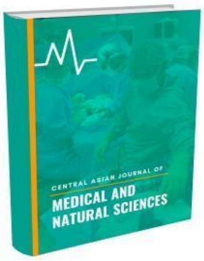

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Article

A Study of the Physical Properties of ZnO Nanoparticles Prepared by Green Synthesis Method for Medical Application

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Abstract: Background: Green-synthesized ZnO nanoparticles possess unique physical properties and biocompatibility, making them promising materials for safe and eco-friendly medical applications. Aims of the study: In this research project, the researcher needs to synthesize zinc oxide nanoparticles through a green procedure and to determine their physical characteristics to determine their applicability in the medical field. Methodology: The Camellia sinensis leaf extract was used as a reducing and stabilizing agent to green-synthesize zinc oxide nanoparticles. The extract was made using hot water and mediated the formation of ZnO under controlled condition of stirring and temperature. Nanoparticles were tested in terms of antimicrobial activity, such as inhibition zone, MIC, MBC, and antibiofilm activity against the selected bacterial and fungal pathogens based on conventional microbiological tests. Result: The ZnO nanoparticles synthesized were of high purity, crystallite size was nanoscale and good crystallinity as seen by XRD and TEM. The UV-vis spectra exhibited a blue shift at approximately 315 nm with an approximate bandgap of 3.9 eV which represented quantum confinements. In microorganisms, biologically prepared ZnO nanoparticles had a good antimicrobial and antibiofilm properties with the highest sensitivity of Klebsiella pneumoniae and low sensitivity to Staphylococcus aureus, indicating microorganism-specific responses. Conclusions: ZnO nanoparticles green-synthesized had good crystallinity and nanoscale and had a high antimicrobial and antibiofilm properties. The effects caused by these are increased surface reactivity, quantum confinement, and generation of ROS that damage microbial membranes and prevent biofilm formation in a dependency on the microorganism.

Keywords: Zinc Oxide Nanoparticles, Green Synthesis, Camellia Sinensis, Antimicrobial Activity, Biofilm Inhibition.

1. Introduction

Nanotechnology has become a revolutionary area of contemporary science and has provided novel solutions into the form of creation of materials at a nanoscale and having distinctive physicochemical and biological characteristics. Zinc oxide nanoparticles (ZnO NPs) are among the other nanomaterials of great interest due to their multifunctionality properties such as high surface area, chemical stability, semiconducting properties, and excellent biological activity [1]. All these characteristics make ZnO NPs very promising in the vast spectrum of medical applications including antimicrobial agents, wound healing systems, drug delivery platforms, bioimaging, and diagnostic platforms [2], [3].

Physical attributes of ZnO nanoparticles especially particle size, morphology, surface area, crystallinity, and optical characteristics determine conclusively the biological performance and medical performance of these nanoparticles [4]. Changes in size and shape in the nanoscale scale can cause major changes in cellular uptake, interaction with biomolecules and antimicrobial efficacies. Further, the photocatalytic activity and the production of reactive oxygen species (ROS) are related closely with the crystalline structure and surface properties of ZnO NPs, which are considered to be major processes underlying antimicrobial and anticancer effects. Thus, complete knowledge on these physical properties is required to ensure the optimization of ZnO nanoparticles as safe and effective medical delivery [5].

To obtain tuned particle properties, the typical ways of synthesizing ZnO nanoparticles are sol solgel, chemical precipitation, hydrothermal synthesis and physical vapor deposition [6]. But these methods are frequently toxic, do consume high energy, require complicated processes and severe reaction environments. These restrictions are important issues of environmental and health concern especially where the manufactured nanoparticles are supposed to be used in the biomedical field. The biocompatibility can be affected by the residual toxic reagents and by-products, which limit the clinical translation [7].

Green synthesis is also a solution to these issues as it is an environmentally safe and sustainable method of nanoparticle synthesis. The natural biological resources used in green synthesis include plant extracts, bacterial, fungal and algal biomass that serves as reducing, capping and stabilizing agents during the formation of nanoparticles. The use of plants, especially in the context of plant-mediated synthesis, has become an important aspect because it is simple, cost-effective, can be scaled, and bioactive phytochemicals are abundant [8], [9]. These phytochemicals, flavonoids, phenolics, terpenoids and alkaloids, do not only aid in the synthesis of nanoparticles but also in stability and possible biological compatibility [10].

