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RESEARCH PAPER

Dried Pomegranate Seed Extract-Mediated Green Synthesis of Silver Nanoparticles with Antibacterial Activity Against Staphylococcus Aureus and Moraxella Catarrhalis

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ABSTRACT

This work outlines a green chemistry-based method for the sustainable synthesis of AgNPs, which involves the use of dried pomegranate seed extract as a natural reducing and stabilizing agent. Confirmatory investigations of the crystal structure and morphology were performed using X-ray diffraction (XRD) and field-emission scanning electron microscopy (FE-SEM). XRD A face-centered cubic (FCC) crystalline configuration having an average crystalline size of 13 nm was discovered through investigation. FE-SEM morphological analysis revealed spherical particles ranging in size from 33.03 to 55.38 nm. A biological study of the nanoparticles towards the bacterial pathogens was conducted. estimated by agar well diffusion method against, the Gram-positive Staphylococcus aureus and the Gram-negative Moraxella catarrhalis Nanoparticles at a dosage of 1000 µg/ml inhibited Moraxella catarrhalis and Staphylococcus aureus with inhibition zones of 26 mm and 24 mm, respectively. The inhibitory activity decreased with increasing concentration and peaked at 125 µg/ml. This indicates an appropriate concentration window for antibacterial action. These findings emphasize the prospect of bioactive silver nanoparticles as prospective antibacterial agents in the realm of Nanomedicine.

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INTRODUCTION

A proven and sustainable technique for creating inexpensive, non-toxic, and environmentally beneficial nanomaterials is called "green synthesis of nanoparticles" [1-2]. Unlike conventional chemical methods, phyto-synthesis uses plant and fruit extracts as stabilizing and reducing agents to produce metallic nanoparticles [3-4]. Because it does not require dangerous chemicals, this method is a safer option for

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medicinal purposes. In particular, because of their unique physicochemical characteristics and their broad-spectrum antibacterial and anticancer potential, silver nanoparticles (AgNPs) continue to garner a lot of interest [5-6]. The formation of stable nanoparticles with high biological activity is facilitated by antioxidant-rich botanical sources, which are responsible for the efficacy of phyto-synthesis. Inedible remnants, such as dried pomegranate seeds, are an underutilized bioresource, despite the fact that edible fruit portions have been the subject of many studies [7-8]. The seeds contain several bioactive polyphenolic compounds, including ellagic acid derivatives, anthocyanins, and punicalagin isomers. These compounds are effective stabilizing and capping agents throughout the synthesis process [9]. Drying seeds from pomegranates have excellent antimutagenic, antibacterial, and free radical scavenging capabilities. [10]. The search for novel antimicrobial medications has escalated as antibiotic resistance has become a global concern. In this field, phyto-synthesized AgNPs have become an effective weapon because they may disrupt bacterial cell membranes and inhibit microbial development [11-12]. The purpose of this research is to evaluate the phyto-synthesis of AgNPs using dried pomegranate seed extracts, with a focus on their inhibitory properties versus a variety of harmful pathogenic microbes, resulting in a green and cost-effective alternative to standard antibiotics.

MATERIALS AND METHODS

Pomegranates were gathered fresh from an orchard in Najaf, Iraq. After separating the seeds, they were stored in a sealed container for 30 days, away from sunlight. Once dried, the seeds were kept in a plastic container at a temperature of 25°C. Silver nitrate ($AgNO_3$) and sodium hydroxide (NaOH) were used in this process.

Use of Pomegranate Seed Extract in the Ecologically Friendly Synthesis of AgNPs

Initially, 100 mL of distilled water and 8.0 g of pomegranate seed powder were combined, and the solution was kept in a water bath at 60 °C for 30 minutes. Whatman filtration was then used to filter the mixture, and the filtrate (extract) was kept at 4 °C until additional analysis. In order to create silver nanoparticles, certain· changes, in accordance with the Suman et al. (2013) technique. In order to get the best results, a newly made aqueous solution of silver nitrate (1 mM) at pH 7 was added in a 1:9 ratio to the seed extract solution at the ideal temperature. After stirring the solution in the sun until it turned reddish-brown instead of white, it was covered with aluminum foil to stop oxidation.

