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Green Synthesis, Characterization, and Antibacterial Activity of Propolis and Pomegranate Peel-Coated Zinc Oxide Nanoparticles Against *Staphylococcus Aureus* and *Escherichia Coli*

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ABSTRACT

The present study aims to synthesize zinc oxide nanoparticles (ZnO NPs) using a green and environmentally friendly approach with propolis and pomegranate peel extracts as natural reducing and stabilizing agents. The synthesized nanoparticles will be characterized using various analytical techniques, including UV–Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), and Dynamic Light Scattering (DLS), to determine their optical, structural, morphological, and physicochemical properties. The antibacterial activity of the prepared nanoparticles will be evaluated against two clinically significant bacterial strains, Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative), using standard microbiological assays. The study is expected to demonstrate that green-synthesized ZnO nanoparticles coated with natural bioactive compounds exhibit enhanced antibacterial efficacy compared with conventional ZnO nanoparticles. This eco-friendly strategy may provide a promising alternative for developing novel antimicrobial nanomaterials for biomedical and pharmaceutical application

KEYWORDS: Green synthesis**;** Zinc oxide nanoparticles (ZnO NPs)**;** Propolis**;** Pomegranate peel extract

INTRODUCTION

The rapid emergence of antibiotic-resistant bacteria has become one of the most significant challenges facing global public health[1]. Pathogenic bacteria, such as Staphylococcus aureus and Escherichia coli, are responsible for a wide range of community- and hospital-acquired infections, including wound infections, urinary tract infections, septicemia, and foodborne illnesses[2]. The increasing prevalence of multidrug-resistant strains has led to a decline in the effectiveness of conventional antibiotics, highlighting the urgent need for new antimicrobial agents[3].

Metal oxide nanoparticles, particularly zinc oxide nanoparticles, have garnered considerable attention due to their broad-spectrum antibacterial activity, chemical stability, biocompatibility, and low toxicity[4]. Zinc oxide (ZnO) nanoparticles exert their antibacterial effects through multiple mechanisms, including the generation of reactive oxygen species (ROS), disruption of bacterial cell membranes, release of zinc ions (Zn^{2+}), and induction of oxidative stress, which reduces the likelihood of bacterial resistance compared to conventional antibiotics[5].

The green synthesis of zinc oxide nanoparticles using natural products has recently emerged as an environmentally friendly alternative to chemical synthesis[6]. Plant extracts contain abundant phytochemicals, such as flavonoids, phenolic acids, tannins, and terpenoids, which act as natural reducing, stabilizing, and encapsulating agents during nanoparticle formation[7]. Propolis is rich in flavonoids and phenolic compounds with well-documented antibacterial and antioxidant properties, while pomegranate peel contains high concentrations of polyphenols, ellagic acid, gallic acid, and tannins, exhibiting potent antimicrobial activity against numerous pathogenic microorganisms[8].

Therefore, this study aimed to synthesize zinc oxide nanoparticles using an electrochemical method, followed by a biolayer of propolis and pomegranate peel extracts. The synthesized nanoparticles were characterized using X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM), and energy-dispersive X-ray spectroscopy (EDX). Their antibacterial activity against Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli was also evaluated. The study further investigated the effect of nanoparticle concentration on antibacterial activity to assess the potential of this environmentally friendly nanocomposite as a promising alternative to antibiotics.

2. MATERIALS AND METHODS

Experimental Procedure for Detecting Bacterial Activity

2.1 Study Design

An experimental laboratory study was applied to investigate the antimicrobial activity of Propolis and Pomegranate Peel after coating with zinc oxide nanoparticles against Staph and E. coli isolates.

2.2 Collection of Microbial Isolates

Isolates of Staph and E. coli were used in this study. The isolates were obtained from laboratory cultures and maintained under appropriate microbiological conditions.