There is also an increased likelihood of ZnO nanoparticles produced by green processes to demonstrate improved biocompatibility and decreased toxicity relative to those made by other chemical processes. Moreover, the decision on the biological source, reaction conditions as well as the conditions under which the synthesis is done have direct effect on the physical properties of the nanoparticles [11]. Particle size distribution, morphology and crystallinity can be controlled by factors like extract concentration, pH, temperature and reaction time. Therefore, it is essential to systematically study the physical characteristics of the green-synthesized ZnO nanoparticles in order to fine-tune the performance of the nanoparticles to certain medical applications [12], [13].

In the given research, the attention is devoted to the synthesis of ZnO nanoparticles by a green method and the specific analysis of their physical characteristics. This study will explain the connection between green synthesis conditions and the behavior of nanoparticles through the study of important properties including particle size, morphology, crystalline structure, and optical properties. The interpretation of these associations is critical to the future development of the rational design of ZnO nanoparticles and the subsequent safe inclusion of these nanoparticles into medicine. The research results of this paper can help make significant contributions into creation of sustainable nanomaterials with high functionality and biomedical interest, complying with the current need in eco-friendly and clinically meaningful nanotechnology.

2. Materials and Methods

Materials

All microbiological media and zinc nitrate hexahydrate ($Zn(NO_3)_2 \bullet 6H_2O$), were purchased at Merck (Germany). The leaves of fresh green tea (Camellia sinensis) were acquired in a local market in Baghdad. All the solutions were made using distilled or deionized water.

Preparation of Camellia sinensis Aqueous Extract

Fresh green tea leaves were well washed using tap water and finally using the double-distilled water to remove any impurities on the surface and then dried in the air. The 50 grams of the dried leaves were placed in 250 mL of hot deionized water and left to steep after 30min until the solution was light yellow. The extract was allowed to cool, filtering Whatman No.1 filter paper, and centrifuged at 3000 rpm so as to get rid of fine particulates. The supernatant was evaporated to get a thick crude extract. To be experimentally used, 0.5 g of the extract was dissolved in 100 mL of deionized water and kept at 4 o C until the next usage.

Green Synthesis of ZnO Nanoparticles

A green method was used to synthesize ZnO nanoparticles with the help of C. sinensis extract. In a nut shell, 6.58 g of zinc acetate dihydrate (0.2 M) was dissolved in 150mL of deionized water under continuous magnetic stirring (1500 rpm). Afterwards, 20 mL of the prepared plant extract was dropwise added and the mixture of reaction stirreded at 40 o C to 2 h. The mixture was centrifuged twice at 4000 rpm during 15 min after cooling, washed with distilled water, and dried at 40 o C. The pale white ZnO nanopowder that was obtained was selected to undergo subsequent analyses and biological assessment.

Microbial Strains and Culture Conditions

Planktonic cultures of Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumoniae, and Candida albicans were prepared from overnight cultures grown on nutrient agar. Colonies were taken individually in nutrient broth and incubated at 37 o C in 18-24 h. The turbidity of both cultures was brought to the 0.5 McFarland standard (≈1.5×10^8 CFU/mL).

Antimicrobial Activity Assay

Antimicrobial activity of ZnO nanoparticles was determined by the agar well diffusion method on a Mueller-Hinton agar. Plates were inoculated with microbial suspensions and 100 μL of the ZnO NP suspension was dispensed into 6 mm wells. The plates were kept at 37 °C for 24 h and the zone of inhibition was measured in mm.

Determination of MIC and MBC

Minimum inhibitory concentration (MIC) was measured by broth microdilution method in 96-well plates. ZnO NPs were serially diluted two-fold in tryptic soy broth with 1% glucose. Microbial suspension (4×10^5 CFU/mL) was added to each well and incubated for 18-24 h at 37 °C. MIC was the lowest concentration with ≥80% inhibition of growth. For minimum bactericidal concentration (MBC), samples from clear wells were spread on Mueller–Hinton agar and the lowest concentration of ZnO NPs that prevented colony formation after incubation was determined as the MBC.

