world, the seeds, leaves, stems, fruit and whole *F. vulgare* plants have been used, both in the past and currently, to treat several diseases, like coughs, colds, renal disease, diarrhea, gastralgia and fever [28–30]. Fennel seed extracts appear relevant to anti-carcinogenic, anti-coagulant, anti-diabetic agent and anti-inflammatory applications, because these appear to contain various biologically active chemical constituents [12, 27, 31–34]. Researchers have studied the essential oils and extracts of this plant to determine its diverse chemical constituents and evaluate their potential antimicrobial and antioxidant activities [35–40]. *Foeniculum vulgare* is rich in proteins, fats, minerals, fibers, carbohydrates, and phytochemical compounds. These phytochemicals, including amino acids, volatile compounds, fatty acids, phenolics, and flavonoids, play a crucial role in the reduction of silver ions (Ag^+) to zerovalent Ag NPs because of their potent antioxidant properties. [41–43] Several studies have demonstrated that the green synthesis of nano-sized materials has been achieved by using extracts of the *F. vulgare* with several of the salts of different metals (Au, Ag, Se), and evaluated their antimicrobial, antioxidant, and catalytic activity [12, 28, 44–46].

The objective of this research is to synthesize Ag NPs using an aqueous extract of *F. vulgare* without the use of a capping agent at two different temperatures, 25 °C and 60 °C, with the aid of ultrasonication. Ultrasonication is known to enhance the synthesis process by providing energy that facilitates the reduction of silver ions and the formation of nanoparticles. This method aims to produce Ag NPs with desirable properties while minimizing the environmental impact. The synthesized Ag NPs will be characterized using various analytical techniques, including UV-Vis spectroscopy, X-ray diffraction (XRD), transmission electron microscopy (TEM), and Fourier-transform infrared spectroscopy (FTIR). These techniques will provide comprehensive information on the size, shape, crystalline structure, and surface chemistry of the nanoparticles. Furthermore, the antimicrobial effects of the prepared Ag NPs will be evaluated against a range of selected microorganisms. The increasing prevalence of antibiotic-resistant bacteria has necessitated the search for alternative antimicrobial agents. Ag NPs have shown promise in this regard due to their ability to disrupt microbial cell membranes and interfere with cellular processes. This study aims to contribute to the growing body of knowledge on green synthesis methods and the potential applications of Ag NPs in antimicrobial treatments. By utilizing *F. vulgare* extract and ultrasonication, this research seeks to develop a sustainable and efficient method for producing Ag NPs with potent antimicrobial properties.

2. Results and Discussion

2.1. UV-vis spectrophotometry of Ag NPs

The process of synthesizing silver nanoparticles (Ag NPs) is a fascinating area of nanotechnology, particularly when using natural compounds like *F. vulgare* (fennel) aqueous extract as a reducing agent. The observation of a color shift from yellow to dark brown is a classic visual indicator of the reduction of Ag^+ to metallic Ag, signifying the formation of Ag-NPs. This color change is typically corroborated by UV-visible spectroscopy, a reliable analytical method to monitor the synthesis and stability of nanoparticles. By scanning across a broad wavelength range, from 180 to 800 nm, researchers can detect the surface plasmon resonance typical of Ag NPs, which confirms their presence in the solution [22]. This technique not only validates the synthesis process but also provides insights into the size, distribution, and concentration of the nanoparticles, which are crucial parameters for their applications in various fields. The Ag NPs display an unusual optical phenomenon known as surface plasmon resonance, which varies based on their size, shape, and morphology. Their absorption mainly appears in the range of 400-500 nm in solution [22]. Based on the absorption spectra, the optimized conditions for synthesizing these Ag NPs are, mixing 6 ml of aqueous extract with 100 ml of 1 mM of AgNO_3 at room temperature. The reaction mixture was sonicated for one hour (refer to Figure 2). This result indicates that the maximum wavelength is approximately 450 nm, which validates the synthetic procedure of Ag NPs and agrees with the findings from other studies [22, 25]. However, it has been recorded that the band was examined at 427nm for the Ag NPs prepared using aqueous extract of *F. vulgaris*. This variation in wavelength may be due to the nature of the reducing agents and their efficiency regarding the plant extract [12]. We assessed the effect of the temperature (40 and 60 °C) on the synthesis of the nanoparticles under constant conditions (concentration and time). The two samples of Ag NPs of *F. vulgare* extract exhibited the same absorption bands at 450 nm but the band at 60 °C was sharper than the others, possibly due to a fast reduction of Ag^+ ions at the higher temperature [16].

