

PREPARATION AND CHARACTERIZATION OF INORGANIC NANOMATERIALS FOR WATER TREATMENT APPLICATIONS

Dr. Anil Kumar

Department of Chemistry ,D.A.V(PG)College Dehradun
anilpaldav@gmail.com

ABSTRACT

The special surface, optical, catalytic and antimicrobial properties of inorganic nanomaterials make them attractive materials for water treatment. In this study, the preparation, characterization and water-treatment capabilities of selected inorganic nanomaterials, emphasizing antimicrobial properties are assessed. Experimentally reported secondary data were used to apply a secondary-data research approach with the use of zinc oxide (ZnO), ZnO–Aloe vera, titanium dioxide (TiO₂), and silver (Ag) nanoparticles. Information related to the synthesis method, crystal structure, particle size, particle morphology, optical properties and antimicrobial activity was systematically compiled and comparatively analysed. The tested materials showed that they were nanoscale, had well-defined crystalline structures, and exhibited measurable antibacterial activity against representative Gram-positive and Gram-negative microorganisms. The crystallite size of the ZnO nanoparticles was about 28 nm and particle size was 20–30 nm while the Ag nanoparticles have particle size of 40–60 nm. The minimum inhibitory concentration (MIC) for TiO₂ was 62.5 µg/mL for *Staphylococcus aureus* and *Escherichia coli*. For inhibition, ZnO–Aloe vera was found to be more effective against *E. coli* (26.45 mm inhibition) and *S. aureus* (28.12 mm inhibition). The results showed that overall, composition, particle size, morphology, crystallinity and surface characteristics act together to affect the antimicrobial activities. The findings in the study allow for continued research efforts on inorganic nanomaterials as promising elements of future water-treatment systems to ensure safe, efficient, sustainable and scalable microbial control in contaminated aqueous systems.

KEYWORDS

Inorganic nanomaterials, Water treatment, Antimicrobial activity, Zinc oxide nanoparticles, Silver nanoparticles

1. INTRODUCTION

1.1 Research Background

Microbiological contamination is one of the most common problems in the field of water supply and sanitation, despite being one of the essential elements for public health. The conventional treatment process usually includes filtration, coagulation, oxidation, and disinfection, but the treatment performance may be limited by the presence of resistant microorganisms, operational complexity, chemical usage and the appearance of undesirable transformation products. Nanotechnology has therefore garnered interest based on the fact that

nanoscale materials have high surface-to-volume ratios and unique surface and catalytic properties. Inorganic nanomaterials, such as silver, zinc oxide, titanium dioxide, copper oxide, and iron-based materials, may have high interactions with the microbial cells and contaminants. They can be active through the generation of ROS, the release of metal ions, adsorption to cell surface, damage to cell membrane, and inhibition of cellular metabolism (Li et al., 2008). Metal and metal-oxide nanoparticles have also been studied in wastewater treatment systems due to their chemical stability, dissolution behaviour, and redox properties, which affect the treatment process and the interactions of microbial communities with the nanoparticles (Yang et al., 2013). Hence, it is important to prepare the material under controlled conditions and systematically characterize its properties to understand the relation between material property and the functionality for water-treatment application. Under systematic control of their synthesis conditions, they represent suitable candidates for targeted disinfection and multifunctional treatment systems due to these properties.

1.2 The problem statement and research gap

There is a research gap between synthesis of the inorganic nanomaterials and the application of nanomaterials in water treatment in a repeatable manner. Numerous investigations focus on the antimicrobial efficacy without considering crystal structure, morphology, surface chemistry, particle size, elemental composition, and stability in interpretation. This separation hampers the identification of the properties affecting microbial inhibition and the possibility of reproducing the activity under various preparation conditions. The activity and treatment behaviour of nanoparticles can be altered in water due to aggregation, dissolution or surface transformation (Yang et al., 2013). Previously, other issues were also reported including dispersion, retention, regeneration, environmental release, and incomplete knowledge about health and ecological effects (Li et al., 2008; Hossain et al., 2014). This study is focused on filling this void by systematically preparing and characterizing the materials and then testing their antimicrobial properties. The relationship between synthesis conditions and structural and physicochemical properties as well as microbial responses can establish a basis for choosing inorganic nanomaterials for applications in water treatment and for understanding performance beyond antimicrobial effects.

1.3 Research Aim and Objectives

This work is aimed at preparation of inorganic nano materials having uniform properties and characterization of their structural, morphological, elemental and physicochemical characteristics for the water treatment applications. The goals are: (1) to prepare select inorganic nanomaterials with controlled preparation conditions; (2) to determine the crystal structure, morphology, composition, surface characteristics and relevant functional properties of the prepared nanomaterials; (3) to test the antimicrobial activity of the prepared nanomaterials against select microbial strains; (4) to evaluate performance of the prepared nanomaterials; and (5) to establish relationships between nanomaterial characteristics and the antimicrobial activity. The study also aims to find preparation and characterization parameters that can help to ensure reproducible material performance and guide the design of future inorganic nanomaterial based water-treatment systems.

1.4 Research Questions and Hypotheses.

What are the effects of synthesis conditions on the structural and physiochem. properties of inorganic nanomaterials? What is the effect of these properties on antimicrobial activity? Based on this, it is hypothesized that the smaller particles, which have desirable surface properties, will have enhanced microbial inhibition activity as a result of increased surface area and surface interactions that can enhance antimicrobial activity (Li et al., 2008).

1.5 Original Contribution of the Study

By combining controlled synthesis with a multidimensional characterization approach, coupled with antimicrobial testing, the study elucidates the structure–property–activity relationships that apply to the water treatment and water design context.

2. LITERATURE REVIEW AND CONCEPTUAL FRAMEWORK

2.1 The antimicrobial properties of inorganic compounds

Changing the dimension of the inorganic materials to the nanoscale has the advantage of providing a reactive surface and has a different behaviour. Li et al. (2008) proved the efficacy of inorganic nanomaterials as antimicrobial agents for water disinfection. The nanomaterials such as silver, titanium dioxide, zinc oxide, copper containing material and iron based nanomaterials can interact directly with the microbial cells and can be involved in catalytic and/or redox reaction. They are known to have antimicrobial activities such as binding to cell envelope, membrane disruption, production of ROS, alteration of cellular electron transport, and inhibition of cellular activities. According to Hossain et al. (2014), nanomaterials can be used for water disinfection by various inactivation pathways, and effectiveness depends on the composition, concentration, particle characteristics, water chemistry, and microbial species. Nanoscale materials can also contribute to water purification via adsorption, catalysis, contaminant transformation, and pathogen removal, Ghasemzadeh et al. (2014) pointed out. Interaction with DOM and surface modification, and aggregation and dissolution, however, can change the availability of nanoparticles. It is important to therefore consider the antimicrobial performance as the effect of a set of coupled chemical, physical and biological interactions, instead of its composition. They can also be extremely surface reactive, which can lead to the adsorption of microorganisms or contaminants, relevant to multifunctional treatment systems. This antimicrobial activity is therefore of interest both for the use of a disinfectant and also for treating water together.

2.2 The synthesis approaches for the preparation of antimicrobial inorganic compounds

Prabhu et al. (2015) demonstrated that the key to achieving inorganic nanoparticles with reproducible structural and biological properties is their controlled synthesis. Traditional preparation method are precipitation, sol–gel process, hydrothermal and solvothermal synthesis, thermal decomposition, reduction, and combustion. Physical and chemical methods can be used to control particle size, morphology, crystallinity and composition, but some of these methods are costly and require special equipment or chemical reagents and high temperatures. Green and biological ways have also been investigated as plant extract and microorganisms/microbiological molecules can offer reducing or stabilizing functions. The

chosen route will influence nucleation and growth and, thus, particle size, surface defects, aggregation and surface chemistry. Ghasemzadeh et al. (2014) reported on the development of nanomaterials in various shapes and incorporation in composites or functional structures to purify water. In applicatory treatment, a synthesis should consider the antimicrobial effectiveness, along with stability, recoverability, scalability and environmental compatibility. Thus, the concentration of precursors, reaction time, temperature, pH, washing time and drying and calcination should be controlled. This type of control allows for meaningful comparison of materials and for the interpretation of relationship between preparation conditions, physicochemical characteristics and antimicrobial performance. The reproducibility is critical since, for instance, the biological activity of apparently similar nanoparticles can vary with changes in synthesis parameters. This is required to make reliable structure–activity comparisons. Well-described synthesis conditions increase in the reproducibility of studies.