Biofilm Inhibition Assay

Biofilm formation was assessed using a microtiter plate assay. Microbial suspensions adjusted to 0.5 McFarland were incubated in TSB containing 1% glucose with sub-inhibitory concentrations (½ MIC) of ZnO nanoparticles for 24 h at 37 °C. Wells were washed with PBS, fixed with methanol, stained with 0.1% crystal violet, and the bound dye was solubilized with ethanol. Absorbance was measured at 570 nm, and biofilm inhibition was expressed relative to untreated controls.

Statistical analysis:

We performed quantitative analysis in SPSS 26. Data are expressed as frequencies and percentages. For normally distributed data, the independent and dependent t-tests (two-tailed). For skewed variables, Mann-Whitney U test, Wilcoxon test, and Chi-square test were used. A p-value of < 0.05 was considered statistically significant.

Ethical approval:

The study protocol was reviewed and approved by a human ethics committee. Patients were given details of the study and written informed consent was obtained. Patient information was kept confidential.

3. Results

X-ray Diffraction studies

The X-ray diffraction (XRD) pattern indicated that the ZnO powder is polycrystalline. XRD was used to determine the crystal structure of the green-synthesized ZnO nanoparticles. All diffraction peaks were well indexed to the hexagonal wurtzite structure of ZnO, with lattice parameters of a = 3.2490 Å and c = 5.2070 Å, showing excellent agreement with the standard JCPDS card No. 01-075-0576. There was no further impurity peak, which is a characteristic of the high purity of the synthesized nanoparticles.

The observable expansion of diffraction peaks is also due to the size of the crystallites per nanoscale that is also normally seen in green-synthesized nanomaterials due to limited crystal growth and high nucleation rates. The average crystallite size was calculated with the help of the Scherrer equation that also confirmed the nanostructured structure of the ZnO particles synthesized. Besides, good crystallinity of the product is reflected in the presence of sharp and intense Bragg reflections.

The improved crystallinity could be attributed to the action of bioactive phytochemicals that are found in the Camellia sinensis extract and served as capping and stabilizing factors in the formation of the nanoparticles. The nucleation and growth were efficiently regulated by these organic compounds resulting in well-dispersed crystalline ZnO nanoparticles. Even though the phytochemicals affected the relative levels of the diffraction peaks, no distinct crystalline stages of organic residues were detected, which verified that they were effectively removed during centrifugation and purification processes.

The obtained structural features are consistent with previously reported studies on green-synthesized ZnO nanoparticles using various plant extracts. Collectively, these findings support the dual role of phytochemicals in both reducing the metal precursor and regulating nanoparticle crystallization, resulting in stable ZnO nanostructures with well-defined crystal phases.

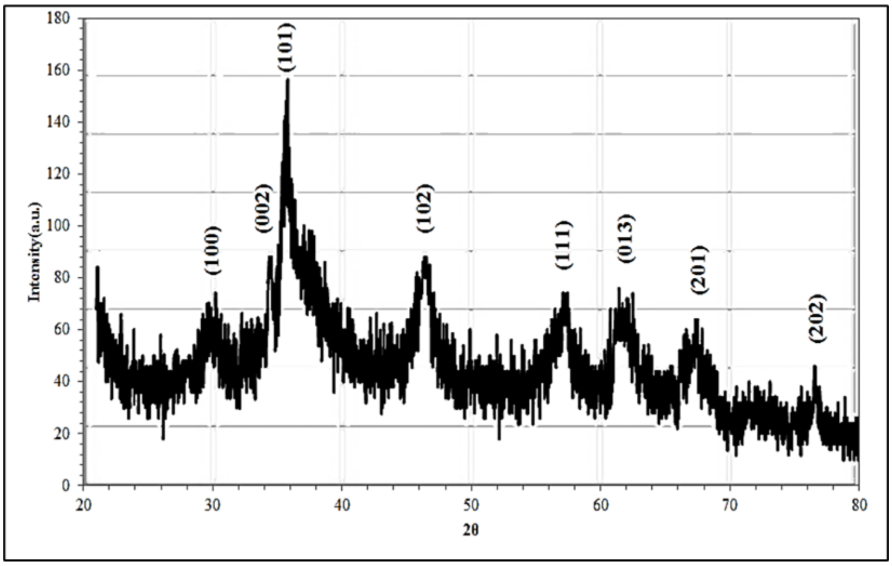

Figure 1. XRD pattern of the as synthesized ZnO Nanoparticles.