Antibacterial Activity of AgNPs

AgNPs were evaluated for their antibacterial efficacy against two pathogenic bacterial strains, Moraxella catarrhalis (Gram-negative) and Staphylococcus aureus (Gram-positive), using the agar well diffusion technique. Using sterile cotton swabs, apply a standardized bacterial suspension (1.5×10^8 CFU/mL, 0.5 McFarland standard) to Mueller-Hinton agar plates. Sterilized corn borers were used to construct 9 mm diameter wells. AgNPs at concentrations of 1000, 500, 250, and 125 μgmL^{-1} were added to each well. Plates were incubated at 37°C for 24 hours, and inhibition zones were measured in millimeters.

RESULTS AND DISCUSSION

Diagnosis Using X-ray Diffraction (XRD)

As illustrated in Fig. 1, the X-ray diffraction (XRD) patterns of biosynthesized silver nanoparticles from pomegranate seed extract at pH 7 showed different diffraction peaks. The measured peaks at 2θ angles of 38.30°, 44.30°, 64.65°, and 77.40° match to the typical card (JCPDS No. 04-0783) and are given to Miller indices (111), (200), (220), and (311), respectively [13, 14]. The additional peaks in the face-centered cubic (FCC) crystal structures are due to the presence of reducing chemicals in the plant extract. Furthermore, the nanoparticle size is affected by both the extract content and the pH of the reaction media [15]. The average crystal size was estimated using the Scherrer-DePay equation, as given in Table 1.

Using a Scanning Electron Microscope (FESEM)

Using scanning electron microscopy with field emission. The nanoparticles' diameters were calculated, and their surface shape was investigated. Fig. 2 shows that the silver nanoparticles (AgNPs) measured between 33.03 and 55.38 nm under the microscope [16]. That FE-SEM pictures demonstrate that the silver nanoparticles are mostly spherical [17]. This discovery is compatible with the Scherrer equation in X-ray analysis [18].

Antibacterial Activity of Silver Nanoparticles

Fig. 3 demonstrates the increased antibacterial activity of biosynthesized silver nanoparticles (AgNPs) generated from dried pomegranate seeds, indicating the synergistic effect of nanoscale delivery.The results demonstrated a considerable inhibition against Moraxella catarrhalis (Gram-negative), with an inhibition zone width of 26 mm, compared with Staphylococcus aureus (Gram-positive), which recorded 24 mm. Gram-negative microorganisms lack the thick layer of peptidoglycan seen in Gram-positive bacteria, which enhances membrane permeation and the accumulation of silver nanoparticles, resulting in damage to key cellular components. This distinction can be explained by structural changes in bacterial cell walls [19].

Moreover, this impact is not exclusive to silver; pomegranate seed-derived bio-capping is crucial. By acting as stabilizing and reducing agents, phenolic compounds and ellagic acid increase the effective surface area of nanoparticles and stop them from aggregating. These results align with the processes suggested in reference [20]. They emphasize how silver causes oxidative stress (ROS), which destroys DNA, and inhibits protein synthesis. Thus, this green synthesis technique is a viable strategy for creating extremely effective nano-antibiotics [21].

The aim of this study was to develop a green chemistry-based method for the synthesis of silver nanoparticles (AgNPs) by utilizing dried pomegranate seed extract as a natural reducing and stabilizing agent. The results confirm that the seeds are rich in polyphenols and flavonoids, which act as effective bioscavengers and inhibit the aggregation of nanoparticles. This supports previous findings that plant-based extracts can

