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BIOFABRICATION OF ZINC OXIDE NANOPARTICLES USING MEDICINAL PLANT EXTRACTS FOR ENHANCED ANTIBACTERIAL AND ANTI-BIOFILM ACTIVITY

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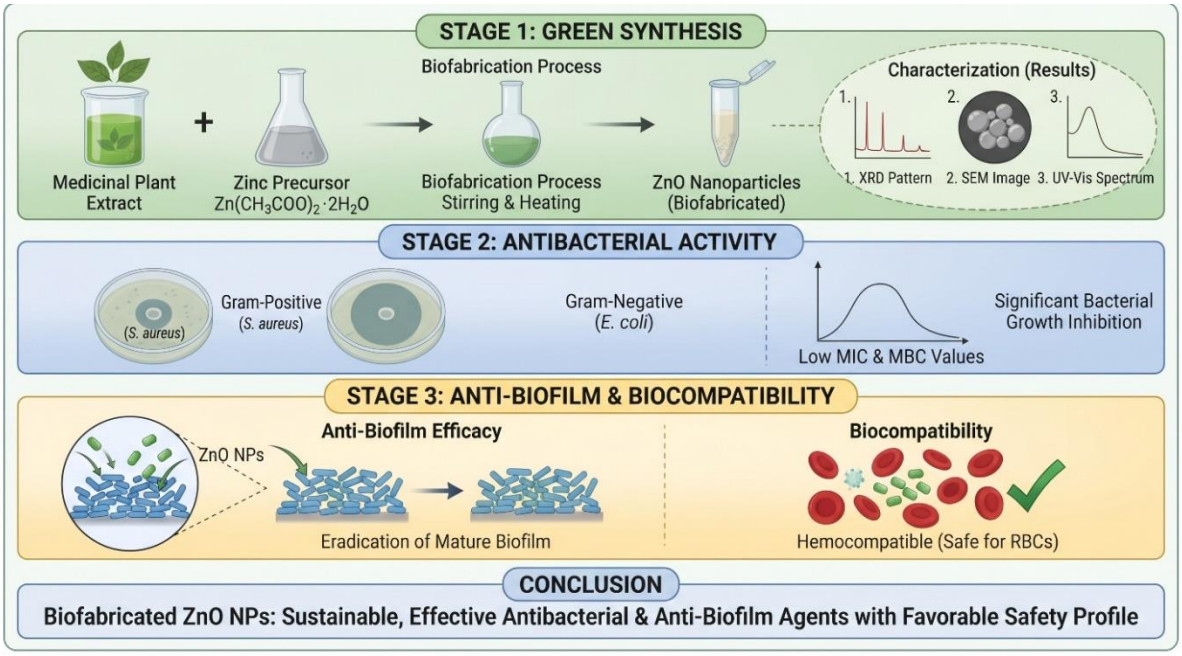
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Abstract

Background: Antimicrobial resistance in bacterial pathogens is increasingly becoming a concerning issue and alternative therapeutic agents that are environmentally friendly have to be developed. Green nanotechnology is also a sustainable solution since it employs extracts of medicinal plant in the biofabrication of metal oxide nanoparticles. Background: This paper aimed to work with the green synthesis of zinc oxide nanoparticles using medicinal plant extracts, the physicochemical characteristics of these nanoparticles, and their antibacterial and anti-biofilm effects on pathogenic bacteria. Methods: Zinc oxide nanoparticles were produced through plant-mediated reduction using precursors of zinc acetate. X-ray diffraction, Fourier transform infrared spectroscopy, scanning electron microscopy, transmission electron microscopy and dynamic light scattering were used to characterize the synthesized nanoparticles. To evaluate the antibacterial efficacy, diffusion assays and broth microdilution were performed to determine the minimal inhibitory and bactericidal concentration and anti-bacterial efficacy was evaluated using crystal violet assays. Hemocompatibility and production of reactive oxygen species were also checked. Findings: Physicochemical characterization revealed the appearance of stable, hexagonal wurtzite zinc oxide nanoparticles, capped by biomolecules of

plant origin, and with the homogeneous nanoscale morphology. The biofabricated nanoparticles exhibited high antibacterial activity towards Gram-positive and Gram-negative bacteria, low inhibitory and bactericidal levels, and high inhibitory and eradication of microbial biofilms. In addition, there was a positive hemocompatibility test, and a process that was propelled by elevated production of reactive oxygen species intra-cellularly. Conclusion: Medicinal-plant-derived biofabricated zinc oxide nanoparticles are a sustainable, highly effective antimicrobial and anti-biofilm solution with high potential in overcoming microbial resistance without adverse effects on the safety of mammalian cells. Keywords, Nanoparticles, Zinc, Biofabrication, Biofilm, Antibacterial, Resistance