Transmission Electron Microscopy (TEM) analysis

Offered more structural evidence for the green-prepared ZnO nanoparticles, which is complementary to earlier findings. As shown in Fig. (6), the TEM results show that the shape of ZnO nanoparticles is approximately quasi-spherical and it shows some agglomeration. The intermediate bright spots found in the images are ascribed to metallic residue particles, affirming subsequent formation of the hexagonal wurtzite crystal structure. The existence of all-round particles is ascribed to an amorphous phase without clear crystal orientation. These nanoparticles are then stabilized by plant extract's biomolecules acting as capping agents. These results are in agreement with other studies that presented analogous morphological character and crystal structure of ZnO NPs prepared by different plant extract. Fouda et al. (2023) synthesized ZnO nanoparticles using Punica granatum peel extract, reporting particle sizes between 10–45 nm, with quasi-spherical shapes and agglomerates observed via TEM. The wurtzite phase was confirmed using XRD. Elsamra et al. (2024) used Carica papaya leaf extract and confirmed the formation of hexagonal wurtzite ZnO NPs through XRD and SAED, along with spherical morphology and minor agglomeration. Punica granatum-mediated synthesis (2022) also reported hexagonal wurtzite crystal structures, with particles in the range of 20–40 nm, and TEM images showing both spherical and hexagonal morphologies.

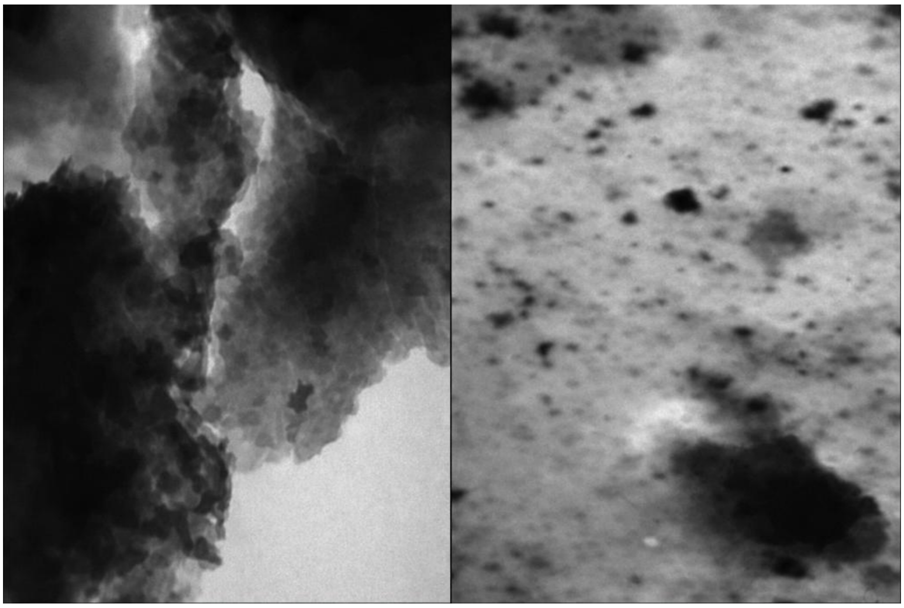

Figure 2. TEM images for green-synthesized ZnO with different magnifications.

