Meta-Analytic Guided Design of Green Synthesized Cu-Zn Nanoparticles from Malunggay (Moringa Oleifera) Leaves' Extract And Its Exploratory Antibacterial Activity Against Klebsiella Pneumoniae

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ABSTRACT

The rapid emergence of multidrug-resistant (MDR) pathogens, particularly Klebsiella pneumoniae, poses a critical global health threat and necessitates alternative antimicrobial strategies. This study explores the green synthesis of bimetallic copper-zinc (Cu-Zn) nanoparticles using aqueous leaf extract of Malunggay (Moringa oleifera) as a natural reducing and stabilizing agent, guided by a meta-analytic review of related literature. Existing studies on copper, zinc, and plant-mediated nanoparticles were systematically examined to identify synthesis parameters and antimicrobial trends relevant to MDR microorganisms. Based on the synthesized evidence, Cu-Zn nanoparticles were produced through an eco-friendly method and characterized in terms of size, shape, and structural properties. The antibacterial activity of the synthesized nanoparticles was then evaluated against Klebsiella pneumoniae at varying metal ratios (1:1, 1:3, and 3:1). Findings from the meta-analysis indicated an overall positive antimicrobial effect of green-synthesized metal nanoparticles despite heterogeneity across studies. Experimental results demonstrated that malunggay-mediated Cu-Zn nanoparticles exhibit inhibitory activity against Klebsiella pneumoniae, suggesting synergistic effects between copper and zinc components. This study highlights the potential of plant-based bimetallic nanoparticles as sustainable and cost-effective alternatives to conventional antibiotics. By integrating meta-analysis with laboratory synthesis, the research contributes to the growing field of green nanotechnology and supports the development of novel antimicrobial agents to combat drug-resistant bacterial infections.

Keywords: *Moringa oleifera* (Malunggay), green synthesis, copper nanoparticles (CuNPs), zinc nanoparticles (ZnNPs), copper–zinc bimetallic nanoparticles (Cu–Zn NPs), *Klebsiella pneumoniae*

INTRODUCTION

Multidrug-resistant (MDR) pathogens have become a significant public health concern worldwide, threatening the effectiveness of existing antimicrobial therapies. Among these pathogens, Klebsiella pneumoniae, a gram-negative bacterium, has been identified as one of the most problematic due to its high adaptivity and resistance to multiple antibiotics, including carbapenems, a last line of defense in many clinical settings (Chen, 2024). The World Health Organization has classified carbapenem-resistant K.

pneumoniae as a critical-priority pathogen, urgently requiring the development of novel treatment strategies.

In the Philippines, infections caused by Klebsiella pneumoniae have become an increasing concern, especially in intensive care units. This bacterium is often behind hospital-acquired pneumonia, bloodstream infections, and urinary tract infections, conditions that can extend a patient's hospital stay, increase treatment costs, and raise the risk of death (Taj-Aldeen et al., 2021). Unfortunately, the development of new antibiotics has not kept pace with the rapid evolution of resistance, making it vital to explore alternative antimicrobial options that are not only effective but also safe and sustainable.

One approach that has been gaining attention is nanotechnology. Metal-based nanoparticles, such as copper (Cu) and zinc (Zn), have shown strong antimicrobial activity. Copper can damage bacterial cell membranes and create oxidative stress, while zinc can disrupt enzymes and interfere with metabolism. When these two metals are combined into bimetallic Cu-Zn nanoparticles, the effects can be stronger than when they are used separately, and this might even help reduce toxicity.

The way nanoparticles are made is also important. Green synthesis uses plant extracts to produce nanoparticles in a safer and more environmentally friendly way compared to traditional chemical methods. In the Philippines, Malunggay (Moringa oleifera) is a good candidate for this. It's easy to grow, inexpensive, and its leaves contain natural compounds like flavonoids and phenolics that can help form and stabilize nanoparticles. On top of that, M. oleifera already has its own antimicrobial properties.

In 2020, copper nanoparticles made from M. oleifera leaf extract were found to be effective against K. pneumoniae, with minimum inhibitory concentrations between 250 and 500 µg/mL. In 2025, researchers produced copper-zinc oxide nanoparticles using the same plant and reported better stability and stronger antibacterial effects, although their tests were done on E. coli. More recently, in 2024, M. oleifera leaf extract was again confirmed to have activity against K. pneumoniae, thanks to its phenolic and flavonoid content.

However, there are still no studies that specifically combine bimetallic Cu-Zn nanoparticles from M. oleifera with testing against K. pneumoniae. This gap is worth addressing, since it could lead to plant-based nanomaterials that are both effective and environmentally sustainable.

In conclusion, the researchers proposed a green synthesis method for producing copper-zinc nanoparticles using aqueous leaf extract of Malunggay (Moringa oleifera) and evaluated their antimicrobial activity against multidrug-resistant Klebsiella pneumoniae through a meta-analysis of relevant studies. By utilizing an eco-friendly and cost-efficient biosynthesis approach for bimetallic nanoparticles, the research seeks to contribute to the development of alternative antibacterial agents, offering promising therapeutic options for combating infections caused by drug-resistant pathogens.

Pathogenic Microorganisms

Pathogenic microorganisms are microscopic entities, including bacteria, viruses, fungi, and parasites, that can cause disease in humans, animals, and plants. Despite their small size, these infectious agents have a profound impact on global health, ranging from mild illnesses to severe, potentially fatal conditions. They spread through various routes, such as direct contact with infected individuals, consumption of contaminated food or water, and airborne transmission (Biology Online, 2023).

Bacteria comprise one of the most medically significant groups of pathogens, though fewer than a hundred species are known to cause human disease (Eisenhofer, 2020). Among these, Klebsiella pneumoniae has emerged as a particularly problematic Gram-negative bacterium. It is an encapsulated, non-motile rod that

acts as an opportunistic pathogen, capable of causing pneumonia, bloodstream infections, urinary tract infections, and wound infections, particularly in immunocompromised individuals and hospitalized patients. In the Philippines, K. pneumoniae infections are most often reported in intensive care units, where they prolong hospitalization, increase treatment costs, and raise mortality rates (Taj-Aldeen et al., 2021).
A major public health concern is the increasing incidence of multidrug-resistant (MDR) K. pneumoniae, especially carbapenem-resistant strains (CRKP). These strains are recognized by the World Health Organization as a critical-priority pathogen due to their resistance to last-resort antibiotics (WHO, 2023). Resistance mechanisms include production of carbapenemases, modification of membrane permeability, biofilm formation, and acquisition of resistance genes via horizontal gene transfer. The ability of K. pneumoniae to form robust biofilms further enhances its persistence on medical devices and surfaces, making infection control in healthcare settings challenging.
Although viral, fungal, and parasitic infections also contribute significantly to the global infectious disease burden, the rise of MDR bacterial pathogens has driven urgent exploration of alternative antimicrobial strategies. In this context, nanotechnology has emerged as a promising approach. Metal-based nanoparticles such as copper (Cu) and zinc (Zn) possess unique physicochemical properties, including high surface-area-to-volume ratios and multiple antimicrobial mechanisms, that make them effective against a broad spectrum of bacteria, including drug-resistant strains. Copper nanoparticles can damage bacterial membranes and induce oxidative stress through reactive oxygen species (ROS) production, while zinc nanoparticles disrupt metabolic processes and enzyme functions (Mubeen et al., 2021).

Emergence of Antimicrobial Resistance

Antimicrobial resistance (AMR) occurs when bacteria, viruses, fungi, or parasites evolve in ways that render the drugs designed to kill them less effective or entirely ineffective. When this happens, once-manageable infections can persist in the body, increasing the likelihood of transmission to others. This not only prolongs illness but also raises the risk of severe disease outcomes and death. Importantly, antibiotic-resistant microorganisms can be carried and transmitted even by individuals who appear healthy, making containment more challenging.
Although often overshadowed by acute health emergencies, AMR represents a slow-moving global crisis with far-reaching implications. Its emergence was first observed shortly after the clinical use of the earliest antibiotics, and since then, resistance has steadily increased. In 2019, the World Health Organization formally recognized AMR as one of the most pressing public health threats of our time. Resistant pathogens complicate infection management, threaten the success of routine medical procedures such as surgeries, and increase the likelihood of fatal outcomes.
Current estimates from the Interagency Coordination Group on Antimicrobial Resistance indicate that drug-resistant infections are responsible for at least 700,000 deaths annually worldwide, with projections suggesting a potential rise to 10 million deaths per year by 2050 if no effective interventions are implemented (EClinicalMedicine, 2021). Among these pathogens, K. pneumoniae has emerged as a major concern due to its increasing resistance to multiple drug classes, including carbapenems. This escalating resistance underscores the urgent need for alternative antimicrobial strategies, such as green-synthesized bimetallic nanoparticles derived from Moringa oleifera, which hold promise for addressing both the clinical and environmental challenges of AMR.

Advent of Nanoparticles for Antimicrobial Resistance

Nanoparticles (NPs) are ultrafine, polymeric or metallic particles ranging in size from 1 to 100 nm, though some definitions extend up to 500 nm. Their spherical morphology and high surface-area-to-volume ratio make them uniquely suited for a wide range of applications (Rahim et al., 2018). Due to their nanoscale dimensions, nanoparticles exhibit distinct physicochemical properties compared to their bulk counterparts, including altered melting points, electrical and thermal conductivity, catalytic activity, and optical behavior. These tunable features have positioned nanoparticles and nanostructured materials (NSMs) at the forefront of scientific research and innovation across medicine, agriculture, environmental science, and materials engineering (Jeevanandam et al., 2018).

The rise of AMR has highlighted the urgent need for alternative therapeutic strategies beyond conventional antibiotics. As emphasized by Arora et al. (2020), antimicrobial tolerance impacts not only public health but also key industries such as food, water, textiles, and pharmaceuticals. Within this context, bimetallic nanoparticles, formed by combining two distinct metals at the nanoscale, have emerged as especially promising. Their unique physicochemical characteristics, synergistic catalytic activity, and multiple mechanisms of antimicrobial action allow them to outperform monometallic nanoparticles. Unlike single-metal nanoparticles, bimetallic systems demonstrate enhanced stability, greater antimicrobial potency, and improved selectivity, making them vital candidates for next-generation antimicrobial materials (AZoNano.com, 2020).

Zinc oxide nanoparticles (ZnO-NPs) are widely studied for their strong antimicrobial properties and essential biological role. They have shown size- and shape-dependent antimicrobial effects, particularly against MDR pathogens, and are effective in disrupting cell walls, generating ROS, and impairing enzymatic function (Rahman et al., 2019; Zhou et al., 2023). Copper nanoparticles (Cu-NPs) also exhibit broad-spectrum antimicrobial activity and have been reported to outperform some conventional antibiotics while also possessing antifungal, antiviral, and anticancer properties (Crisan et al., 2021).

Combining copper and zinc into bimetallic nanoparticles (Cu-Zn NPs) can enhance antimicrobial effects, improve stability, and reduce toxicity compared to their monometallic forms. When synthesized through plant-mediated green methods, such as using Moringa oleifera leaf extract, these nanoparticles offer an eco-friendly and biocompatible solution for addressing the growing crisis of AMR, particularly in drug-resistant K. pneumoniae.

Development of Green Synthesis

In recent years, green synthesis of metallic nanoparticles has emerged as a sustainable and important approach in nanotechnology. This method offers several advantages over conventional chemical and physical synthesis, including simplicity, cost-effectiveness, shorter reaction times, and the production of environmentally benign by-products. Green synthesis also produces nanoparticles with good stability and high biocompatibility, making it highly suitable for biomedical and antimicrobial applications. In contrast, traditional chemical synthesis often leaves behind toxic residues on nanoparticle surfaces, raising safety and environmental concerns (Bedlovičová, 2022).

