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Research Article

Green Synthesis of Silver Nanoparticles using Egyptian Date Palm (*Phoenix dactylifera* L.) Seeds and Their Antibacterial Activity Assessment

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Abstract
A simple, cost-effective and eco-friendly synthesis technique of silver nanoparticles (AgNPs) using the aqueous extracts of Egyptian date palm (*Phoenix dactylifera L.*) seeds and their antibacterial activity assessment have been conducted. The aqueous extract was used as reducing and stabilizer agents in the synthesis of AgNPs. Characterization of AgNPs was done using different methods including ultraviolet-visible spectroscopy (UV-Vis), Fourier transform infrared (FTIR), field emission-scanning electron microscope (FE-SEM), and X-ray diffraction (XRD). UV-Vis spectrum of the aqueous medium containing AgNPs showed an absorption peak at around 432 nm. FTIR spectra had shown that the biomolecules were responsible for the reduction and capping agents of AgNPs. XRD study showed the particles to be crystalline with a face-centered cubic (fcc) structure. The AgNPs exhibited significant anti-bacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Enterococcus faecalis*, and *Staphylococcus aureus*. Overall, these findings suggest that biosynthesized AgNPs may be used as a potential therapeutic formulation against bacterial infections.

Keywords: antibacterial, *Phoenix dactylifera* L., seed, silver nanoparticles

1. INTRODUCTION

Silver has a long history of being used as an antimicrobial agent either on its own or in combination with other technologies. It has been extensively researched for its ability to inhibit bacterial growth and has been incorporated into various products and applications. These include creams and dressings for treating burns and ulcers, food packaging to prevent contamination, home appliances like refrigerators and washing machines, and numerous industrial uses [1]. With the advent of nanotechnology, the focus shifted to exploring the antibacterial capacity of silver nanoparticles (AgNPs). AgNPs are nanoscale particles with dimensions ranging from 1 to 100 nm, exhibiting unique electrical, optical, and catalytic properties [2]-[4]. The exceptional antibacterial activity displayed by AgNPs attracted significant attention from researchers and industries. AgNPs have demonstrated antimicrobial activity against a wide range of microorganisms, including multidrug-resistant bacteria. This enhanced antibacterial activity is relevant in medical and healthcare applications, where AgNPs have been studied for incorporation into surgical tools, clothing, cosmetics, dental products, catheters, and dressings. The potential of AgNPs as antibiotics stems from their various mechanisms of action, allowing them to attack microorganisms on multiple fronts and effectively combat different types of bacteria [5]-[7].

Various synthesis methods have been developed for the production of AgNPs, including physical, chemical, and green synthesis techniques [8]. Physical methods involve the use of expensive equipment, high temperatures, and high pressures. On the other hand, chemical methods utilize toxic chemicals that can have detrimental effects on the environment and living organisms [9]. Consequently, the use of physical and chemical methods is restricted due to these drawbacks. As a result, green synthesis has emerged as a more environmentally friendly and cost-effective alternative. Green synthesis involves the utilization of plants, bacteria, fungi, algae, and other natural sources to produce AgNPs. This approach offers several advantages over traditional methods, as it is more sustainable and poses fewer risks to the environment and human health [10].

