ASSESSMENTOF THE ANTIBACTERIAL ACTIVITY OF ZINC OXIDE NANOPARTICLES AGAINST SOME SELECTED BACTERIAL ISOLATES

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BENIN CITY

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF B.Sc. (HONS) IN MICROBIOLOGY, UNIVERSITY OF BENIN, BENIN CITY.

AUGUST, 2023.

CERTIFICATION

This is to certify that this project work was carried out by **Emmanuel Ogheneochuko MOWARIN** in the department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under my supervision.

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MR. G. O. ORIBHABOR **DATE**

(Project Supervisor)

APPROVAL

This project work was carried out by **Emmanuel Ogheneochuko MOWARIN** in partial fulfillment of the award of a Bachelor of Science, B.Sc (Hons) degree in the Department of Microbiology, University of Benin, Benin City.

_____ _____

PROF. (MRS.) F.I. AKINNIBOSUN **DATE**

(Head of Department**)**

DEDICATION

This project work is dedicated to God Almighty for his love, faithfulness and mercy and also to my Mother for her love, understanding, and financial support.

ACKNOWLEDGEMENT

I want to first appreciate God for His mercies and grace that has brought me so far; for helping me persevere and achieve my goals in the university. To him be all glory.

To my supervisor, MR. G. O. ORIBHABOR, for being very patient, advice and motivation, I say thank you. May the Almighty always continue to provide the best for you and your family and may you achieve your hearts desires.

To my project coordinator, PROF. H. O. SHITTU, for his guidance, support and kindness, throughout the duration of this project, I also say thank you. May the Almighty continue to provide and may he reward you for your work. To all my lectures in the Department of Microbiology I say thank you.

My appreciation also goes to my Mother, Mrs. MOWARIN FATIMA for always believing in me and for her unending love and support. I pray God gives her long life to enjoy the fruits of her labour.

Also special appreciation to my sister Maria for her support and to my able coursemates and friends. May God bless you all abundantly.

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ABSTRACT

Zinc oxide nanoparticles (ZnONPs) have been investigated for their great antimicrobial effect against multiple pathogens. Bacterial pathogens used for this research were isolated from African black soap samples and characterized using the phenotypic method. The antibacterial activity of biologically synthesized zinc oxide nanoparticles (ZnONPs) against the isolates; *Pseudomonas amygdali* 35-1 CP084212, *Micrococcus* sp. and *Staphylococcus aureus* was investigated *in-vitro*. Nanoparticle treatments were applied at different concentrations (25%, 50%, and 100%) and an antibacterial agent Ampicillin was used as a control. The zones of inhibition were measured in millimeters. ZnONPs at all concentrations had insignificant inhibitory effect on *Micrococcus* sp. and *Staphylococcus aureus* ($p>0.05$) compared to the control. However, ZnONPs (100%) demonstrated significantly higher inhibitory activity on *Pseudomonas amygdali* 35-1 CP084212 ($p<0.05$) compared to the control. The zones of inhibition ranged from 3.33 ± 0.54 to 18.00 ± 0.94 mm while the zones of inhibition for the control were 6.33 ± 1.09 mm (*Pseudomonas amygdali* 35-1 CP084212), 18.67 ± 1.09 mm (*Micrococcus* sp.) and 16.00 ± 4.10 (*Staphylococcus aureus*). The findings obtained from this investigation demonstrated the antimicrobial efficacy of zinc oxide nanoparticles against the selected bacterial isolates.

CHAPTER ONE

1.0 INTRODUCTION

Nanotechnology is a hotspot for study in contemporary materials science. This technology has the potential to provide a wide range of unique applications, including revolutionary fabric chemicals, food processing and agricultural production, and advanced pharmaceutical approaches (Sahoo, 2010). Nanoparticles (NPs) are atomically regulated or modified particles (1-100 nm). They exhibit sized-related characteristics that differ greatly from bulk materials (Buzea *et al*., 2007). Nanoparticles feature bigger structures than their counterparts due to their tiny size. This feature enables them potential applications in a wide range of domains, including biosensors, nanomedicine, and bionanotechnology (Ashe, 2011).

The inherent characteristics of metallic nanoparticles, such as zinc oxide (ZnO), titanium oxide (TiO_2), and silver, are primarily defined by their size, content, crystallinity, and shape. The chemical, mechanical, electrical, structural, morphological, and optical characteristics of materials can be altered by shrinking them to the nanoscale. These changed properties enable nanoparticles to interact in a novel way with cell biomolecules, facilitating the physical transport of nanoparticles into inner cellular structures. Nanostructured materials contain a higher fraction of atoms on their surface, resulting in greater surface reactivity. Thus, nanomaterials have lately gained prominence in basic and applied sciences, as well as bionanotechnology (Rasmussen *et al*., 2010).

Zinc oxide nanoparticles (ZnONPs) are among the most explored studies due to their capacity to be used in a variety of downstream applications (Mohan and Renjanadevi, 2016). Zinc oxide nanoparticles are the second most prevalent metal oxide after iron, and they are affordable, safe, and simple to make (Kaplana *et al*., 2018). Zinc oxide nanoparticles (ZnONPs) are white

powders that are insoluble in water and have an energy band of 3.37 eV and a bonding energy of 60 meV, resulting in outstanding chemical, electrical, and thermal stabilities (Watanabe *et al*., 2018).

Zinc oxide nanoparticles (ZnONPs) have been used for many years as multifunctional semiconductor photoconductive antibacterial treatments. They've been employed as active components in antibacterial creams, lotions, and ointments (such as Sudocrem), as well as mouthwashes and paints. They have also been employed as a biofilm inhibitor in surface coatings (Jones *et al*., 2008). Despite the fact that zinc oxide nanoparticles (ZnONPs) have antibacterial action, aquatic organisms might be extremely susceptible to dissolved zinc (Franklin *et al*., 2007).

Zinc oxide nanoparticles (ZnONPs) have been studied for their antibacterial activity against a variety of food-borne pathogens and pollutants, including enterotoxigenic Escherichia coli and Botrytis cinerea (He *et al*., 2011). Zinc oxide nanoparticles have also been shown to be particularly effective against multi-drug resistant infections and pathogens associated with biofilms, such as Enterococcus faecalis and Staphylococcus epidermis (Ranghar *et al*., 2014).

The antibacterial function of zinc oxide nanoparticles was shown to be attributable to the generation of reactive oxygen species (ROS), which causes the destruction of bacterial cell components as well as damage to the electron transport chain and the bacterial cell membrane (Allaker, 2010). Zinc oxide nanoparticles are often produced by mechanochemical techniques such as sol-gel and spray pyrolysis (Espitia *et al*., 2012). The photocatalytic generation of reactive oxygen species such as hydrogen peroxide H2O2 is primarily responsible for the antibacterial action (Li *et al*., 2009). When activated by UV light, it also produces Zn^{2+} ions, which harm and destroy bacteria cells (Bumbudsanpharoke and Ko, 2015).

1.1 AIMS AND OBJECTIVES

The aim of the study was to demonstrate the antibacterial activity of zinc oxide nanoparticles on selected human pathogens.

The specific objectives of this research were to:

1. biologically synthesize zinc oxide nanoparticles using *Moringa oleifera* leaf extract.
2. characterize the zinc oxide nanoparticles synthesized
3. test and compare the antibacterial effect of the zinc oxide nanoparticles against the selected bacterial isolates

CHAPTER TWO

LITERATURE REVIEW

2.1 HISTORY OF NANOTECHNOLOGY

In 1959, Nobel Prize-winning American physicist Richard Feynman first proposed the idea of nanotechnology. At the California Institute of Technology (Caltech), Feymann gave a presentation titled "There's Plenty of Room at the Bottom" during the American Physical Society's annual meeting. The question "Why can't we write the entire 24 volumes of the Encyclopedia Britannica on the head of a pin?" was posed by Feynman in this lecture, and he also sketched out a vision of utilizing machines to build smaller machines all the way down to the molecular level (Hulla *et al*., 2015).