Biosynthesis approaches have gained momentum due to their use of biological materials such as plant extracts, bacteria, fungi, and algae. Plant-based synthesis is especially attractive because plants contain a diverse array of phytochemicals, including phenolics, flavonoids, alkaloids, terpenoids, and proteins, that act both as reducing and stabilizing agents during nanoparticle formation (Suhag et al., 2022). This eliminates the need for harsh chemicals while enabling the synthesis of nanoparticles with tailored properties.

Moringa oleifera, commonly known as malunggay in the Philippines, has drawn significant interest as a plant-mediated synthesis agent for metallic nanoparticles. Its leaves are rich in bioactive compounds that not only facilitate nanoparticle formation but also provide inherent antimicrobial activity. Studies have demonstrated that M. oleifera extracts can synthesize stable silver, gold, zinc oxide, and copper nanoparticles with strong antimicrobial effects (Das et al., 2021; Singh et al., 2022). Its abundance in tropical and subtropical regions, including the Philippines, makes it an accessible and sustainable choice for large-scale nanoparticle production.

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Application of Meta-Analysis

In nanotechnology research, particularly in the development of metallic nanoparticles, meta-analysis serves as a powerful statistical tool to consolidate findings from multiple independent studies. It aggregates outcomes from prior research on similar questions, increasing statistical robustness by boosting sample size, enhancing population diversity, and pooling effect sizes from different experiments (Salters-Pedneault, 2022). When studies include randomized controlled trials (RCTs), meta-analysis represents the highest level of evidence, followed by systematic reviews (Himmelfarb Health Sciences Library).

In antimicrobial research, meta-analysis can assess how variables such as particle size, synthesis method, surface functionalization, and metal composition influence efficacy. This approach is especially valuable for bimetallic systems like Cu-Zn NPs, where synergistic effects may depend on synthesis conditions and the target pathogen.

For example, Herrera et al. (2023) conducted a meta-analysis on metal nanoparticles for pesticide degradation, compiling 408 observations from 94 studies. Although their work focused on environmental applications, it demonstrated how systematic data synthesis can identify the most effective nanoparticle types and configurations. Applying a similar approach to M. oleifera-mediated Cu-Zn nanoparticles could determine optimal synthesis parameters for combating MDR K. pneumoniae.

Synthesis

The reviewed literature highlights the substantial global health threat posed by MDR K. pneumoniae, a major cause of hospital-acquired infections with increasing resistance to multiple antibiotic classes, including carbapenems. This underscores the urgent need for novel, effective, and sustainable antimicrobial strategies. Nanotechnology offers a promising avenue, with metallic nanoparticles demonstrating potent antimicrobial activity through multiple mechanisms. Bimetallic Cu-Zn nanoparticles are of particular interest for their enhanced antimicrobial efficacy, improved stability, and potential reduced toxicity compared to monometallic forms. Green synthesis, particularly using Moringa oleifera leaf extract, aligns with sustainability goals while leveraging the plant's phytochemicals to facilitate nanoparticle formation and enhance bioactivity. Combining laboratory experimentation with meta-analysis can provide a stronger evidence base for the optimal design and application of these nanoparticles against MDR K. pneumoniae. This integrated approach may accelerate the development of plant-based nanomaterials as viable alternatives to conventional antibiotics.

Statement of the Problem

This study focuses on the green synthesis of Copper-Zinc (Cu-Zn) nanoparticles using a Malungggay (Moringa Oleifera) extract as a natural reducing agent. These nanoparticles are known for their strong antimicrobial properties even at low concentrations, making them a promising alternative to other medications. The current study involves a comprehensive analysis of the synthesized nanoparticles' potential in combatting bacterial infections, particularly those caused by Klebsiella Pneumoniae. This study aims to answer:

1. How can existing studies be collected & analyzed to determine the antimicrobial effects of green-synthesized Cu-Zn nanoparticles against multidrug-resistant microorganisms like Klebsiella Pneumoniae?
2. Can Cu-Zn nanoparticles be successfully synthesized using a Malunggay (Moringa Oleifera) extract as a reducing agent?
3. What are the size, shape, and structural characteristics of the synthesized Cu-Zn nanoparticles?
4. How effective are the synthesized Cu-Zn nanoparticles at controlling the growth of Klebsiella Pneumoniae?

Objectives

The goal of this study is to synthesize Cu-Zn Nanoparticles using Malunggay extract and evaluate its antibacterial activity against Klebsiella Pneumoniae.

1. To review & analyze existing research papers on the antimicrobial activity of Cu-Zn Nanoparticles against Klebsiella Pneumoniae through a meta-analytic approach.
2. To synthesize Cu-Zn Nanoparticles using Malunggay (Moringa oleifera) extract as a natural reducing agent.
3. To determine the size, shape, and structural characteristics of the synthesized nanoparticles.
4. To assess the antifungal activity of the synthesized Cu-Zn nanoparticles against Klebsiella Pneumoniae across varying concentrations (1:1, 1:3, & 3:1)

Hypotheses

The researchers dealt with the analysis of collated related studies for meta-analysis of the antibacterial activity of green-synthesized copper, zinc, and copper-zinc nanoparticles.

H_0 : The collated studies do not exhibit an overall positive direction of effects across multiple pathogens, despite heterogeneity.

H_A : The collated studies exhibit an overall positive direction of effects across multiple pathogens, despite heterogeneity.

Scope and Limitation

This study focused on the green synthesis of bimetallic Cu-Zn Nanoparticles using Malunggay (Moringa Oleifera) leaf extract and on evaluating their antibacterial activity against Klebsiella Pneumoniae. The experiment involves preparing the Malunggay extract, mixing it with liquid Copper Sulfate and Zinc Chloride, and testing the antibacterial properties of the resulting nanoparticles. The malunggay leaves samples were collected locally, and extracts were obtained from the leaves of the plant. The experiment was conducted at one of the researcher's residences, and at the Physics Laboratory of Manila Science High School to gain access to proper lab equipment that were necessary for the experiment. The study is delimited to testing antibacterial results that are against Klebsiella Pneumoniae and did not account for other bacterial pathogens.

Medical Professionals

Healthcare professionals could gain access to more effective strategies for managing Klebsiella Pneumoniae infections. These strategies may support better patient recoveries, and make it easier to manage infections that don't respond to typical/traditional treatments.

Government

This study could help the government address the growing concern of the drug-resistant Klebsiella Pneumoniae bacterial infections. It may also help the government to develop better policies and manage funds or resources more effectively.

Pharmaceutical Scientists

This study could inspire the development of new antimicrobial treatments. It could also help them understand how plant-based nanoparticles work against drug-resistant bacteria like Klebsiella Pneumoniae.

Future Researchers

This study may help future researchers to understand how plant-based nanoparticles can be used against Klebsiella Pneumoniae and pave the way to make new approaches to antimicrobial treatments.

Theoretical Framework

This study is anchored on three interrelated theoretical foundations that explain the synthesis, characteristics, and antimicrobial efficacy of green-synthesized bimetallic nanoparticles.\

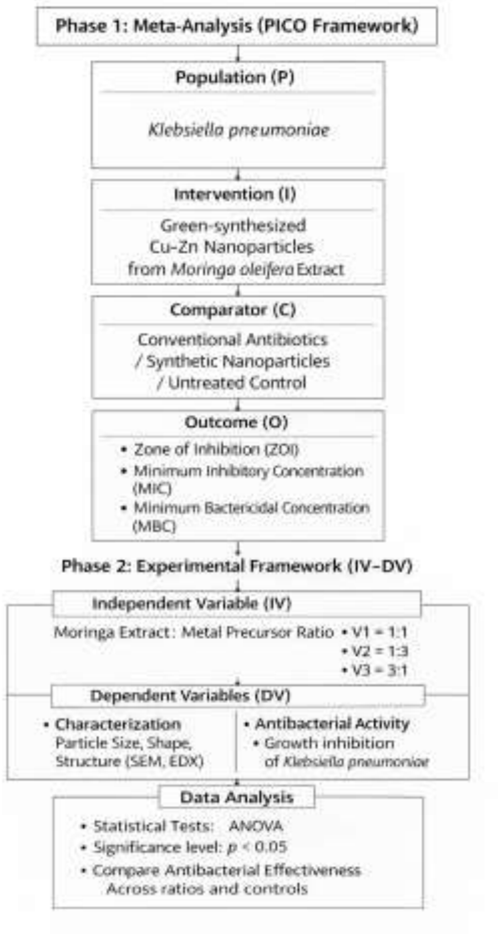
First, the framework is grounded in Green Synthesis, which posits that biological materials such as plant extracts can function as reducing and stabilizing agents in nanoparticle formation (Ying, et al., 2022). In this study, Moringa oleifer leaf extract serves as the independent biological mediator due to its high concentration of phytochemicals, particularly phenolics and flavonoids. These compounds facilitate the reduction of copper (Cu^{2+}) and zinc (Zn^{2+}) ions into their nanoscale metallic forms while simultaneously preventing agglomeration. Within a quantitative paradigm, the concentration of plant extract and metal precursors are treated as controlled independent variables influencing nanoparticle formation efficiency, size distribution, and stability, which are measurable physicochemical outcomes.

Second, the study is supported by the Bimetallic Synergy Principle, which asserts that nanoparticles composed of two metals exhibit enhanced physicochemical and biological properties compared to monometallic systems (Zhao, et al., 2025). The interaction between copper and zinc at the nanoscale is theorized to produce synergistic effects, improving surface reactivity, ion release behavior, and antimicrobial potency (Dehkordi, 2021). Nanoparticle composition (Cu-Zn ratio) functions as an independent variable, while antimicrobial activity (measured through zone of inhibition, minimum inhibitory concentration, or bacterial growth reduction rate) serves as the dependent variable.

Third, the framework incorporates established Mechanisms of Antimicrobial Action to explain the causal relationship between nanoparticle exposure and bacterial inhibition. Copper nanoparticles are known to induce membrane disruption and oxidative stress through reactive oxygen species (ROS) generation, while zinc nanoparticles interfere with enzymatic and metabolic processes (Ma, et al., 2022). These mechanisms collectively predict a measurable decrease in the viability of Klebsiella pneumoniae. Thus, nanoparticle concentration and exposure time are treated as predictor variables influencing bacterial survival rate as the primary quantitative outcome.

Conceptual Framework

The conceptual framework for this study illustrates the relationship between the green synthesis of bimetallic copper-zinc (Cu-Zn) nanoparticles using Moringa oleifera leaf extract and their antibacterial activity against multidrug-resistant Klebsiella pneumoniae. It is designed to show how plant-mediated nanoparticle synthesis, informed by meta-analytic evidence from previous studies, influences the structural characteristics of nanoparticles and their subsequent effectiveness in inhibiting bacterial growth. By visually mapping the independent, dependent, and control variables, the framework provides a structured representation of the research process, guiding both the synthesis methodology and the evaluation of antimicrobial outcomes.



Conceptual Framework

Definition of Terms

For clarity, the following terms are defined as they are used in this study to avoid ambiguity and ensure consistency throughout the research paper.

Aqueous Extract

A solution obtained by boiling or soaking plant material in distilled water to extract bioactive compounds. In this study, Malunggay (*Moringa oleifera*) leaves were used to produce the aqueous extract, which acts as a reducing and stabilizing agent during nanoparticle synthesis.