Recent studies have focused on the synthesis of

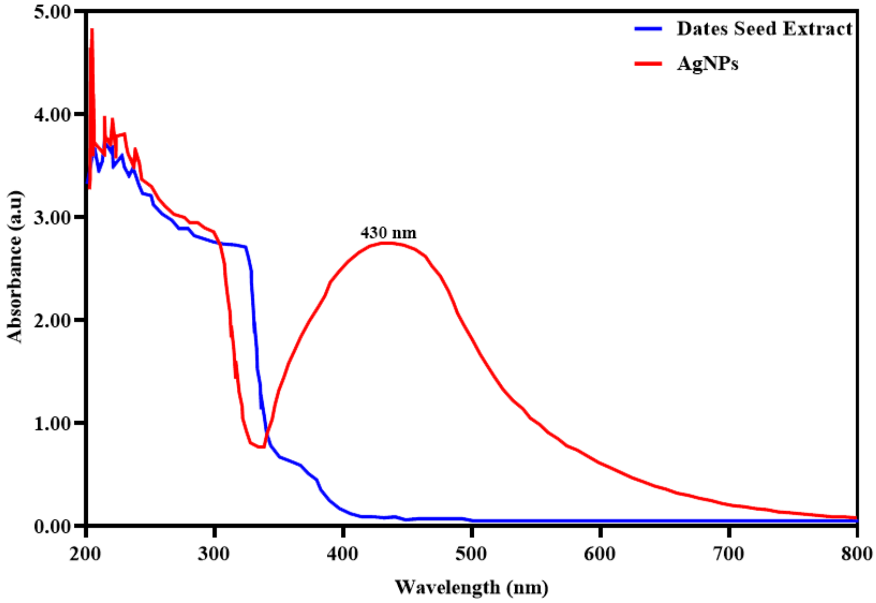

Figure 1. UV-Vis spectra of silver nanoparticles synthesized using Egyptian date palm seed extract.

AgNPs using natural extracts derived from leaves [11], seeds [12], and roots [13] of various plant species. These plants include *Allium ampeloprasum* [14], *Aerva lanata* [15], *Scutellaria barbata* [16], *Annona glabra* [17], etc. However, in these studies, the reduction of Ag(I) ions took a considerably long time. To compete with chemical methods and achieve faster synthesis rates with high monodispersion, there is a need for improved biosynthesis methods of AgNPs. Utilizing seed extracts of plants is a promising approach to meet this objective. Several research papers have reported the synthesis of AgNPs using seed extracts, including *Phyllanthus amarus* [18], *Tectona grandis* [19], *Piper nigrum* [20], *Artocarpus hirsutus* [21], and *Nigella sativa* [22] demonstrating their potential in achieving faster and more efficient synthesis rates.

The date palm tree (*Phoenix dactylifera*) is a tropical and subtropical tree that holds a significant place in human history as one of the oldest cultivated plants. For over 7,000 years, it has played a crucial role in the daily lives of people. The cultivation of date palm trees spans across 35 countries globally, covering approximately 2.9 million acres of land. The worldwide production of date fruit is estimated to exceed 7,000,000 metric tons. Notably, Iran is the second-largest producer, accounting for 14% of global production, with Egypt being the leading producer at 17% of global production [23]. Date seeds, referred to as stones or pits, are an essential component of the date fruit and account for approximately 10 to 15% of its weight. These seeds of *P. dactylifera* are known to contain significant bioactive compounds, making their utilization highly desirable. Date seeds contain a variety of phytochemicals such as flavonoids, polyphenols, phytosterols, and carotenoids, similar to the fruit flesh [24]. This rich phytochemical composition makes date seeds promising candidates for deriving extracts that can be utilized in the synthesis of nanoparticles. In a study by Bhattacharjee et al., date seed extracts were used in the synthesis of FeS nanoparticles using $FeSO_4 \cdot 7H_2O$ precursors. The researchers suggested that the extract played a role as a stabilizing agent for the particles and potentially acted as a reducing agent during the synthesis process [25]. Another study by Dash et al. employed date seed extracts to reduce Au(III) ions, resulting in the synthesis of gold nanoparticles with sizes ranging from 10 to 15 nm, all achieved at room temperature [26]. The main purposes of this work are to evaluate the potential of the seed extract of *P. dactylifera* for the biosynthesis of AgNPs and investigation of their antibacterial activities against *Bacillus subtilis*, *Escherichia coli*, *Enterococcus faecalis*, and *Staphylococcus aureus*.

