Research Article

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Synthesis of Ag_2O Nanoparticles via Fresh Pomegranate Peel Extract for Bioapplications

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Abstract

Fresh pomegranate peel extract was employed to synthesize silver oxide nanoparticles (Ag_2O NPs). Rapid formation of stable Ag_2O NPs was observed on exposure to the aqueous fresh pomegranate peel extract with solution of $AgNO_3$. The Ag_2O NPs were characterized by X-ray analysis, scanning electron microscopy (SEM), ultraviolet–visible (UV–Vis) spectroscopy, and Fourier transform infrared spectroscopy (FTIR). The X-ray diffraction (XRD) confirmed that the forming Ag_2O NP has a crystalline size of 37 nm, while SEM micrographs revealed a comparatively spherical shape, with the size of ~ 64 nm. The Ag_2O spectrum displayed a peak in the visible range and a blue shift at 461 nm corresponding to the Plasmon absorbance of silver nanoparticles. Four bacterial strains and one type of fungus were tested using Ag_2O NPs. The results showed the negative influence of Ag_2O NPs on the growth rate, thus implying the significance of the present study in production of biomedical products.

Keywords: silver oxide nanoparticles (Ag_2O NPs); ultraviolet–visible (UV–Vis) absorption; fresh pomegranate peel; antibacterial

Introduction

Several metallic nanoparticles were synthesized using green chemicals without external chemicals that could contaminate the environment [1–3]. The green approach is more favourable because of its simplicity, purity, and cost-effectiveness when synthesizing nanoparticles with specific attributes [4, 5]. Plant extracts contain bioactive substances such as starch, proteins, terpenoids, phenolic acids, alkaloids, polysaccharides, and polyphenols. By acting as reducing and capping agents, these chemicals can aid in the formation of nanoparticles [6, 7]. Silver, a noble metal, stands out among the many metallic nanoparticles studied in nanomaterial science because of its unique qualities that can be used in various sectors. The antimicrobial characteristics of silver oxide nanoparticles (Ag_2O NPs), extensively utilized in antibacterial and antifungal applications, are due to the electrical changes in the bacterial membrane when they come into contact. These changes significantly boost AgNP's surface reactivity [8].

Furthermore, metal nanoparticles' resistance to degradation under culture conditions and their capacity to retain their effectiveness for extended

periods without degrading enhance their bactericidal efficacy [9]. Nanoparticles' antibacterial effect is the most likely because electrostatic contact with Bacteria's cell membrane and internalization of Ag_2O NPs in the cell results in reactive oxygen species (ROS) and membrane damage [10, 11]. ROS are oxidizing chemicals that cause DNA damage by oxidizing lipids and proteins present in the cell. The lack of critical proteins causes oxidative stress and disrupts normal cellular functions, damaging DNA. Bacteria metabolism and respiratory cycles are also impacted or inhibited. Cell death and, as a result, bacterial growth suppression occurs as a result of these mechanisms [12–14]. The biological applications of green Ag_2O syntheses by fresh pomegranate peel are evaluated in this work.

Experimental

Preparation of Ag_2O NPs

After thoroughly washing the plant pomegranate peel under running tap water, 40 g of fresh peel was added to 100 mL of deionized water using a magnetic stirrer and set on a hot plate at 60 °C for 0.5 h. After cooling, the pomegranate peel extract was filtered 3 times with Whatman filter paper No.1 to get a pure aqueous extract, as shown in Fig. 1. $AgNO_3$ and the fresh pomegranate peel extract used the green synthesis method as precursors to synthesize Ag_2O NPs. 1.6 g of $AgNO_3$ was added to 100 mL of deionized water and was placed on a hotplate-magnetic stirrer at 60 °C for 1 h. Then, 5 mL of the plant extract was mixed with 100 mL of $AgNO_3$ solution by constant and continuous stirring for 1 h under normal atmospheric pressure. The color change indicated that Ag_2O NPs had been synthesized (Fig. 2).