Copper Sulfate ($CuSO_4$)
An analytical-grade metal salt used as a precursor for copper nanoparticles. It provides Cu^{2+} ions that undergo reduction in the presence of plant extract to form copper nanoparticles.

Zinc Chloride ($ZnCl_2$)
An analytical-grade metal salt used as a precursor for zinc nanoparticles. It provides Zn^{2+} ions that participate in nanoparticle formation when combined with plant extracts.

Green Synthesis
An environmentally friendly method of producing nanoparticles using natural biological agents, such as plant extracts, which serve as reducing and stabilizing agents, thereby avoiding harmful chemicals.

Cu-Zn Nanoparticles (Cu-Zn NPs)
Nanoscale particles composed of copper and zinc metals, synthesized via green synthesis. These particles have potential antimicrobial activity and are characterized by unique optical and chemical properties.

Metal Precursor Solution
A solution containing metal salts ($CuSO_4$ and $ZnCl_2$ in this study) that serves as the source of metal ions for nanoparticle formation.

Variation Ratios (V1, V2, V3)
Different volume ratios of Malunggay leaf extract to metal precursor solution used in this study to optimize nanoparticle formation:

- V1 = 1:1 ratio of extract to precursor solution
- V2 = 1:3 ratio of extract to precursor solution
- V3 = 3:1 ratio of extract to precursor solution

Hot Plate Drying
A method used to remove residual moisture from synthesized nanoparticles by applying controlled heat (60–80 °C) to obtain stable, dry nanoparticles for characterization and testing.

Antimicrobial Activity
The ability of synthesized nanoparticles to inhibit the growth of microorganisms. This property is often evaluated to determine potential biomedical applications of nanoparticles.

REVIEW OF RELATED LITERATURE

The Review of Related Literature (RRL) provides a comprehensive examination of previous studies on multidrug-resistant Klebsiella pneumoniae, the antimicrobial properties of copper, zinc, and bimetallic nanoparticles, and the green synthesis of nanoparticles using plant extracts, particularly Moringa oleifera. By synthesizing findings from past research, it highlights existing knowledge, identifies gaps, and establishes the rationale for this study's focus on eco-friendly Cu-Zn nanoparticles as potential alternatives to conventional antibiotics.

Pathogenic Microorganisms and Klebsiella pneumoniae

Pathogenic microorganisms, including bacteria, viruses, fungi, and parasites, are agents capable of causing disease in humans, animals, and plants (Biology Online, 2023). Among bacterial pathogens, Klebsiella pneumoniae has emerged as a major global health concern due to its adaptability and resistance to multiple antibiotics (Eisenhofer, 2020). This Gram-negative, encapsulated, non-motile bacterium can cause pneumonia, bloodstream infections, urinary tract infections, and wound infections, particularly among immunocompromised individuals and hospitalized patients. In the Philippines, K. pneumoniae is frequently implicated in intensive care unit infections, prolonging hospitalization and increasing both healthcare costs and mortality risk (Taj-Aldeen et al., 2021).

The rise of multidrug-resistant (MDR) strains of K. pneumoniae, especially carbapenem-resistant strains, has prompted the World Health Organization to classify it as a critical-priority pathogen (WHO, 2023). Resistance mechanisms include carbapenemase production, membrane permeability alterations, biofilm formation, and horizontal gene transfer. The capacity for biofilm formation enhances persistence on medical devices and complicates infection control efforts.

Emergence of Antimicrobial Resistance

Antimicrobial resistance (AMR) arises when microorganisms evolve mechanisms to withstand drugs designed to kill them, resulting in infections that are harder to treat and control. AMR represents a slow-moving yet severe global crisis, responsible for at least 700,000 deaths annually and projected to increase to 10 million by 2050 if unaddressed (EClinicalMedicine, 2021). MDR K. pneumoniae is particularly concerning due to resistance to multiple antibiotic classes, including last-resort carbapenems. Consequently, exploring alternative antimicrobial strategies is critical to address this escalating threat.

Nanoparticles as Antimicrobial Agents

Nanoparticles (NPs), particles with dimensions between 1 and 100 nanometers, possess unique physicochemical properties such as high surface-area-to-volume ratios, altered electrical and thermal conductivity, and catalytic activity that enable diverse biomedical applications (Rahim et al., 2018; Jeevanandam et al., 2018). These features allow metal-based nanoparticles, particularly copper (Cu) and zinc (Zn), to exert strong antimicrobial effects. Cu nanoparticles disrupt bacterial membranes and induce oxidative stress, while Zn nanoparticles interfere with metabolic enzymes and other cellular functions (Mubeen et al., 2021).

Bimetallic nanoparticles, combining two metals at the nanoscale, provide enhanced antimicrobial activity compared to monometallic nanoparticles. The synergistic effects of Cu-Zn nanoparticles include greater stability, reduced toxicity, and improved selectivity (AZoNano.com, 2020). Such nanoparticles are especially promising for targeting MDR pathogens like K. pneumoniae.

Green Synthesis of Nanoparticles

Traditional chemical synthesis of nanoparticles often involves hazardous reagents, generating toxic residues and raising environmental concerns. In contrast, green synthesis uses biological agents, such as plant extracts, bacteria, fungi, and algae, as reducing and stabilizing agents, producing nanoparticles in an eco-friendly, cost-effective, and biocompatible manner (Bedlovičová, 2022; Suhag et al., 2022).

Plant-based synthesis is particularly advantageous due to the presence of bioactive phytochemicals, including phenolics, flavonoids, alkaloids, and terpenoids, which aid in nanoparticle formation. Moringa oleifera (Malunggay) is abundant in tropical regions, including the Philippines, and is rich in these compounds, making it an ideal candidate for green nanoparticle synthesis (Das et al., 2021; Singh et al., 2022). Previous studies have demonstrated that M. oleifera extracts can successfully mediate the formation of copper, zinc oxide, silver, and gold nanoparticles with strong antimicrobial activity.

Meta-Analysis in Nanoparticle Research

Meta-analysis is a statistical method that synthesizes results from multiple independent studies to identify trends, consolidate evidence, and strengthen conclusions (Salters-Pedneault, 2022). In antimicrobial nanoparticle research, meta-analysis can reveal how factors such as particle size, metal composition, synthesis method, and surface functionalization affect efficacy. For instance, Herrera et al. (2023) employed meta-analysis to determine the most effective metal nanoparticles for environmental applications, highlighting its potential to guide synthesis strategies in biomedical contexts. Applying meta-analytic insights to M. oleifera-mediated Cu-Zn nanoparticles can help optimize antibacterial performance against MDR K. pneumoniae.

Integration and Implications

The reviewed literature underscores the urgent need for effective alternatives to conventional antibiotics due to rising MDR pathogens, particularly K. pneumoniae. Metal-based nanoparticles, especially green-synthesized bimetallic Cu-Zn nanoparticles, show promise due to their synergistic antimicrobial activity, stability, and biocompatibility. Using M. oleifera leaf extract as a reducing and stabilizing agent aligns with sustainable practices and leverages the plant's inherent antimicrobial properties. Integrating meta-analytic findings with laboratory synthesis offers a robust approach for developing effective, eco-friendly, and cost-efficient antimicrobial agents capable of addressing multidrug-resistant infections.

METHODOLOGY

This part described the methods used to prepare the Malunggay (Moringa oleifera) leaves extract and metal precursors, and the experimental procedures used to treat Klebsiella pneumoniae UPCC 1382 (ATCC 13883) bacteria.

Research Design

This study employed a two-phase quantitative approach: a systematic review with meta-analysis followed by a two-group experimental design. In the preliminary phase, a systematic review of multiple databases was conducted to gather evidence regarding optimal conditions for the green synthesis of Cu, Zn, and Cu-Zn nanoparticles, as well as their antimicrobial activities against various bacteria, including Klebsiella pneumoniae UPCC 1382 (ATCC 13883). The findings from this synthesis were used to develop the conceptual and theoretical frameworks guiding the experimental phase.

In the second phase, a two-group experimental design was used to investigate the antibacterial activity of green-synthesized Cu-Zn nanoparticles against K. pneumoniae (ATCC 13883). The experimental group consisted of three varying ratios of plant extract to $CuSO_4$-$ZnCl_2$ metal precursor solution. These variations were compared against a control group containing only the plant extract solution and a positive control of chloramphenicol (30 µg).

Locale

This study was conducted at a member's residence in Imus, Cavite, and at the Science Laboratory of Manila Science High School, where the Malunggay (Moringa Oleifera) leaf samples used in this study were collected from San Pedro, Laguna. The synthesized (fine powdered) nanoparticles were brought to the *iNano Research Facility* of the De La Salle University, Manila for SEM and EDX analysis to determine the characterization (size, structure, composition, & image) of the nanoparticles, while the antibacterial testing against Klebsiella pneumoniae\italicized was performed at the *Natural Sciences Research Institute (NSRI)* of the University of the Philippines, Diliman.

Population

This study focused on green-synthesized Cu-Zn nanoparticles and their potential antibacterial properties. Since antibacterial testing was not conducted in the experimental phase, the population was defined through a meta-analysis of published studies that examined the antimicrobial activity of copper, zinc, or copper–zinc nanoparticles synthesized through green methods against Klebsiella pneumoniae\italicized

Phase: Meta-Analysis

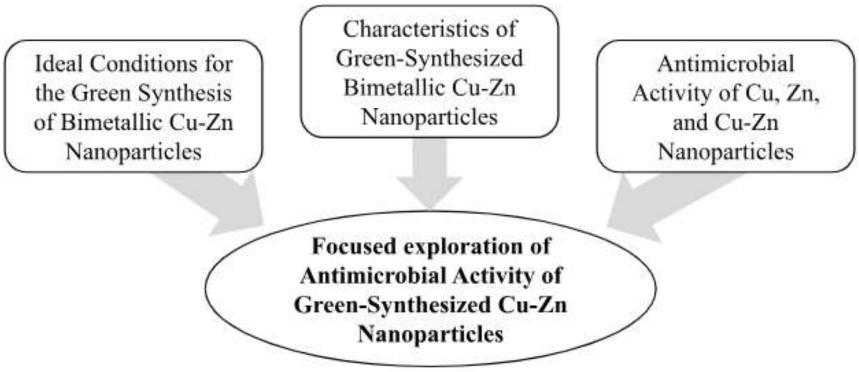


Figure 1. Conceptual framework for the Meta-Analysis of the antibacterial activity of green-synthesized Cu-Zn Nanoparticles

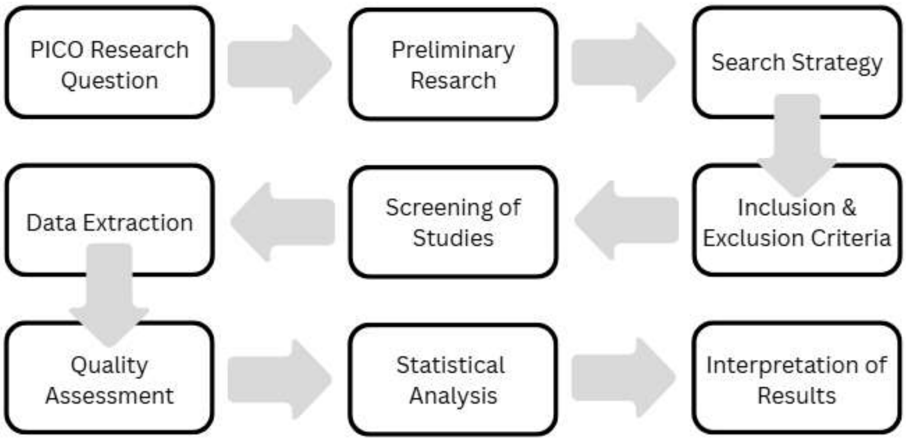


Figure 2. Theoretical framework for the Meta-Analysis of the antibacterial activity of green-synthesized Cu-Zn Nanoparticles

A. PICO Framework

In line with the suggested frame work—**P**opulation - studies using bacterial strains, particularly Klebsiella pneumoniae UPCC 1382 (ATCC 13883); **I**ntervention - green-synthesized Cu-Zn nanoparticles (Cu-Zn NPs) derived from Moringa oleifera extract; **C**omparator - conventional antibiotics, chemically synthesized NPs, or untreated controls; and **O**utcome - antibacterial activity measured by zone of inhibition, MIC, MBC, or bacterial growth reduction—the following research question will guide the meta-analysis.