2.3Electrochemical Preparation of Zinc Oxide Nanoparticles (NPs)

The electrochemically prepared zinc oxide nanoparticles consist of two electrodes: a pure metal (Zn) for synthesizing ZnO nanoparticles, which serves as the positive electrode, and a graphite electrode as the negative electrode. All electrodes are connected to a DC power source. Both the positive and negative electrodes are washed with ethanol and acetone, followed by deionized water. The electrodes were immersed in a 200 mL solution containing 5 mL of potassium chloride (KCl) as the electrolyte, 10 mL of a stabilizer (polyvinyl alcohol (PVA)), and 185 mL of distilled water, as shown in Figure 1. Upon application of a voltage, the reaction began. The solution was stirred for 1 hour at approximately 30 °C, resulting in the formation of a white precipitate of zinc hydroxide (ZnOH) nanoparticles. After centrifugation, the precipitate was washed several times with deionized water, acetone, and ethanol, then poured into a drying vessel and dried at 100 °C for 1 hour, followed by calcination at 700 °C for 1 hour[9, 10].

Figure 1. Electrochemical preparation of zinc oxide (NPs)

2.4 Preparation and Extraction

Initially, the mixture was prepared by combining 15 grams of raw propolis with 5 grams of pomegranate peel. This mixture was then dissolved in 100 ml of 70% ethanol and stirred with a magnetic stirrer for one hour. For extraction, the solution was stored in a dark-colored bottle in a dark place for ten days. Afterward, the mixture was filtered using filter paper, and the resulting filtrate was concentrated in a water bath until a thick, waxy, viscous mass was obtained. This mass was left at room temperature for two additional days to ensure the ethanol had completely evaporated. The final formulation was obtained by adding 50 ml of olive oil to every 5 grams of the extracted waxy material[11].

Figure 2. Extraction process of Propolis and Pomegranate Peel

2.5 Coating Zinc Oxide Nanoparticles with Propolis and Pomegranate Peel Extract

The synthesized ZnO nanoparticles were characterized using XRD for crystal structure analysis, FESEM for surface morphology, and EDX for elemental composition. All measurements were performed according to the manufacturers' operating procedures under standard laboratory conditions.
[12].

Figure 3. Sonication process of Propolis and Pomegranate Peel

2.6 Antibacterial Activity of Zinc Oxide Nanoparticles Coated with Propolis and Pomegranate Peel

Preparation of the Bacterial Inoculum

Clinical isolates of Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative) were cultured on nutrient agar and incubated at 37°C for 24 hours. Fresh bacterial colonies were resuspended in sterile saline, and the turbidity was adjusted to 0.5 McFarland, which is approximately equivalent to 1×10^8 colony-forming units/ml.

Preparation of the Nanoparticle Suspension

Zinc oxide nanoparticles coated with propolis and pomegranate peel were dispersed in sterile distilled water containing 5% dimethyl sulfoxide (DMSO) to improve dispersion. The suspension was sonicated for 10–15 minutes before testing. Three concentrations were prepared:

50 µg/ml
75 µg/ml
100 µg/ml

Agar Diffusion Test

Antibacterial activity was evaluated using the agar diffusion method. Mueller-Hinton agar (MHA) plates were homogeneously inoculated with the bacterial suspension prepared using a sterile cotton swab. Sterile cork plugs were used to create 6 mm diameter wells in the agar. Each well was filled with 100 µL of the nanoparticle suspension at the specified concentration.

Control Samples

A well containing sterile distilled water with 5% dimethyl sulfoxide (DMSO) was used as a negative control, while a standard antibiotic tablet (e.g., ciprofloxacin, 5 µg) was used as a positive control.

2.7 Ethical and Biosafety Statement

Clinical bacterial isolates of Staphylococcus aureus and Escherichia coli were obtained from routine laboratory cultures and handled according to institutional biosafety regulations. No human participants, personal patient information, or animal experiments were involved in this study. Therefore, formal ethical approval was not required.

2.8 Statistical Analysis

All experiments were performed in triplicate (n = 3). Data are expressed as mean ± standard deviation (SD). Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Differences were considered statistically significant at **$P < 0.05$**.