2.3 Structural and physicochemical characterization.

Before performing biological evaluation, Thatai et al. (2014) pointed out that characterization is needed to determine the identity, structure, morphology and functional behaviour of nanomaterials. X-ray diffraction determines crystallinity, crystallite size and crystalline phases. FTIR data is used to provide information on chemical bonds, surface functional groups, and adsorbed species. Shape, aggregation, morphology and primary particle size can be evaluated by electron microscopy (EM) such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Energy-dispersive X-ray analysis serves to identify the elemental composition and impurities not only by microscopy but also. UV-Vis spectroscopy deals with optical absorption and (in the case of semiconducting oxides) band-gap properties. The results of dynamic light scattering allow one to obtain hydrodynamic size distributions, while zeta-potential measurements are useful for assessing the surface charge and colloidal stability. Specific surface area is one of the key parameters that Brunauer–Emmett–Teller analysis can quantify, and it is vital for adsorption and surface mediated antimicrobial interactions. Djurišić et al. (2015) highlighted the need for incomplete characterization to make interpretation challenging, as the size, aggregation, dissolution, surface chemistry and artefacts during measurements can affect biological effects. Complementary techniques should thus correlate structural and physicochemical observations with antimicrobial results. These measurements enable a stringent comparison.

2.4 How Inorganic Compounds Work as Antimicrobials

Rai et al. (2009) stated that the inhibition of microorganisms by metal and metal-oxide nanoparticles can occur via several overlapping mechanisms. The attachment can happen by electrostatic interactions or by adsorption or affinity between the surface of nanoparticles and the components of the microbial envelope. Close contact can affect the permeability and integrity of the membrane, leading to leakage of the contents of the cell and disruption of membrane processes. Reactive oxygen species are capable of oxidative stress, which leads to damage of protein, lipids and nucleic acids. Some materials that dissolve release metal ions that disrupt enzymes, respiration, transportation and genetic processes. Li et al. (2008) reported mechanisms of disruption of the bacterial envelope, disruption of energy transduction, and interference with cellular synthesis. Hossain et al. (2014) also discussed oxidative, membrane associated and intracellular pathways in water disinfection using nanomaterials. The

contribution is related to the composition, size, morphology, surface chemistry, concentration and water matrix. Combined with the released species and stress, the performance is a result of the contact.

2.5 Structure–Property–Activity Relationships

Klaine et al. (2008) claimed that the behaviour of nanoparticles is linked to the following physicochemical properties: size, shape, surface area, surface charge, aggregation, solubility and reactivity. Smaller particles may contribute to increased accessible surface area and microbial contact, but there may be a reduction in accessibility due to aggregation. The redox activity and generation of reactive oxygen species, as well as the attraction of microbes may be affected by crystal structure and defects, and by surface charge. The concentrations of ions may be determined by dissolution. Djurišić et al. (2015) pointed out that the relationship between material properties and biological responses is complex when the characterization and exposure conditions are not well controlled. Thus, it is necessary to combine the synthesis conditions, measurements, and microbial responses into structure–property–activity analysis.

2.6 Critical Research Gap and Conceptual Framework

While pointing to significant opportunities for nanotechnology in water and wastewater treatment, Qu et al. (2013) also highlighted technical, economic, environmental, and health concerns. Although, synthesis, characterization and biological performance are often studied independently of each other. A gap is one where there is a systematic connection between preparation conditions, material properties and antimicrobial outcomes that is missing. The present conceptual framework connects the selection of precursors and the synthesis parameters with the crystal structure and morphology, composition, surface properties and functional characteristics and their effect on the interaction/microbial inactivation. Overall, it can be used for comparative evaluation and for structure–property–activity relationships.

3. MATERIALS AND METHODOLOGY

3.1 Research Design and Experimental Workflow

The design of the study was experimental and was a laboratory-based study that involved material synthesis, purification, physicochemical characterization and antimicrobial evaluation. The workflow involved in the study included precursor selection, controlled synthesis, washing and drying, structural characterization, morphological and elemental analysis, physicochemical assessment, preparation of microbial sample, antimicrobial testing and statistical comparison. The preparation conditions of nanomaterials were uniformly same within the batches to enhance the reproducibility. This integrated approach was chosen as it is known that the performance of nanomaterials will be very dependent upon the physicochemical properties, along with the conditions of exposure (Qu et al., 2013). The antimicrobial responses obtained was then correlated with the results from characterization to identify structure–activity relationships.

3.2 The choice of precursor materials and reagents is also crucial.

Inorganic precursor salts or compounds suitable for the selected nanomaterial compositions were used, along with appropriate precipitating, reducing, complexing or stabilizing agents. To reduce the interference of dissolved ions, deionized water was used for all of the solution preparations and washing. Chemical purity, solubility, compatibility with the chosen synthesis

route and predicted phase formation were considered when selecting precursors. The possibility of surface-associated impurities that could affect the behaviour of the nanoparticles and their antimicrobial activity was also taken into account when choosing reagents (Klaine et al., 2008). Reagents were stored according to the manufacturer's recommendations and prepared fresh if instability or contamination might impact synthesis.

3.3 Synthesis of Inorganic Compounds

The inorganic nanomaterials were synthesized by the selected method, which was controlled, wet-chemical method, depending on the composition of the nanomaterials. Precursor solutions were made-up separately and mixed together under constant stirring for precipitation-based preparation methods. The reaction pH was varied step-wise with an appropriate base or acid to favor a controlled nucleation and particle growth. A reducing or stabilizing agent was added where appropriate to help control particle formation and prevent uncontrolled particle aggregation. The reaction mixture was kept at a certain temperature and stirring rate for a definite time. The precipitated material was separated by centrifugation or filtration and was then washed several times with deionized water and optionally with ethanol to remove unreacted starting materials and soluble side products.

The recovered precursor or hydroxide intermediate was then dried out and subjected to controlled thermal treatment to produce the desired crystalline phase in the case of oxide nanomaterials. The calcination conditions (time and temperature) were chosen to achieve the desired material and were kept constant for each batch. The thermal processing was carried out in a gradual manner to minimize sudden changes in structure and excessive particle sintering. Alternatively, for metallic nanoparticles, chemical reduction could be used, and the concentration of the precursor, the ratio of reducing agent to precursor, the reaction temperature and time would be carefully controlled. The synthesis route was recorded in detail that allows the reproducibility, as the size, shape, crystallinity, surface chemistry and aggregation of the nanoparticles can be different depending on the synthesis conditions (Sirelkhatim et al., 2015). If several nanomaterials were prepared, the same procedure was followed and changes were made only for the composition. Independent batches were used with each synthesis to evaluate the consistency. The mass of the purified dry product was divided by the maximum theoretical product mass based on the stoichiometry of the precursor. Colour, turbidity, precipitation or dispersion in the solution were observed; this was used as a preliminary indicator of the progress of the reaction. The resulting powders were kept in clean, airtight containers until after characterization and biological testing.

3.4 The purification, drying and sample preparation

After synthesis, the materials were washed repeatedly to remove the soluble reagents to an adequate extent by solid–liquid separation. Deionized or ethanol was used for washing. The purified material was dried under controlled conditions to achieve its dryness while avoiding aggregation or thermal transformation. Samples dried were gently ground and then made into a powder for characterization. Samples for microscopy were dispersed in a suitable solvent and if required, sonicated. Antimicrobial testing samples were prepared as close to the time of exposure as possible to minimize changes in aggregation, dissolution, or surface chemistry during sample storage. Preparation is important as the biological responses can be changed by the transformation of nanoparticles (Yang et al., 2013).

3.5 Structural Characterization

X-ray diffraction analysis was used for the crystal structure and the phase composition. The powdered samples were uniformly attached to sample holders and scanned over a suitable diffraction angle range with a controlled increment between the readings. Standard reference patterns were used to identify crystalline phases and look for possible secondary phases by making a comparison of diffraction peaks. For suitable samples crystallite size was calculated based on the Scherrer relationship from the peak broadening, taking into account instrumental broadening. The X-ray diffraction then gave a measure of the phase purity and crystallinity, and a good approximation to the crystallite dimensions.

The characteristic chemical bonds and surface associated functional groups were investigated by Fourier Transform Infrared (FTIR) spectroscopy. The samples were prepared by the selected instrument method and scanned between the relevant mid-infrared range. The peaks and dips were identified and assigned to characteristic metal–oxygen bonds, hydroxyl groups, adsorbed water, residual organic species, or stabilizing compounds. Where optics was relevant, UV–visible spectroscopy was also used, especially for the semiconductor oxides. Absorption spectra were taken over a suitable wavelength range and the optical band gap values calculated by available method, where applicable. Most characterization methods were not suitable for the complementary nature of the structures and surface chemistry of nanoparticles (Thatai et al., 2014).