In bacterial strains of Klebsiella pneumoniae UPCC 1382 (ATCC 13883) (P), do green-synthesized copper-zinc nanoparticles derived from Moringa oleifera leaves (I), compared to other treatments or controls (C) , in terms of inhibition zones, Minimum Inhibitory Concentration (MIC), or Minimum Bactericidal Concentration (MBC) (O)?

B. Preliminary Search

Before officially starting the manual search, the researchers conducted a preliminary search to identify if there are enough articles for conducting the analysis, and also to ensure the validity of the proposed idea to avoid duplication. In this case, the search string ("Moringa oleifera" OR malunggay OR "plant extract") AND ("copper nanoparticles" OR "zinc nanoparticles" OR "Cu-Zn nanoparticles" OR "bimettalic nanoparticles") AND ("green synthesis") AND ("Klebsiella pneumoniae (ATCC 13883)") AND ("antibacterial" OR "antimicrobial") was searched in Google Scholar, PubChem, MDPI, and PubMed. Figures 1, 1.1, and 1.2 show that the search string produced enough articles related to this study.

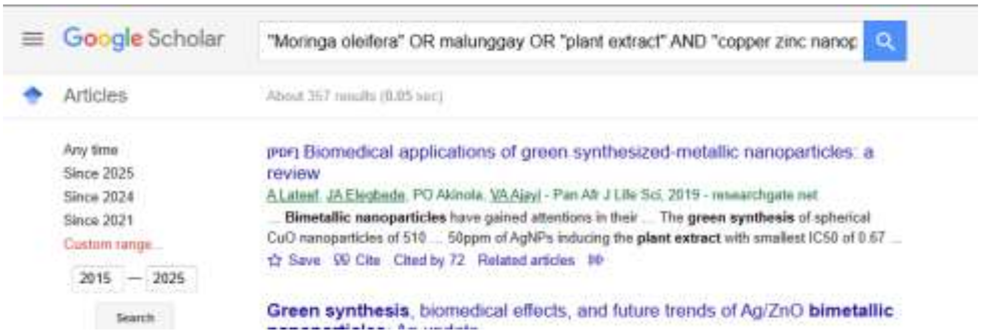


Figure 3. Search string result in Google Scholar

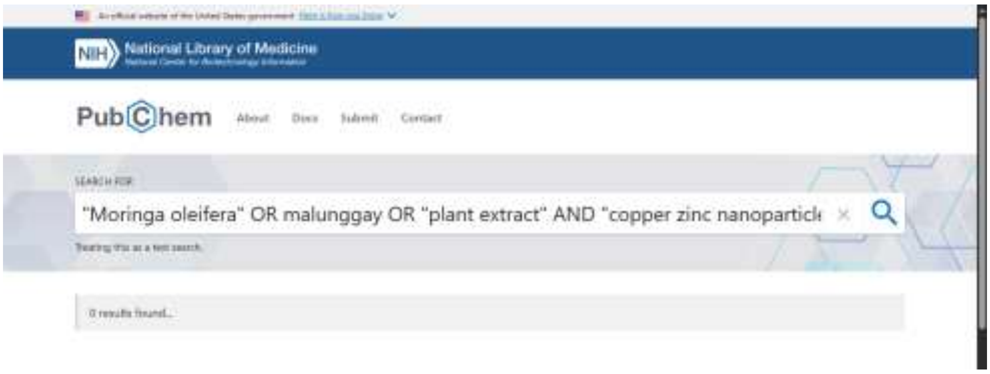


Figure 3.1 Search string result in PubChem

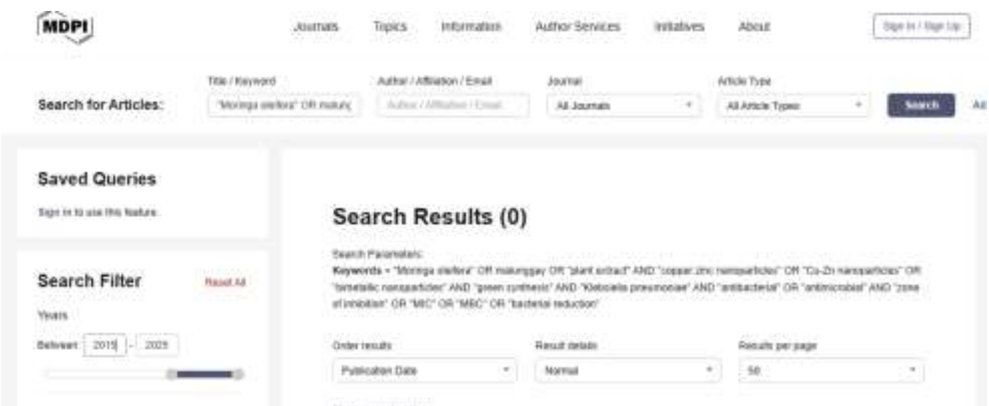


Figure 3.2 Search string result in MDPI

Figure 3.3 Search string result in PubMed

C. Investigation Strategy for Meta-Analysis

To ensure each search string is targeted and focused, the search terms are built based on the PICO research question. In the search strategy, the outcome would not be used as part of the search terms as it would hinder the database from retrieving eligible studies. To narrow down results, limiters and boolean operators were used. With the use of limiters, the search was filtered out 5 years back. With the use of boolean operators, these are the search strings generated: (“Moringa oleifera” OR malunggay OR “plant extract”) AND (“copper nanoparticles” OR “zinc nanoparticles” OR “Cu-Zn nanopartciles” OR “bimettalic nanoparticles”) AND (“green synthesis”) AND (“Klebsiella pneumoniae UPCC 1382 (ATCC 13883)”) AND (“antibacterial” OR “antimicrobial”) , ("Moringa oleifera" OR malunggay OR "plant extract") AND ("copper zinc nanoparticles" OR "Cu-Zn nanoparticles" OR "bimetallic nanoparticles") AND ("green synthesis") AND ("Klebsiella pneumoniae UPCC 1382 (ATCC 13883)") AND ("antibacterial" OR "antimicrobial") AND ("zone of inhibition" OR "MIC" OR "MBC" OR "bacterial reduction"). Since after using the previous earch strings, the search results were too broad, the researchers narrowed it down by using these search strings: “Green Synthesis” AND “copper nanoparticles”, “Green Synthesis” AND (“copper nanoparticles OR “zinc nanoparticles” OR “Cu-Zn nanopartcicles” OR “bimetallic nanoparticles”), “Green Synthesis” AND (“zinc nanoparticles”), “Plant extract” AND “copper nanoparticles”, “Plant extract” AND “zinc nanoparticles”, (“Green-synthesis” OR “plant extract”) AND (“copper nanoparticles” OR “zinc nanoparticles” OR “Copper-Zinc nanoparticles” AND “antibacterial activity” (“Green-synthesis” OR “plant extract”) AND (“copper nanoparticles” OR “zinc nanoparticles” OR “Copper-Zinc nanoparticles” AND "antimicrobial activity”.

D. Inclusion and Exclusion Criteria

For the inclusion and exclusion criteria, articles were included if (a) they discuss a process of nanoparticles in inhibiting the growth of any multidrug-resistant microorganism; (b) they present information on the effectivity of bimetallic nanoparticles against any multidrug-resistant microorganism; (c) they provide results of SEM and EDX Analysis of particular nanoparticles; (d) they are in English language; (e) they are original and peer-reviewed, and (f) the full-text version of the articles could be sourced; (g) experimental studies (in-vitro or in-vivo) on Cu, Zn, or Cu-Zn nanoparticles synthesized from plant extracts; (h)provides quantitative data (mean, SD, sample size).

On the contrary, articles were excluded if (a) they are not related to nanoparticles as antimicrobials; (b) they do not supply any information regarding size and morphology, elemental composition, green synthesis, and antimicrobial activity ; (c) they discuss the rate of effectivity based on statistical analyses

only and not with experimentation; and (d) they are gray literature, like theses, conferences, and review papers; (e) Non-plant-based synthesis; (f) the study was published beyond five years back.
The researchers used the developed criterion while stating certain "identifiers" from the manuscript to choose which articles were evaluated in this study. Table1 shows a checklist that was modified by Porrit et al. (2014), wherein these criteria served as a reference in determining which studies to include and which to leave out. The researchers applied the inclusion and exclusion criteria to systematically identify relevant studies.

Table 1.
Criteria and Identifiers for the Selection of Studies

Criteria	**Details**	**Mark**
Date	Studies published five years back will only be included	✓
Language	Articles in the English Language only	✓
Peer-review	Only peer-reviewed studies should be included	✓
Setting	Information on the size and morphology, elemental composition, green synthesis, and antimicrobial/antibacterial activity of a particular nanoparticle is provided	✓
Methodology	The study will only include researches that are relevant to the objectives of this study	✓
Publication	Only articles found in the following databases should be reviewed for this study: ResearchGate, WIPO Patentscope, Google Scholar, Science Direct, PubMed, Frontiers, and MDPI.	✓

E. Screening of Studies
After confirming the feasibility of a meta-analysis through a preliminary search, the researchers conducted a manual search across seven databases. According to the Assessment of Multiple Systematic Reviews (AMSTAR) guidelines, at least two databases must be searched for a meta-analysis. However, to obtain more accurate and comprehensive results, additional databases were included. Using the formulated search strings and the selected databases, the researchers listed the retrieved articles in an Excel sheet. These articles were then subjected to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) flow diagram process. The PRISMA flow chart consists of four major levels of screening. The first level, Identification, accounts for records removed before screening due to duplication or incomplete data. The second level, Screening, requires researchers to examine the title and abstract of each article to assess the study's title, year of publication, and author(s). The third level, Eligibility, involves applying the inclusion and exclusion criteria to evaluate the appropriateness of each study. At this stage, researchers must also document the reasons for excluding any articles. Finally, the included level comprises the

articles that met all inclusion criteria and were deemed suitable food meta-analysis.