Fifteen years later, at a conference in 1974, Norio Taniguchi, a Japanese scientist from Tokyo University of Science, became the first person to use and define the term "nanotechnology" to describe semiconductor processes with characteristic control on the order of a nanometer, such as thin film deposition and ion beam milling. "Nano-technology" is primarily the processing, separation, consolidation, and deformation of materials by one atom or one molecule, according to his definition. The phrase was not used once more until 1981, when Eric Dexler published his first work on nanotechnology (Ranjit and Kenneth, 2012). At that time, Dexler was not aware of Taniguchi's earlier use of the phrase. As a result, nanomedicine has emerged as one of the primary subfields of nanotechnological research. Today, the scope of nanotechnology has expanded beyond materials to include the usage in machines and devices as well as in medicine. Its current focus is on creating novel techniques for preventing, detecting, and treating various diseases (Zdrojewicz *et al*., 2015).

2.2 NANOPARTICLE

According to Vert *et al*., (2012), a matter particle with a diameter of between 1 and 100 nanometers (nm) is referred to as a nanoparticle or ultrafine particle. Since the wavelengths of visible light (400–700 nm) are substantially larger than the size of nanoparticles, electron microscopes must be used to view them. In order to separate nanoparticles from liquids, unique nanofiltration techniques are needed because ordinary filters, such as typical ceramic candles, are easily penetrated by nanoparticles (Simonis and Basson, 2011).

Numerous fields, including chemistry, physics, geology, and biology, investigate nanoparticles because they are present in nature on a large scale. They frequently display phenomena that are not seen at either size because they are at the interface between bulk materials and atomic structures. They are a significant contributor to atmospheric pollution and essential components of a variety of industrial goods, including paints, plastics, metals, ceramics, and magnetic goods. A subfield of nanotechnology is the creation of nanoparticles with particular characteristics (Ibrahim *et al*., 2019).

2.3 SYNTHESIS OF NANOPARTICLES

The three kinds of approaches for the synthesis of nanoparticles are;

1. Physical methods
2. Chemical methods
3. Biological methods

Depending upon the need, the methods for synthesis of nanoparticles are selected (Krishnappa *et al*., 2018).

2.3.1 PHYSICAL METHODS

2.3.1.1 Ball milling

The types of mills utilized in this approach include rod, tumbler, planetary, and vibrating mills. The container contains steel or carbide-based hard balls. This technique is used to produce nanocrystalline cobalt (Co), chromium (Cr), tungsten (W), silver (Ag), and iron (Fe). Balls to materials are arranged in a 2:1 ratio. Inert gas is placed into the container, which is then rapidly rotated around its axis. Between the container's walls and the balls, the materials are compressed. In order to create nanoparticles of the ideal size, milling time and speed are crucial factors (Konrad *et al*., 2001).

2.3.1.2 Pulse laser ablation

The target sample is placed inside a vacuum chamber. The high-pulsed laser beam is focused on the sample and plasma is generated, which is formerly transformed into a colloidal solution of nanoparticles. The second harmonic group type laser is widely employed to create nanoparticles (Ponon *et al*., 2015).

2.3.1.3 Pulsed wire discharge method

The most popular technique for creating metal nanoparticles is this one. Pulsed current causes a metal wire to evaporate, producing a vapour that is then cooled by ambient gas to form nanoparticles. The fabrication speed and energy productivity of this plan may be high. Nitride nanoparticles are an example (Boddolla and Thodeti, 2018).

2.3.1.4 Melt mixing

Mixing turbulence from nanoparticles with high-velocity streams of molten metals. In a glass, nanoparticles are detained. Glass is an amorphous material that lacks symmetrical atom or molecule order. Metals can create amorphous solids-metallic glasses when they are cooled rapidly (Rastogi, 2017).

2.3.1.5 Chemical vapour deposition (CVD)

At a temperature between 300 and 1200 °C, a thin coating of a gaseous reactant is applied to the substrate. A thin film of the product is produced on the surface of the heated substrate as a result of a chemical reaction between the substrate and the combining gas. The applied pressure fluctuates between 100 and 105 Pa. There are numerous CVD variations, including Plasma Enhanced CVD, Atomic Layer Epitaxy, Vapor Phase Epitaxy, and Metallo Organic CVD.

The manufacturing of stiff, homogeneous, strong, and extremely pure nanoparticles is a benefit of this method. In order to remove the by-products from the substrate, they must be transported back to the gaseous phase. Substrates are heated using two different techniques: cold wall and hot wall. Even the reactor walls are susceptible to deposition in the hot wall configuration. The cold wall method avoids this. The development rate and quality of the film are ultimately influenced by gas pressure and substrate temperature (Dikusar*et al*., 2009).

2.3.1.6 Laser pyrolysis

Laser pyrolysis is the procedure used in creating nanoparticles in this manner. When there is an inert gas present, such as helium or argon, an intense laser beam is concentrated to break down the mixture of reactant gases. The gas pressure has a crucial role in influencing the particle sizes and their distribution (Behera and Nayak, 2013).

2.3.1.7 Ionized cluster beam deposition

The process was created in 1985. The primary goal of this technique is to produce excellent single-crystalline thin films. A source of evaporation, a nozzle through which material can expand into the chamber, an arrangement to accelerate the clusters, an electron beam to ionize the clusters, and a substrate on which a nanoparticle layer can be formed are all included in the arrangement.Collections become ionized following contact with an electron beam. The clusters are concentrated close to the substrate because of the hastening voltage utilized. By keeping an

eye on the accelerating voltage, it is probable to be able to regulate the energy with which the clusters impact the substrate. Certain materials' stable clusters would like to remain as small as clusters of particles because doing so would require a lot of energy. As a result, it is possible to create nanocrystalline material films using an ionized cluster beam (Karthikeyan and Loganathan, 2013).

2.3.2 CHEMICAL METHODS

2.3.2.1 Sonochemical synthesis

By sonochemically fusing copper salt with palladium and water, palladium copper (II) oxide (Pd-CuO) nanohybrids have been successfully created. Switch metal salts could be converted into their oxides in the presence of palladium and water by using ultrasonic waves. The sources of palladium are either palladium salts or pure metallic palladium (Ziylan *et al*., 2015).

2.3.2.2 Sol-gel method

Metal alkoxides or metal precursors in solution are condensed, hydrolyzed, and thermally decomposed. The result is the formation of a stable solution, or sol. The gel's viscosity increases as a result of hydrolysis or condensation. By adjusting the precursor concentration, temperature, and pH levels, the particle size can be observed. In order to facilitate the growth of solid mass, a mature stage is necessary. The solvent elimination, Ostwald ripening, and phase transformation could all take a few days. Nanoparticles are created by detaching the unstable chemicals (Li *et al*., 2016).

2.3.2.3 Inert gas condensation method

This approach allows for the mass production of metal nanoparticles. It had been popular to make fine nanoparticles using the inactive gas compression approach, which creates nanoparticles by causing a metallic source to vanish in an inert gas. At a temperature that is attainable, metals evaporate at a tolerable pace. Copper metal nanoparticles are created by

vaporizing copper metal inside a container containing argon, helium, or neon. By cooling the vaporized atom with an inert gas after it boils out, the atom quickly loses its energy. Liquid nitrogen is used to cool the gases, creating nanoparticles in the range of 2-100 nm (Perez *et al*., 2008).