UV-Visible Spectra of Zinc Oxide

The UV–Vis absorption spectrum of the ZnO nanoparticles prepared is shown in Fig. (6). The generation of ZnO nanoparticles was evidenced by the blue shift that appeared in the absorption peak, which occurred around 315 nm. On the other hand, the absorption peak of bulk ZnO is usually expected to occur at about 388 nm. This blue shift reflects differences in electronic excitation, because it is the UV or visible absorption processes by which electrons are transferred to higher energy states and when this occurs, changes in optical response occur. (Dhayalan et al., 2022). The observed blue shift can be attributed to Surface Plasmon Resonance (SPR), where conduction electrons on the nanoparticle surface resonate with the incident electromagnetic field, particularly at the interface of regions with opposite permittivity. Additionally, this shift is often correlated with reduced particle size and quantum confinement effects, which become significant at the nanoscale and lead to an increase in the optical bandgap energ. In this study, the estimated optical bandgap for the green-synthesized ZnO nanoparticles was found to be around 3.9 eV, calculated using the Tauc relation. Furthermore, the slight variation in absorption peaks (ranging from 325 nm to 365 nm) across different synthesis batches can be attributed to the use of diverse plant extracts during green synthesis. These extracts act as both reducing and stabilizing agents and contribute to minor differences in nanopa rticle morphology and size (Rani et al., 2021)

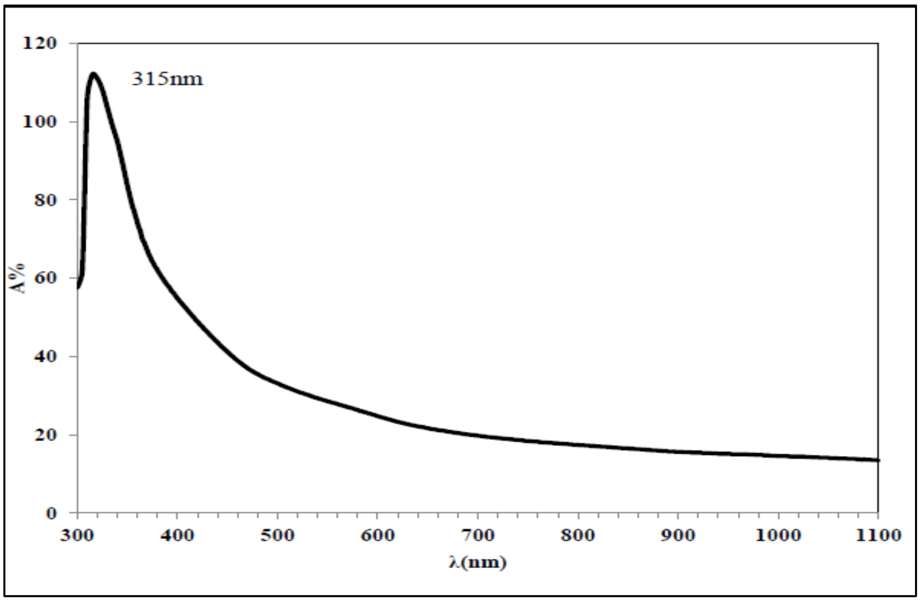

Figure 3. Absorption Spectrum of ZnO Nanoparticles.

The minimum inhibitory concentrations (MICs) of ZnO nanoparticles were assessed against E. coli, S. aureus, S. epidermidis, K. pneumoniae, and C. albicans

ZnO nanoparticles were evaluated against E. coli, S. aureus, S. epidermidis, K. pneumoniae and C. albicans using their minimum inhibitory concentrations (MICs). These MIC findings have been confirmed with minimum bactericidal concentration (MBC) findings, which verified the antimicrobial and antifungal activities of the nanoparticles.

Table 1. Minimum inhibitory concentrations (MICs) of ZnO nanoparticles.

Microorganism	MIC (mg/ml) – ZnO NPs
C. albicans	0.18
K. pneumoniae	0.035
S. epidermidis	0.045
S. aureus	0.55
E. coli	0.08
Synthesis Method	Green Tea (*Camellia sinensis*) Leaf Extract with Magnetic Stirring

Bactericidal Activity of Green Tea–Synthesized ZnO Nanoparticles Against Selected Microorganisms

The table demonstrates that zinc oxide nanoparticles prepared by the green synthesis method through the use of green tea extract have a distinct bactericidal effect on all the microorganisms tested with a significant difference in minimum lethal concentration (MBC) values. Klebsiella pneumoniae registered the least MBC (0.015 mg/ml), which implies the greatest sensitivity to ZnO NPs, followed by Staphylococcus epidermidis (0.03 mg/ml), and Candida albicans (0.04 mg/ml). On the contrary, Escherichia coli exhibited a higher relative resistance in terms of MBC value (0.05 mg/ml) and Staphylococcus aureus had the highest MBC value (0.25 mg/ml), which meant that it was less sensitive than the other two isolates. These findings indicate the capacity of green-prepared ZnO nanoparticles to generate an effective organism-type-specific lethal impact, which is probably connected with the dissimilarity in cell wall framework and oxidative stress reaction systems between bacterial species and yeast ones.