2. MATERIALS AND METHODS

2.1. Materials

Date palm (*Phoenix dactylifera* L.) fruits (Tamr stage) were collected from Alexandria markets, Alexandria Governorate, Egypt. Laboratory grade silver nitrate ($AgNO_3$) and Mueller-Hinton agar were purchased from Merck. Double distilled de-ionized water wasused for the experiments. All glass wares were properly washed with distilled water and dried in an oven.

2.2. Methods

2.2.1. Preparation of date seed aqueous extract

The date fruits were first de-pitted and the obtained date seeds were washed carefully with distilled water to remove any adhering flesh. The seeds were thereafter dried in an oven at 50 °C for 24 h. The dried seeds were then ground to powder. The obtained fine powder was stored in sealed glass containers at 4 °C until further use. To prepare the date seed extract conducted concerning the method reported Bhattacharjee et al. [25], 5 g of date seed powder was added to 50 mL of deionized water, and boiled for 10 min. The suspension was thereafter centrifuged at 5,000 rpm for 5 min to remove suspended particles and then the supernatant was filtered using a 0.45 μm filter to obtain a clear brownish solution of date seed extract with total solids (TS) concentration of 7 g/L.

2.2.2. Green Synthesis of AgNPs

In a typical reaction procedure, 10 ml of the seeds extract was added to 90 ml of 10^{-3} M aqueous $AgNO_3$ solution. The flask (aqueous phase) was then incubated at room temperature overnight. Any color changes in the solution were observed [18].

2.2.3. Characterization of AgNPs

The formation and stability of AgNPs were observed by measurement of UV-Vis spectra on diluted samples. Distilled water served as the reference blank solution. The absorbance spectra of the AgNPs solution were recorded using a UV-Vis spectrophotometer within the wavelength range of 200 to 800 nm. The AgNPs produced were characterized using different instrumental techniques. To detect surface functional groups, the samples were characterized using a Fourier Transform Infrared spectrophotometer (FTIR, Shimadzu IR Prestige 21). The surface morphology of AgNPs was examined by field emission-scanning electron microscopy (FE-SEM, Carl Zeiss SIGMA). The crystal structure was studied using X-ray diffraction (XRD, PAN Analytical Expert Pro) techniques.

2.4. Antibacterial Activity Assessment

The antibacterial assays were assessed on Gram-positive and Gram-negative pathogens such as *Bacillus subtilis* (ATCC 6051), *Escherichia coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 29212), and *Staphylococcus aureus* (ATCC 25923), which obtained from Cairo-MIRCEN (Microbiological Resource Center), Faculty of Agriculture, Ain Shams University by using the standard well-diffusion method. The antibacterial activity was measured based on the inhibition zone

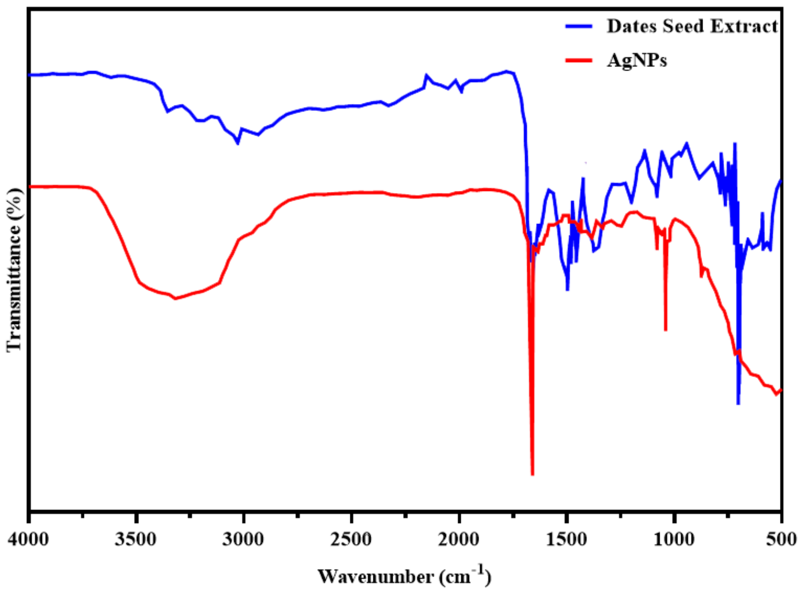

Figure 2. FTIR spectra of silver nanoparticles synthesized using Egyptian date palm seed extract.