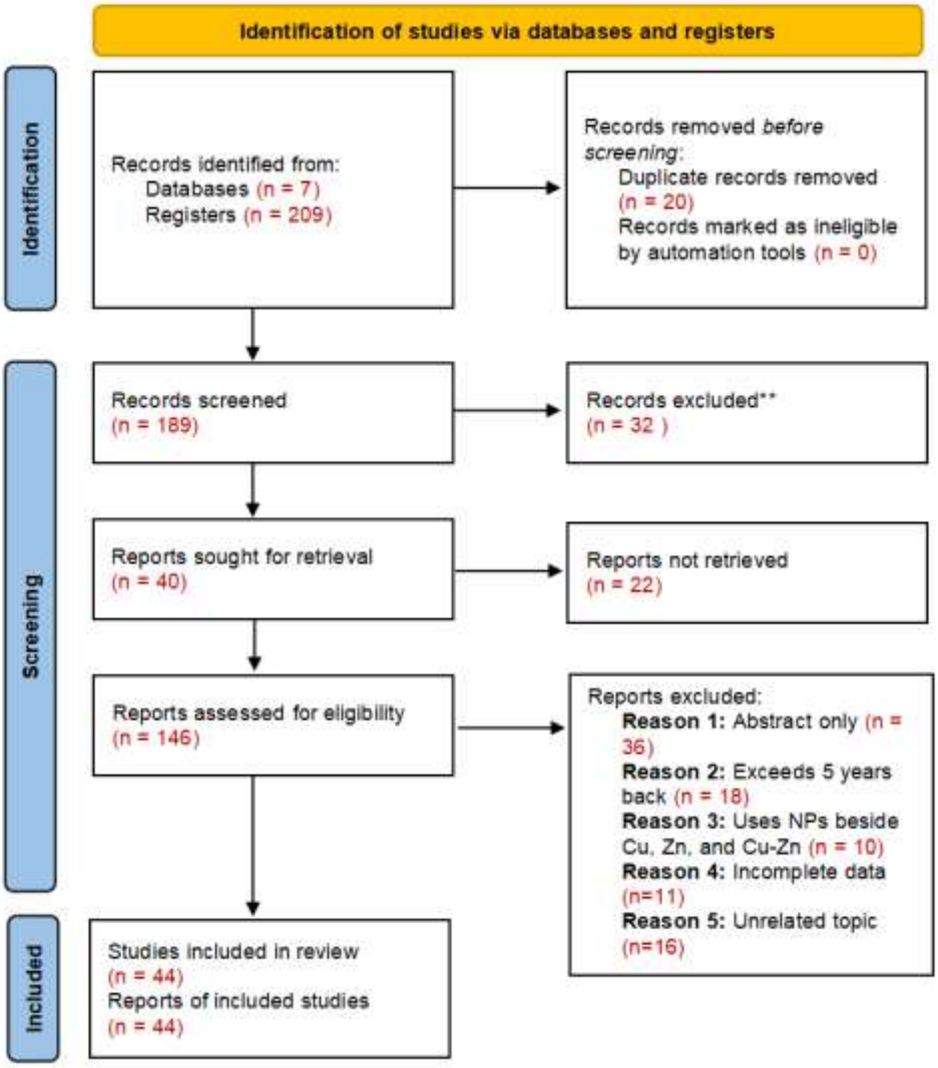


Figure 4. PRISMA 2020 Flow Diagram

For the meta-analysis of the green synthesis of Cu-Zn nanoparticles, 189 studies were initially screened, and 44 studies met the inclusion criteria. In the current study, Cu-Zn nanoparticles were synthesized using fresh *Moringa oleifera* leaves as the reducing and stabilizing agent, with copper sulfate ($CuSO_4 \cdot 5H_2O$) and zinc sulfate ($ZnSO_4 \cdot 7H_2O$) as metal precursors. The leaves were first washed thoroughly with distilled water to remove dust and impurities. The aqueous extract was prepared by boiling 100g of the chopped leaves in 100 mL of distilled water for 30 minutes. The resulting extract was cooled to room temperature and used directly for nanoparticle synthesis

F. Data Extraction

From all the 44 studies these following information were all recorded and summarized in a table: average size (nm) of the green-synthesized Cu, Zn, or Cu-Zn nanoparticles, number of trials, mean of the zone of inhibition (ZOI) (mm), and the standard deviation.

Table 2.

Summary of the Data Extracted for the Antibacterial Activity of Cu-Zn Nanoparticles

Bacteria	Average size (nm)	Number of Trials	Mean of the ZOI (mm)	Standard Deviation	Author/s	Year	Type of NPs
E.coli	50	3	21	1	Jamshidi &Sazegar	2020	Zn
S.aureus	50	3	23	1	Jamshidi &Sazegar	2020	Zn
S.mutans	32	3	20		Khatak et.al.	2020	Cu-Zn
S.aureus	32	3	21		Khatak et.al.	2020	Cu-Zn
B.substilis	32	3	28.33		Khatak et.al.	2020	Cu-Zn
E.coli	32	3	26.33		Khatak et.al.	2020	Cu-Zn
P.aeuriginosa	32	3	27		Khatak et.al.	2020	Cu-Zn
B.substilis	20.3	3	13		Teklu et al.	2023	Cu
E.faecalis	20.3	3	8		Teklu et al.	2023	Cu
E.coli	20.3	3			Teklu et al.	2023	Cu
V.cholera	20.3	3			Teklu et al.	2023	Cu
P.aeuriginosa		3	17.0	4.24	Ifeoluwa Peter Oyekunle et. al	2022	Cu
S.aureus		3	8.0	2.83	Ifeoluwa Peter Oyekunle et. al	2022	Cu
S.typhi		3	6.0	2.83	Ifeoluwa Peter Oyekunle et. al	2022	Cu
E.coli		3	8.0	2.83	Ifeoluwa Peter Oyekunle et. al	2022	Cu
Shigella sp.		3	16	5.66	Ifeoluwa Peter Oyekunle et. al	2022	Cu
E.coli	28	3	25	1	Amer & Awwad	2021	Cu
S.aureus	28	3	20	0.8	Amer & Awwad	2021	u
S.aureus (25 µg/ml)	7.38	3	10	1.1	K.E. Alsamhary	2023	Cu
S. aureus (100 µg/ml)	7.38	3	13	0.9	K.E. Alsamhary	2023	Cu
E.faecalis	7.38	3	13	0.8	K.E. Alsamhary	2023	Cu
E.coli	7.38	3	15	0.7	K.E. Alsamhary	2023	Cu
K.pneumonniae (25 µg/ml)	7.38	3	12	1.2	K.E. Alsamhary	2023	Cu
K.pneumonniae (100 µg/ml)	7.38	3	14	0.9	K.E. Alsamhary	2023	Cu
K.species		14	14.67	2.31	Vazhacharickal & Krishna	2022 Mungbean (Vigna radiata) & braased exudate	Cu
S.typhi		14	21.67	6.66	Vazhacharickal & Krishna	2022 Mungbean (Vigna radiata) & braased exudate	Cu
P.aeruginosa		14	19	3.61	Vazhacharic	2022 Mungbean (	Cu

					kal & Krishna	Vigna radiata) 6 hrsseed exudate	
E.coli		13	20	4.58	Vazhacharickal & Krishna	2022 Mungbean (Vigna radiata) 6 hrsseed exudate	Cu
S.aureus		14	25.33	7.02	Vazhacharickal & Krishna	2022 Mungbean (Vigna radiata) 6 hrsseed exudate	Cu
K.species		12	24.33	2.08	Vazhacharickal & Krishna	2022 Mungbean (Vigna radiata) 6 hrsseed exudate	Zn
S.typhi		14	23	2.65	Vazhacharickal & Krishna	2022 Mungbean (Vigna radiata) 6 hrsseed exudate	Zn
P.aeruginosa		14	18.67	2.31	Vazhacharickal & Krishna	2022 Mungbean (Vigna radiata) 6 hrsseed exudate	Zn
E.coli		13	24.67	3.51	Vazhacharickal & Krishna	2022 Mungbean (Vigna radiata) 6 hrsseed exudate	Zn
S.aureus		14	30.00	6.00	Vazhacharickal & Krishna	2022 Mungbean (Vigna radiata) 6 hrsseed exudate	Zn
S.aureus	25	3	26.53		Albrahim et	2025	ZnCl2
					al.		
B.subtilis	25	3	27.93		Albrahim et al.	2025	ZnCl2
E.coli	25	3	27.6		Albrahim et al.	2025	ZnCl2
K.pneumoniae	25	3	15.9		Albrahim et al.	2025	ZnCl2
E.faecallis	25	3	14.9		Albrahim et al.	2025	ZnCl2
MRSA	25	3	No Inhibition		Albrahim et al.	2025	ZnCl2
P.aeruginosa	25	3	No Inhibition		Albrahim et al.	2025	ZnCl2
K.species		13	3	2	Vazhacharickal & Krishna	2022 Cowpea (Vigna unguiculata) 6 hrsseed exudates	Cu
S.typhi		11	26	1.53	Vazhacharickal & Krishna	2022 Cowpea (Vigna unguiculata) 6 hrsseed exudates	Cu
P.aeruginosa		15	12.33	1.53	Vazhacharickal & Krishna	2022 Cowpea (Vigna unguiculata) 6 hrsseed exudates	Cu
E.coli		13	22.67	4.62	Vazhacharickal & Krishna	2022 Cowpea (Vigna unguiculata) 6 hrsseed exudates	Cu
S.aureus		13	27.67	7.21	Vazhacharickal & Krishna	2022 Cowpea (Vigna unguiculata)	Cu

						6 hrsseed exudates	
K.species		13	21.67	2.52	Vazhacharickal & Krishna	2022 Cowpea (Vigna unguiculata) 6 hrsseed exudates	Zn
S.typhi		12	21	4.58	Vazhacharickal & Krishna	2022 Cowpea (Vigna unguiculata) 6 hrsseed exudates	Zn
P.aeruginosa		15	21	4.58	Vazhacharickal & Krishna	2022 Cowpea (Vigna unguiculata) 6 hrsseed exudates	Zn
E.coli		13	24.33	5.51	Vazhacharickal & Krishna	2022 Cowpea (Vigna unguiculata) 6 hrsseed exudates	Zn
S.aureus		13	28	7.21	Vazhacharickal & Krishna	2022 Cowpea (Vigna unguiculata) 6 hrsseed exudates	Zn

G. Statistical Analysis

Data extracted from the 44 studies were analyzed using OpenMetaAnalyst software. We calculated standardized mean differences (SMD; Cohen's d) as the effect size metric because the included studies employed diverse measurement methods to assess antibacterial activity despite sharing the same research objective. Given the anticipated heterogeneity across studies (e.g., variations in bacterial strains, concentrations, and methodologies), we employed a random-effects model using the DerSimonian-Laird method to pool effect sizes.

The meta-analysis revealed a significant pooled standardized mean difference (SMD = 3.71, 95% CI [2.35, 5.07], $p < .001$), indicating that green-synthesized nanoparticles exerted a strong, statistically significant antibacterial effect compared with controls. The confidence interval excluded zero, reinforcing the robustness of this finding.

However, the analysis demonstrated substantial heterogeneity ($I^2 = 72.87\%$, $Q = 51.60$, $p < .001$), suggesting considerable variability across included studies. Such heterogeneity likely arises from differences in study design, bacterial strains tested (e.g., Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae UPCC 1382 (ATCC 13883)), intervention concentrations (25 µg/mL vs. 100 µg/mL), and methodological inconsistencies across publication years (2020–2023). This variability necessitates cautious interpretation of the pooled results, as effect sizes were not uniform across all contexts.

At the individual study level, several estimates exhibited wide confidence intervals, reflecting variability in sample sizes or effect magnitudes. Notably, studies examining E. coli (2021) and S. aureus (2021) reported extremely large mean differences, substantially influencing the pooled effect size. Conversely, studies with narrower confidence intervals (e.g., S. aureus at 25 µg/mL and 100 µg/mL, 2023) provided more precise estimates that remained consistent with the overall significant effect.

The forest plot indicated that most included studies demonstrated effect sizes favoring the intervention, supporting the overall significant result. Despite substantial heterogeneity, the consistently positive direction of effects suggests that green-synthesized nanoparticles generally produce beneficial antimicrobial outcomes across multiple pathogens and experimental conditions.