Table 2. Minimum Bactericidal Concentration (MBC) Values of ZnO Nanoparticles Prepared by Green Synthesis.

Microorganism	MBC (mg/ml) – ZnO NPs (Green Tea Synthesis)
Candida albicans	0.04
Klebsiella pneumoniae	0.015
Staphylococcus epidermidis	0.03
Staphylococcus aureus	0.25
Escherichia coli	0.05

Inhibitory Effect of Green Tea–Synthesized ZnO Nanoparticles on Bacterial Biofilm Formation

As indicated in the table, the nanoparticles of zinc oxide produced using the green tea extract had high capacity to prevent the formation of biofilms in the examined bacterial isolates, and the relative differences in the inhibition rate between the species could be observed. Klebsiella pneumoniae had the best biofilm inhibition value of 79.0% followed by Escherichia coli with 77.21 and this means the nanoparticles are very effective against gram- negative bacteria. The inhibition rate of Staphylococcus epidermidis and Staphylococcus aureus was 70.04 and 65.33, respectively. Such findings indicate the helpfulness of ZnO nanoparticles in preventing bacterial adhesion and the extracellular matrix development, probably because it disrupts the cell membrane integrity and causes oxidative stress, which restricts the capacity of bacterial cells to develop stable biofilms.

Table 3. Percentage Inhibition of Biofilm Formation by ZnO Nanoparticles Prepared via Green Synthesis.

Bacterial Isolates	Inhibition of Biofilm Formation (%)
Klebsiella pneumoniae	79.0%
Escherichia coli	77.21%
Staphylococcus epidermidis	70.04%
Staphylococcus aureus	65.33%

4. Discussion

The present work is a detailed evaluation of bio-synthesized zinc oxide nanoparticles (ZnO NPs) using Camellia sinensis leaf extract, highlighting the relationship between the structure, optical properties and biomedical properties of the nanoparticles. The commentary combines the findings of the X-ray diffraction (XRD), Transmission electron microscopy (TEM), UV- visible spectral characterisation and antimicrobial and anti biofilm studies to explain the success of the green synthesis approach and biomedical potential.

The XRD results confirmed the nanoparticles of ZnO are poly crystalline with hexagonal wurtzite crystal structure as shown by sharp peaks in the XRDs which match the standard JCPDS card No. 01-075-0576 [14]. The narrowness and absence of the extra impurity peaks, which indicate phase purity, demonstrate that the green synthesis route produces crystalline ZnO nicely, without any other phases. The broadening of the peaks is a general feature of nanocrystalline materials and a reduction in crystallite size, which is usually the case for green synthesis due to the high nucleation rate and small crystallite growth [15], [16]. The calculation of the crystallite size of the product by the Scherrer equation also suggested that the product was in the nanoscale [17]. The peaks are well defined which indicates a good level of crystallinity and therefore, the phytochemicals present in the green tea extracts were used to provide stability and direction for the synthesis process. These findings are consistent with other literature studies which have reported similar crystallographic properties of ZnO nanoparticles prepared from plant extracts which has confirmed the double role of the phytochemicals acting both as reducing and capping agents [18].

TEM analysis given also provided an additional information on the morphology and size distribution of the synthesized ZnO nanoparticles. The quasi-spherical shapes of the nanoparticles with a low level of agglomeration were observed, which is typical in metal oxide nanoparticles since they have a high surface energy [19]. This can be explained by Van der Waals agglomeration between nanoparticles even though the plant-derived biomolecules have been used to stabilize them. The dark and bright contrast areas of the TEM images imply the difference in thickness and crystallinity, and the existence of the rounded particles without obvious orientation indicates the partial amorphous areas stabilized by organic capping layers [20]. The findings of these observations are in line with previous reports in which green-synthesized ZnO nanoparticles exhibited either spherical or quasi-spherical morphologies, with sizes that are in the range of 10-45 nm. The agreement between the TEM and XRD findings also serves as confirmation that ZnO in nanoscale with a well-defined wurtzite structure was formed [21].