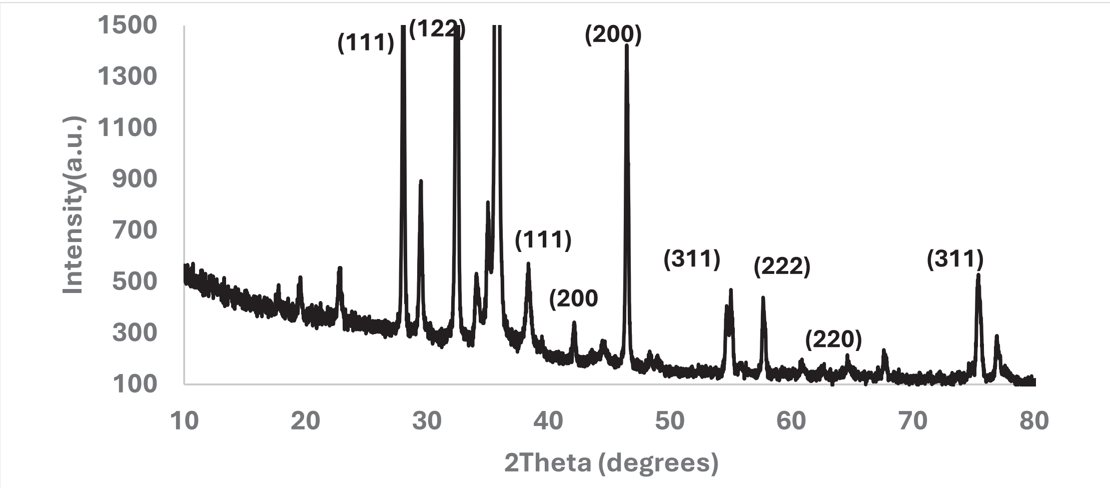


Fig. 1. X-ray diffraction pattern of AgNPs

Table 1: Scherrer-Debye equation values for AgNPs prepared at pH 7.

2 Theta	FWHM	D (nm)	Micro strain
27.92	0.1197	51.8	0.23884
32.42	0.1909	51.8	0.26615
38.30	0.4586	13	0.90407
42.08	0.1768	55.2	0.19415
46.43	0.1927	57.6	0.16967
54.65	0.217	46.3	0.18118
57.68	0.1882	62.2	0.12832
64.65	0.8435	65	1.11626
77	3.1968	17	3.53765

$D= (K\times\lambda)/(\beta\times\cos(\theta))$

replace traditional, toxic chemical reagents in the preparation of nanoparticles.

X-ray diffraction (XRD) showed that the synthesized AgNPs formed a face-centered cubic (FCC) crystal structure, and the calculated average crystal size was 13 nm. This is in line with other plant-based syntheses that have reported similar crystal structures. FE-SEM analysis morphologically confirmed that the particles were mainly spherical and ranged in size from 33 to 55 nm. The agreement between the results of these two methods confirms the reliability of the synthesis protocol.

Antibacterial experiments showed that AgNPs were effective against both Gram-positive Staphylococcus aureus and Gram-negative Moraxella catarrhalis. The inhibition zones were larger for the Gram-negative bacterium, which can be explained by its thinner peptidoglycan layer and thus easier penetration of the nanoparticles into the cell membrane. This observation supports the notion that AgNPs may be particularly useful in combating Gram-negative pathogens, which often cause difficult-to-treat infections.