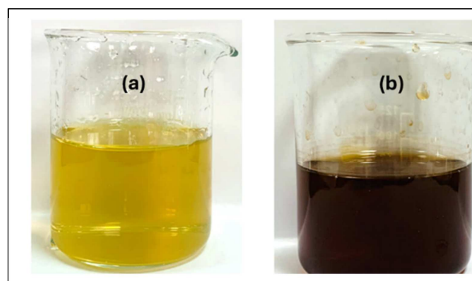


Figure 1: A (a) Plant extracts with AgNO_3 and (b) Ag NPs (right) synthesized from *Foeniculum vulgare* L.

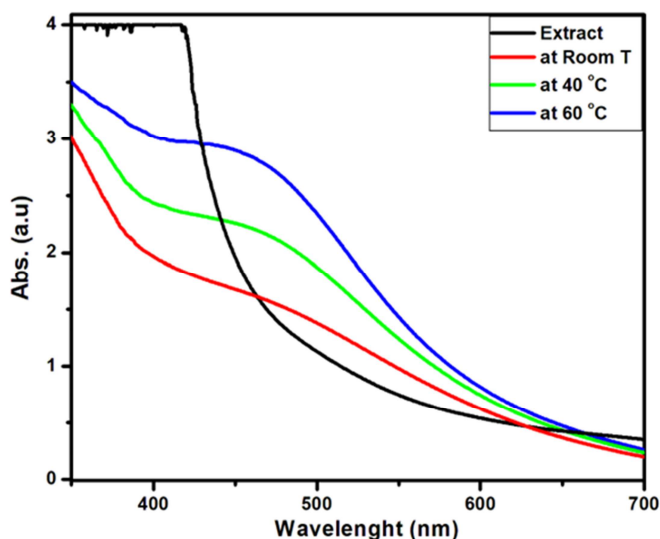


Figure 2: Ultraviolet–visible spectra of synthesized Ag NPs with extract of *F. vulgare*, (black) extract, (red) at 25°C (Room T), (blue) at 40 °C and (green) at 60 °C.

2.2. Fourier transform infrared spectroscopy (FT-IR)

FT-IR is considered a valuable technique for clarifying the convolution of the surface functional groups in metal interactions. This tool is used to determine the groups that are present in *F. vulgare* and predict their behavior during the synthesis of Ag nanoparticles [50]. The FT-IR spectrum for silver nanoparticles (Ag NPs) synthesized using *Foeniculum vulgare* (fennel) extract at 25 °C and 60 °C revealed distinct peaks at 3251.60, 2908.32, 2653.07, 1539.1, 1371.23, and 1046.07 cm^{-1} , respectively. These peaks provide valuable insights into the functional groups present in the synthesized nanoparticles and the role of the plant extract in the synthesis process. This peak corresponds to the stretching 3251.60 cm^{-1} vibrations of the OH group, which are characteristic of phenols and alcohols. The presence of this peak indicates that hydroxyl groups from the *F. vulgare* extract are involved in the reduction and stabilization of Ag NPs.: The weak band at 2908.32 cm^{-1} is attributed to the C–H stretching vibrations of proteins. This suggests that proteins in the plant extract play a role in the synthesis process, possibly acting as reducing agents or stabilizers. This weak band 2653.07 cm^{-1} is associated with N–H or C–O stretching vibrations. The presence of these functional groups indicates that amines or carboxyl groups from the plant extract are involved in the capping of Ag NPs. The strong peak at 1539.1 cm^{-1} is linked to the stretching vibrations of C=O and C=C bonds. This suggests the presence of carbonyl groups, which are known to interact strongly with metal ions, aiding in the reduction and stabilization of Ag NPs. These peaks 1371.23 cm^{-1} and 1046.07 cm^{-1} suggest the presence of alcohols, carboxylic acids, ethers, and esters in the plant extract. These functional groups contribute to the capping and stabilization of the nanoparticles.[51, 52] The ability of biological molecules to naturally undertake the role of nanoparticle formation and stabilization is a significant finding. This biogenic approach to nanoparticle synthesis offers several advantages, including eco-friendliness, cost-effectiveness, and the potential for large-scale production. The study by Showmya et al. (2012) provides a scientific basis for further exploration into the use of biological molecules in nanotechnology.[33] The insights gained from this study have broad implications for the development of new nanotechnologies and their applications in various fields, including medicine and materials science. For instance, the biogenic synthesis of Ag NPs can be leveraged for antimicrobial applications, drug delivery systems, and the development of novel materials with enhanced properties.