In the process of green synthesis of Ag_2O NPs, the plant extract functions as both reducing and capping/stabilizing agent. The first step in the creation of nanoparticles is the blending of plant extract with metal salt solution. The reaction color changes as a result of the biochemical reduction of the metal salt. Phytochemicals (flavonoids, polysaccharides, alkaloids, proteins, phenolic compounds, and cellulose) and secondary metabolites found in plant extracts are used to create nanoparticles that have the potential to reduce Ag ions [6]. The reduction of metal ions to make metallic nanoparticles most likely involves the oxidation of polysaccharide hydroxyl groups to carboxyl groups. Metal ions are initially activated from their monovalent or divalent oxidation state to zero valent state, and the reduced metal atoms are then formed. A nanoparticle can aggregate to form many shapes. The synthesis of varied size, shape, and morphological of nanoparticles is caused by variations in the composition and concentration of reducing agents in plant extracts [7].

Fig. 1 Pomegranate peel extract.

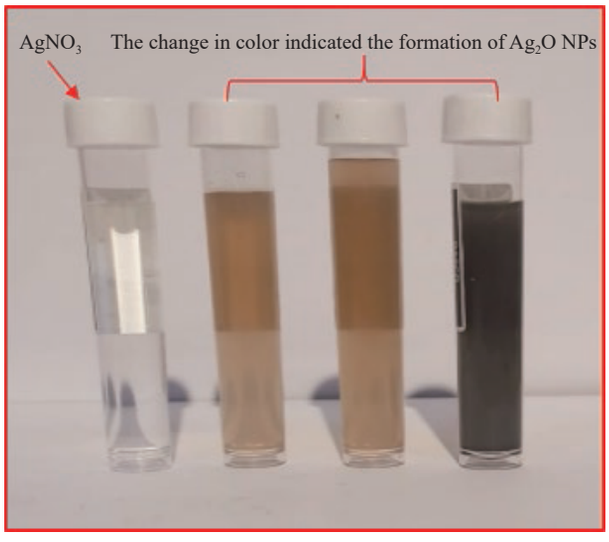

Fig. 2 The stage of solution color change.

Preparation of microbial suspension (inoculum)

The antimicrobial activity of synthesized Ag_2O NPs was evaluated against (*Staphylococcus aureus*, *S. epidermis*, and *Escherichia coli*) and one fungal (*Candida albicans*) by well diffusion method. Muller-Hinton agar (MHA) plates were prepared per manufacturer's instructions. The test microorganisms were seeded over the MHA plates using sterile cotton swabs. Wells of 6 mm in diameter were punched over the agar plates using a sterile puncher. After 150 µL of Ag_2O NPs being added to each pore, the plates were incubated at 37 °C (bacteria) and 30 °C (*C. albicans*) for 24 h. After incubation, inhibition zones around the wells confirmed the antimicrobial activity.

The same procedure was repeated for all the test strains. The clearance zones formed around each well were measured, and the inhibition zone's average diameter was taken to evaluate the antimicrobial activity.

X-ray diffraction (XRD) analysis of Ag_2O NPs

Figure 3 exhibits XRD analysis of strong peaks of the thin film (Ag_2O nanostructure) in a polycrystalline structure. XRD patterns display diffraction peaks at 2θ of 44.2°, 64.4°, and 77.4°, corresponding to cubic phase structure with crystal planes (200), (220), and (311), respectively; this result agrees with the card number (00-004-0783) [15–17]. Whereas the peak at 2θ of 37.94° is related to (200) plane of Ag_2O F.C.C. crystalline (No. 01-076-1393) [18]. Using Scherrer formula, the crystallite size of the produced NPs was estimated to be (37 ± 1) nm [19–21].