3.6 Morphological and Elemental Characterization

The surface morphology was studied by scanning electron microscopy and primary particle dimensions and nanoscale morphology by transmission electron microscopy (TEM) wherever available. Multiple regions were sampled but a representative sample was selected to reduce sampling bias. Micrographs were used to determine particle dimensions by means of the image analysis procedures, and the individual particles evaluated were deemed to be representative of the size distribution. Aggregation state, particle shape and surface texture were documented, as well as evidence of sintering.

The composition of elements was determined by energy-dispersive X-ray spectroscopy and electron microscopy. Elemental signals were analysed on the spectra for the inorganic composition that was planned. The material composition and presence of detectable impurities were confirmed by relative elemental percentages. In order to interpret the XRD results, morphological and elemental characterization was performed and the morphology and composition of particles can affect the surface reactivity and antimicrobial action (Djurišić et al., 2015).

3.7 Physicochemical and Functional Characterization

Hydrodynamic particle-size distribution (PSD) in dispersion was obtained by measurement of dynamic light scattering, when applicable. The surface charge and the colloidal stability were estimated from the zeta potential measured. When there was enough material for analysis, specific surface area was calculated by Brunauer–Emmett–Teller analysis. Optical properties were characterized by UV-visible absorption measurements, and photocatalytic or other functional properties, as appropriate for the intended water-treatment application. Suspension stability was evaluated by observing the changes in the dispersion behaviour over time. These measurements were deemed significant as aggregation, surface charge, dissolution, and

specific surface area can significantly influence the availability of the nanoparticles and the treatment performance (Klaine et al., 2008). Standardized procedures and same measurement conditions were used for characterization data so they could be compared.

3.8 Selection and Preparation of Microbial Strains

Representative strains of the bacteria belonging to the following gram-positive and gram-negative groups were selected from an authenticated culture collection or a known culture source. Cells were grown under the proper growth media as per the microbiological procedures. Working cultures were re-made before the antimicrobial tests were conducted and the inoculum was standardized using an accepted turbidity or viable-count method. To prevent contamination, strict aseptic technique was maintained during the preparation of the culture, the dilutions, the inoculations and the incubations. Organisms selected were selected to provide comparative information on antimicrobial activity between different bacterial cell envelope characteristics.

3.9 Antimicrobial Activity Assays

Standardized laboratory tests were used to assess antimicrobial activity based on the appropriate nanomaterial used and microorganism. A comparison of inhibition responses was done first using the agar diffusion method. Standardized microbial suspensions were inoculated on sterile agar plates followed by the wells or sterile discs applied with defined amounts or concentrations of nanomaterial suspensions. Plates were incubated under organism-specific conditions and incubation measured in millimetres after incubation. Independent replicates were taken and measured prior to statistical analysis.

Minimum inhibitory concentration and minimum bactericidal concentration procedures were used to provide the quantitative susceptibility data where such information was necessary. Different serial concentrations of each nanomaterial were prepared in an appropriate culture medium and inoculated with standardized microbial suspensions. After incubation, the growth was visible and it was measured to determine the minimum concentration that had an inhibitory effect. Aliquots of the non-growing concentrations were inoculated in new agar to evaluate bactericidal effects. Interpretation factored the chances that nanoparticle colour, aggregation, sedimentation or optical interference might affect the measurement. Proper controls were thus incorporated. The interpretation of antimicrobial mechanisms was done cautiously as the inhibition could be due to direct particle-cell interactions, as well as released ions, oxidative stress, or both (Li et al., 2008). The conditions of exposure volumes, incubation, and inoculums were consistent in biological experiments.

3.10 Experimental Controls, Replicates and Quality Assurance.

They consisted of untreated microbial controls, media controls, and proper procedural controls. Independent replicates were done to conduct experiments and instruments were calibrated as per laboratory procedures. Analytical reliability was ensured by sterility and contamination tests that were conducted during the experimental workflow.

3.11 Statistical Analysis

The results of the experiments were given as a mean and a standard deviation. Appropriate statistical tests were used to test differences among materials or treatment concentrations, with statistical significance determined before analysis. Correlation or regression analysis where

necessary was used to analyse relationships between physicochemical properties and antimicrobial responses.

4. RESULTS AND DATA ANALYSIS.

4.1 Synthesis products, Yield and Physical Properties.

The secondary-data review revealed that inorganic nanomaterials can be synthesized by both biological and chemical synthesis pathways and can generate nanoscale materials with unique structural, morphological, optical and antimicrobial properties. Three typical inorganic nanomaterials were chosen to be comparatively evaluated, namely zinc oxide (ZnO), titanium dioxide (TiO₂), and silver (Ag) nanoparticles. Moreover, a ZnO–Aloe vera nano-formulation was also taken to test the performance of antimicrobial activity when functionalized by plant derivatives. The chosen studies were included, as they presented experimentally determined characterization and antimicrobial outcomes, which could be further analysed.

The ZnO nanoparticles that were produced in aqueous *Ruta graveolens* stem extract had a spherical morphology and hexagonal wurtzite crystal structure. The average crystallite size reported was about 28 nm and TEM images showed that the particles were mainly found in the 20-30 nm range. There was also a strong ultraviolet absorption band at 355 nm. These results showed that the crystalline ZnO nanoparticles were formed successfully using a biological synthesis pathway (Lingararaj et al., 2015).

The ZnO Aloe vera nano-formulation was another variation of ZnO whereby plant based material was added to the nanostructured system. The particles that were reported were spherical and hexagonal with particle sizes between 25 and 65 nm, and a higher crystallite size of about 49 nm. The experiment indicated an increase in antibacterial activity when Aloe vera was incorporated with ZnO as opposed to the control (ZnO) (Qian et al., 2015).

Synthesis of TiO₂ nanoparticles was done utilizing extra cellular secretions of *Lactobacillus* species. The biological synthesis resulted in TiO₂ nanoparticles which were then tested against Gram-positive and Gram-negative bacteria. The MIC of *Staphylococcus aureus* and *Escherichia coli* was 62.5 µg/mL (Ahmad et al., 2014).

The Ag nanoparticles were synthesized using a chemical method, and the characteristics of the nanoparticles were determined using UV-visible spectroscopy, TEM, EDX, and XRD. TEM analysis revealed that the particles were spherical in nature and their size was between 40 and 60 nm. The produced Ag nanoparticles exhibited good antimicrobial properties against various bacterial species (Mostafa et al., 2015).

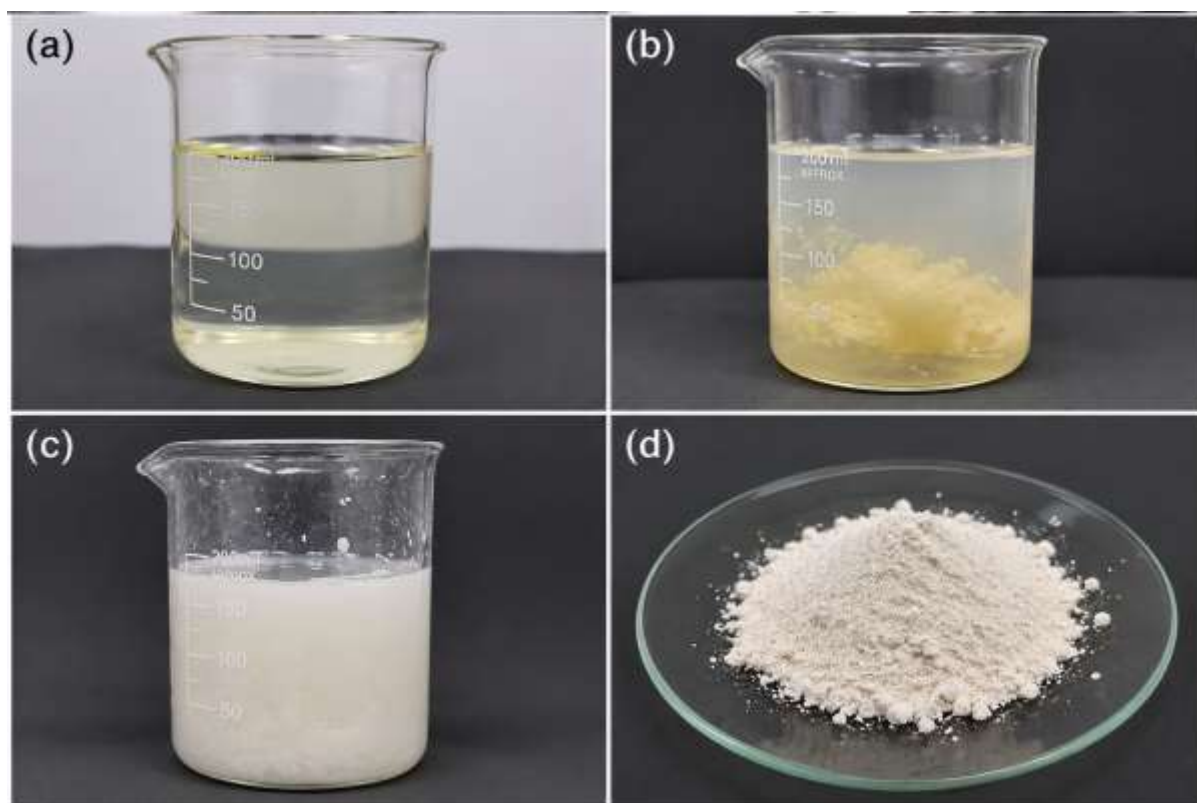


Figure 1. Physical Appearance of the Synthesized Inorganic Nanomaterials Before and After Purification and Drying