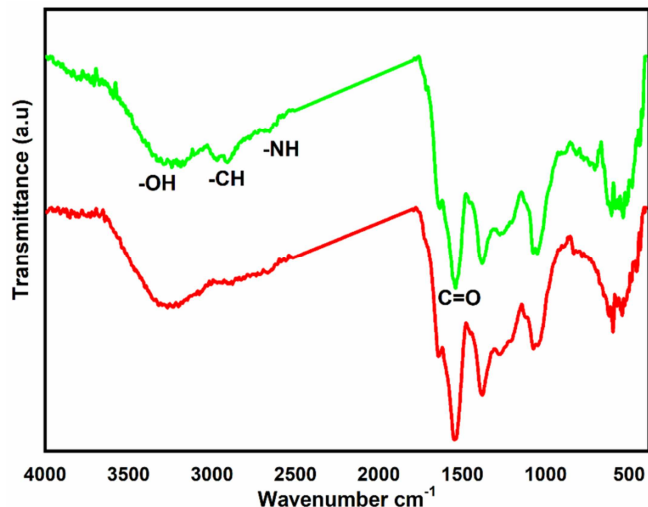


Figure 3: Fourier transform infrared spectra of synthesized Ag NPs, (red) at 25°C, and (green) at 60 °C.

2.3. X-Ray diffraction (XRD)

X-ray diffraction (XRD) examination was used to confirm the crystalline structure of Ag nanoparticles produced using *F. vulgare* extract (Fig. 4). The XRD pattern of the Ag nanoparticles revealed three distinct diffraction peaks at 2θ values of 38.20°, 45.60°, and 64.65°, which correspond to the (111), (200), and (220) planes, respectively, indicating a face-centered cubic structure of the biosynthesized Ag NPs, as confirmed by (JCPDS), file number 31-1238. The clarity of the diffraction peaks indicated that the Ag crystalline particles were in the nanoparticle range. The average crystal size of the produced Ag NPs was calculated to be 27 ± 5 nm using the Debye-Scherrer formula.

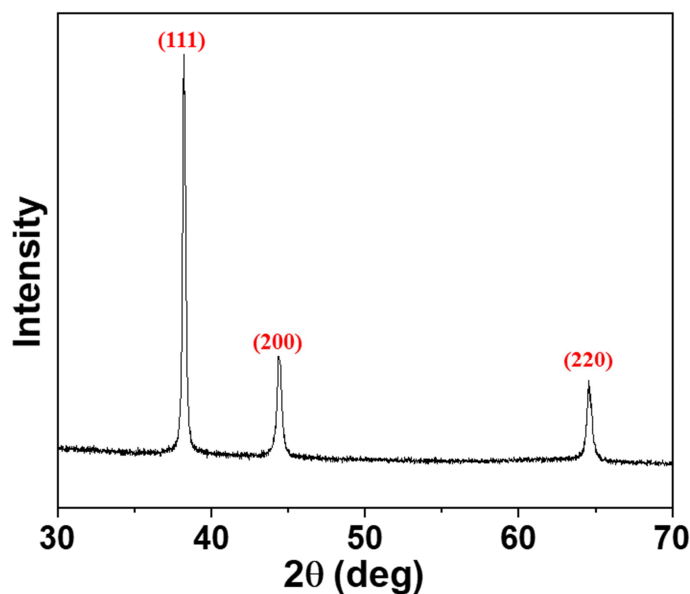


Figure 4: The X-ray diffraction (XRD) pattern of the green-synthesized AgNPs using the *F. vulgare* extract at 60 °C.