2.3.2.4 Hydrothermal synthesis

It is one of the techniques for making nanoparticles that is most frequently employed. It is primarily based on chemical reactions. For the synthesis of nanoparticles, hydrothermal synthesis uses a wide temperature range from ambient temperature to extremely high temperatures. Comparing this strategy to physical and biological ones has a number of benefits. Higher temperature ranges may cause the hydrothermal synthesis-produced nanomaterials to become unstable (Banerjee *et al*., 2008).

2.3.2.5 Co-precipitation method

It is a solvent displacement method and is a wet chemical procedure. Ethanol, acetone, hexane, and nonsolvent polymer are examples of polymer solvents. Polymer phases can be either synthetic or natural. By mixing the polymer solution last, fast diffusion of the polymer-solvent into the nonsolvent phase of the polymer results. Interfacial stress at two phases is what creates nanoparticles (Das and Srivasatava, 2016).

2.3.3 BIOLOGICAL METHODS

2.3.3.1 Synthesis using microorganisms

Due to their affordability and environmental friendliness, microorganism-based nanoparticle production has attracted increased attention in recent years. Extracellular biosynthesis and intracellular biosynthesis are the two processes used to create nanoparticles from microorganisms, respectively. Metal ions can be separated by some microorganisms. *Pseudomonas stuzeri* AG295 accumulates silver inside or outside of cell walls, which enables it

to be often discovered in silver mines. Reductase enzymes come in a variety of varieties in microorganisms. As a result, they may detoxify and store heavy metals. Cadmium sulfide CdS nanoparticles can be made using *Klebsiella pneumonia* (Pandey *et al*., 2012).

2.3.3.2 Synthesis using plant extracts

The production of nanoparticles demonstrates the critical role played by plant extracts. This procedure is also known as a green synthesis or an environmentally friendly method of producing nanoparticles. The geranium plant (*Pelargonium graveolens*) has leaves that have been utilized to make gold nanoparticles. To create silver nanoparticles, 1 ml of a 1 mmol aqueous silver nitrate solution is added to 5 ml of the plant extract. The same process is used to create the product from an alcoholic extract. The plant extract and silver nitrate are shaken in the dark at 150 rpm.

2.3.3.3 Synthesis using algae

Preparation of algal extract in an organic or aqueous solvent through heating or boiling for a set amount of time. Preparation of an ionic metallic complex one molar solution. Algae solution and molar solution of ionic metallic complexes are incubated under controlled circumstances, either with continuous stirring or without stirring for a predetermined amount of time. Peptides, pigments, and polysaccharides are biomolecules that are responsible for the reduction of metals. Some seaweeds, including *Sargassum wightii* and *Fucus vesiculosus*, can be utilized to create silver nanoparticles (AgNPs) of various sizes and forms (Vinay *et al*., 2020).

2.4 CLASSIFICATION OF NANOPARTICLES

In general, nanoparticles can be divided into three categories: organic, inorganic, or a combination of the two. Lipid-based nanoparticles like liposomes and nanoemulsions, as well as polymeric and carbon-based nanoparticles, are examples of organic nanoparticles. Metallic nanostructures like quantum dots are examples of inorganic nanoparticles (Mishra *et al*., 2010).

2.4.1 ORGANIC NANOPARTICLES

2.4.1.1 Polymeric nanoparticles

A biocompatible and biodegradable polymer that can be sourced from both natural and artificial sources serves as the foundation for polymeric nanoparticles. The majority of synthetic polymers, such as poly (lactic acid), polyglycolic acid, poly (lactic-co-glycolic acid), polymethyl methacrylate, poly (caprolactone), and poly (amino acid), are employed to create sustainable polymers (Li and Tuan, 2005). The flexibility and chemical properties of polymeric nanoparticles make them ideal for combining with biomaterials like genetic components and growth factors as well as for target distribution to support tissue regeneration (Saravanan *et al*., 2017).

2.4.1.2 Liposomes

Liposomes have attracted a lot of attention over the past few decades (Lee et al., 2015). Liposomes are concentrated lipid bilayer nanocarriers with an aqueous core and a surfactant formed of phospholipids that can be either synthetic or natural. Liposomes can be categorized into three classes based on their structural characteristics: multilamellar vesicles (MLVs), oligo lamellar vesicles (OLVs), and unilamellar vesicles (ULVs). The diameters of small unilamellar vesicles (SUVs), medium unilamellar vesicles (MUVs), large unilamellar vesicles (LUVs), and giant unilamellar vesicles (GUVs) range from 20 to 100 nm, 100 nm, and 1,000 nm, respectively. Immunoliposomes, virosomes, stealth lipid membranes, and archaeosomes are examples of liposome-based carrier systems with biocompatible lipid bilayers that may improve the solubility and stability of the core component (Alavi *et al*., 2014; Merino *et al*., 2018; Mineart *et al*., 2018).

2.4.1.3 Dendrimers

Usually having many arms extending from the centre, dendrimers are branching macromolecules. Whether natural or synthetic, they are often produced using ingredients

including sugars, nucleotides, and amino acids. They are able to change molecules with a very consistent tree pattern, a certain molecular mass, and a distinctive number of peripheral groups thanks to their stepwise synthesis. You can create dendrimers from monomers by using divergent or convergent polymerization. The target size and form of a dendrimer can be demonstrated using a variety of units, including as chitin, melamine, polyamidoamine, poly L-glutamic acid, polyethylene glycol, and polypropylenimine, by demonstrating how the repeating unit's percentage of branching units affects these properties (Duncan and Izzo, 2005).

2.4.1.4 Carbon based Nanoparticles

Taking the shape of a graphene layer that has been coiled into a cylinder or closed at both ends to create a Bucky ball form, the carbon nanotube is a carbon-based tube-like structure (Duncan and Izzo, 2005). The two carbon-based structures are single-walled nanotubes (SWNT) and multi-walled nanotubes (MWNT). Contrary to single-walled nanotubes, which are made up of a single graphene cylinder, multi-walled nanotubes are composed of more than two circular cylindrical shells of graphene layers encircling a central hollow core (Keservani *et al*., 2017). The division of the nanotubes into target-based, ligand-attached, solvent-dispersed, and surfactant-grafted categories is done further in the creation. In addition to tubular variations, fullerenes are common carbon-based nanocarriers with geometric prison-like structures comprised of pentagonal and hexagonal carbon faces (Ezzati *et al*., 2011).

2.4.2 INORGANIC NANOPARTICLES

2.4.2.1 Quantum Dots

Quantum dots are artificial fluorescent semiconducting atom nanoparticles with a size range of 2–10 nm. A core and an aqueous zinc sulphide shell make up the semiconducting compound cadmium selenide, which inhibits the core to enhance optical properties. It is possible to create quantum dots that emit light with wavelengths from ultraviolet to infrared. The radiated

wavelengths are potent enough to be recognized at the microscopic level (Qu *et al*., 2017). Quantum dots also provide a constant and inert delivery method since proteins can be attached to the outer aqueous shell (Mo *et al*., 2017).

2.4.2.2 Nanoemulsion

Nanoemulsions provide a number of potential advantages over conventional emulsions for specialized uses in food and beverage products. According to Tadros*et al*., (2004), nanoemulsions are more resilient to mechanical separation and particle aggregation than traditional emulsions. According to the mutual spatial arrangement of the water and oil phases, nanoemulsions—droplets with diameters ranging from 10 to 100 nm—are divided into two groups (McClements and Rao, 2011). The usual oil and water nanoemulsion is moderately turbid to transparent and kinetically stable. Nanoemulsion particles are suitable for incorporation into optically clear products such as fortified soft drinks and waters, whitening cosmetics, and soups due to their minimal light scattering (Boonme*et al*., 2009; Silva *et al*., 2011).