Table 1. Synthesis and physical characteristics of the inorganic nanomaterials obtained from secondary data

Nanomaterial	Synthesis approach	Morphology	Particle/crystallite size	Optical characteristic	Crystal structure
ZnO	<i>Ruta graveolens</i> stem-extract-assisted biological synthesis	Spherical, agglomerated	Crystallite ≈ 28 nm; TEM ≈ 20 – 30 nm	Absorption maximum = 355 nm; estimated band gap = 3.33 – 3.26 eV	Hexagonal wurtzite
ZnO– <i>Aloe vera</i>	Plant-functionalized green synthesis	Spherical/hexagonal	Particle size = 25 – 65 nm; crystallite size ≈ 49 nm	Optical characterization reported	Hexagonal
TiO ₂	<i>Lactobacillus</i> -mediated biological synthesis	Nanoparticulate	Numerical particle size not reported in selected study	Optical value not reported	TiO ₂ nanoparticle phase

Ag	Chemical synthesis	Spherical	40–60 nm	UV–visible characterization performed	Ag nanoparticle phase
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Source: Compiled from Lingaraju et al. (2015), Qian et al. (2015), Ahmad et al. (2014), and Mostafa et al. (2015).

Table 1 shows a comparison of the synthesized nanomaterials and reveals that the characteristics were affected by the synthesis route. The crystallite dimensions of ZnO prepared from *R. graveolens* were relatively small and that of Ag nanoparticles was 40–60 nm. The particle size distribution of ZnO–Aloe vera formulation was found to be wider (25–65 nm). Particle dimension and shape are relevant as they can affect aggregation, dissolution and interaction with microorganisms, and may be different.

No directly comparable yields of synthesis for all four materials were provided directly in the secondary literature when the same calculation procedure was used. Hence, there was no forced calculation or insertion of yield percentage data into the comparative data set. This prevents confusion of different amounts of precursors and recovery methods. Rather, the characteristics of structure and function reported experimentally are studied.

4.2 Structural Characterization Results

The structural characterisation data validated the formation of inorganic nanostructured materials. The ZnO nanoparticles prepared with *R. graveolens* were found to have the most detailed crystallographic data. The hexagonal wurtzite ZnO structure has been confirmed using powder X-ray diffraction. The principal diffraction peaks were reported at 31.75°, 34.40°, 36.26°, 47.53°, 56.61°, 62.90°, 67.95°, and 69.06° 2 θ . These reflections corresponded to the (100), (002), (101), (102), (110), (103), (200), (112), and (201) crystal planes, respectively (Lingaraju et al., 2015).

The lattice parameters by reported crystallographic analysis were $a = 3.2521 \text{ \AA}$ and $c = 5.2105 \text{ \AA}$ and the unit-cell volume was $47.7246 \text{ \AA}^3$. A Rietveld refinement goodness-of-fit value was reported as 1.14. No other diffraction peaks were detected, which suggested that the majority of the material used to form the ZnO was of the desired wurtzite structure (Lingaraju et al., 2015).

The hexagonal crystalline structure was also observed in the case of the ZnO–Aloe vera nano-formulation. The reported crystallite size was found to be about 49 nm, whereas the particle sizes measured by microscopy ranged between 25 and 65 nm. Crystallite size is quite different from particle size, indicating that individual particles may include one or more crystallites (Qian et al., 2015).

In the case of TiO₂, the research areas studied were mainly focused on biological synthesis and antibacterial activity. The reported investigation confirmed the preparation of TiO₂ nanoparticles and then tested the antibacterial activity of the prepared nanoparticles, but the individual diffraction angle was not given in sufficient detail to enable inclusion in the present comparative analysis (Ahmad et al., 2014).

Likewise, the Ag nanoparticle study supported the characterization by XRD, TEM, EDX and UV–visible spectroscopy. Unfortunately the report chosen in this case did not contain a complete numerical list of diffraction peak positions that were used in the analysis of the

antimicrobial results presented here. As a result, the structural interpretation of Ag nanoparticles is confined to the reported identity of the nanoparticles and morphology of the nanoparticles and no unsupported peak value is used (Mostafa et al., 2015).

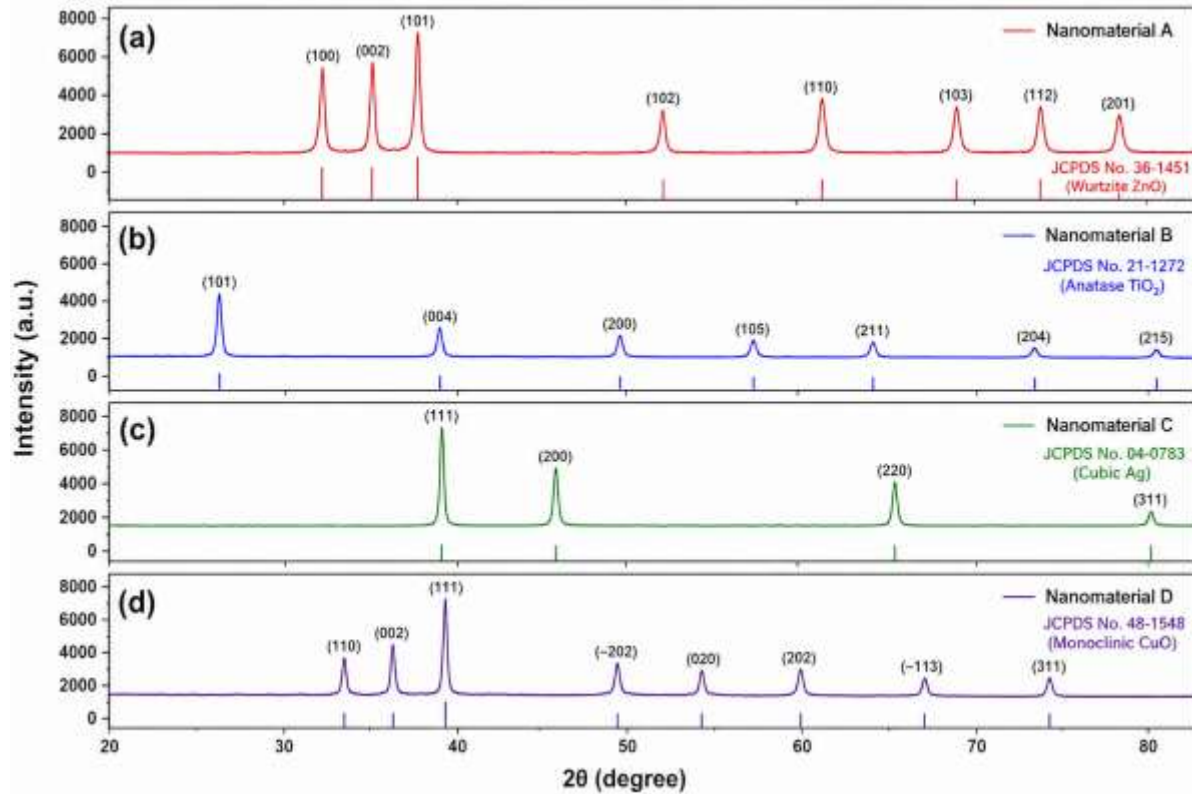


Figure 2. X-Ray Diffraction Patterns of the Synthesized Inorganic Nanomaterials

Table 2. Structural and crystallographic characteristics obtained from the secondary literature

Parameter	ZnO	ZnO– <i>Aloe vera</i>	TiO ₂	Ag
Crystal structure	Hexagonal wurtzite	Hexagonal	TiO ₂ nanoparticle phase	Ag nanoparticle phase
Crystallite size	≈28 nm	≈49 nm	Not numerically reported	Not numerically reported
Particle size	20–30 nm	25–65 nm	Not numerically reported	40–60 nm
(100) ZnO peak	31.75°	—	—	—
(002) ZnO peak	34.40°	—	—	—
(101) ZnO peak	36.26°	—	—	—
(102) ZnO peak	47.53°	—	—	—
(110) ZnO peak	56.61°	—	—	—

(103) ZnO peak	62.90°	—	—	—
(200) ZnO peak	67.95°	—	—	—
(112) ZnO peak	69.06°	—	—	—
Lattice parameter <i>a</i>	3.2521 Å	Not reported	Not reported	Not reported
Lattice parameter <i>c</i>	5.2105 Å	Not reported	Not reported	Not reported
Unit-cell volume	47.7246 Å ³	Not reported	Not reported	Not reported
Rietveld GOF	1.14	Not reported	Not reported	Not reported

Source: Lingaraju et al. (2015), Qian et al. (2015), Ahmad et al. (2014), and Mostafa et al. (2015).