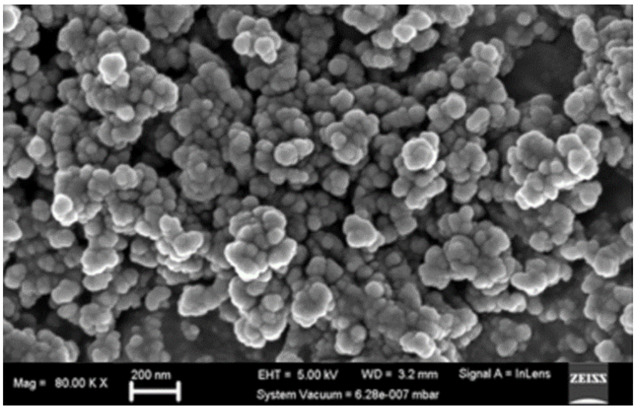

Figure 3. FESEM image of silver nanoparticles synthesized using Egyptian date palm seed extract

around the well impregnated with plant extract and synthesized AgNPs.

3. RESULTS AND DISCUSSIONS

3.1. UV-Vis Characterization

The biosynthesis of AgNPs from *P. dactylifera* extract was confirmed by UV-Vis spectroscopy, which is a commonly used method to characterize biosynthesized metal/metal oxide nanoparticles. The maximum absorption was observed at 430 nm (Figure 1), which validated the presence of biosynthesized AgNPs in the solution. Similar results emerged from a study by Allemailem et al. that recorded the peak at 432 nm for AgNPs synthesized from Ajwa dates seed extract [27]. Ansari and Alzohairy showed that the maximum absorbance peak was seen at 429 nm for date seed extract. It is generally recognized that UV-Vis spectroscopy could be used to examine the size and shape-controlled nanoparticles in aqueous suspensions [28]. Farhadi et al. showed that, with the decrease in the amount of the extract, the size of AgNPs became smaller and temperature was indirectly proportional to the size of AgNPs [29].

3.2. FTIR Analysis

FTIR spectroscopy was performed to identify the biomolecules responsible for capping and effectively stabilizing AgNPs. The significant peaks, corresponding wavenumbers, and possible functional group interpretations can be seen in Figure 2 of the FTIR spectra for both the dates seed extract and AgNPs. In the extract from *P. dactylifera* seeds, peaks were observed at 665, 1019, 1421, 1632, 2854, and 3410 cm^{-1}. Subsequently, after reacting with $AgNO_3$, these peaks shifted towards higher wavenumbers, while their intensities decreased, specifically at 1074, 1382, 1592, 2925, and 3381 cm^{-1}. The presence of a band at 1019 cm^{-1} indicates the N–H bonding vibration of amides. The bands between 1019 and 665 cm^{-1} in the seed extract signify the C=C stretching mode in aromatic compounds, suggesting the presence of certain aromatic compounds like flavonoids. Egyptian date palm seed powder contains various bioactive compounds such as flavonoids, terpenoids, alkaloids, and glycosides. Additionally, researchers have demonstrated the existence of hydroxyl, carboxyl, and carbonyl functional groups in carbohydrates, flavonoids, tannins, and phenolic acids found in palm date fruit and seed extracts, which may contribute to the reduction of Ag(I) ions and stabilization of AgNPs. The involvement of flavonoids and phenolic compounds in the reaction mechanism during the formation of AgNPs can be attributed to their ability to donate electrons or hydrogen, with the keto form present in the backbone of flavonoid compounds being responsible for the reduction of Ag(I) to Ag(0).