2.4. Scanning Electron Microscope (SEM) analysis

SEM images were carried out to identify the shape of Ag NPs that had been generated using *F. vulgare* aqueous extract. The SEM images exhibit agglomerated microscale spheres in all samples of 10–25 nm in size, that assembled regularly and in close proximity to each other (Fig. 5). The hydroxyl and other functional groups may be responsible for the formation of the agglomerated structures of Ag NPs [28].

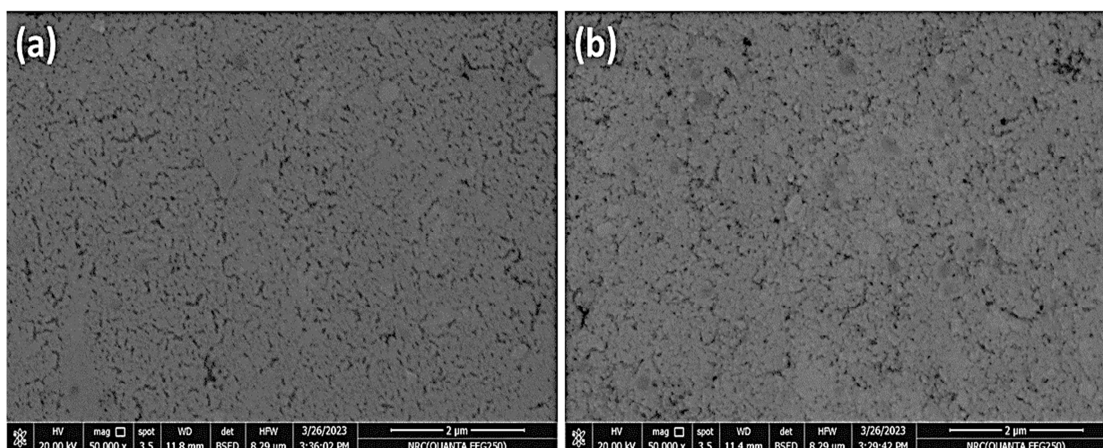


Figure 5: SEM images of synthesized Ag NPs (a) at 25 °C and (b) at 60 °C.

2.5. TEM and Selected Area Electron Diffraction (SAED) studies

The particle size, crystallinity, and morphology of the produced Ag NPs were identified by using SAED and TEM investigation. Typical TEM images of Ag NPs generated in a green process are shown in Figure 6(a-f). The images reveal that the Ag NPs were predominantly spherical and polydisperse in nature, with only a small number of particles exhibiting irregular morphologies. Such size and shape variations in the biosynthesized nanoparticles are relatively common [45, 53]. Additionally, TEM analysis demonstrated that the majority of the Ag NPs, synthesized at both 25 °C and 60 °C, had an average size of approximately 15–20 nm and 15–30 nm, respectively. Figure 6 (c) and (f) showed a histogram depicting the particle size distribution that was generated using ImageJ software to analyze approximately 35 particles. These figures show that the typical size of the Ag NPs particles, synthesized at both 25 °C and 60 °C, is approximately 20 ± 3 nm and 15 ± 3 , respectively, and that the nanoparticles that vary in size from 5 to 12 nm account for approximately 90% of all of the particles examined. The circular white dot-like fringe patterns observed in (SAED) are indicative of the crystalline nature of nanoparticles, in this case, silver nanoparticles (Ag NPs). These fringe patterns are characteristic of the diffraction from spherical crystalline structures, as the uniformity of the rings represents the regular, repeating atomic structure of the crystalline material. Figures 6 (b) and (e) suggest a high degree of crystallinity within the Ag NPs, which is essential for various applications that require consistent and predictable properties from the nanomaterials.