UV-vis spectroscopy was used to determine the optical characteristics of the ZnO nanoparticles synthesized. Compared with bulk ZnO which usually absorbs concentration at 388 nm, the absorption spectrum showed high blue shift with a peak of highest absorption at approximately 315 nm. It is a sign of the nanoscale size effects and quantum confinement effect, whereby decreasing the size of the particle, the bandgap energy increases [22], [23]. This is also confirmed by the estimated optical bandgap of about 3.9 eV calculated by the Tauc relation. Moreover, surface plasmon resonance (SPR) on the surface of nanoparticle can also be part of the modified optical response. The fact that absorption peaks may vary slightly in one synthesis bunch to another may be explained by the fact that nanoparticles change in size, morphology, and surface chemistry during plant-extract-mediated synthesis. The results are consistent with the past research which has shown that the optical behavior of ZnO nanoparticles is highly conditioned by green synthesis conditions [24], [25].

In addition to physics chemical characterization, one of the main results of this research is the biological performance of green-synthesized ZnO nanoparticles. The antibiotic tests showed a great bactericidal and fungicidal activity against various pathogenic microorganisms, including Gram-negative (Klebsiella pneumoniae, Escherichia coli), Gram-positive (Staphylococcus aureus, Staphylococcus epidermidis) and fungi (Candida albicans). The outcome of the MIC and MBC tests showed that the K. pneumoniae was the most vulnerable organism with the lowest MIC and MBC values [26], [27]. This increased susceptibility may be associated with the structural features of Gram-negative bacteria, which have a thinner peptidoglycan layer and a more permeable outer membrane, which makes it easier to enter nanoparticles and cause damage with the help of ROS [28].

Intermediate antimicrobial activity was tested against S. epidermidis, C. albicans, and E. coli with S. aureus with the greatest resistance of all organisms test. We can say that the decreased vulnerability of S. aureus is explained by thick peptidoglycan layer, which serves as a mechanical barrier to the infection by nanoparticles, and its effective reaction to oxidative stress [29]. These findings are being consistent with the prior researches that show discerning sensitivity of Gram-positive and Gram-negative bacteria to ZnO nanoparticles. The involvement of the antimicrobial effect is generally explained by the fact that reactive oxygen species (ROS) are formed, cell membrane integrity is disturbed, Zn^{+2} are released, and intracellular metabolism is disrupted [30], [31].

Besides planktonic antimicrobial effect, the ZnO nanoparticles exhibited strong antibiofilm effects. The best inhibition of biofilm-formation was found with K. pneumoniae and E. coli, then with S. epidermidis and S. aureus. Of acute significance is the discovery of biofilm inhibition because biofilms have been credited to amplify the resistance of microbes to standard antibiotics and host immune responses. This disruption of biofilm formation is probably due to ZnO nanoparticles affecting their ability to interact with bacteria adhesion, extracellular polymeric substance (EPS) generation, and quorum sensing [32]. The increase of anti-browning effect against microbes in the form of antibiofilms in this study can also be affected by the phytochemicals, which are present in green tea extract, e.g., catechins and polyphenols, which have inherent antimicrobial and antibiofilm activities and exhibit synergistic activity with ZnO nanoparticles [33].

5. Conclusion

Comprehensively, the results of the given research prove that green synthesis with the help of the Camellia sinensis extract is a promising approach to ZnO nanoparticles synthesis with the desired structural, optical, and biological characteristics. The green-synthesized nanoparticles of ZnO have better biocompatibility, a lower environmental effect, and a better antimicrobial effect as a result of the bioactive plant-based compounds. These strengths make the ZnO nanoparticles synthesized through green methods one of the potential areas in biomedical applications such as antimicrobial coating, wound dressings, and noninfectious biofilm formation. Further research projects ought to be geared towards determining cytotoxicity of nanoparticles in mammalian cells, size and dispersion optimization of the nanoparticles, and experiments in vivo to further confirm the safety and therapeutic prospects of the nanoparticles.

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