2.4.2.3 Hydrogel nanoparticle

The 3-D polymer networks known as hydrogels can absorb large volumes of biological fluid or water. The hydrogels' capacity to absorb water is determined by the presence of hydrophilic groups, such as -OH, $-CONH^{-}$, $-CONH_2^{-}$, and $-SO_3H$ (Pachioni *et al*., 2016). Crosslinks come in two different forms: chemical tie-points and junctions and physical linkages or crystallites. To build cross-linked networks for drug delivery systems, polymers like alginate, chitosan, poly (vinyl alcohol), poly (ethylene oxide), and poly-N-isopropylacrylamide are frequently used. These networks are impacted by the electric field, light intensity, pH, and temperature (Lin *et al*., 2009; Xiao *et al*., 2016).

2.5 APPLICATIONS OF NANOPARTICLES

2.5.1 NANOREMEDIATION OF SOIL

The settlement of *Homo sapiens* during the shift from hunter-gatherers to farmers, nature was permanently altered. The monopolization of the wheat industry, first as a means of subsistence and then as a mode of economic exchange, had an impact on the extinction of animal and plant species, the alteration of river courses, soil erosion, and contamination. As a result, the emergence and expansion of industrialization as well as excessive urbanization have sped up soil degradation and contamination (Kumar *et al*., 2021).

Due to their high surface-to-volume ratio, surface functionalization, and ability to alter physical characteristics like size, shape, porosity, and chemical composition, nanomaterials have recently gained popularity for soil remediation. The combination of these attributes enables efficiency and selectivity in the capture of contaminants. Due to the in situ application, the intercalation of nanoparticles in the soil enables the cleaning of large areas while reducing costs and time. Metallic and magnetic nanoparticles, carbon nanotubes, and nanoscale zero-valent iron have dominated nanoremediation for soil contamination (Mukhopadhyay *et al*., 2021).

2.5.2 NANOREMEDIATION OF WATER

Environmental researchers are concerned about the issue of contaminated groundwater because it poses serious hazards to various ecosystems, which is crucial for the survival of living things (Schweitzer and Noblet, 2018). Many ions, heavy metals, petroleum hydrocarbons, pesticides, radioactive chemicals, as well as developing pollutants including medicines and personal care products, are capable of contaminating water supplies (Jhadav *et al*., 2015; Zamora-Ledezma *et al*., 2021). Metallic nanoparticles, biopolymeric membranes, and materials produced from carbon are the main types of nanomaterials used in water remediation (Saikia *et al*., 2019).

2.5.3 NANOREMEDIATION OF GAS PHASE

Air pollution is one of the most serious issues confronting the world this century, affecting both climate change and human health. Particulate matter, nitrogen oxides, sulfur dioxide, carbon monoxide, lead, and ground-level ozone, which is generated by chemical interactions between nitrogen oxides and volatile organic compounds (VOCs), are the six most frequent and dangerous outdoor air pollutants (Manisalidis *et al*., 2020). Secondary particulate matter precursors include nitrogen oxides, sulfur oxides, volatile organic molecules, and ammonia (NH3). Several approaches have been investigated to address this issue, including the use of graphene oxides and carbon nanotubes with highly reactive surface sites, as well as mesoporous silica materials with an ordered and tunable porous structure, high surface area, large pore volume, and thermal stability (Guerra *et al*., 2018).

2.5.4 AGRICULTURE

The application of nanotechnology in agriculture could be multifaceted. Using this technology, pesticides encapsulated in nanomaterials for controlled release, bio-pesticide stabilization, gradual release of nanomaterial assisted fertilizers, bio-fertilizers, and micronutrients for efficient usage, and field applications of agrochemicals are all possible. The detection of agricultural host pathogens and pesticide compounds on the host using nanosensor technology has significant potential in the health sector as well as cross-country export and import. It was also reported that nanoparticles, particularly porous hollow silica-based, clay-polyester, plastic starch coated or cemented nanoparticles, are important for soil conservation, and silver nanoparticles, as antifungal and antibacterial agents, play an important role in agricultural crop protection, where these particles also regulate proper nutrition to plants (Ghormade *et al*., 2011).Furthermore, the use of this technology may be contemplated in improving the quality of agricultural products based on pesticide usage, disease detection, nanofertilizer, chemical and bio-pesticide application,

residual quantity detection in agro-products, content of nucleic acid using nanosensor, and soil structure maintenance by producing natural nano-clays, among other things. Thus, it may be claimed that appropriate agricultural sector innovations have the potential to revolutionize agricultural and food production while also assisting in the right sustainability of agricultural products (Azarpour *et al*., 2011).

2.6 NANOPARTICLES AS ANTIBACTERIAL AGENTS

Antibacterial activity of nanoparticles of various metallic oxides and other metallic compounds has also been demonstrated. Metal oxide has been shown to be a promising combatant against a wide spectrum of pathogenic microorganisms, including multidrug resistant (MDR) isolates. Calcium oxide (CaO) nanoparticles inhibit bacterial cell integrity and damage the cell membrane of *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*, resulting in antibiotic activity. Magnesium oxide nanoparticles suppress growth significantly in a collection of multidrug resistant Gram-positive and Gram-negative microorganisms at a dose of 7.81 g/ml. Though the mechanism remains unknown, it suggests reactive oxygen species (ROS) liberation and interaction with the bacteria cell wall (El-Sayyad *et al*., 2018).

Furthermore, nanoparticles appear to successfully prevent bacterial biofilm formations. Biogenic selenium nanoparticles, for example, inhibit the biofilm formation of Gram-negative *Pseudomonas aeruginosa* (Cremonini *et al*., 2016).

2.7 NANOPARTICLES AS RENEWABLE ENERGY SOURCE

Nanotechnology has gained popularity in recent years because to its applications in science, engineering, and technology. Nanotechnology, like many other sciences, has opened up new avenues for solving issues. Nanotechnology is projected to drastically reduce global energy consumption, which is one of the most pressing issues of our day. While countries try to reduce

natural resource usage, research is being performed to develop and make new energy resources available. Nanotechnology has applications in energy efficiency, storage, and production, as well as the detection and removal of environmental pollutants (Verbruggen *et al*., 2017).

Nanotechnology has opened up new avenues for addressing pressing issues in the energy sector. One of the most pressing issues in energy resources today is the scarcity of alternative energy resources, or the inability of existing resources to achieve the needed degree of efficiency. Energy losses occur as a result of issues that arise during the storage and transfer of the obtained energy. In order to solve these challenges, intensive effort is being carried out in accordance with the advancements brought about by nanotechnology (Sia, 2017).

Nanotechnology-based insulating materials save 30% more energy than standard materials. These insulating materials are applied as a thin coating on any surface or squeezed between solid panels. The use of hydrogen sensors and nanotechnological membranes can improve the efficiency of fuel cells (Abdalla *et al*., 2020).

2.8 GOLD NANOPARTICLE

Gold nanoparticles (AuNPs) or (GNPs) are minuscule gold particles with diameters ranging from 1 to 100 nm that are also known as colloidal gold when distributed in water. Gold nanoparticles differ from gold particles in that gold particles are a yellow inert solid, whereas gold nanoparticles are a wine-red substance with antioxidant capabilities. Gold nanoparticles (AuNPs) exhibit different sizes ranging from 1 nm to 8 um, and they also simultaneously present distinct forms like suboctahedral, spherical octahedral, icosahedral multiple twined, decahedral, multiple twined, tetrahedral, irregular shape, nanotriangles, hexagonal platelets, nanorods and nanoprisms (Kazutami *et al*., 2017). Gold nanoparticles have been created utilizing numerous

environmentally friendly methods, including seed-mediated growth, synthesis in the presence of ionic liquids, and other reduction nanoparticles (Shalini and Pragnesh, 2021).