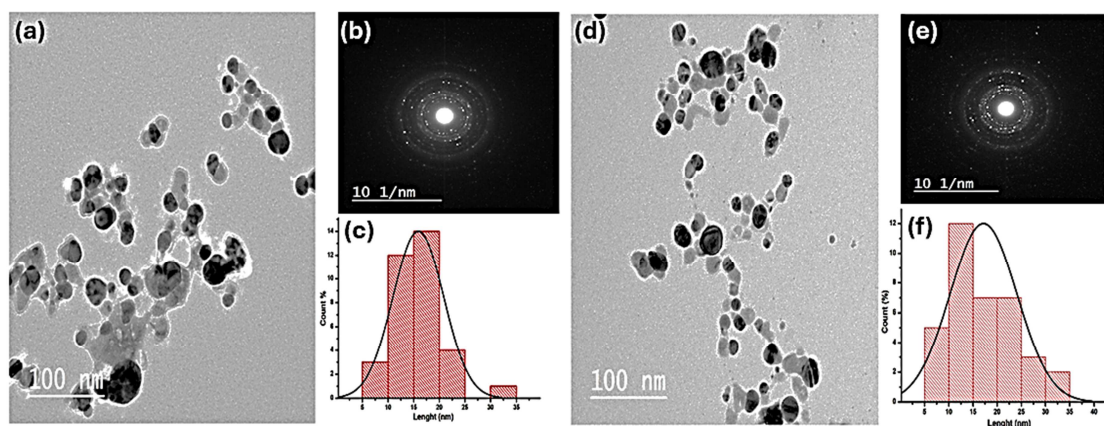


Figure 6: TEM of synthesized Ag NPs with *F. vulgare* aqueous extract (a-c) at 25 °C and (e-f) 60 °C.

2.6. Antimicrobial Activity

The green-synthesized Ag NPs, which were synthesized at 25 °C and 60 °C, were assessed against the variability of bacteria's Gram-negative and Gram-positive as well as fungi, as indicated in Table 1. The evaluation was carried out using the method of diffusion on an agar well. The results indicate that the Ag NPs had a positive effect on the Gram-positive bacteria, particularly on *Escherichia coli*, than *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

Furthermore, the inhibition zone (IZ) values of the Ag NPs against *E. coli* were 26 mm and 17 mm for samples 1 and 2, respectively, when compared to the standard antibiotic. Meanwhile, the efficacy of the Ag NPs against the Gram-negative bacteria and fungi appeared to exert a moderate effect on the Gram-negative bacteria (*Staphylococcus aureus* and *Streptococcus mutans*) and a weak effect on the strain test fungi. Notably, sample 2 of the Ag NPs also exhibited activity against *Aspergillus ochraceus* compared to the standard antibiotic. These findings suggest that Ag NPs have the potential for use as an effective antimicrobial agent against a variety of harmful microorganisms. These findings are in line with a previous study published by Bonde S. in 2011, which demonstrated that the phyto-synthesized Ag nanoparticles of *F. vulgare* exhibited antibacterial activity against *S. aureus* and *E. coli*. Furthermore, the study found that the combination of these nanoparticles with commercially available antibiotics resulted in remarkable antibacterial activity against two human pathogenic bacteria [8]. These results suggest that Ag NPs could represent a promising tool for combatting bacterial infections, especially when used in combination with conventional antibiotics. Recent research shows that the Ag nanoparticles synthesized using *F. vulgare* displayed a good antibacterial influence against clinical isolates of MRSA, both in vitro and in vivo [51, 54]. These findings suggest the potential use of these nanoparticles in plant-based alternative and complementary medicine

Table 1: Inhibition zones of the tested Ag NPs synthesized *Foeniculum vulgare* extract against some microorganisms.