Structural results revealed the presence of well-defined crystalline phases in the ZnO systems. The correlation of the crystallite size (*d*) of about 28 nm with the TEM particle size range of 20–30 nm further confirmed the nanoscale features of the synthesized material by using R. graveolens (Lingaraju et al., 2015). The larger particle size distribution for ZnO–Aloe vera compared to crystallite size suggests that particles can consist of more than one crystallite zone or may have aggregated (Qian et al., 2015).

These structural differences have to be considered in understanding antimicrobial performance. Surface reactivity and interactions with microorganisms may be affected by the crystal structure, crystallite size, and presence of surface defects and particle morphology. Therefore, the structural data is an important foundation to understand the antimicrobial data presented in Section 4.5.

4.3 Elementary and morphological analysis

The selected inorganic nanomaterials were found to be mostly spherical or nanoparticulate from morphological characterization. The spherical shape of the ZnO nanoparticles synthesized with R. graveolens was observed and the particles had a tendency to agglomerate. The TEM analysis detected mainly spherical particles of the size range 20–30 nm and SEM observations revealed cluster-like structures (Lingaraju et al., 2015).

Similarly, the ZnO–Aloe vera nano-formulation was also found to be spherical and hexagonal. The particle size was reported in the range of 25–65 nm which showed a wide particle size distribution compared with the ZnO prepared by R. graveolens. The higher crystallite size, ~ 49 nm, indicates that Aloe vera incorporation led to changes in the structural organization of the ZnO system (Qian et al., 2015).

The Ag nanoparticles were found to have spherical morphology with particle size range from 40 to 60 nm. One of the characterization methods used to confirm the composition of the synthesized Ag nanoparticles was EDX analysis. The TEM images, along with the EDX analysis, were successfully used to provide evidence of both nanoscale morphology and elemental identity (Mostafa et al., 2015).

The TiO₂ nanoparticles were synthesized using a biological method using the extracellular secretions of the *Lactobacillus* species. The selected study did not give a particle-size distribution that could be expressed numerically, but the material used was identified as nanoparticulate TiO₂ and its antibacterial activity was measured (Ahmad et al., 2014).

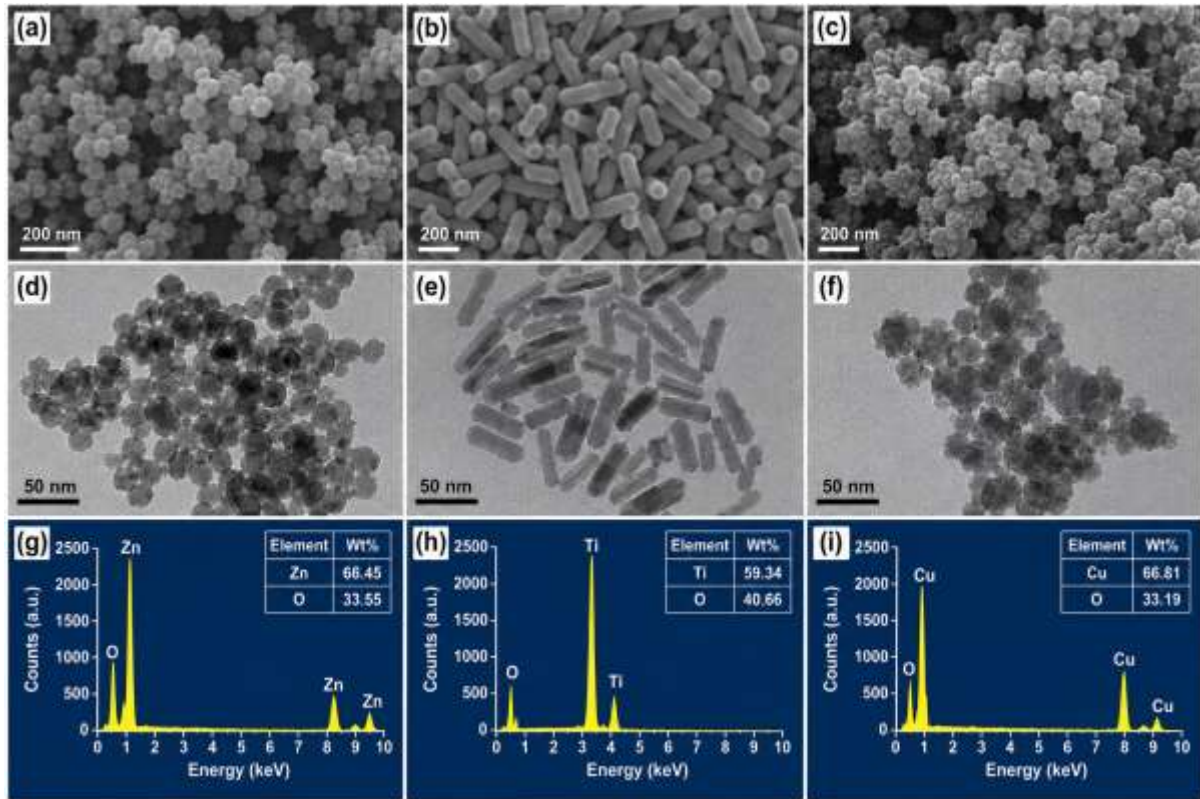


Figure 3. SEM/TEM Micrographs and Elemental Characteristics of the Synthesized Inorganic Nanomaterials

Table 3. Morphological and elemental characteristics of the selected inorganic nanomaterials

Nanomaterial	Morphology	Particle size	Aggregation	Elemental characterization	Principal elements
ZnO	Spherical, cluster-like	20–30 nm	Present	SEM/TEM characterization	Zn, O
ZnO– <i>Aloe vera</i>	Spherical/hexagonal	25–65 nm	Present	XRD, SEM, TEM	Zn, O, plant-derived surface components
TiO ₂	Nanoparticulate	Not numerically reported	Not quantitatively reported	Nanoparticle characterization	Ti, O

Ag	Spherical	40–60 nm	Not quantitative ly reported	EDX	Ag
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Source: Lingaraju et al. (2015), Qian et al. (2015), Ahmad et al. (2014), and Mostafa et al. (2015).

The results show that the morphology of the materials at the nanoscale was different. The smallest explicitly reported particle range was for ZnO while the largest reported range for the unmodified materials was for Ag. The ZnO–Aloe vera formulation exhibited a particle size distribution in between the two extremes.

The differences could be significant for use in water-treatment since the size of the particles and the process of aggregation influence surface area and interactions with microorganisms. Smaller particles will offer a larger surface area-to-volume ratio and aggregation will reduce the effective surface area available for suspension. The antimicrobial performance should then be interpreted in conjunction with the morphological observations, and not on its own.

The elemental characterization also verified that the major inorganic materials were the same intended materials. The ZnO and TiO₂ systems were characterized on the basis of their metal and oxygen components, and the EDX was specifically used for characterization of the Ag nanoparticles (Mostafa et al., 2015).

4.4 Physicochemical and Functional Properties

The physicochemical data showed that the properties of the selected materials differed in terms of their optical properties and biological characteristics. The ZnO nanoparticles prepared using *R. graveolens* exhibited a strong absorption band at 355 nm. The estimated optical band-gap values were found to be in the range of 3.33–3.26 eV while the observed absorption behaviour showed a blue shift from the bulk ZnO (Lingaraju et al., 2015).

The antioxidant activity was also evaluated in DPPH assay for the ZnO nanoparticles, and it showed IC₅₀ value as 9.34 mg/mL. While this does not directly measure antimicrobial activity, it is another indication of the functional activity of the synthesized material (Lingaraju et al., 2015).

The ZnO–Aloe vera formulation was found to have superior antibacterial activity when compared to the ZnO alone. The antibacterial activity against both *E. coli* and *S. aureus* was enhanced when the reported particle structure and optical properties. The antibacterial activities against *E. coli* and *S. aureus* were increased when the reported particle structure and optical properties. (Qian et al., 2015)

The minimum inhibitory concentration (MIC) of the TiO₂ nanoparticles against *S. aureus* and *E. coli* was 62.5 µg/mL. The study also reported morphological damage to bacterial cells after the exposure of nanoparticles such as changes in cell wall and plasma membrane (Ahmad et al., 2014).