Gold nanoparticles are being studied all around the world due to their potential use in fields such as electronics, nanotechnology, and biomedicine. In comparison to the other metal nanoparticles listed above, they are reported to have modest antibacterial action. However, very small gold nanoparticles (approximately 2 nm) have demonstrated significant antibacterial action. The gold nanoparticle's antibacterial activity can be related to the production of reactive oxygen species (ROS), which increases the oxidative stress of microbial cells. Photothermal energy (PPT) can be used to enhance the antibacterial properties of gold nanoparticles. Gold nanoparticles can generate heat when exposed to laser light due to electron excitation. They can now be employed as anticancer or antibacterial agents. When gold nanoparticles are subjected to laser radiation, they exhibit an improved bactericidal action against Staphylococcus aureus (Mohammad *et al*., 2019).

Because of their diverse surface chemistry, gold nanoparticles can be coated with a variety of compounds such as tiny molecules, biomolecules, and polymers. As a result, they have numerous uses in a variety of sectors, including catalysis, sensory probes, drug delivery, and therapeutic agents. Recently, gold nanoparticles have been identified as an appropriate electrode surface modification in the production of electrochemical sensors. Because of their excellent properties such as tunable physiochemical properties, outstanding electrical conductivity, oxidation resistance, high stability, simple preparation, narrow size distribution, excellent biocompatibility, surface modification capacity, large surface area, and excellent catalytic activities, gold nanoparticles are widely used in electrochemical fabrication (Ali *et al*., 2022).

CHAPTER THREE

MATERIALS AND METHODS

3.0 COLLECTION OF REAGENT

Zinc nitrate hexahydrate [$Zn(NO_3)_2.6H_2O$] precursor salt was purchased from Solutions Biotechnology Laboratory, at University of Benin, Benin City, Edo State, Nigeria.

3.1 PREPARATION OF NUTRIENT AGAR FOR STOCK CULTURE

The antibacterial experiment was performed on nutrient agar (NA). The medium was made in accordance with the manufacturer's instructions. In 1000 ml of distilled water, 28g of nutritional agar powder was completely dissolved. The resultant mixture was then placed into a conical flask, covered with aluminum foil paper, and sterilized for 15 minutes in an autoclave at 121^{o}C (Wiley, 2005).

3.2 ISOLATION OF BACTERIA FROM THE SAMPLES

The surfaces of the soap samples were swabbed with a sterile swab stick, and the swab sticks were dipped in 10 ml of normal saline and briskly shaken. The wash of the black soap swabs was then plated aseptically onto separate Petri dishes, pour plated with nutritional agar, and incubated in duplicate. The plates were incubated for 24 hrs at 37°C. The bacterium colonies formed after incubation were subcultured to create pure cultures for subsequent bacteriological identification (Zeiny, 2009).

3.3 IDENTIFICATION OF BACTERIAL ISOLATES

3.3.1 Gram staining

Gram staining was used to identify the sub-cultured bacterial strain. A few drops of crystal violet were applied to the bacterial smear on a sterile glass slide for 60 seconds, the excess stain

was then washed off with running water, and Lugols iodine solution was applied for 60 seconds before being rinsed off with flowing water. The smear was decolored with 70% ethanol, and the counterstain Safranin was applied for 60 seconds before being washed off. The stained slide was air dried before adding a drop of immersion oil and seeing it with the oil immersion objective lens of a bright field microscope (Kalchev and Murdjeva, 2022).

3.3.2 Colonial morphological examination

Identification of bacterial isolates was also carried out using colonial morphological characteristics such as texture, colour, elevation and margin.

3.3.3 Biochemical tests

Biochemical tests were further used to identify the bacteria isolates down to the specie level and was achieved using Indole, citrate catalase and motility tests.

- **Catalase test**

This test was used to distinguish between bacteria that generate the catalase enzyme. A tiny number of bacteria were gathered and deposited on a clean glass slide using a sterile wire loop. On the glass slide, 10 droplets of hydrogen peroxide were dropped. For a positive test, it was then examined for immediate active bubbling (Makowski *et al*., 2021).

- **Oxidase test**

A small amount of culture was streaked smoothly on an oxidase disc (Tetra methyl Para PhenyleneDiaminoDihydrochloride) with a wire loop. A positive reaction was indicated by an intense deep purple colour appearing with in five to ten seconds (Makowski *et al*., 2021).

- **Motility test**

The motility test measures an organism's ability to move around. While certain motile cocci do exist, bacilli account for the vast bulk of mobile organisms. An organism was suspended on a slide in this experiment, and a drop of common saline was added; the emulsified slide was then examined under a microscope. Movement in various directions resulted in a positive test (Palma *et al*., 2022).

- **Citrate test**

According to the manufacturer's instructions, a slant of Simmons citrate agar was made. The test organism was then poked into the medium with an inoculating needle and spread across the medium's surface with an inoculating loop. After that, the test tube was incubated for 48 hrs. The creation of a blue color as a result of citrate breakdown suggests a favorable outcome (Jeroen, 1993).

- **Indole test**

On the confirmation of gram positive bacteria, Kovac's reagent, which includes 4(p)-dimethyl aminobenzaldehyde, combines with the indole to generate a red coloration compound (Makowski *et al*., 2021).

3.4 DNA EXTRACTION

750 µl of lysis solution was added into the tube containing 100 mg of the bacteria that have been suspended in up to 200 µl of isotonic buffer (pbs). It was secured in a beater fitted with a 2 ml tube holder assembly and centrifuged in a micro-centrifuge at 1000xg for 1 minute. 400 µl of supernatant was then transferred to a zymo-spin TM IV Spin Filter in a collection tube and centrifuged at 7000xg for 1 minute. The base of the zymo-spin IVTM filter was snapped off and

1,200 µl of bacterial DNA binding buffer was added to the tube. 800 µl of the mixture was added to a zymo-spin TM IIC column and centrifuged at 10,000xg/min before it was transferred to a 1.5 ml micro centrifuge tube. DNA elution buffer was added directly to the column matrix and centrifuged at 10,000xg for 30 seconds to elute the DNA.

3.5 DNA AMPLIFICATION PROTOCOL

(1) The PCR master mix was gently centrifuged after thawing.

(2) The thin walled PCR tubes were then placed on ice and the following components were added for each 25µl reaction

- One Taq One Step PCR master mix (2x) - (12.5µl)
- Forward primer (20µM) - (1.25µl)
- Reverse primer (20µM) - (1.25µl)
- Template DNA – (5µl)
- Nuclease free water (5µl)

(3) The samples were then gently spun.

(4) PCR was then performed using the thermal cycling conditions outlined below.

Step	**Temperature**	**Time**	**Number of Cycles**
Initial denaturation	94^{o}C	3mins	1
Denaturation	94^{o}C	30sec	
Annealing	54^{o}C	30sec	35
Extension	72^{o}C	1min	
Final extension	72	7mins	1
Hold	4^{o}C	∞	

(5) 10 µl of the PCR product was analysed on a 1.0% agarose gel electrophoresis, stained with ethidium bromide

3.6 PREPARATION OF 1% AGAROSE GEL

100ml of TBE buffer was measured into a flask. 1g of agarose gel powder was then weighed and added to the buffer. The buffer was heated for 3 minutes to dissolve the powder and allowed to cool to 56^{o}C. 5 µl of ethidium bromide was then added and the molten gel solution was poured in a gel mould in which the gel comb has been appropriately inserted. The gel was then allowed to solidify for 45 minutes at room temperature.