Microorganism	Sample 1	Sample 2	Standard antibiotic
Gram-negative bacteria			Gentamicin
<i>E. coli</i> (ATCC:10536)	26.0±1.0c	17.0±1.0a	27±1.0c
<i>K. pneumonia</i> (ATCC:10031)	16.6±0.7b	14.6±0.7a	25.3 ±0.7c
<i>P. aeruginosa</i> (ATCC:27853)	11.6±0.7a	12.6±0.7a	28±1.0b
Gram-positive bacteria			Ampicillin
<i>S. aureus</i> (ATCC:13565)	13.3±0.7b	11.3±0.7a	21.3±0.7c
<i>S. mutans</i> (ATCC:25175)	14.3±0.7a	14.6±0.7a	28.3±0.7c
Fungi			Nystatin
<i>Candida albicans</i> (ATCC:10231)	NA	NA	21.6±0.7
<i>Asperagillus ngar</i> (ATCC:16404)	NA	NA	19.3±0.7

- Mean ± Standard deviation (mm) is used to show the Zone of inhibition
- NA: No activity
- Sample 1 prepared at 25 °C, Sample 2 prepared at 60 °C.

3. Experimental

3.1. Materials and methods

The aerial parts of *F. vulgare* were collected from a local garden in Al Mukhwah City, Southern Saudi Arabia, with great care, to ensure optimal quality. The samples were meticulously air-dried for six days at room temperature, then ground to a fine powder and securely stored in closed bottles.

3.2. Preparation of the plant extract

To prepare the aqueous extract of *F. vulgare*, 20 g of the plant material was heated in 100 mL double-distilled water and stirred at 150 rpm for 2 hours. The solution is then filtered using a normal filter paper to isolate the solid material, then filtered again through the Whatman filter paper No. 1. The resultant extract is kept in the refrigerator at 5 °C and is used as a reduction and stabilization agent to synthesize silver nanoparticles.

3.3. Green synthesis of AgNPs

To prepare Ag nanoparticles, 6 mL of *F. vulgare*'s aqueous extract was added to 100 mL of 0.01 M of AgNO₃ and sonicated for 1 hour at 25 °C. The experiment was also conducted at 60 °C using ultrasound radiation. These processes were performed at pH 7 to detect how temperature affects the properties of Ag-NPs. The formation of Ag NPs was proven by a change in the mixture's color from yellow to dark brown, which is a characteristic of Ag-NPs formation, as reported in previous studies [47, 48]. The synthesized Ag NPs were then centrifuged and dried in oven at 40 °C for 24 hours before being

subjected to characterization techniques to evaluate their physiochemical properties and also were tested for their antimicrobial activity.

3.4. Ag NPs Characterization

The absorption spectrum of the Ag NP suspension was determined using UV-Vis spectra (Shimadzu Spectrophotometer UV-1800). Using quartz cells with a 1 nm resolution, the absorbance of the materials was monitored over a 200–800 nm wavelength range in order to construct plasmonic curves. FTIR analysis of the synthesized Ag NPs was performed using the potassium bromide (KBr) pellet (FTIR grade) method in a 1:100 ratio. The spectrum was registered using a JASCO FT/IR-6300 spectrometer prepared with a JASCO IRT-7000 Intron Infrared Microscope using transmittance mode operating at a resolution of 4 cm⁻¹ (JASCO, Tokyo, Japan). The morphology of the obtained Ag NPs was analyzed using a scanning electron microscope (SEM) (Quanta FEG 250 with field emission gun, FEI, Netherlands) and high-resolution transmission electron microscopy (HR-TEM), along with elemental fingerprinting after sonicating Ag NPs for 1 h in ethanol (JEM-2100, JEOL, Tokyo). Powder X-ray diffraction (XRD) was carried out using a Bruker D8 Advance and a Bruker Xpert diffractometer with Cu-K α radiation ($\lambda = 1.54178 \text{ \AA}$) at room temperature.