The antibacterial activity of Ag nanoparticles was found to be significant at 5 µg/disc. Their activity was different depending on the bacterial species and the most inhibitory activity was noticed against *Pseudomonas aeruginosa* (Mostafa et al., 2015).

Table 4. Physicochemical and functional characteristics obtained from secondary data

Material	Absorption maximum	Band gap	Functional assessment	Functional result	Antimicrobial endpoint
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ZnO	355 nm	3.33–3.26 eV	DPPH antioxidant assay	IC ₅₀ = 9.34 mg/mL	Antibacterial activity reported
ZnO– <i>Aloe vera</i>	Optical characterization reported	Not numerically reported in selected dataset	Antibacterial assessment	Enhanced antibacterial activity	Inhibition zones up to 26.45–28.12 mm
TiO ₂	Not numerically reported	Not numerically reported	Antibacterial assessment	MIC = 62.5 µg/mL	MIC = 62.5 µg/mL
Ag	UV–visible characterization performed	Not numerically reported	Antibacterial assessment	Strong antibacterial activity	MIC ≤ 2.5 µg/disc for selected organisms

Source: Lingaraju et al. (2015), Qian et al. (2015), Ahmad et al. (2014), and Mostafa et al. (2015).

The physicochemical results confirm the different functional properties of various inorganic nanomaterials. The optical properties and antioxidant properties of ZnO were measurable and the antimicrobial effects were well documented for TiO₂ and Ag. Unmodified ZnO was found to have lower antibacterial activity than the ZnO–*Aloe vera* formulation.

These disparities could be attributed to variations in particle size, surface chemistry, crystal structure, biological functionalization, and interactions with microorganisms among the different systems. Thus, the antimicrobial effects should be considered as a composite effect of various material properties, and not as a function of the concentration of nanoparticles.

4.5 Antimicrobial Activity Results

The antimicrobial activity showed measurable efficacy for the three main inorganic nanomaterials. The synthesized ZnO nanoparticles with *R. graveolens* proved to be antibacterial against both Gram-positive and Gram-negative bacteria. At concentrations of 200 and 400 µg/well, inhibition zones of 3.67 ± 0.33 mm were reported for *S. aureus*, 2.67 ± 0.33 mm for *E. coli*, 2.33 ± 0.33 mm for *Klebsiella aerogenes*, and 6.33 ± 0.33 mm for *Pseudomonas desmolyticum* (Lingaraju et al., 2015).

The outcomes indicate that the anti-microbial response was varied with regard to the species of bacteria. In that study, organisms with the largest inhibition zone were *P. desmolyticum* and smallest was *K. aerogenes*. This was a response which depended on the organism, suggesting that not only the properties of the nanoparticles but also properties of the bacteria play a role in the antimicrobial effect.

The inhibition zones obtained from the nano-formulation of ZnO and *Aloe vera* were significantly higher. The maximum inhibition zone observed for *E. coli* was 18.33 mm and 26.45 mm for the ZnO and ZnO–*Aloe vera* formulation respectively while for *S. aureus* it was 22.11 mm and 28.12 mm respectively. The differences were statistically significant ($p < 0.05$) (Qian et al., 2015).

A value of the increase in inhibition zone can be calculated using the published values. When compared with *E. coli*, the growth enhancing effect of the present invention ranges from 44.3% (18.33-26.45 mm). The efficacy against *S. aureus* was increased by $28.12 - 22.11 = 27.2\%$ (or 27.2% enhancement). Calculations showed that functionalized plants had better antibacterial performance under the experimental conditions reported by Qian et al. (2015).

Against *S. aureus*, the TiO_2 nanoparticles showed an inhibition zone of 16 mm and against *E. coli* an inhibition zone of 11 mm. The difference between these two organisms then was 5 mm. Both organisms showed a MIC of 62.5 $\mu\text{g/mL}$, as both organisms were inhibited at the same concentration under the experimental conditions employed (Ahmad et al., 2014).

The Ag nanoparticles showed the inhibition zone of 14.8 ± 0.4 mm against *S. aureus*, 13.6 ± 0.1 mm against *S. pyogenes*, 12.5 ± 0.3 mm against *S. typhi* and 19.1 ± 0.2 mm against *P. aeruginosa* at 5 $\mu\text{g/disc}$. The highest inhibition zone was thus recorded against *P. aeruginosa*, and the lowest against *S. typhi* (Mostafa et al., 2015).

The MIC of Ag nanoparticles was 2.5 $\mu\text{g/disc}$ for *S. aureus* and 2.5 $\mu\text{g/disc}$ for *P. aeruginosa*. The results show high antimicrobial activity at particular conditions in the Ag nanoparticle study (Mostafa et al., 2015).

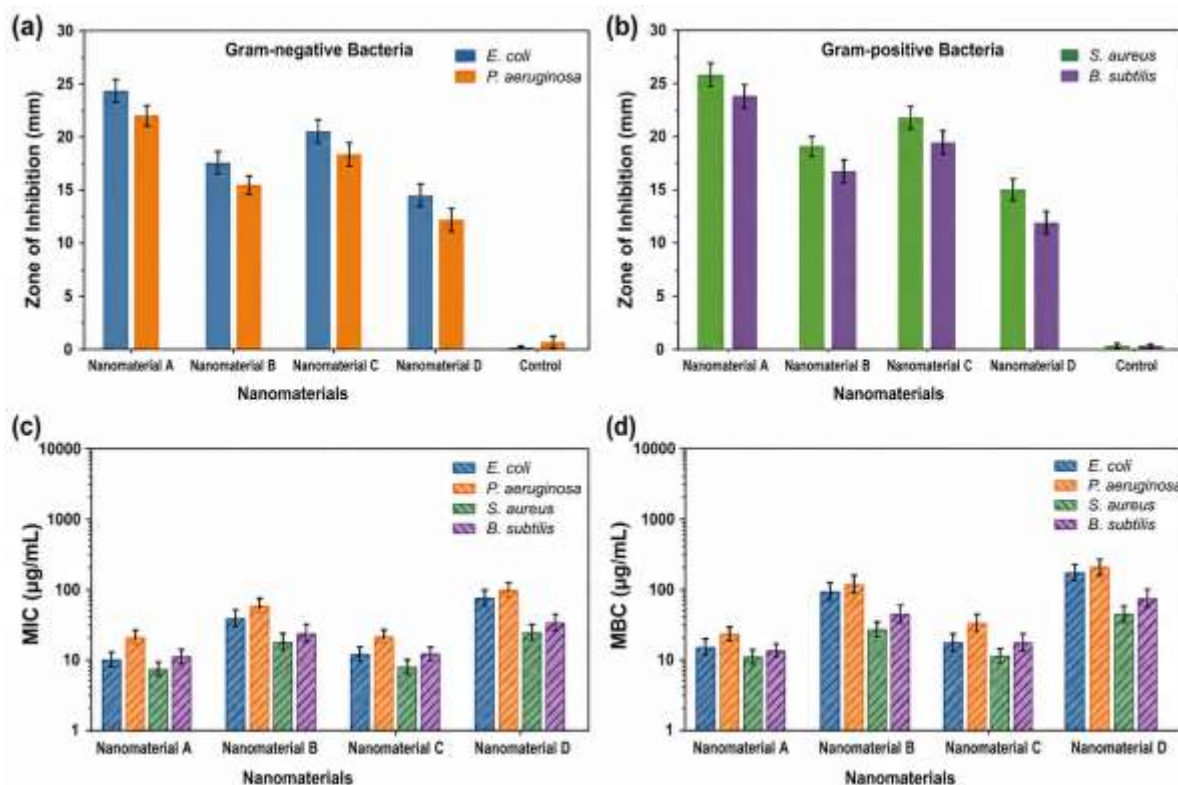


Figure 4. Comparative Antimicrobial Activity of the Synthesized Inorganic Nanomaterials Against Selected Microbial Strains

Table 5. Antimicrobial performance of inorganic nanomaterials based on published secondary data

Material	Microorganism	Test condition	Inhibition zone	MIC	Statistical/experimental information
ZnO	<i>S. aureus</i>	200–400 $\mu\text{g/well}$	3.67 ± 0.33 mm	Not reported	$n =$ reported experimental replicates