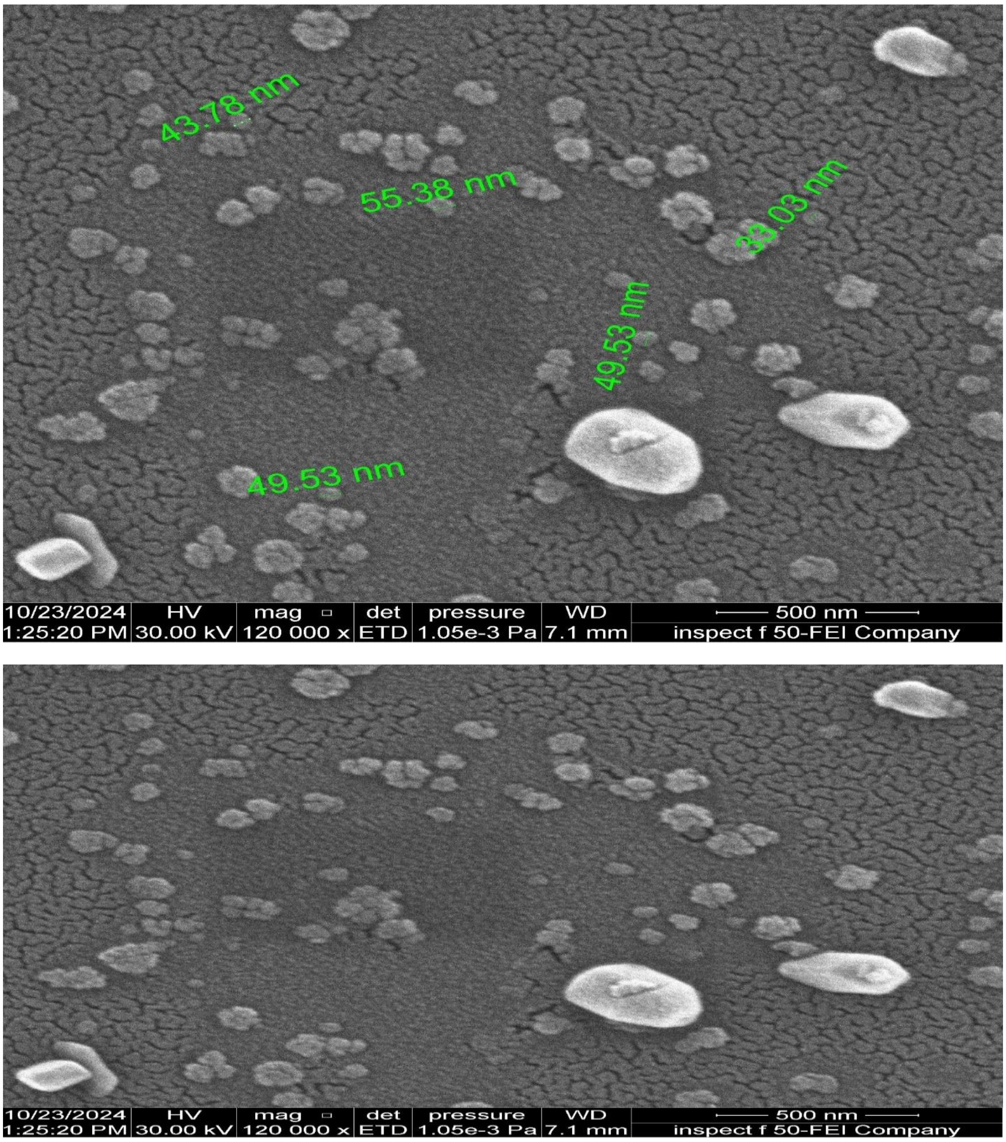
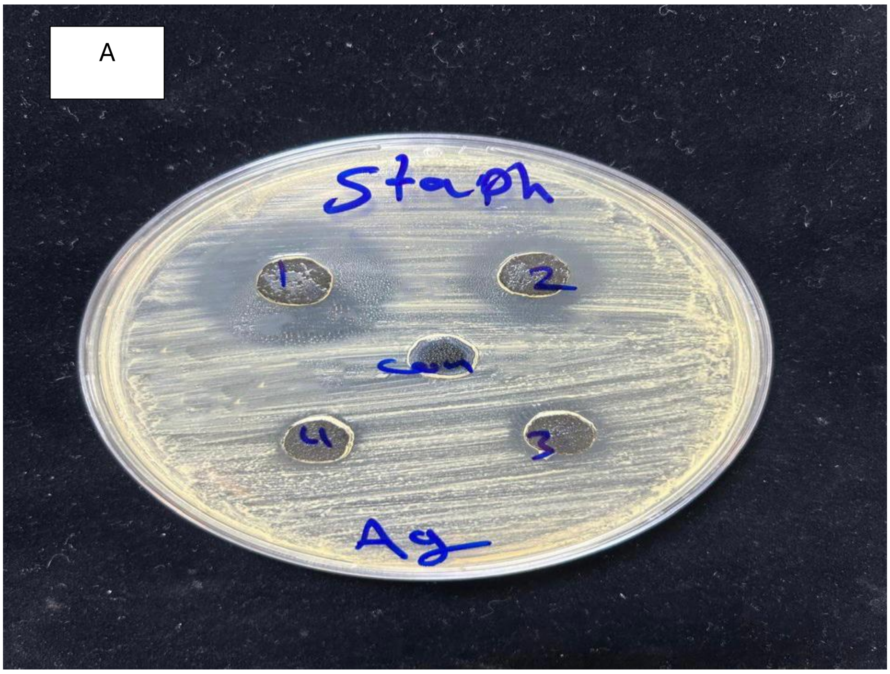


Fig. 2. Ag Nps nanoparticle under scanning electron microscope (FESEM).