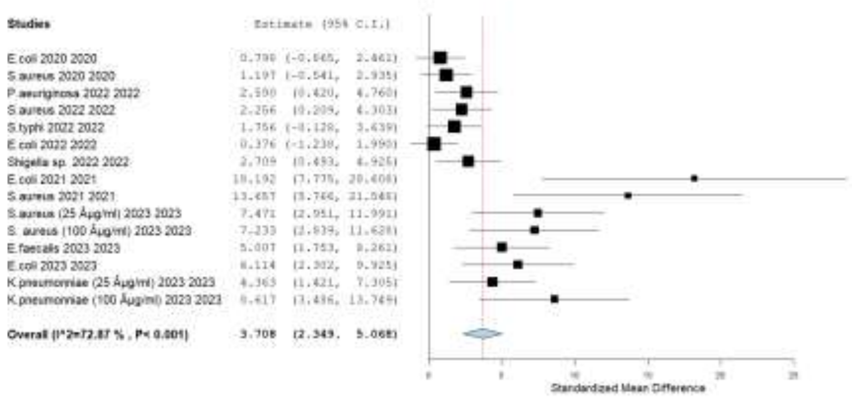


Figure 5. Forest Plot of Standardized Mean Difference Metric using Dersimonian-Laird Method - Open Meta Analysis

The results of the meta-analysis, using a continuous random-effects model, revealed a significant pooled standardized mean difference (SMD = 3.708, 95% CI: 2.349–5.068, $p < 0.001$). This indicates that, overall, the intervention under the used studies have a strong and statistically significant effect compared with the control or baseline.

H. Interpretation of Results

These findings support the potential application of green-synthesized Cu, Zn, and Cu-Zn nanoparticles as effective antimicrobial agents. Nevertheless, the observed heterogeneity warrants further investigation through subgroup analyses or meta-regression to identify potential moderators. Factors such as publication year, bacterial species, dosage concentration, and methodological differences should be examined to better understand sources of variability in effect sizes.

Phase 2: Experimental

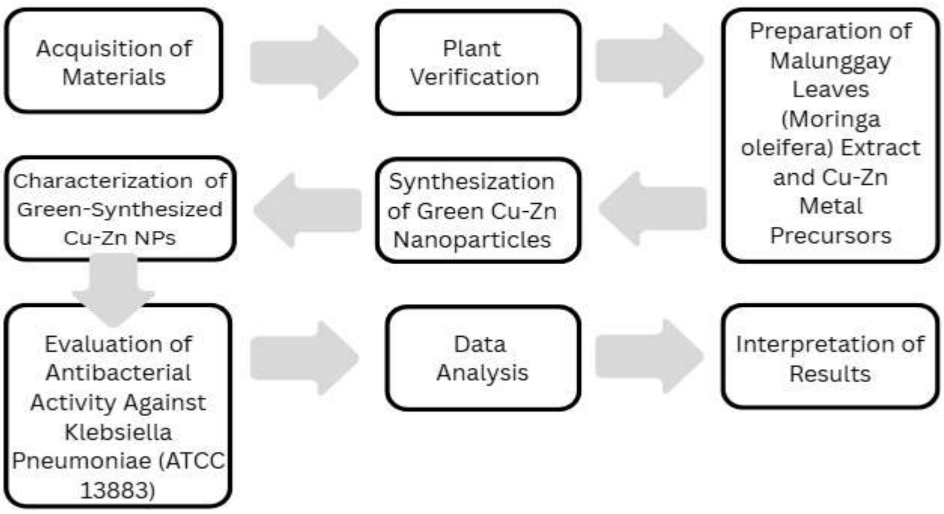


Figure 6. Methodological Framework

A. Materials and Reagents

Laboratory materials, including Erlenmeyer flasks, a beaker, a watch glass, and a hot plate, were borrowed from the junior high school chemistry laboratory of Manila Science High School. Additional materials, including a stirring rod, laboratory tongs, a funnel, latex gloves, and four 50 mL bottles, were purchased from PulJed's Laboratory Store in Bambang, Manila. The metal salt precursors, copper sulfate ($CuSO_4$) and zinc chloride ($ZnCl_2$), were also purchased from the same laboratory store. Malunggay (*Moringa oleifera Lam.*) leaves were collected from the researcher's residence in San Pedro, Laguna. Other materials, including distilled water, qualitative filter paper, and ethyl alcohol, were purchased from a local superstore and national bookstore in San Pedro, Laguna.

B. Preparation of Extracts and Solutions

Prior to use in the experiment, the Malunggay (Moringa oleifera Lam.) leaves were submitted online to the Bureau of Plant Industry for verification. Identification was confirmed through morphological characteristics. Following the acquisition of all required materials, the aqueous leaf extract and metal precursor solution were prepared. Following the method described by Rivero (2019), 100 g of fresh leaves, with stems removed, were rinsed with distilled water. Two hundred mL of distilled water were heated in a 250 mL beaker on a hot plate until the temperature reached 80 °C. The 100 g of Malunggay (*Moringa oleifera Lam.*) leaves were then added and maintained at 80 °C for 20 min. The mixture was stirred occasionally to ensure thorough extraction of phytochemicals.

After heating, the mixture was allowed to cool for 10 min and was subsequently filtered using qualitative filter paper to separate the aqueous extract from the leaf residues. The filtrate was stored at room temperature until further use. The metal precursor solution was prepared by dissolving 25 mL each of copper sulfate ($CuSO_4$) and zinc chloride ($ZnCl_2$) in 100 mL of distilled water in a 250 mL beaker. The solution was stirred continuously using a glass stirring rod for 30 min and then allowed to stand for 40 min to ensure homogeneity.

C. Synthesis of Cu-Zn Nanoparticles

Adapting the ratio described by Staples et al. (2023), three treatment variations were prepared using different ratios of metal precursor solution to leaf extract. These consisted of a 1:1 ratio, combining 12 mL of leaf extract and 12 mL of metal precursor solution; a 3:1 ratio, combining 12 mL of leaf extract and 36 mL of metal precursor solution; and a 1:3 ratio, combining 36 mL of leaf extract and 12 mL of metal precursor solution.

Each mixture was subjected to a water bath at 80 °C for 18 min. During heating, a color change and partial coagulation were observed. The solutions were then allowed to cool at room temperature until visible particle formation occurred at the bottom of the flasks.

Figure 7. Variations in the experimental group, negative control, and positive control

D. Characterization of Green-synthesized of Cu-Zn Nanoparticles

The three aqueous variations were submitted for drying and analysis via scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDX) at De La Salle University–Manila's iNano Research Facility. SEM analysis was employed to determine particle size and morphology, while EDX was used to ascertain elemental composition. The same three variations, along with a control sample of 36 mL malunggay extract, were submitted to the University of the Philippines–Diliman Natural Sciences Research Institute (NSRI) to evaluate antibacterial activity against Klebsiella pneumoniae UPCC 1382 (ATCC 13883) (ATCC 13883).

NSRI conducted the culturing of *Klebsiella pneumoniae UPCC 1382 (ATCC 13883)* (ATCC 13883) and applied the three treatment variations and one control group provided by the researchers, along with an additional positive control group administered by NSRI, to the cultured bacteria. From their sent report, the bacterial suspension was generated from a culture that had grown for 18 to 24 hours, with 0.1% peptone water serving as the suspension medium. Commercially prepared Mueller Hinton Agar plates, with a depth of roughly 3 mm, served as the growth medium for bacterial seeding. Inoculation was performed by sweeping the agar surface with a culture-laden swab. A sterile cotton swab affixed to a wooden stick was submerged in the bacterial mixture and twirled repeatedly. To eliminate surplus liquid, the swab was pressed and twirled forcefully along the interior of the tube wall positioned above the suspension. The treated swab was then drawn across the agar in an even pattern. This sweeping action was duplicated twice more, with each repetition involving a 60° turn of the plate to promote even bacterial spread across the surface. Using a cork borer, three evenly spaced cavities measuring 10 mm across were punched into the solidified agar. Two hundred microliters of test substance were transferred into every cavity. All seeded MHA plates underwent incubation at 35 °C for one day. Upon completion of the incubation period, clear zones surrounding the wells where bacterial growth had been prevented were quantified in millimeters.

E. Statistical Analysis

A one-way analysis of variance (ANOVA), also known as the F-test, was conducted to determine statistically significant differences in antimicrobial activity among the groups. The F-statistic was calculated as follows:

$$F = \frac{mean square for treatments (MST)}{mean square for errors (MSE)}$$

where F is based on degrees of freedom of (k − 1) for treatments and (n − k) for errors

with k representing the number of groups and n the total sample size. Following significant ANOVA results, Tukey's honestly significant difference (HSD) post hoc test was employed to identify which specific means differed significantly.

F. Risk and Safety

The researchers handled the laboratory equipment and chemical

IV. Discussions

A. Green-Synthesized of the Cu-Zn Nanoparticles

Three samples of Cu-Zn Nanoparticles were prepared with different ratios of Cu-Zn using Green synthesis. The metals were obtained from Copper Sulfate and Zinc Chloride. On the other hand, Malunggay leaf extract (Moringa Oleifera) was used as natural reducing agent to convert the metal into nanoparticles (specifically Cu-Zn nanoparticles). Three samples were sent for testing with the ratio of copper to zinc V1 (1:1), V2 (1:3), & V3 (3:1) to examine the effect of the ratio of the copper to the zinc on the nanoparticles.

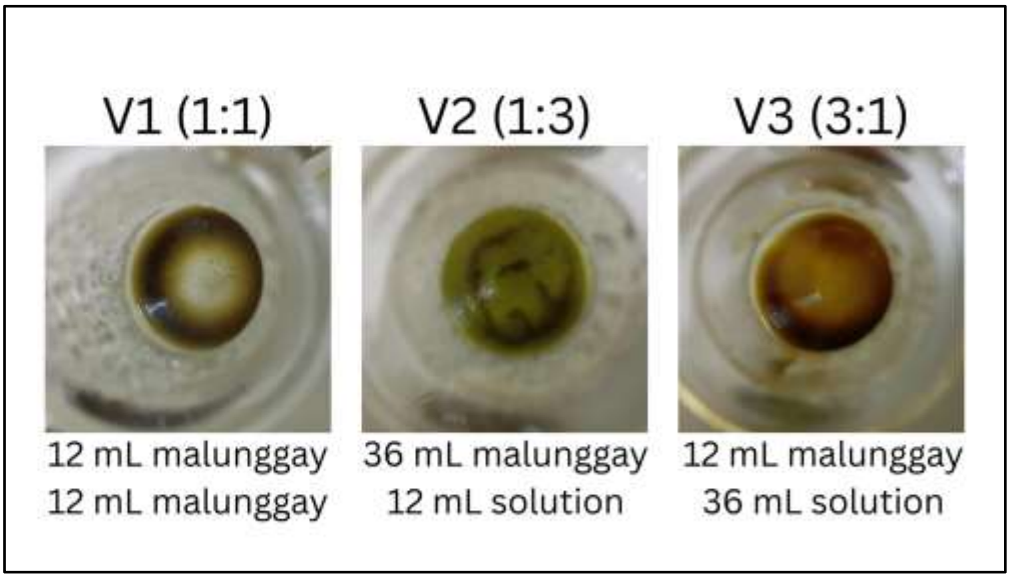


Figure 8. Variations after subjecting to bathwater in 80°C

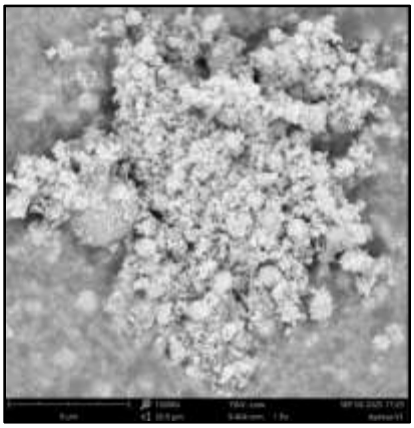
As can be seen in the figure above, V1(1:1) formed a dark brown 'product' along the sides of the flask. V2 (1:3) turned into a light green 'product' with some uneven patches. Lastly, V3 (3:1) produced a brownish-green 'product' that looked more solid but had a smoother surface.