3.5. Ag NPs-Antimicrobial profile

The way of agar well diffusion was employed to assess the antibacterial ability of the Ag NPs. The antibacterial properties of the Ag NPs were evaluated in vitro using a nutrient agar medium against the Gram-negative bacteria *Escherichia coli* (ATCC:10536), *Pseudomonas aeruginosa* (ATCC:27853) and *Klebsiella pneumonia* (ATCC:10031), as well as the Gram-positive (*Staphylococcus aureus* (ATCC:13565) and *Streptococcus mutans* (ATCC:25175)). Additionally, a Sabouraud dextrose agar medium was used to assess the antifungal activity against *Candida albicans* (ATCC:10231) and *Aspergillus niger* (ATCC:10231). The standard medications for Gram-negative and Gram-positive bacteria were gentamicin and ampicillin, respectively. Nystatin was employed to combat the strains of fungus. DMSO was employed as a solvent (negative) control. Compounds were examined at a concentration of 15 mg/ml against bacterial and fungal strains.

3.5.1. Well diffusion study

Sterilized 20-25 mL Petri dishes were occupied with the sterile media and left to harden at room temperature. A microbial suspension was prepared in sterilised saline equivalent to 0.5 McFarland standard solution ($1.5 \times 10^5 \text{ CFU mL}^{-1}$); its turbidity was set at OD = 0.13 utilising a spectrophotometer at 625 nm. A sterile cotton swab was then dipped into the suspension and spread on the dried agar surface of a Petri plate within 15 minutes of adjusting turbidity. The plate was covered and left to dry for another 15 minutes. Afterward, six-millimeter holes were drilled into the solidified media using a sterile auger.

3.5.2. Statistical analysis

To compare the variation among groups of the same microbial species, we applied one-way ANOVA to the data. This statistical test allowed us to determine whether the differences between the samples were significant or not.[49].

4. Conclusions

In conclusion, this study successfully demonstrates a facile, biomimetic, and green method for synthesizing stable silver nanoparticles (Ag NPs) using an aqueous extract of the *Foeniculum vulgare* plant. The phytochemicals present in the extract played a crucial role in both the reduction of Ag⁺ ions and the stabilization of the resulting nanoparticles. The synthesis process, conducted at 25 °C and 60 °C, was thoroughly characterized using UV-visible spectrophotometry, FT-IR, SEM, and TEM analyses. TEM analysis revealed that the green-synthesized Ag NPs were predominantly polydisperse with minimal agglomeration, exhibiting spherical morphologies with sizes ranging from 10 to 20 nm at 25 °C and 10 to 25 nm at 60 °C. The antibacterial activity of these Ag NPs was notable, displaying significant efficacy against Gram-positive bacteria and moderate effects against Gram-negative bacteria. This highlights the potential of these nanoparticles as effective antibacterial agents, particularly in the development of antibiotics derived from natural sources. The findings of this study underscore the importance of further research to isolate and identify the most active components within the *Foeniculum vulgare* extract, which could enhance the antibacterial properties of the synthesized nanoparticles. Moreover, this green synthesis approach aligns with the principles of sustainability and environmental friendliness, offering a viable alternative to conventional chemical synthesis methods that often involve toxic reagents and generate hazardous by-products. The use of plant extracts not only reduces the environmental impact but also leverages renewable resources, making this method highly attractive for large-scale production. Future studies should focus on optimizing the synthesis parameters to achieve more uniform nanoparticle sizes and exploring the full spectrum of biological activities of the synthesized Ag NPs. Additionally, investigating the mechanisms underlying the antibacterial action of these nanoparticles could provide deeper insights into their potential applications in medical and pharmaceutical fields. Overall, this research contributes significantly to the field of green nanotechnology, providing a robust foundation for the development of eco-friendly and sustainable methods for nanoparticle synthesis. The promising antibacterial properties of the Ag NPs synthesized using *Foeniculum vulgare* extract pave the way for their potential use in various biomedical applications, including the development of new antibacterial agents and the enhancement of existing therapeutic strategies.

5. Conflicts of interest

“There are no conflicts to declare”.

6. Acknowledgments

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