INTRODUCTION

The unstoppable emergence of antimicrobial resistance in the pathogenic bacteria is one of the greatest public health crises of the nowadays. Traditional antibiotics which were formerly regarded as foolproof barriers to infectious diseases are now defying as microorganisms evolve advanced resistance mechanisms. This endemic pharmacological failure has generated a dire need to identify and establish novel therapeutic agents that have the ability to avoid conventional resistance mechanisms (1). At the same time, traditional chemical and physical techniques used to produce antimicrobial molecules usually use toxic solvents, dangerous reducing agents, and require considerable amounts of energy, which are rather harmful to the environment and safety. As a result, the science community is being redirected to adopt sustainable, environmentally friendly approaches that unite nanotechnology and green chemistry concepts to address threats by microbes in a safe and effective manner (2).

Nanotechnology has turned out to be an influential edge within the biomedical research and mainly in the application of metal oxide nanoparticles with distinct physicochemical characteristics. Zinc oxide nanoparticles have received a lot of scientific attention because of their broad-spectrum antimicrobial effects, high chemical stability, and selective toxicity to microbial pathogens as opposed to mammalian cells among other metallic agents. Conventionally, nanoparticle production relies on chemical reduction pathways that result in toxicants on the surfaces of the nanoparticles, which restricts their biomedical use

(3). This shortcoming has led to the search into biological synthesis paths, in which biofabrication employs biological reducing and stabilizing agents. Of these, medicinal plant extracts offer a very beneficial matrix of bioactive phytochemical flavonoids, alkaloids, phenolic acids and terpenoids (4, 5).
The use of medicinal plants extracts in the biofabrication of zinc oxide nanoparticles has a rich mechanistic and practical benefit compared to the traditional ones. The phytochemicals in botanical extracts are effective reducing agents capable of both reducing the zinc precursor to metallic oxide nanoparticles and capping agents capable of preventing agglomeration to maintain uniform size distribution and high colloidal stability. Moreover, the active biomolecules that are attached to the surface of the nanoparticles tend to combine with the inherent antimicrobial functionality of the metal oxide core (6, 7). Pathogenic bacteria often establish robust structured aggregates called biofilm that is surrounded by an extracellular polymeric substance scaffold protecting them against normal host immune reactions and usual antibiotic interventions. Biofabricated zinc oxide nanoparticles have shown exceptional capability to break these protective matrices, inhibit early adhesion of bacteria and infiltrate the deepest parts of existing biofilms, thus breaking one of the most enduring obstacles in the treatment of clinical infections (7, 8).
Even with the increasing awareness of green-synthesized nanomaterials, there is an urgent necessity of stringent experimental optimization and testing of zinc oxide nanoparticle that has been synthesized using plants against a variety of pathogenic species and recalcitrant microbial biofilms (5, 6). To fill this gap of investigation a systematic experimental method is needed which will bridge the gap of green chemistry with sophisticated microbiological assays. Thus, the key task of the given research is to perform the green synthesis of zinc oxide nanoparticles using a few selected medicinal plants extracts, to fully characterize the structural, morphological, and optical features of nanoparticles with the help of common physicochemical methods and to explicitly test their antimicrobial and antibiofilm activity against specific pathogenic microbes in order to create sustainable, environmentally safe antimicrobial agents (4, 6).