3.7 AGAROSE GEL ELECTROPHORESIS

10µl of ready to use DNA ladder (molecular marker) was mixed with loading dye was loaded in the first well of the solidified gel immersed in TBE buffer in Gel electrophoresis chamber. 90 volts was run through for 60 minutes and it was viewed under gel documentation system with UV transilluminator.

3.8 COLLECTION OF PLANT SAMPLE

Moringa oleifera leaves were harvested from Moringa tree at Faculty of Agriculture garden, University of Benin, Benin City, Nigeria.

3.9 PREPARATION OF *Moringa oleifera* AQUEOUS EXTRACT

10g of the Moringa leaves was weighed, washed with distilled water, sterilized with 70% ethanol and then air-dried at room temperature (36^{o}C). The dried leaves were macerated in a mortar and then transferred into a beaker containing 100ml of sterile distilled water and boiled for 10

minutes for optimal extraction of the phytochemical components. The extract was then sieved, labelled and refrigerated at 4ºC (Adam *et al*., 2021).

3.10 SYNTHESIS OF ZINC OXIDE NANOPARTICLES

To make a 0.1M solution of the zinc oxide nanoparticles, 2.9749 g of zinc nitrate hexahydrate [$Zn(NO_3)_2.6H_2O$] precursor salt was dissolved in 100 ml of distilled water and agitated. Then, using a magnetic stirrer, 50 ml of *Moringa oleifera* leaf extract solution was injected dropwise into a conical flask holding 50 ml zinc nitrate solution while swirling rapidly at 80ºC for 3 hrs to facilitate the creation of zinc oxide nanoparticles. Eventually, the solution turned hazy yellow. After then, the solution was incubated for 24 hrs before being characterized (Adam *et al*., 2021).

3.11 CHARACTERIZATION OF THE BIOLOGICALLY SYNTHESIZED ZINC OXIDE NANOPARTICLES

A UV-Vis spectrophotometer with different ranges was used to characterize the produced nanoparticles. The nanoparticles were described one by one, with each nanoparticle being 100% concentrated and placed in a cuvette, with two cuvettes positioned on the spectrophotometer equipment. One cuvette held the nanoparticle, while the other held water. Water was used as a control, and measurements were taken to determine the absorbance value of the nanoparticle solution (Vijayakumar, 2018).

3.12 ANTIMICROBIAL ASSAY

Agar well diffusion was used to test the antibacterial activity of biologically produced nanoparticles. Wells of 8 mm diameter were excavated using a cork borer and injected with varying concentrations of nanoparticles (25%, 50%, and 100%) and an antibacterial agent (ampicillin). After a 24-hour incubation period, the zone(s) of inhibition were assessed (Vijayakumar, 2018).

3.13 STASTITCAL ANALYSIS

The data obtained in this investigation were expressed as mean values and standard error of triplicate. The data was then subjected to One-way Analysis of Variance (ANOVA) using SPSS version 27.0 software and mean values were differentiated using Duncan Multiple Range (DMR) test (Ogbeibu, 2005).

CHAPTER FOUR

RESULTS

The results from the morphological description in Table 1 reveals that the suspected organisms were *Pseudomonas* sp., *Micrococcus* sp., and *Staphylococcus aureus*. Colonial features such as shape, size, margin, surface texture, optical activity and margination were recorded. The isolate identified as *Pseudomonas* sp. was then subjected to molecular analysis which revealed the isolate was *Pseudomonas amygdali* 35-1 CP084212. The other two isolates were subjected to biochemical tests. Results are represented in Table 2.

Zinc oxide nanoparticles were synthesized using zinc nitrate hexahydrate precursor solution and *Moringa oleifera* leaf extract. The nanoparticle solution was subjected to UV-Vis spectrophotometric analysis. The absorbance values of the biologically synthesized zinc oxide nanoparticles measured after 24 hrs of synthesis are represented in Figure 1. The highest peak of the absorbance values was at 350 nm indicating nanoparticle synthesis.

Table 3 shows the Antibacterial activity of the biologically synthesized zinc oxide nanoparticles against the selected bacterial isolates represented by the zones of inhibition (mm). The experiment was carried out in triplicate and the zones of inhibition were measured in millimeters for 25%, 50% and 100% concentrations of the biologically synthesized nanoparticles. Ampicillin was used as a positive control and the results were recorded after a 24 hour incubation period.

Table 1: Morphological description of the bacterium isolated from black soap on nutrient agar

Morphology	**Isolate 1**	**Isolate 2**	**Isolate 3**
Shape	Irregular	Irregular	Circular
Size	Large	Large	Medium
Surface	Smooth	Rough	Smooth
Colour	Creamy-white	Yellow	Golden-yellow
Opacity	Opaque	Opaque	Opaque
Elevation	Convex	Flat	Convex
Margin	Wavy	Wavy	Entire
Suspected organism	*Pseudomonas* sp.	*Micrococcus* sp.	*Staphylococcus aureus*