B. Analysis on SEM Results

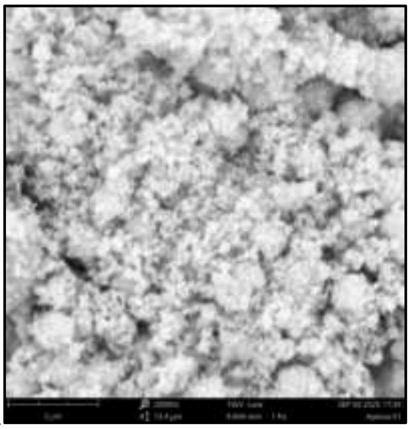
The morphological characteristics of the resulting nanoparticles produced by green synthesis method show significant variation (as seen by SEM images) per the combination of various composition of the mixtures used for Cu/Zn particle synthesis as well as the relative amount was made from either Cu or Zn during synthesis. Sample V1 (Cu:Zn in a ratio of 1:1) produced highly clustered and agglomerated particles with a bulky texture, resulting in irregularly shaped, densely packed, and strongly agglomerated particles. The SEM images of sample V2 (Cu:Zn in a ratio of 1:3) had a relatively smoother appearance with slight aggregations and more dispersed and less dense agglomerated particles compared to sample V1. Additionally, the morphology of sample V3 showed a much smoother and more solid appearance signifying huge aggregated structures due to the higher concentration of Copper (Cu) used compared to the other samples (1:1 and 1:3). It can be deduced that synthesis at a varying concentration of Cu:Zn ratio yielded nanoparticles with drastically different morphological appearances, especially with respect to agglomeration characteristics and surface properties.

Figures 9.1 & 9.2

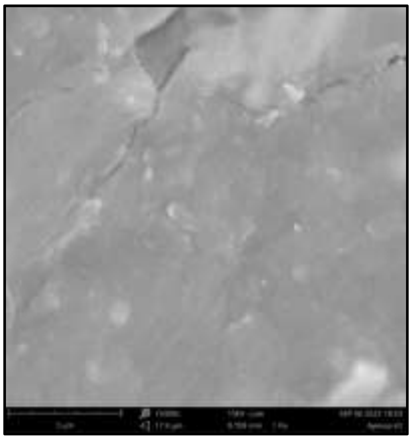
SEM images of the Green-Synthesized Cu-Zn Nanoparticles in various magnifications



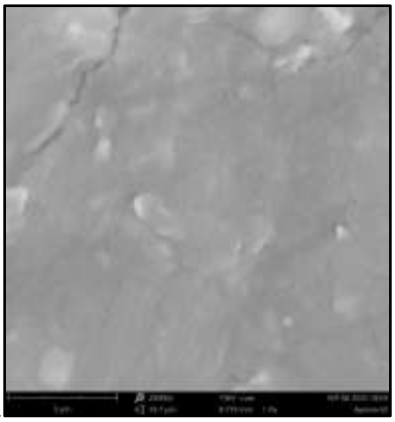
a. 1



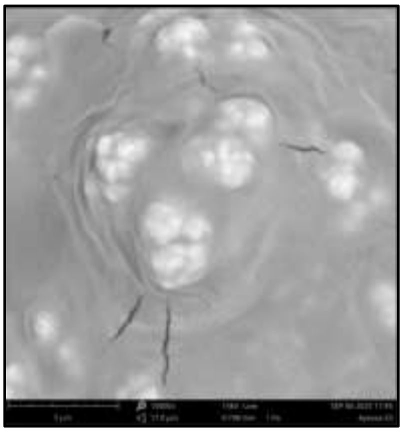
a. 2



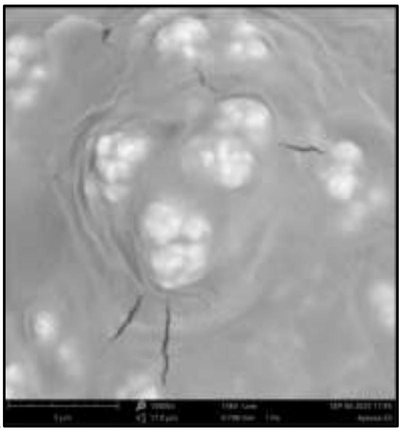
b. 1



a. 2



b. 1



c. 2

C. Analysis on EDX Results

Sample V1 (1:1)

Figure 10.1 shows that, according to EDX analyses, the dominant element in the material is zinc (84.27%), followed by oxygen (15.44%), and only a trace of copper (0.29%). The high level of zinc indicates that it has undergone more successful reduction and incorporation into the nanoparticles than copper. The presence of oxygen also implies that there may be oxides (ZnO) formed. The extremely low percentage of copper suggests that there was very little copper incorporated at this relative ratio.

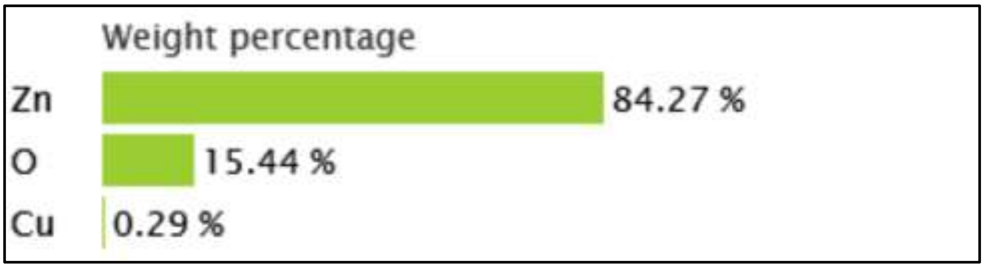


Figure 10.1. EDX Analysis of the Elemental Composition of the Cu-Zn Nanoparticles for V1.

Sample V2 (1:3)

EDX analysis results shown in another table (see Figure 10.2) demonstrate that oxygen (50.10%) has been identified as the most significant component of the sample, which is accompanied by other elements such as carbon (36.32%), calcium (6.88%), potassium (4.45%), zinc (1.63%), and copper (0.62%). The high percentage of both oxygen and carbon in combination with organic compounds suggests that the final result is greatly influenced by malunggay leaf extracts; however, since both of these elements are present in some amount as chemical components in the extract, it may be concluded that the calcium and potassium associated with this material possibly originated from these chemical sources. Also, the low percentages of both zinc and copper indicate that there are few metal nanoparticles associated with these elements, and therefore, nanoparticle metal formation will have occurred to a lesser extent than with the oxygen and carbon.

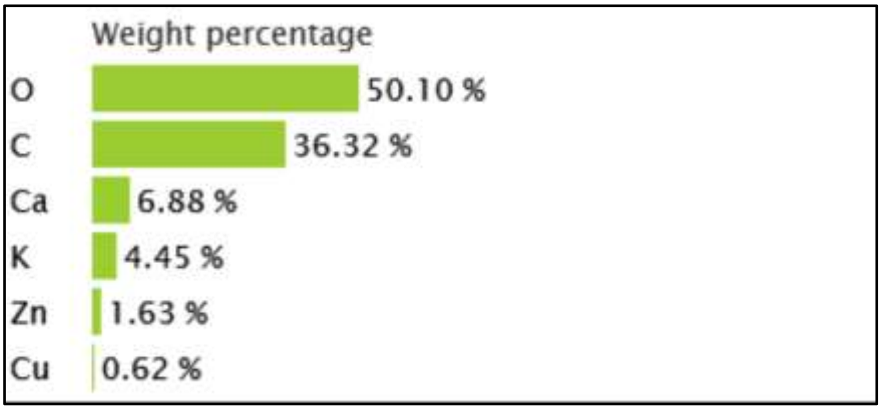


Figure 10.2. EDX Analysis of the Elemental Composition of the Cu-Zn Nanoparticles for V2.

Sample V3 (3:1)

As shown in the EDX analysis results for Sample V3 (Figure 10.3), it was composed mostly of the element oxygen (33.69%), then carbon (28.11%), copper (23.60%), and zinc (14.61%). The high composition of oxygen suggests that during the green synthesis, metal oxides may have formed (e.g., CuO or ZnO). The carbon seen can be attributed to the organic compounds in the malunggay leaf extract used as a reducing and stabilizing agent in synthesizing CuZnO materials. Lastly, although copper is present in a higher composition (23.60%) compared to previous samples, this increase is to be expected because this sample was synthesized with a much greater precursor ratio of copper (3:1 Cu:Zn) to encourage copper to be incorporated into the nanoparticles. Similarly, zinc is present in a lesser composition (14.61%) compared to copper, but it is still present at a sufficient composition based on the intentional increase of copper in this sample.In summary, the elemental analysis from Sample V3 shows that changing the Cu:Zn precursor ratio has a strong effect on the resulting chemical composition of the nanoparticles formed during synthesis (i.e., increasing the copper precursor ratio increases the copper composition of the nanoparticles).

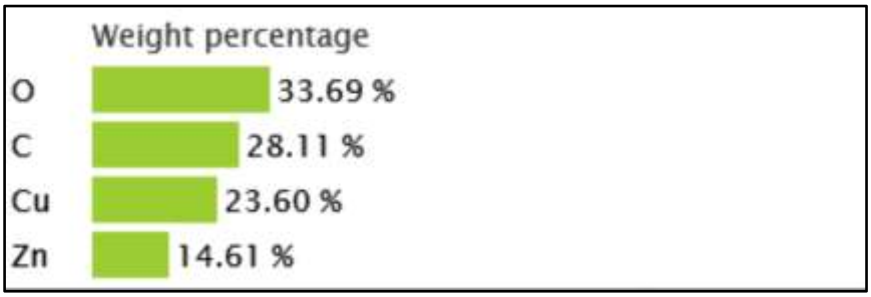


Figure 10.3. EDX Analysis of the Elemental Composition of the Cu-Zn Nanoparticles for V3.

D. Discussion on the Antibacterial Activity of CuZn Nanoparticles

From 12 studies in this category, 12 studies of 93 studies that are manually searched, have studied the antibacterial activity of Cu-Zn NPs against gram positive and gram negative bacteria.

Cu-NPs were synthesized using aqueous sumaq (Rhus coriaria L.) (Ismail, 2020). Fruit extract as a stabilizing agent and hydrazine hydrate as a reducing agent. TEM, FTIR, XRD and UV–visible were used to characterize the synthesized Cu-NPs. An absorption peak was observed at 50 nm. TEM characterized the particles as spherical in shape with a diameter in the range 22–27 nm. The mean crystallite size is 18nm, hence it was considered as nanosized (100 > size > 1nm). Cu-NPs are found to possess antibacterial activity against gram positive and gram negative bacteria E. coli, P. aeruginosa, B. cereus and S. aureus.

A comparable research was done recently by (Teklu et al., 2023) where CuO NPs were prepared by Balanites aegyptiaca stem bark extract and its antibacterial properties were tested against gram positive and gram negative bacteria B. subtilis, E. faecalis, E.coli and V. cholerae. CuO-NPs showed maximum zone of inhibition of 13mm against B. subtilis and 8mm against E. faecalis of gram positive bacterial strains at the concentration of 100 µg/mL. Other antibacterial activities of zinc based NPs have also been investigated.