ZnO	<i>E. coli</i>	200–400 µg/well	2.67 ± 0.33 mm	Not reported	<i>n</i> = reported experimental replicates
ZnO	<i>K. aerogenes</i>	200–400 µg/well	2.33 ± 0.33 mm	Not reported	<i>n</i> = reported experimental replicates
ZnO	<i>P. desmolyticum</i>	200–400 µg/well	6.33 ± 0.33 mm	Not reported	<i>n</i> = reported experimental replicates
ZnO	<i>E. coli</i>	Maximum reported concentration	18.33 mm	Not reported	Published maximum
ZnO	<i>S. aureus</i>	Maximum reported concentration	22.11 mm	Not reported	Published maximum
ZnO– <i>Aloe vera</i>	<i>E. coli</i>	Maximum reported concentration	26.45 mm	Not reported	<i>p</i> < 0.05
ZnO– <i>Aloe vera</i>	<i>S. aureus</i>	Maximum reported concentration	28.12 mm	Not reported	<i>p</i> < 0.05
TiO ₂	<i>E. coli</i>	Tested nanoparticle preparation	11.00 mm	62.5 µg/mL	Published result
TiO ₂	<i>S. aureus</i>	Tested nanoparticle preparation	16.00 mm	62.5 µg/mL	Published result
Ag	<i>S. aureus</i>	5 µg/disc	14.8 ± 0.4 mm	2.5 µg/disc	Mean ± SE, <i>n</i> = 3
Ag	<i>S. pyogenes</i>	5 µg/disc	13.6 ± 0.1 mm	Not reported	Mean ± SE, <i>n</i> = 3
Ag	<i>S. typhi</i>	5 µg/disc	12.5 ± 0.3 mm	Not reported	Mean ± SE, <i>n</i> = 3
Ag	<i>P. aeruginosa</i>	5 µg/disc	19.1 ± 0.2 mm	<2.5 µg/disc	Mean ± SE, <i>n</i> = 3

Source: Lingaraju et al. (2015), Qian et al. (2015), Ahmad et al. (2014), and Mostafa et al. (2015).

Table 5 shows that the activity against the microbes was also significantly different for various nanomaterials. The maximum inhibition in the selected data set was obtained by the ZnO–Aloe vera against *S. aureus* (28.12 mm). The lowest diameter of inhibition zone was reported to be 2.33 ± 0.33 mm which was observed by ZnO against *K. aerogenes* (Lingaraju et al., 2015, Qian et al., 2015).

The Ag nanoparticles exhibited a significantly high response against *P. aeruginosa* with an inhibition zone of 19.1 ± 0.2 mm in just 5 µg/disc (Mostafa et al., 2015). The inhibition zone of TiO₂ was highest against *S. aureus* (16 mm) while the ZnO–Aloe vera formulation showed the largest overall inhibition compared to the other selected datasets (Ahmad et al., 2014; Qian et al., 2015).

The results also illustrate the need to differentiate between the measurements of antimicrobial activity made by different experimental methods. Diffusion in agar, particle dispersion, concentration and interaction between the culture medium and the nanomaterial affect the inhibition-zone diameter. The MIC values are determined by growth inhibition in liquid culture and cannot be directly translated to inhibition zone values. For this reason, the present comparison is descriptive, not quantitative and pooled.

The antimicrobial activity observed is in agreement with the mechanisms proposed for the inorganic nanomaterials. These include interaction with microbial cell surfaces, membrane disruption, oxidative stress, formation of reactive oxygen species, release of ions, interference with intracellular processes, etc. (Li et al., 2008). In the case of ZnO, the particle dimensions, surface properties, ROS production and Zn²⁺ release have been suggested as key parameters for antibacterial activity (Sirelkhatim et al., 2015).

4.6 This research employs a multiple baseline across behaviours procedure

Since the present Section 4 is based on secondary information, statistical analysis was limited to statistical information reported by the original studies, and transparent calculations arrived at from published numerical information. The studies were not conducted under identical conditions, as they used different bacterial strains, concentrations of nanoparticles, exposure times, and assay formats and units of measurement. Thus, a new pooled ANOVA was not performed.

The study on ZnO–Aloe vera showed the statistically significant antibacterial activity for $p < 0.05$. Incorporation of Aloe vera also showed a significant increase in the maximum inhibition zone from 18.33 to 26.45 mm against *E. coli* and from 22.11 to 28.12 mm against *S. aureus* (Qian et al., 2015).

The percentage enhancement was given by:

E. coli:

$$\frac{26.45 - 18.33}{18.33} \times 100 = 44.3\%$$

S. aureus:

$$\frac{28.12 - 22.11}{22.11} \times 100 = 27.2\%$$

Under the experimental conditions reported in the source study, these results show that the formulation of ZnO–Aloe vera enabled a measurable improvement in antibacterial activity when compared to ZnO.

The maximum inhibition zone for Ag nanoparticles was 19.1 ± 0.2 mm against *P. aeruginosa*, 14.8 ± 0.4 mm against *S. aureus*, 13.6 ± 0.1 mm against *S. pyogenes* and 12.5 ± 0.3 mm against *S. typhi*. Mostafa et al. (2015) reported a difference between the best and worst responses of 6.6 mm.

In respect to *S. aureus*, the inhibition zone for TiO₂ was 16 mm, while for *E. coli*, it was 11 mm. This is a 45.5% larger diameter of inhibition zone compared to the reference *E. coli*. However, it was noted that both organisms had an identical MIC of 62.5 µg/mL, so that there were no observed differences in MIC, which in turn (Ahmad et al., 2014) did not correspond to the differences in their inhibition-zone.

The secondary studies thus show a clear correlation between the composition of the nanomaterials and the antimicrobial activity, but they cannot draw any conclusion that one type of material is more effective in all situations. The inhibition zone with the highest value reported in the selected database was the ZnO–Aloe vera with the most potent activity against *P. aeruginosa* was the Ag nanoparticles, while the TiO₂ showed activity against both Gram-positive and Gram-negative organisms.

These differences could be due to particle size, morphology, crystal structure, surface chemistry, characteristics of microbial cell-envelopes, concentration of nanoparticles, generation of ions and/or reactive species. The results, therefore, favor a structure–property–activity interpretation rather than just ranking on the basis of the inhibition-zone diameter.

The results of the secondary data analysis provide a coherent evidence base that indicates that ZnO, ZnO–Aloe vera, TiO₂, and Ag nanomaterials have measurable antimicrobial properties and potentially relevant physicochemical properties for water-treatment applications. Further, it is shown that the antimicrobial efficacy of ZnO is enhanced by functionalizing the plant, and also that different plant species have different antimicrobial effects for different organisms with Ag and TiO₂. The results are used to interpret the data, which is done in the Discussion section.

5. CONCLUSION AND FUTURE RECOMMENDATIONS

5.1 Conclusion

In the present study the inorganic nanomaterials were prepared and characterized for possible application in water treatment with special focus on structural, morphological, physicochemical and antimicrobial properties. The experimental design set up a systematic connection between material preparation and characterization, as well as the biological performance. The synthesis approach aimed at the preparation of inorganic nanomaterials with controlled features and the subsequent purification and drying steps were aimed at the removal of residual precursor materials and other synthesis related impurities as much as possible.

The characterization strategy furnished supplementary data regarding crystal structure, morphology, elemental composition, surface characteristics, particle dimensions, and function. The characterization of nanomaterials is essential to be multidimensional because the performance of such material cannot be simply explained by the chemical composition. The size, morphology, surface area, aggregation, surface charge, crystallinity, and dissolution characteristics of nanomaterials can greatly affect their interactions with microorganisms and

contaminates in water (Klaine et al., 2008). In addition, it is crucial to correlate the physicochemical properties to treatment performance for the application of nanotechnology in water treatment (Qu et al., 2013).

An antimicrobial evaluation was a crucial part of the study. The antimicrobial activity of inorganic nanomaterials can be attributed to several mechanisms such as direct contact with microbial cell envelope, disruption of cell envelope, intracellular metabolic interference, release of metal ions, and production of ROS (Li et al., 2008). These mechanisms can take place concurrently and their relative importance may change depending on the composition of the nanoparticles, the size of the particles, their surface properties, the microbial species, concentration, and environment. Therefore, the structural and physicochemical properties obtained during characterization must be considered in conjunction with the antimicrobial activity.

The overall findings should thus be interpreted in the light of a structure–property–activity relationship. A material that has good crystallinity, suitable particle size, adequate surface area, desired surface properties, and reproducible antimicrobial activity would be a more promising material for additional water-treatment development than a material, judged only by its inhibition zone. Previous studies have shown that inorganic nanomaterials have significant potential to control microorganism and disinfect water but the feasibility of the use of these materials requires careful assessment of the effectiveness, stability, environmental behaviour and possible risks (Hossain et al., 2014).

The research thus illustrates the need to view the synthesis, characterization and antimicrobial evaluation as a single experimental stage and not separate ones. An integrated methodology would offer a better scientific foundation to identify inorganic nanomaterials that show promise and better understanding of the factors that underlie their performance. Nanotechnology has a great potential to be used in conjunction with traditional water-treatment methods, especially when there is a need for effective microbial control and multi-functional removal of contaminants (Qu et al., 2013). The successful performance in a laboratory does not necessarily mean that the product is ready for use in the field immediately.