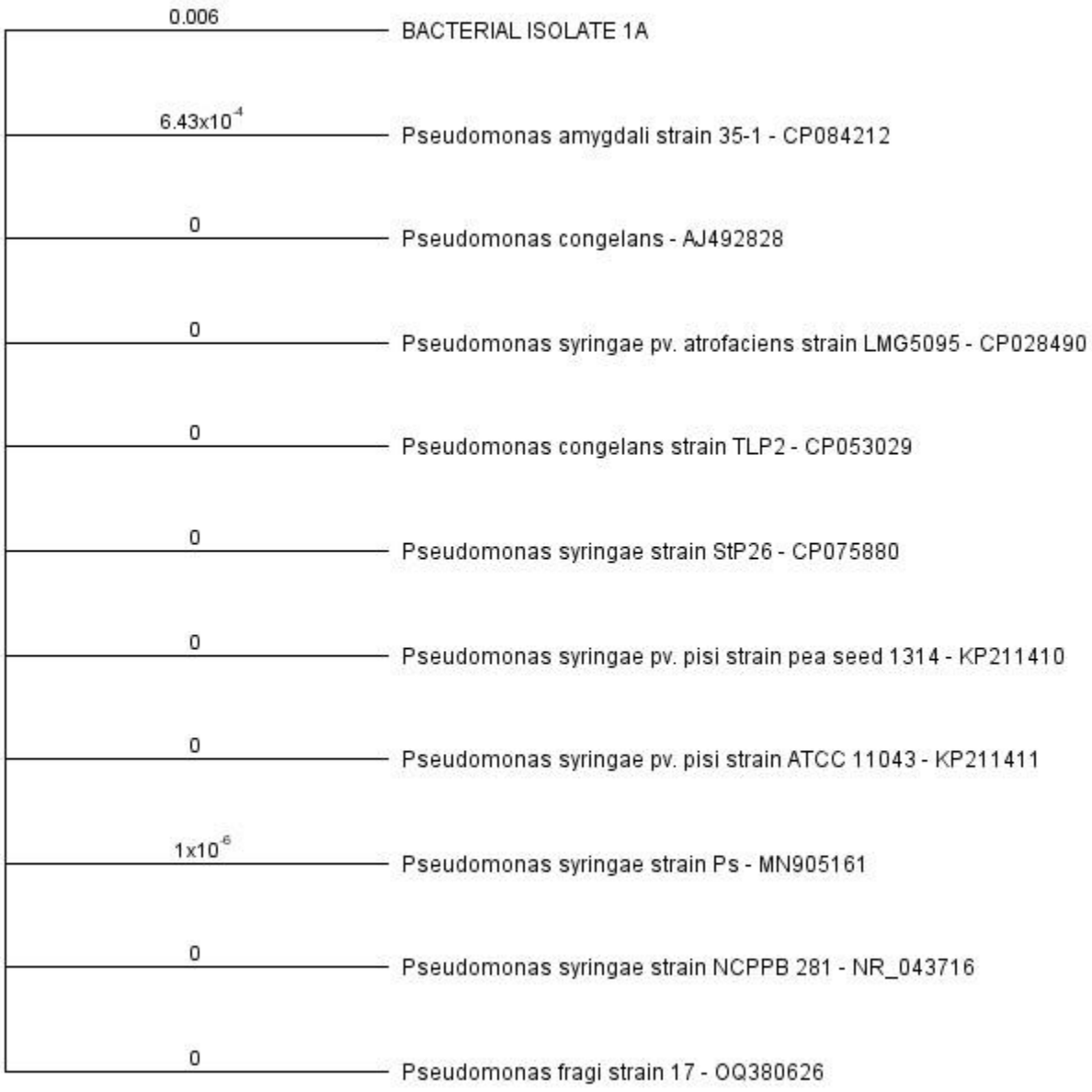

Figure 1: Phylogenetic analysis of isolate based on the nucleotide sequence of part of the 16S rRNA nucleotide sequence of bacteria. The phylogenetic tree was constructed by the Neighbor-Joining method program in the Geneious package (version 9.0.5). The numbers at the forks show the numbers of occurrences of the repetitive groups to the right out of 100 bootstrap samples. The isolate has a similar sequence with *Pseudomonas amygdali* strain 35-1 with accession number CP084212.

Table 2: Biochemical characteristics of the bacterial isolates

Test	**Isolate 2**	**Isolate 3**
Gram reaction	+	+
Cell type	Cocci	Cocci
Catalase	+	+
Oxidase	+	-
Motility	-	-
Indole	-	-
Citrate	-	+
Supected organism	*Micrococcus* sp.	*Staphylococcus aureus*

Legend

+: Positive to test

-: Negative to test

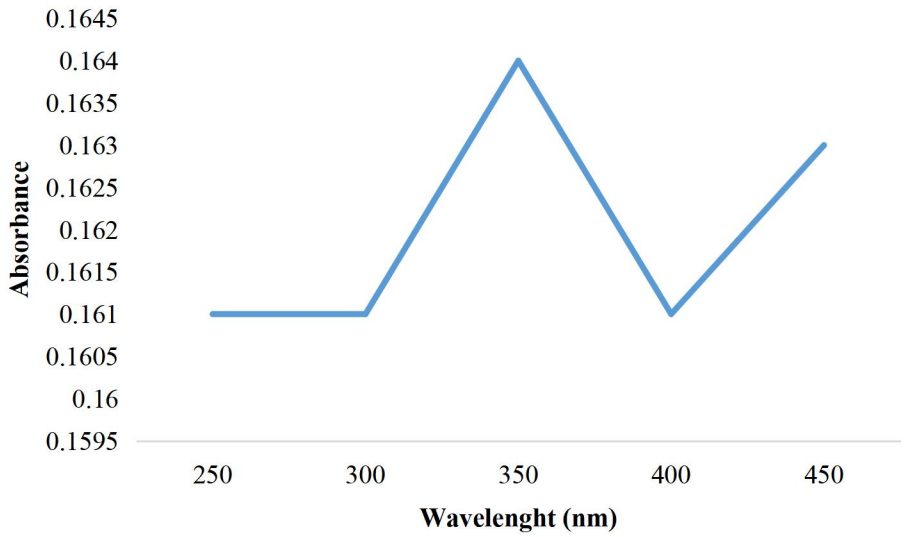

Figure 2: Absorbance values of the biologically synthesized zinc oxide nanoparticles after 24 hrs of synthesis. The highest peak of absorbance of the zinc oxide nanoparticle solution was recorded at 350 nm, indicating nanoparticle synthesis.

Table 3: Antibacterial activity of the biologically synthesized zinc oxide nanoparticles against the selected bacterial isolates represented by the zones of inhibition (mm).

Selected organism	25%	50%	100%	Control
***Pseudomonas amygdali* 35-1 CP084212**	4.67 ± 1.09^{a}	7.33 ± 1.25^{b}	10.67 ± 1.89^{c}	6.33 ± 1.09^{ab}
***Micrococcus* sp.**	3.33 ± 1.09^{a}	13.33 ± 3.31^{ab}	16.67 ± 4.25^{b}	18.67 ± 1.09^{b}
Staphylococcus aureus	3.33 ± 0.54^{a}	6.67 ± 1.09^{a}	18.00 ± 0.94^{b}	16.00 ± 4.10^{b}

*Values are in mean ± standard error of triplicate and mean values were differed statistically using Duncan multiple range test; a-c: Different superscripts in the same rows indicate values with significant difference ($p<0.05$).

CHAPTER FIVE

DISCUSSION AND CONCLUSION

The study demonstrated that zinc oxide nanoparticles are highly efficient against a wide range of biofilm-related pathogenic bacteria. The formation of zinc oxide nanoparticles in this study was confirmed after 24 hrs with a UV-VIS spectrophotometer and the peak of absorption was recorded at 350 nm. This is in line with a study carried out by Cheng *et al*., (2019), where they reported that the synthesis of the zinc oxide nanoparticles was due reducing ability of phytochemicals such as flavones, ketones and amines present in the plant extract used.

The findings of this study is also in agreement with a study carried out by El-Sayyad *et al*., (2018), where they demonstrated that nanoparticles of various metallic oxides and other metallic compounds have also shown potent antibacterial efficacy and present a solution to challenges arising from pathogenic microorganisms including multi drug resistant (MDR) bacterial isolates. Nanotherapy seems to have a significant effect on biofilms of bacteria and fungi imparting vague mechanisms. The same result was observed for TiO_2 nanoparticles against fungal biofilms as reported by (Haghighi *et al*., 2013).

The inhibitory mechanism that is followed by the nanoparticles is not correctly assembled and fully explained. However, evidence from studies by Gurunathan *et al*., (2012) and Liu *et al*., (2011) shows that the inhibitory activity is due to induced oxidative stress and released metal ions. Other mechanisms such as microbial cell penetration, the generation of reactive oxygen species (ROS), damaged DNA and proteins, loss of cellular integrity are the underlying mechanisms that cause inhibition for bacteria, fungi, and likewise viruses. Cell penetration is often the initial step in the stages involved in some microbial cell inhibition process before other mechanisms are adopted.

It was observed that the zinc oxide nanoparticles also exhibited inhibitory activity on the isolates used in this research at all concentrations with zones of inhibition ranging from from 4.67 ± 1.09 to 10.67 ± 1.89 mm (*Pseudomonas amygdali* 35-1 CP084212); 3.33 ± 1.09 to 16.67 ± 4.25 (*Micrococcus* sp.) and 3.33 ± 0.54 to 18.00 ± 0.94 (*Staphylococcus aureus*). This is in accordance with the reports of Pramanik *et al*., (2012) and Mai-Prochnow *et al*., (2016), that nanoparticles manifest antibacterial activity on both Gram-positive and Gram-negative bacterial strains. In the case of gram-negative strains, the cell wall composition shows a thin layer of peptidoglycan polymer with surface carrying negative charges. These features are directly linked to the antibacterial activity exhibited by the nanoparticles, the thin cell-wall ensures better penetration into the bacterial cell and, the negatively charged surface provides high electrostatic interaction between cells and nanoparticles leading to the formation of reactive oxygen species (ROS) and oxidative stress on the cells.

5.1 CONCLUSION

The study's findings indicate that biologically synthesized zinc oxide nanoparticles, which were synthesized with *Moringa oleifera* leaf extract, displayed some antibacterial activity against bacterial pathogens and the inhibitory activity of the nanoparticles increases with concentration in solution. The future direction of this study is to screen other nanoparticles for antimicrobial activities as a means of controlling human bacterial pathogens.