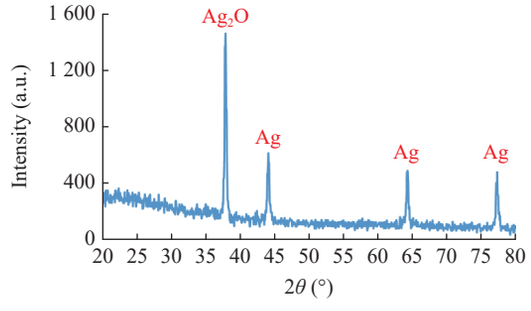

Fig. 3 XRD pattern of Ag_2O nanostructure.

Scanning electron microscopy (SEM) micrographs revealed a comparatively spherical structure (Fig. 4) with a size of ~ 64 nm. The separation of these nanoparticles could be due to protein capping.

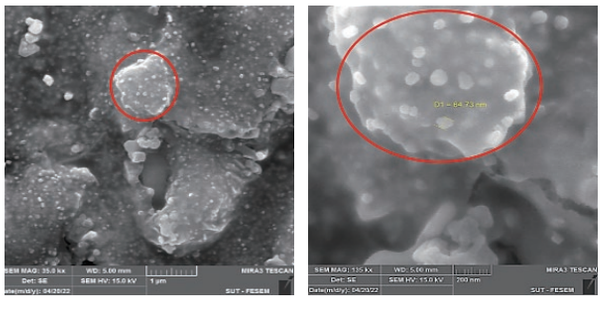

Fig. 4 SEM images of Ag_2O thin film.

Ultraviolet–visible (UV–Vis) spectroscopy studies of Ag_2O NPs and Tauc's relation

The spectra of the Ag_2O NPs' absorbance over the range of 200–900 nm can be seen in Fig. 5(a). The wavelengths at which the apex is observed are 461 nm. The quantum size effect caused the band to change to longer wavelengths as the nanoparticles grew in size. This caused the band to move up the frequency spectrum. The surface plasmon bands that correspond to the completely or roughly spherical shape of the Ag_2O NPs have been allocated to these bands. These bands each reflect a different frequency of collective oscillation for the electrons in the conduction band. The wavelengths of the incident electrons are much shorter than those of the incident light waves, which corresponds to the sizes of Ag NPs. Electrons, in this scenario, submit to the electromagnetic field and propagate like a plasmon wave [22–24]. In addition, the electric field generated by light, denoted by the symbol Eo, remains unchanged. Because the nuclei of the atoms do not move, the oscillation of the electrons results in a periodic charge separation. It creates oscillating dipoles, the most significant amplitude at the surface of the nanoparticles (Fig. 5(b)). When resonance occurs, there is an increase in the ratio of the amplitude of the local electric field in the particle to the amplitude of the applied field.

The energy band gap (E_g) of colloidal Ag_2O NPs is determined by Tauc's formula:

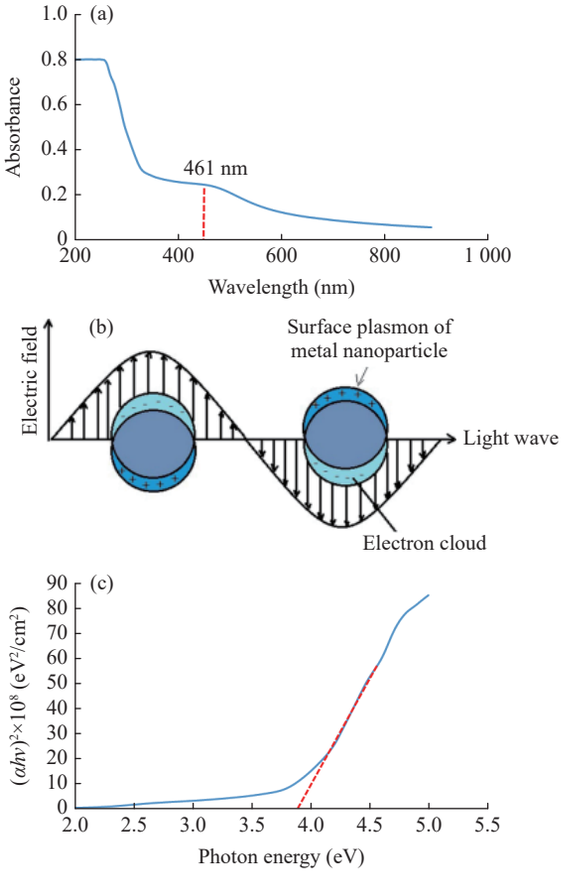

Fig. 5 (a) Absorption spectrum of Ag_2O NPs. **(b)** Oscillating dipoles induced by light radiation of the Ag_2O NPs. **(c)** Graph of $(\alpha hv)^2$ vs. photon energy (hv).

$$\alpha h\nu = C(h\nu E_g)^n \quad (1)$$

where C is a constant, α is the absorption coefficient, $h\nu$ is the photon energy, and n indicates the type of electronic transition.

The energy gap of Ag_2O NPs was 3.9 eV, as seen in Fig. 5(c). The energy bandgap of Ag_2O NPs colloidal solution is more remarkable than previously published values; this could be attributed to particle size and quantum confinement effect [25, 26].

The chemical bonds and functional groups of colloidal Ag_2O NPs produced by fresh pomegranate peel extract were analyzed using Fourier transform infrared spectroscopy (FTIR) spectroscopy at 500–4 000 cm^{-1}, as displayed in Fig. 6. The bands in colloidal Ag_2O NPs are visible at 3 363 cm^{-1}. The peak corresponds to the hydroxyl group's stretching vibration (H-bonded O—H stretch) [27, 28]. The signal at 2 359.27 cm^{-1} corresponds to aliphatic amines group C—N stretching. Ag_2O NPs reveal an Ag═O bending vibration mode at 763 cm^{-1}, suggesting metal-oxygen bonding formation [29, 30]. O—H bending vibration of an adsorbed water molecule on the surface of Ag_2O NPs is at 1 658 cm^{-1} [31, 32].

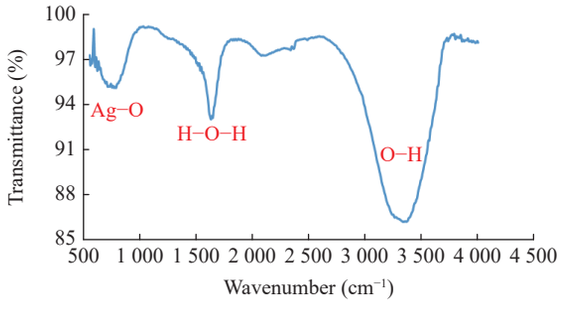

Fig. 6 FTIR spectra of Ag_2O NPs.

This study tested pathogenic microbial (*S. aureus*, *S. epidermis*, and *E. coli*) and fungal (*C. albicans*) species using Ag_2O NPs colloidal solution prepared from fresh pomegranate peel extract. The concentration of Ag_2O NPs employed in this test was (0.1 mol/L). The halo around the well suggests that Ag_2O NPs have an antibacterial action [33, 34]. The respiratory process in the microbial cell walls is inhibited by Ag_2O NPs' interaction with the respiratory enzymes in the cell walls. Figures 7 and 8 show the inhibition zone of bacteria and fungi; the diameter of inhibition zone for Gram-negative bacteria *E. coli* and *Klebsiella* is 13 and 15 mm, respectively. While the inhibition zone of Gram-positive *S. aureus* and *S. epidermis* (15 mm in diameter) and a *Candida* (15 mm in diameter) is shown in Fig. 9. X-ray and SEM results show that the Ag_2O prepared by the green synthesis on the nanoscale demonstrates that NPs can quickly enter the cell wall. This result supports the idea that NPs can quickly pierce the cell wall. Antimicrobial activity is attributed to the high surface area-to-volume ratio between silver NPs and cells. Also, free radicals such as OH is verified by FTIR, the release of Ag^+ ions, and ROS production [35–39].