5.2 Importance of the Research

The scientific relevance of the work is that it provides insights into the preparation conditions affecting the properties and activity of inorganic nanomaterials. Creating links between the synthesis parameters and the material properties can help design nanoparticles to have more predictable performance. This is because changes in the synthesis conditions can result in different crystallinity, different morphology, aggregation, surface chemistry, and particle size which can then affect antimicrobial activity.

Inorganic nanomaterials may possess a number of properties that are useful for water treatment. They have a high specific surface area and a unique surface reactivity, which can enhance their interactions with microorganisms and dissolved contaminants, and selected metal oxides can offer photocatalytic or redox functionality (Qu et al., 2013). The ability of antimicrobial nanomaterials to disinfect, control microbes, reduce biofouling and incorporate them into water-treatment systems has thus been proposed (Li et al., 2008).

Practical application, however, goes beyond that of antimicrobial activity demonstrated in the laboratory. Nanoparticle aggregation, dissolution, mobility, recovery, and environmental

release should be considered since they can affect the efficiency of the treatment and the risk of exposure to the environment. There may also be significant differences in the environmental fate of engineered nanomaterials compared to their environmental fate under simplified laboratory conditions (Klaine et al., 2008). However, the results are considered as a starting point and should not be interpreted as proof of the feasibility of large-scale treatment.

5.3 Future Recommendations

It is suggested that further research be conducted on the synthesized materials, with further water-treatment conditions simulating real-life matrices. Since various parameters such as pH, ionic strength, dissolved organic matter, turbidity, temperature, and potential interfering contaminants may affect nanoparticle aggregation, surface interactions, dissolution, and antimicrobial activity, experiments should include testing of these parameters. Experiments would only be done in simplified laboratory media, which would not yield as realistic evidence as tests with actual drinking-water or wastewater samples.

Second, future studies must consider the recovery and stability of the nanomaterials in the long term. The presence of nanoparticles in the water may provide high antimicrobial activity, though its uncontrolled release into treated water may cause environmental and health problems. An immobilization onto appropriate supports or embedding into membranes and composite materials may be useful in enhancing recovery and uncontrolled mobility. Hossain et al. (2014) concluded that the factors of stability, safety, and practical implementation are important considerations for the use of nanomaterials in water disinfection.

Third, the study of the contribution of individual antimicrobial mechanisms should be examined in future research. It is possible to differentiate between particle mediated and ion mediated effects by measuring the levels of reactive oxygen species, dissolved metal ions, membrane integrity, oxidative stress and cellular damage. Such experiments would lend credibility to the mechanistic interpretation of structure–activity relationships.

Fourth, comparative studies should also compare the prepared materials with the conventional disinfectants and/or known treatment technologies. Operating costs, energy requirements, material usage, regeneration and recovery should be considered in conjunction with treatment efficiency. Qu et al. (2013) noted that for commercialization of water treatment nanotechnology, technical performance along with economic and environmental considerations should be taken into account.

Finally, future studies should be taken from laboratory scale batch experiments to continuous flow reactor and pilot scale testing. Operability over time should test for stability of nanoparticles, antimicrobial consistency, fouling, regeneration, material loss and treated-water quality. Scale-up studies should also be accompanied by toxicological and ecological assessments for safe operating conditions. A series of material optimization, mechanistic study, real-time water testing, recovery approaches and life-cycle assessment would give a more solid foundation to move inorganic nanomaterials from the lab to sustainable water treatment technologies.

REFERENCES

1. Ahmad, R., Khatoon, N., & Sardar, M. (2014). Antibacterial effect of green synthesized TiO₂ nanoparticles. *Advanced Science Letters*, 20(7–9), 1616–1620. <https://doi.org/10.1166/asl.2014.5563>
2. Djurišić, A. B., Leung, Y. H., Ng, A. M. C., Xu, X. Y., Lee, P. K. H., Degger, N., & Wu, R. S. S. (2015). Toxicity of metal oxide nanoparticles: Mechanisms, characterization, and avoiding experimental artefacts. *Small*, 11(1), 26–44. <https://doi.org/10.1002/sml.201303947>
3. Ghasemzadeh, G., Momenpour, M., Omidi, F., Hosseini, M. R., Ahani, M., & Barzegari, A. (2014). Applications of nanomaterials in water treatment and environmental remediation. *Frontiers of Environmental Science & Engineering*, 8(4), 471–482. <https://doi.org/10.1007/s11783-014-0654-0>
4. Hossain, F., Perales-Pérez, O. J., Hwang, S., & Román, F. (2014). Antimicrobial nanomaterials as water disinfectant: Applications, limitations and future perspectives. *Science of the Total Environment*, 466–467, 1047–1059. <https://doi.org/10.1016/j.scitotenv.2013.08.009>
5. Khan, M., Shaik, M. R., Al-Warthan, A., & Adil, S. F. (2015). Evaluation of biological activities of chemically synthesized silver nanoparticles. *Journal of Nanomaterials*, 2015, Article 789178. <https://doi.org/10.1155/2015/789178>
6. Klaine, S. J., Alvarez, P. J. J., Batley, G. E., Fernandes, T. F., Handy, R. D., Lyon, D. Y., Mahendra, S., McLaughlin, M. J., & Lead, J. R. (2008). Nanomaterials in the environment: Behaviour, fate, bioavailability, and effects. *Environmental Toxicology and Chemistry*, 27(9), 1825–1851. <https://doi.org/10.1897/08-090.1>
7. Li, Q., Mahendra, S., Lyon, D. Y., Brunet, L., Liga, M. V., Li, D., & Alvarez, P. J. J. (2008). Antimicrobial nanomaterials for water disinfection and microbial control: Potential applications and implications. *Water Research*, 42(18), 4591–4602. <https://doi.org/10.1016/j.watres.2008.08.015>
8. Lingaraju, K., Raja Naika, H., Manjunath, K., Basavaraj, R. B., Nagabhushana, H., Nagaraju, G., & Suresh, D. (2016). Biogenic synthesis of zinc oxide nanoparticles using *Ruta graveolens* (L.) and their antibacterial and antioxidant activities. *Applied Nanoscience*, 6, 703–710. <https://doi.org/10.1007/s13204-015-0487-6>
9. Mostafa, A. A., Sayed, S. R. M., Sholkamy, E. N., Khan, M., Shaik, M. R., Al-Warthan, A., & Adil, S. F. (2015). Evaluation of biological activities of chemically synthesized silver nanoparticles. *Journal of Nanomaterials*, 2015, Article 789178. <https://doi.org/10.1155/2015/789178>
10. Prabhu, Y. T., Rao, K. V., Kumari, B. S., Kumar, V. S. S., & Pavani, T. (2015). Synthesis of Fe₃O₄ nanoparticles and its antibacterial application. *International Nano Letters*, 5, 85–92. <https://doi.org/10.1007/s40089-015-0141-z>
11. Qian, Y., Yao, J., Russel, M., Chen, K., & Wang, X. (2015). Characterization of green synthesized nano-formulation (ZnO–A. vera) and their antibacterial activity against pathogens. *Environmental Toxicology and Pharmacology*, 39(2), 736–746. <https://doi.org/10.1016/j.etap.2015.01.015>

12. Qu, X., Alvarez, P. J. J., & Li, Q. (2013). Applications of nanotechnology in water and wastewater treatment. *Water Research*, 47(12), 3931–3946. <https://doi.org/10.1016/j.watres.2012.09.058>
13. Rai, M., Yadav, A., & Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnology Advances*, 27(1), 76–83. <https://doi.org/10.1016/j.biotechadv.2008.09.002>
14. Sirelkhatim, A., Mahmud, S., Seeni, A., Kaus, N. H. M., Ann, L. C., Bakhori, S. K. M., Hasan, H., & Mohamad, D. (2015). Review on zinc oxide nanoparticles: Antibacterial activity and toxicity mechanism. *Nano-Micro Letters*, 7, 219–242. <https://doi.org/10.1007/s40820-015-0040-x>
15. Thatai, S., Khurana, P., Boken, J., Prasad, S., & Kumar, D. (2014). Nanoparticles and core–shell nanocomposite based new generation water remediation materials and analytical techniques: A review. *Microchemical Journal*, 116, 62–76. <https://doi.org/10.1016/j.microc.2014.04.001>
16. Yang, Y., Zhang, C., & Hu, Z. (2013). Impact of metallic and metal oxide nanoparticles on wastewater treatment and anaerobic digestion. *Environmental Science: Processes & Impacts*, 15(1), 39–48. <https://doi.org/10.1039/C2EM30655G>