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APPENDIX

MEDIA PREPARATION AND COMPOSITION

MEDIA COMPOSITION

NUTRIENT AGAR

Meat peptone	5g
Beef extract	3g
Sodium chloride	5g
Agar	15g
Water	1L

28 g was suspended in 1L in distilled water. The mixture was stirred and autoclaved at 121^{o}C for 15 minutes.

NUTRIENT BROTH

Peptone	5g
Beef extract	3g
Sodium chloride	5g
Final pH	$7.4^{+}0.2$
Water	1L

18 g was suspended in 1000 ml of distilled water. The mixture was stirred and autoclaved at 121^{o}C for 15 minutes.

INDOLE TEST (KOVAC'S REAGENT)

P-dimethylaminocinnamaldehyde	10.0gm
Hydrochloric acid	100ml
Distilled water	900ml

OXIDASE TEST

1% tetramethyl-p-phenylenediaminedihydrochloride mixed in water.

CATALASE TEST

Drops of 3% hydrogen peroxide on a smear of a single colony on a filter paper

GRAM STAINING

CRYSTAL VIOLET

Crystal violet	2g
95% ethyl alcohol	20ml
Distilled water	

Mix crystal violet and ammonium oxalate monohydrate to make the crystal violet stain.

IODINE

Iodine	1g
Potassium iodide	2g
Sodium bicarbonate	3g
Water	300ml

ALCOHOL

95% ethanol

95% acetone

Mix together ethanol and acetone

SAFRANNIN

Safranin	2.5g

Mix 2.5g of safranin in 1L of distilled water

BIOCHEMICAL TESTS

Catalase test

A Pasteur pipette is used to place a drop of hydrogen peroxide on a clean glass slide. A sterile wire loop is used to collect a small portion of the test organism; it is then placed in the drop of

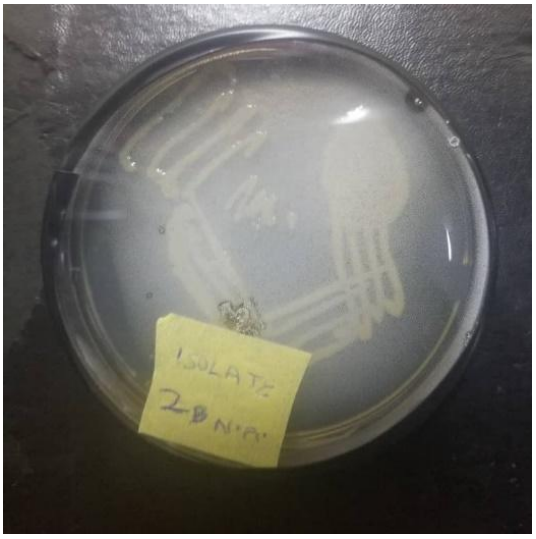
hydrogen peroxide on the slide and emulsified. Positive result gave effervescence while negative result gave no effervescence.

Oxidase test

Two drops of oxidase reagent were placed on a piece of filter paper, a colony of the test organism was collected and smeared on the emulsified filter paper. A positive reaction turned the paper dark purple within 10-20 secs while negative reaction shows no immediate colour change. This test is used to identify *Pseudomonas* species among other gram negative bacilli.

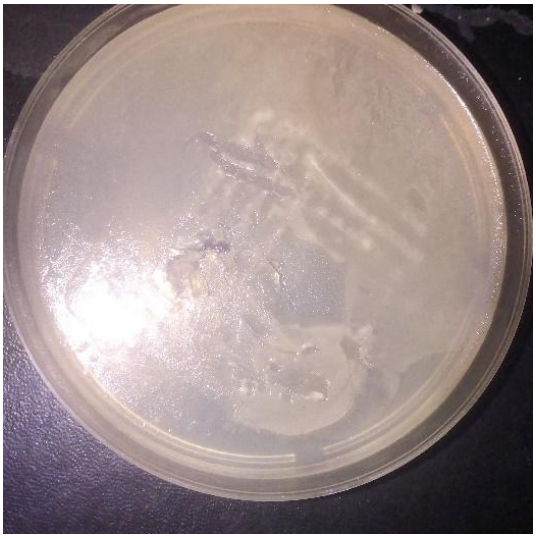
Indole test

The test was done on gram negative bacilli for the identification of Enterobacteriaceae: *Escherichia coli*, *Proteus* species and *Klebsiella* species. The test organism was inoculated in a test tube that contained 3ml of Kovac's reagent was added and shaken gently. The product was examined for a red colouration in the surface layer within 10mins. A positive result gave no red surface layer.



A

B



C

Plate 1: Pictorial representation of the test organisms

Legend:

A: Pure culture of *Pseudomonas amygdali* 35-1 CP084212

B: Pure culture of *Micrococcus* sp.

C: Pure culture of *Staphylococcus aureus*

A

B

C

D

Plate 2: Biological synthesis of zinc oxide nanoparticles.

Legends:

A: Zinc nitrate hexahydrate precursor solution; **B:** *Moringa oleifera* leaf extract

C: Zinc oxide nanoparticles after 1 hour of synthesis **D:** Zinc oxide nanoparticles after 24 hrs of synthesis.

DESCRIPTIVES

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean
						Lower Bound
Pseudomonas	Control	3	6.3333	1.15470	.66667	3.4649
amygdali 35-1	25%	3	4.6667	1.15470	.66667	1.7982
CP084212	50%	3	7.3333	.57735	.33333	5.8991
	100%	3	10.6667	1.15470	.66667	7.7982
	Total	12	7.2500	2.45412	.70844	5.6907
Micrococcus	Control	3	18.6667	2.30940	1.33333	12.9298
sp.	25%	3	3.3333	2.30940	1.33333	-2.4035
	50%	3	13.3333	7.02377	4.05518	-4.1147
	100%	3	16.6667	9.01850	5.20683	-5.7365
	Total	12	18.6667	2.30940	1.33333	12.9298
Staphylococcus	Control	3	3.3333	.57735	.33333	1.8991
aureus	25%	3	6.6667	1.15470	.66667	3.7982
	50%	3	16.0000	5.19615	3.00000	3.0920
	100%	3	19.3333	2.30940	1.33333	13.5965
	Total	12	11.3333	7.27803	2.10099	6.7091

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Pseudomonas amygdali 35-1 CP084212	Between Groups	417.333	3	139.111	3.937	.054
	Within Groups	282.667	8	35.333		
	Total	700.000	11			
Micrococcus sp.	Between Groups	57.583	3	19.194	17.718	.001
	Within Groups	8.667	8	1.083		
	Total	66.250	11			
Staphylococcus aureus	Between Groups	514.667	3	171.556	20.183	.000
	Within Groups	68.000	8	8.500		
	Total	582.667	11			

DUNCAN

	Treatment	N	Subset for alpha = 0.05		
			1	2	3
Pseudomonas amygdali 35-1 CP084212	25%	3	4.6667		
	Control	3	6.3333	6.3333	
	50%	3		7.3333	
	100%	3			10.6667
	Sig.		.086	.273	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

DUNCAN

	Treatment	N	Subset for alpha = 0.05	
			1	2
Micrococcus sp.	25%	3	3.3333	
	50%	3	13.3333	13.3333
	100%	3		16.6667
	Control	3		18.6667
	Sig.		.073	.323

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

DUNCAN

	Treatment	N	Subset for alpha = 0.05	
			1	2
Staphylococcus aureus	Control	3	3.3333	
	25%	3	6.6667	
	50%	3		16.0000
	100%	3		19.3333
	Sig.		.199	.199

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.