3 RESULTS AND DISCUSSION

3.1 XRD Analysis of zinc oxide nanoparticles.

The values 72.58°, 69.191°, 68.051°, 66.52°, 62.983°, 56.683°, 47.645°, 36.374°, 34.552° and 31.873°, related to the Miller indices ((202), (004), (201), (112), (200), (110), (102), (101), (002) and (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202), indicate that the phase of the zinc oxide nanoparticles is wurtzite, as shown in Figure 4 [13]

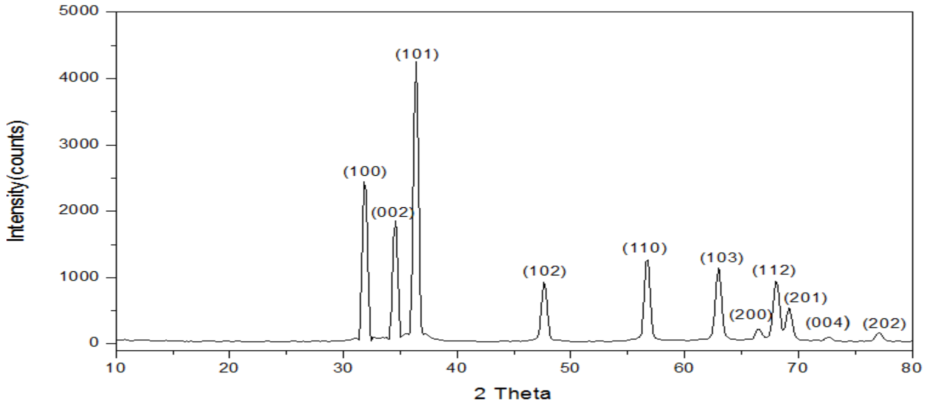

Figure 4. X-Ray Diffraction pattern of ZnO NPs.

3.2 Analysis of Zinc Oxide Nanoparticles Using Field Emission Scanning Electron Microscopy (FESEM).

The morphology, size, and shape of zinc oxide nanoparticles were characterized using field emission scanning electron microscopy (FESEM), which provides high-resolution images of the zinc oxide nanoparticle surface. The FESEM images of the zinc oxide nanoparticles show a rice-grain-like shape[14], and agglomeration is also observed, as illustrated in Figure 5.

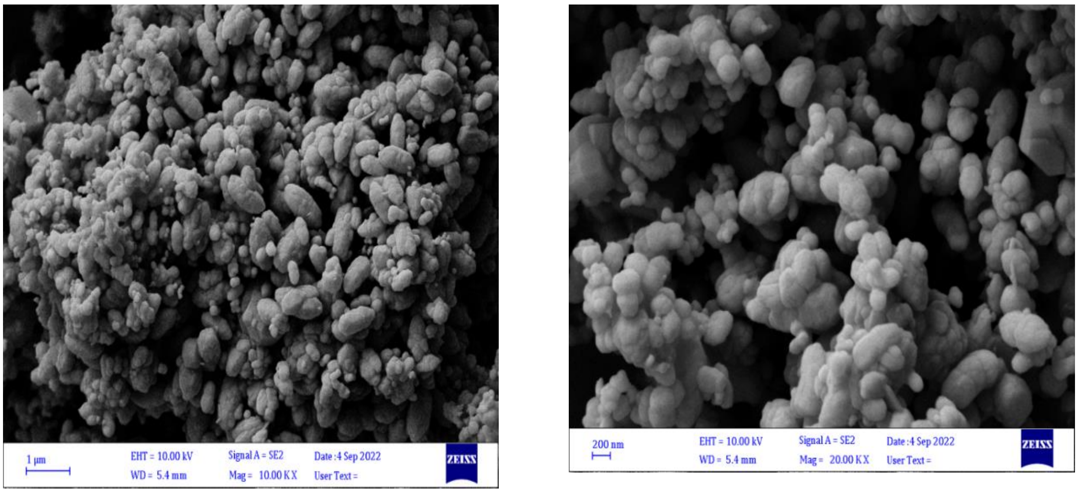

Figure 5. FESEM images of ZnO NPs.

3.3 EDX Analysis of Zinc Oxide Nanoparticles

The purity and composition of the electrochemically synthesized zinc oxide nanoparticles were determined using EDX measurements, as shown in Figure 6. Oxygen and zinc signals are present, indicating the purification of the zinc oxide nanoparticles from impurities. The composition of the zinc oxide nanoparticles was as follows: oxygen = 11.6%, zinc = 88.4%. Gold, used as a coating material according to the measurements, was also detected[13] .