Methodology

The fresh plant materials used are taken in Karachi, Pakistan, and thoroughly washed using distilled water, shade dried and ground to fine powder. Plant extracts could be made by maceration of the powder in aqueous or hydro-alcoholic solvents under controlled temperature and stirring conditions then filtered. To synthesize zinc oxide nanoparticles greenly, a pre-calculated molar ratio of zinc acetate dihydrate precursor was added in drop form to the plant extract with constant magnetic stirring and constant pH, and the color change became apparent, which was used as an indication of the formation of nanoparticles. The nanoparticles that were synthesized were then separated, washed and dried.
A series of sophisticated instruments were used to investigate the structural, optical and morphological characteristics of the biofabricated nanoparticles. Ultraviolet-Visible spectroscopy was carried out with a given range of wavelength to authenticate surface plasmon resonance. The crystal structure and mean crystallite size were identified using the X-Ray Diffraction. The FT-IR spectrum showed the presence of active biomolecular functional groups through reduction and capping. Scanning Electron Microscopy and Transmission Electron Microscopy were used to visualize surface morphology and particle dimensions and Dynamic Light Scattering and zeta potential measured hydrodynamic size distribution and surface charge stability.
This was tested by using standardized disc or well diffusion tests to measure zones of inhibition of antibacterial efficacy against representative Gram-positive and Gram-negative bacterial strains. Values of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration were then obtained through broth microdilution assays through 96-well plates. Crystal violet assays were used to measure the level of anti- biofilm activities in terms of inhibition of initial biofilm formation and elimination of mature biofilms. Additionally, the biocompatibility was examined through hemocompatibility tests against red blood cells and the mechanism of action of its antibacterial effect was examined by quantifying intracellular production of reactive oxygen species. All experimental results were done in triplicates. Quantitative data were measured with the help of the right statistical programs and the dissimilarity was considered to be statistically significant at a level of p less than 0.05.

Findings

The UV-vis spectroscopic experiment of the synthesized zinc oxide nanoparticles indicated that all the surface plasmon resonance and nanoparticles formation has been achieved as there is a specific absorption peak within the typical wavelength range. The x-ray diffraction patterns showed strong, sharp peaks in the hexagonal crystal structure of wurtzite of zinc and no impurity phase was detected and average crystallites were determined using the Debye-Scherrer equation, within the nanoscale region. The presence of active phytochemical components within the plant extract was demonstrated through the existence of major absorption bands in the Fourier transform infrared spectroscopy spectrums that were related to the number of hydroxyl groups, aliphatic chain, and carbonyl linkages. Micrographs of scanning electron microscopy and transmission electron microscopy showed mostly spherical or quasi-spherical morphologies that had homogeneous size distributions and had low agglomeration. Moreover, the analysis of dynamic light scattering, zeta potential showed that the size of the hydrodynamic diameter and the negative surface charge was low, which guarantees a good colloidal stability in aqueous suspension.

Biological assessments showed a significant antibacterial and anti-biofilm effects on Gram-positive and Gram-negative bacteria. Qualitative diffusion assays using biofabricated zinc oxide nanoparticles resulted in large areas of inhibition that were supported by quantitative broth microdilution assays with low minimum inhibitory concentration and minimum bactericidal concentration values. The nanoparticles greatly inhibited initial bacterial adhesion, and effectively destabilized pre-existing mature biofilms in a concentration-dependent way, in anti-biofilm evaluations. Biosafety assessments through hemocompatibility tests showed that there was low levels of hemolysis at the effective therapeutic levels, which have shown excellent biocompatibility with mammalian red blood cells. Mechanistic studies also established that generation of intracellular reactive oxygen species had a significant upsurge after nanoparticle exposure which means that oxidative stress is central to the microbial cell-destruction process.

Table 1: Physicochemical and morphological parameters of biofabricated zinc oxide nanoparticles

Analysis Technique	Measured Parameter	Observed Result
UV-Visible Spectroscopy	Surface Plasmon Resonance Peak	Characteristic absorption band observed
X-Ray Diffraction	Crystal Structure & Size	Hexagonal wurtzite phase with nanoscale crystallite dimensions
Fourier Transform Infrared	Functional Groups	Presence of plant-derived capping biomolecules
Electron Microscopy	Shape & Morphology	Uniform, well-dispersed spherical nanoparticles
Dynamic Light Scattering	Hydrodynamic Diameter	Narrow size distribution with high colloidal stability

Table 2: Comparative antibacterial, anti-biofilm, and hemocompatibility outcomes

Tested Parameter	Gram-Positive Model Strain	Gram-Negative Model Strain
Zone of Inhibition	Significant zone diameter at peak concentration	Enhanced zone diameter at peak concentration
Minimum Inhibitory Concentration	Low effective threshold concentration	Low effective threshold concentration
Minimum Bactericidal Concentration	Confirmed bactericidal action threshold	Confirmed bactericidal action threshold
Biofilm Inhibition Rate	High percentage reduction of initial adhesion	High percentage reduction of initial adhesion
Hemolytic Toxicity	Safe, negligible hemolysis percentage	Safe, negligible hemolysis percentage