3.3. FE-SEM analysis

The FE-SEM image highlighted that the formed nanoparticles were spherical in shape and were fine and uniform in size (Figure 3). The green synthesized AgNPs from various plant extracts have also been described previously. Our results are consistent with those obtained by Allemailem et al. [27] who showed that the AgNPs prepared from Ajwa-dates seed extracts were mostly spherical in shape and 15 to 80 nm in diameter.

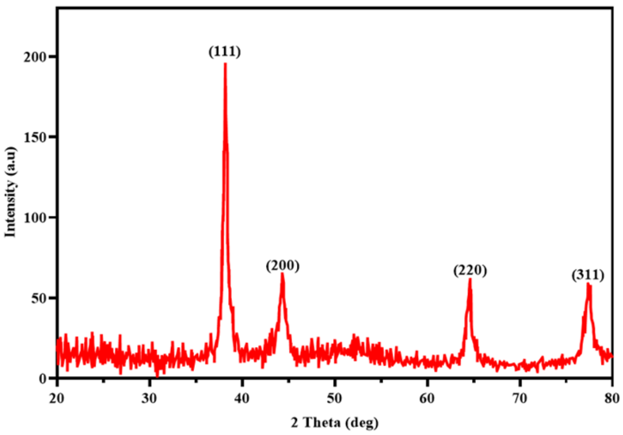

Figure 4. XRD pattern of silver nanoparticles synthesized using Egyptian date palm seed extract

Table 1. Antibacterial activity of silver nanoparticles synthesized using Egyptian date palm seed extract.

Bacterium Name	Zone of Inhibition (mm)
Bacillus subtilis	17
Escherichia coli	19
Enterococcus faecalis	20
Staphylococcus aureus	20

3.4. XRD analysis

The nanoparticles synthesized in this method are characterized using powder XRD to confirm the particles as silver and to know the structural information. Figure 4 shows the XRD pattern of AgNPs. The pattern clearly shows the main peaks at 2θ of 36.74, 44.28, 62.82 and 77.47 corresponding to the (111), (200), (220) and (311) planes, respectively. By comparing JCPDS (file no: 89-3722), the typical pattern of green-synthesized AgNPs is found to possess a face-centered cubic (fcc) structure. A similar result was observed by Anandalakshmi et al. [30] who identified crystalline peaks (38.19, 44.37, 64.56 and 77.47).

3.5. Antibacterial Activity Assessment

The activity of AgNPs against bacterial strains was observed at 100 µg/mL, as indicated in Table 1. These results show that AgNPs display an evident significant effect against the tested bacterial strains. The green synthesized AgNPs demonstrated its antibacterial activity against both gram-positive and gram-negative bacteria, it was found that the zone inhibition of AgNPs against *Bacillus subtilis*, *Escherichia coli*, *Enterococcus faecalis*, and *Staphylococcus aureus* was 17, 19, 20 and 20 mm, respectively.

These findings are supported by an earlier study that showed that the highest antimicrobial activity was exhibited against *Enterococcus faecalis* and *Staphylococcus aureus*, whereas the lowest antimicrobial activity was noted against *Bacillus subtilis* and *Escherichia coli*. These bacterial group incubations around the wall are due to the release of diffusible inhibitory compounds from silver nanoparticles. The green synthesis of AgNPs has also paved a better methodological approach in the medical field.

4. CONCLUSIONS

The green synthesis method is eco-friendly, of low cost and capable of producing AgNPs at room temperature. The current study reveals that the Egyptian date palm (*Phoenix dactylifera L.)* seeds extract may be a viable source for the green synthesis of AgNPs. The antimicrobial activity is well demonstrated against *Bacillus subtilis*, *Escherichia coli*, *Enterococcus faecalis*, and *Staphylococcus aureus*. The present study showed a simple, rapid, and economical route to synthesize AgNPs.

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Conflicts of Interest

The authors declare no conflict of interest.

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