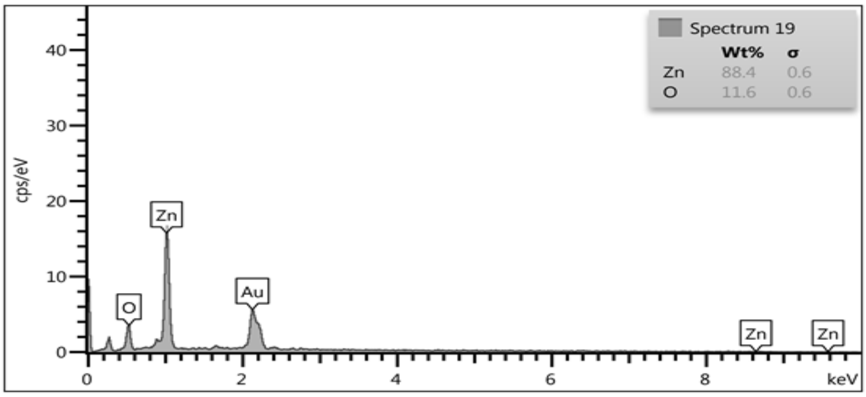

Figure 6. EDX -ray of ZnO NPS.

3.4 Antibacterial Activity Analysis

The antibacterial activity of zinc oxide nanoparticles (ZnO NPs) coated with propolis and pomegranate peel was evaluated against Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli using the agar diffusion method. The results showed a clear concentration-dependent antibacterial effect. For Staphylococcus aureus, the inhibition zones increased from 13 mm at 50 µg/ml to 15 mm at 75 µg/ml, and then to 18 mm at 100 µg/ml. In contrast, Escherichia coli showed no significant inhibition at 50 µg/ml, while inhibition zones of 12 mm and 14 mm were observed at 75 µg/ml and 100 µg/ml, respectively. No inhibition zone was observed in the negative control sample, confirming that the observed antibacterial activity is due to the coated zinc oxide nanoparticles, not the solvent.

The greater antibacterial activity observed against Staphylococcus aureus compared to Escherichia coli can be explained by differences in bacterial cell wall structure. Gram-positive bacteria possess a thick peptidoglycan layer but lack the lipopolysaccharide outer membrane found in Gram-negative bacteria. The additional outer membrane of E. coli acts as an effective permeability barrier, reducing nanoparticle penetration and thus decreasing its sensitivity to antibacterial agents. Consequently, Gram-positive bacteria are generally more sensitive to zinc oxide nanoparticles than Gram-negative bacteria.

The antibacterial mechanism of action of zinc oxide nanoparticles involves multiple, complementary pathways. Zinc oxide nanoparticles produce various reactive oxygen species, including hydroxyl radicals, superoxide anions, and hydrogen peroxide, which induce oxidative stress and damage bacterial proteins, lipids, and nucleic acids. Furthermore, zinc ions (Zn^{2+}) released from the nanoparticle surface interfere with essential metabolic processes and increase membrane permeability, ultimately leading to bacterial cell death. The small size and large surface area of zinc oxide nanoparticles enhance their interaction with bacterial cells, thus improving their antibacterial activity.

The antibacterial activity observed in this study may also be attributed to the bioactive phytochemicals present in propolis extracts and pomegranate peel. Propolis contains flavonoids, phenolic acids, and caffeic acid derivatives with well-documented antibacterial properties, while pomegranate peel is rich in ellagic acid, gallic acid, tannins, and other polyphenolic compounds that disrupt bacterial membranes and inhibit key bacterial enzymes. The bioencapsulation of zinc oxide nanoparticles with these natural extracts likely resulted in a synergistic antibacterial effect, combining the oxidative damage caused by the zinc oxide nanoparticles with the antimicrobial action of the phytochemicals. This synergy may also enhance the stability and dispersion of the nanoparticles, increasing their contact with bacterial cells and promoting their antibacterial activity.

Overall, these results suggest that zinc oxide nanoparticles encapsulated in propolis and pomegranate peel possess promising broad-spectrum antibacterial activity, particularly against Staphylococcus aureus. Its environmentally friendly manufacturing process, coupled with its potent antibacterial activity, suggests that this nanocomposite may be a promising candidate for future biomedical and pharmaceutical applications, wound dressings, and antimicrobial coatings**.**