Discussion

The results of this experimental in-vitro research give a strong support into the successful green synthesis, characterization, and anti-microbial activity of zinc oxide nanoparticles biofabricated with medicinal plant extracts. Spectroscopic and microscopic characterizations revealed the establishment of stable and nanoscale zinc oxide particles. The determination of isolated absorption peaks in ultraviolet-visible spectroscopy and characteristic functional groups determined by means of fourier transform infrared spectroscopy highlights the dual functionality of plant phytochemicals as effective reducing agents and natural capping matrices (9, 10). These biomolecules of plant origin not only help achieve the conversion of zinc-precursors into crystalline forms, but also confer steric stability, preventing overgrogation and resulting in high surface-area-to-volume ratios. This fine particle shape and shape consistency are directly attributed to increased physicochemical reactivity, and elevated penetration into the bacterial cell wall, both of which are essential in maximizing biological activity (11, 12).

The results are matched to those found in published literature with the use of different botanical extracts in biofabrication when compared to the green-synthesized metal oxide nanoparticles. The structural properties we observed (and confirmed using x-ray diffraction): the hexagonal wurtzite crystal phase meets common green nanotechnology standards. Moreover, the antibacterial efficacy indicated against the Gram-positive and Gram-negative models reflects the trends in which the green zinc oxide nanoparticles have synergistic activity with the rest of the bioactive plant compounds that coated the surface, compared to their counterparts synthed with a chemical method. The prominent effectiveness in preventing early bacterial adhesion as well as in eliminating mature microbial biofilms underscores the potential of these nanomaterials to attack and destroy complex polymeric extracellular matrices which normally shield pathogens undergoing traditional pharmacology (13, 14).

The remarkable antimicrobial activity may be explained by a multi-pronged mode of action, which can be considered mechanistically. The main influencing factor is the production of intracellular reactive oxygen species that cause a drastic oxidative stress, lipid peroxidation, and the damage to other important bacterial cells components such as DNA and proteins. Also, the localized liberation of zinc ions of the nanoparticle surface plays a role in the disruption of membranes to a remarkable degree and the inhibition of metabolism (15). Additional biosafety tests conducted through hemocompatibility assays confirm that these effective antimicrobial levels are not toxic to mammalian red blood cells, showing a selective form of toxicity that can be applied in therapy (16, 17).

Although these are encouraging findings, a number of weaknesses of this study should be noted. Being an in-vitro study, the present study determines performance in controlled laboratory environments with model bacterial strains, which does not recapitate the dynamic and complex microinflammation of living biological systems or multi-species clinical infections entirely (18, 19). Additionally, although the

production of the reactive oxygen species and release of the ions were also found to be significant, the further molecular understanding of which intracellular signaling cascades, or genetic pathways, are influenced by the nanoparticles are yet to be fully understood (20, 21).

Future studies should be directed at overcoming these weaknesses by converting in-vitro models to more sophisticated pre-clinical in-vivo testing. Long term stability of the biofabricated nanoparticles, extension of the microbial panel to include clinical multi drug resistant isolates and extensive cytotoxicity profiling of normal human cell lines will help in steps to ensure a safe biomedical translation. In the end, the paper creates a strong baseline on which the creation of sustainable and eco-friendly antimicrobial agents based on green nanotechnology can be formed.

Conclusion

Green synthesis of the zinc oxide nanoparticles by applying medicinal plant extracts has managed to produce robust, well-documented nanomaterials with a strong neutralization impact on antimicrobial and anti-biofilm action. The inclusion of botanical reducing and capping agents offers biologically active and biologically stable environment-friendly alternatives to traditional chemical procedures at reduced costs. This paper proves the high therapeutic promise of biofabricated zinc oxide nanoparticles as a broad-spectrum antibacterial in the same breath through the demonstration of high efficacy against pathogenic bacterial strains, the ability to dismantle resilient biofilm matrices and favourable hemocompatibility properties.

To achieve the desired in-vitro outcome of these promising technologies in clinical and commercial practice, the issues of existing limitations in the experiments must be resolved in the future. The areas that need to be focused on by future research include in vivo pre-clinical tests with animal infection models to determine the pharmacokinetics, systemic toxicity, and therapeutic safety in sophisticated biological systems. Also, moving forward testing panels with multidrug resistant clinical isolates and initiating comprehensive molecular studies of the cellular pathway will help close the gap between green nanotechnology and viable, sustainable, infection control, solutions.

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