In addition, bioactive compounds derived from pomegranate seeds, such as ellagic acid derivatives and punicalagins, not only stabilized the particles but also enhanced their biological activity. This synergistic effect was particularly evident at the highest concentrations (1000 µg/mL), where significant growth inhibition was observed. However, the antibacterial efficacy decreased at lower concentrations, suggesting the existence of an optimal dose window.

A limitation of the study is that the experiments were conducted on only two bacterial species. In



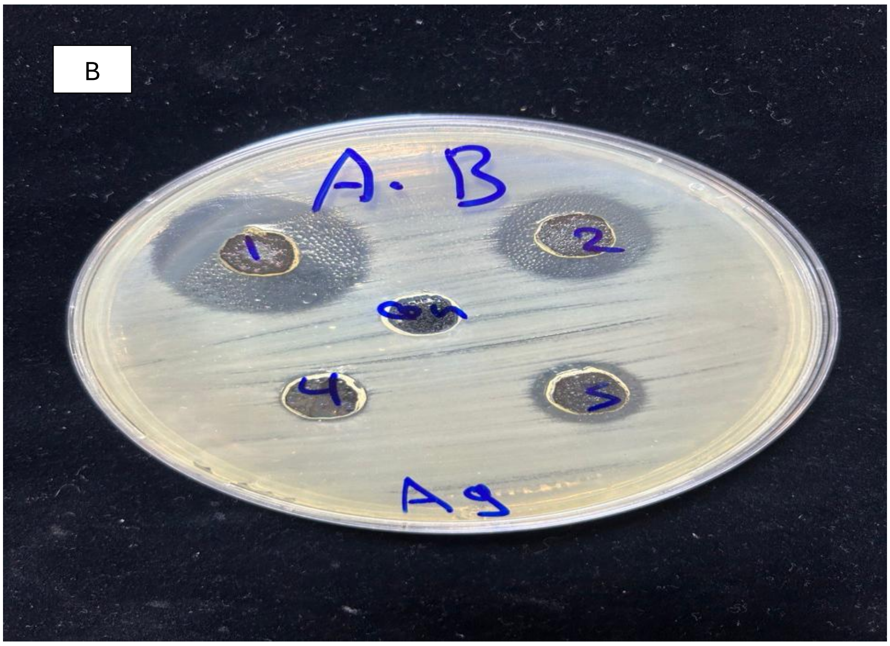


Fig. 3. AgNPs ability to inhibit bacterial growth in both gram-positive and gram-negative organisms at 1000 µg/ml (1), 500 µg/ml (2), 250µg/ml (3) and 125 µg/ml (4). (A) *staphylococcus aureus(B) Moraxellacea*.

Table 2. Antibacterial activity and inhibition zone diameters (mm) of synthesized silver nanoparticles (Ag NPs) against *Staphylococcus aureus* and *Moraxellaceae* at various concentrations.

Kind of used Bacteria	1000 μg/ml	500 μg/ml	250 μg/ml	125 μg/ml
Staph. aureus	24	17	13	11
moraxellaceae	26	18	14	zero

the future, it would be necessary to expand the study to more clinically relevant pathogens and to evaluate the toxicity of AgNPs in cell cultures and animal models. In addition, the lack of a control group of healthy controls limits the comparability of the results.

Overall, this work demonstrates that the utilization of dried pomegranate seeds offers a cost-effective, environmentally friendly and biologically active approach for the preparation of silver nanoparticles. The results support the potential of AgNPs as future nanoantibiotics and highlight the importance of plant-based raw materials in the development of sustainable nanotechnology.

CONCLUSION

The current work used a cost-effective, sustainable, and ecologically safe method to create silver nanoparticles. The study showed how sunlight may be used to decrease silver ions, which has a major impact on the production of AgNPs from pomegranate seed extract and silver nitrate solution. Pomegranate seed extract's polyphenols and flavonoids are thought to be important in the sunlight-induced decrease of silver ions, which stops AgNPs from aggregating. This approach is attractive as a green and sustainable way to deal with environmental issues because of its ease of use, affordability, and dependence on environmentally friendly raw ingredients and solvents. The special qualities of the biosynthesized silver nanoparticles call for more research into their possible uses in a variety of biological and environmental domains. Additionally, it was shown that AgNPs exhibit a stronger antibacterial effect on Gram-negative bacteria than Gram-positive bacteria.

CONFLICT OF INTEREST

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

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