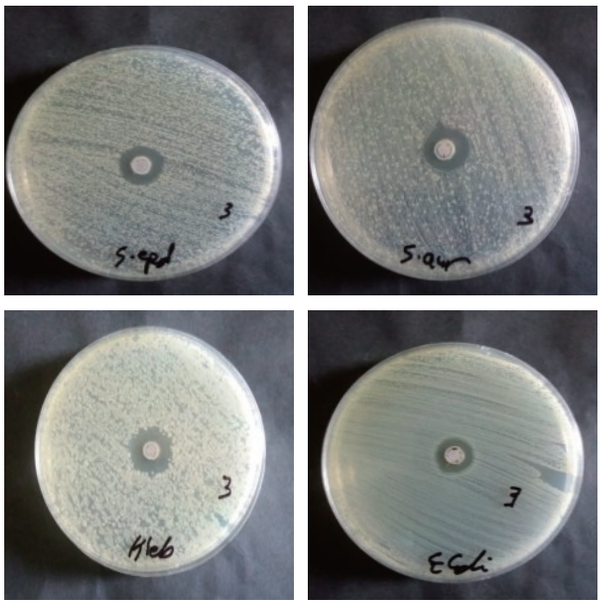

Fig. 7 Evaluation of the effectiveness of Ag_2O NPs as antibacterial treatment.

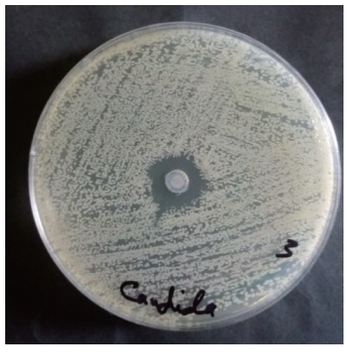

Fig. 8 Evaluation of the effectiveness of Ag_2O NPs as antifungal.

Because silver has a broad-spectrum activity and a suggested mechanism of action that targets multiple bacterial components, there is not a widespread development of silver resistance [34]. This can be attributed to the fact that silver resistance does not occur frequently. Even though resistance to Ag is a possibility, the rate of development appears to be

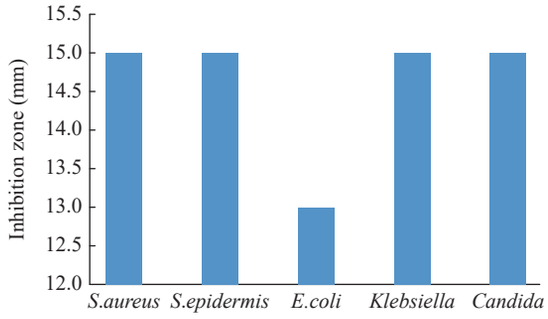

Fig. 9 Diameter of inhibition zone of Ag_2O NPs.

more delayed than that of alternative antimicrobial agents. This suggests that there is a hope for the future development of Ag-based antimicrobial therapies that can be used in combinatorial and mixed-therapy applications [37]. However, the restricted solubility of pure silver metal in aqueous solutions has been one of the primary barriers to using silver as an antimicrobial agent or in combination approaches [38]. This has been one of the most significant barriers. Our previous research and the research presented here demonstrate a workable approach for developing silver-based coatings that get around the solubility problem by using silver oxide (Ag_xO) [39].

Conclusion

We succeeded in synthesizing nanoparticles of Ag_2O using fresh pomegranate peel extract. This method is simple and environmentally friendly. It also saves many nanomaterials, where Ag_2O has proven its efficacy as an antibiotic, as shown by the results in this research work. It can be used in many applications such as anticancer treatment and treatment of worm damage that cause great economic loss.

CRediT Author Statement

Wedian K. Abad: conceptualization, data analysis, visualization, and manuscript preparation. **Ahmed N. Abd**: experimental, resources and methodology. **Nadir Fadhil Habubi**: supervision, counselling, reviewing, and editing.

Conflict of Interest

The authors declare that they have no conflict of interest.

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