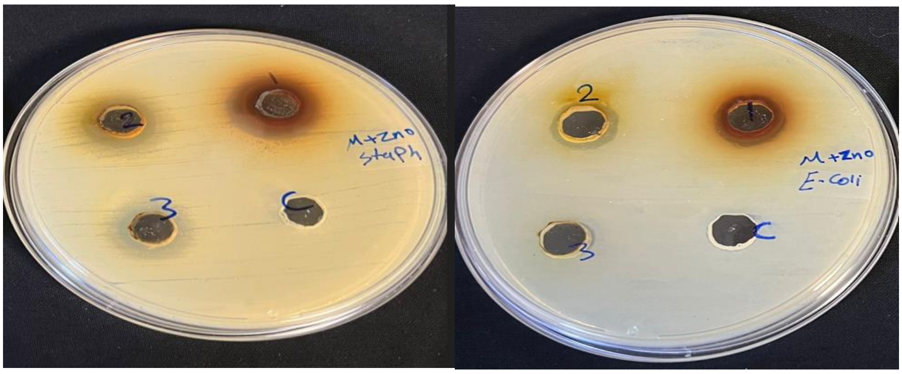

Figure 7. Representative agar well diffusion assay showing the antibacterial activity of propolis and pomegranate peel-coated ZnO nanoparticles against *Staph and E. coli* at different concentrations.

Table 2. Antibacterial activity of propolis and pomegranate peel-coated zinc oxide nanoparticles against *Staph and E.coli* at different concentrations.

Sample	Bacteria	100	75	50	control
Propolis and Pomegranate Peel -zinc oxide nanoparticles	Staph	18	15	13	0
	E.coli	14	12	R	0

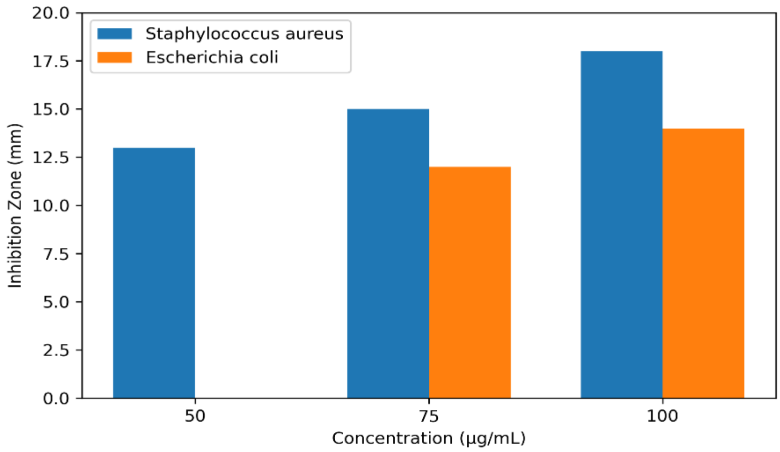

Figure 8. Antibacterial activity of propolis and pomegranate peel-coated ZnO nanoparticles against *Staphylococcus aureus* and *Escherichia coli* at different concentrations (50, 75, and 100 μg/mL)

4.CONCLUSION

In the present study, zinc oxide nanoparticles (ZnO NPs) were successfully synthesized using an environmentally friendly electrochemical approach and subsequently coated with propolis and pomegranate peel extracts. Structural characterization by XRD, FESEM, and EDX confirmed the successful formation of crystalline ZnO nanoparticles with high purity and appropriate morphology. The coated nanoparticles exhibited concentration-dependent antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli*, with greater inhibition observed against the Gram-positive strain. The enhanced antibacterial performance is likely attributed to the combined effects of ZnO nanoparticles and the bioactive phytochemicals present in the natural extracts. These findings demonstrate that propolis and pomegranate peel-coated ZnO nanoparticles possess promising antibacterial properties under laboratory conditions and may serve as a sustainable platform for the development of eco-friendly antimicrobial nanomaterials. Further investigations, including cytotoxicity assessment, mechanistic studies, and in vivo evaluation, are recommended to validate their potential for future biomedical applications.

5. CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this manuscript.

6. FUNDING

This research received no external funding.

7. AUTHOR CONTRIBUTIONS

Conceptualization: Israa Sami Hadi.
Methodology: Israa Sami Hadi.
Investigation: Israa Sami Hadi.
Formal analysis: Israa Sami Hadi.
Writing—original draft: Israa Sami Hadi.
Writing—review and editing: Israa Sami Hadi.
Supervision: Israa Sami Hadi.

8. DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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