Jamshidi and Sazegar (2020) investigated the activity of Zn-MSN nanoparticles and CGZM-bio suspensions against E. coli and S. aureus by using disk diffusion in MH agar to determine the MIC and Minimum Bactericidal Concentration (MBC) values. An opposite correlation was found between the particle size and antibacterial activity. The disk concentrations used were 5, 10 and 100 µg/mL. The MIC value for Zn-MSN and CGZM-bio against E. coli were found to be 10 µg/mL and 5 µg/mL, respectively. Moreover, both materials tested had an MIC of 10 µg/mL against S. aureus. Preparation and antibacterial activity of Cu nanoparticles using Citrus limon fruit extract.

Amer and Awwad (2021) reported CuNPs green synthesis by Citrus limon fruit extract and its antibacterial activity against E. coli (ATCC 25922) and S. aureus (ATCC 29213). UV-Vis spectroscopy (absorption peak at 579 nm), FTIR (functional groups have participated for the stabilization of CuNPs), XRD (peaks at 111, 200, 220 were seen indicating the crystalline structure of CuNPs) and SEM/TEM (average size of spherical CuNPs 18-28 and the range 5-28 nm) analysis confirmed the formation of nanoparticles. The antibacterial activity of CuNPs was evaluated by agar well diffusion. The inhibition zones were found to be 25 and 20 mm against E. coli and S. aureus, respectively. Meanwhile, the Citrus limon extract on its own displayed weak activity (4.5 and 2.2 mm, respectively), and chloramphenicol displayed inhibition zone values of 20 and 22 mm, respectively. The results of the MIC test showed that E. coli was more sensitive to CuNPs than S. aureus, probably because the structure of bacterial cell walls is different. The authors concluded that Citrus limon extract was an effective medium for green synthesis of nanoscale CuNPs with strong antibacterial activity.

E. Statistical Analysis

The researchers applied a Standardized Mean Difference and Dersimonian-Laird Method

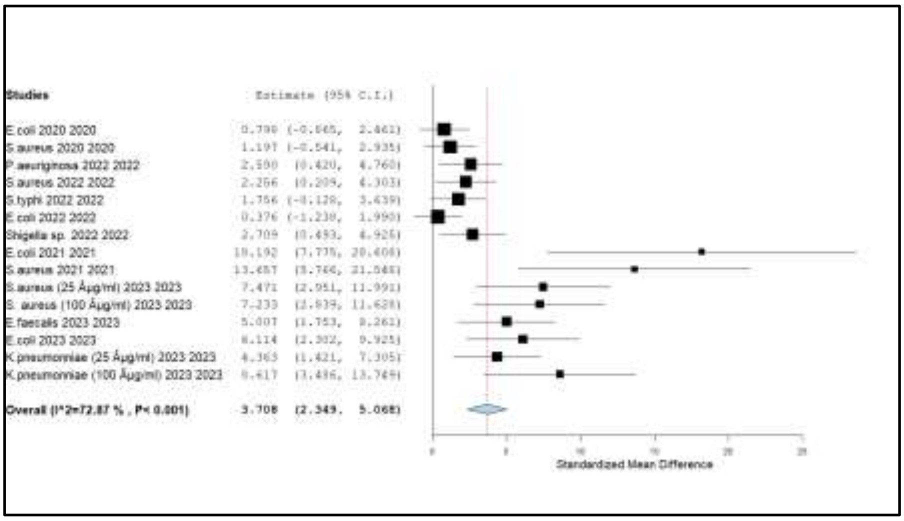


Figure 11. Forest Plot of Standardized Mean Difference Metric using Dersimonian-Laird Method - Open Meta Analyst

Meta-analysis of antibacterial activity was carried out, consisting of evaluation of antibacterial effects of nanosilver-containing wound protection lotion using in vitro and ex vivo methods. A continuous random-effects model was used to calculate the pooled effect. A total of 26 studies were identified, and 23 studies were included in the meta-analysis. The pooled standardized mean difference (SMD) was 3.708, 95%CI: 2.349–51.068, p< 0.001. Furthermore, heterogeneity was substantially high, I^2 = 72.87%, Q = 51.603, p< 0.001. The results of this meta-analysis showed that the average effect of the intervention was more than the average effect of control or baseline. This could be significant. However, there was a significant amount of heterogeneity between studies. In the present study, effects from studies with narrow CI's (i.e. S. aureus 25 µg/ml and 100 µg/ml, 2023) helped provide more accurate estimation and consistent significant effects. The direction of the effects also showed that most studies favoured the treatment/intervention (i.e. consistent significant effects). In other words, effects from studies included tended to be in the same direction. Considering these findings from the forest plot analysis, the tested treatment/intervention may have the potential to be applied as an antimicrobial treatment. Nonetheless, heterogeneity from the studies should be explored by performing subgroup analysis or meta-regression. For instance, effect size could be examined by using moderator variables such as year of publication, type of bacterial species, dosage concentration and study methodology.

F. Antimicrobial Activity of Malunggay-Mediated Cu–Zn Nanoparticles Against Klebsiella pneumoniae UPCC 1382 (ATCC 13883)

All antimicrobial assays in this study were carried out at the University of the Philippines Natural Sciences Research Institute (UP NSRI) Microbiological Research and Services Laboratory using the agar well diffusion method.The antimicrobial activity of malunggay *Moringa oleifera*–mediated Cu–Zn nanoparticle solutions was tested against *Klebsiella pneumoniae UPCC 1382 (ATCC 13883) UPCC 1382 ATCC 13883* by measuring zones of inhibition and computing the antimicrobial index AI.Results showed that Samples 1, 2, and 3 exhibited antimicrobial activity. Sample 4, which contained malunggay extract alone, showed no inhibition of bacterial growth.Among the nanoparticle formulations, Sample 3 with a 1:3 extract-to-metal precursor ratio showed the highest antimicrobial activity AI = 1.Samples 1 and 2 both recorded AIs of 1, while Sample 6 recorded a different value.This shows that raising the concentration of $ZnCl_2$ and $CuSO_4$ compared to the plant extract improved antibacterial effectiveness.The lack of activity in the malunggay-only extract suggests that the aqueous extract alone was insufficient to inhibit *K.pneumoniae.* This shows the plant extract serves mainly as a reducing and stabilizing agent in nanoparticle synthesis, rather than acting on its own as an antimicrobial.The positive control, chloramphenicol (30 µg), produced the highest AI (3).5), confirming the validity of the assay.

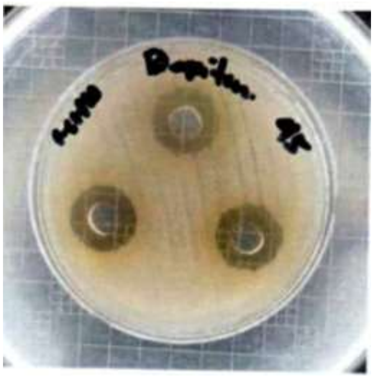
Table 3.
UP-NSRI Antibacterial Result

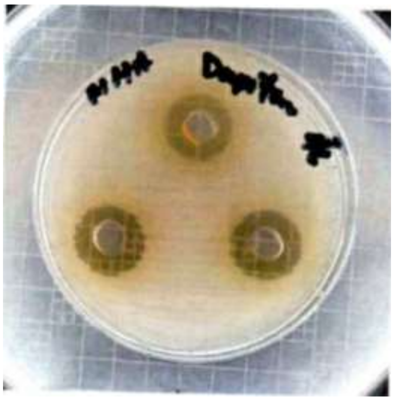
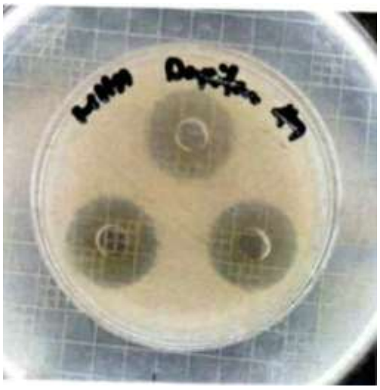
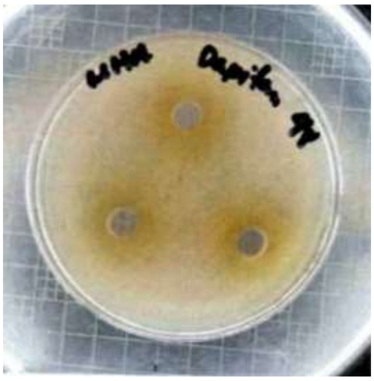
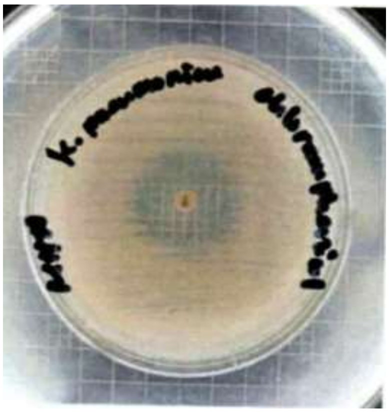
Test Organism	**Sample**	**Clearing Zone, mm**			**Mean of the Clearing Zone (mm)**	**Antimicrobial Index (AI)**
		1	**2**	**3**		
K. pneumoniae UPCC 1382 (ATCC 13883)	1. V1(1:1), - 12 mL of Malunggay extract, - 12 mL of Zinc Chloride and Copper Sulfate solution, - TOTAL: 24 mL Cu–Zn nanoparticle solution	20	21	20	20.33	1.0
	2. V2 (3:1), - 36 mL malunggay extract, - 12 mL Zinc Chloride and Copper Sulfate solution, - TOTAL: 48 mL Cu-Zn nanoparticles solution	20	20	19	19.67	1.0
	3. V3 (1:3), - 12 mL malunggay extract, - 36 mL Zinc Chloride and Copper Sulfate	27	26	26	26.33	1.6

	solution, - TOTAL: 48 mL Cu-Zn nanoparticles solution					
	4. Malunggay Extract (36 mL), - 100 g of malunggay leaves boiled in 200 mL of distilled water at 80 degrees Celsius	-[b]	-	-	0	0
	Chloramphenicol, 30 µg	27				3.5

*Results were from UP-Diliman NSRI
Test Method: Agar Well Method
Legend:
[a] - ATCC derivative
[b] - No clearing zone; no inhibition of growth of test organism
[c] Antibiotic disc (6 mm diameter)

Table 4
Results of the Antibacterial Assay of Samples 1–3

Test Organism	Sample	
K. pneumoniae UPCC 1382 (ATCC 13883)	1. V1(1:1), - 12 mL of Malunggay extract, - 12 mL of Zinc Chloride and Copper Sulfate solution, - TOTAL: 24 mL Cu–Zn nanoparticle solution	

	2. V2 (3:1), - 36 mL malunggay extract, - 12 mL Zinc Chloride and Copper Sulfate solution, - TOTAL: 48 mL Cu-Zn nanoparticles solution	
	3. V3 (1:3), - 12 mL malunggay extract, - 36 mL Zinc Chloride and Copper Sulfate solution, - TOTAL: 48 mL Cu-Zn nanoparticles solution	
	4. Malunggay Extract (36 mL), - 100 g of malunggay leaves boiled in 200 mL of distilled water at 80 degrees Celsius	
	Chloramphenicol, 30 µg	

The results demonstrated that the samples with Cu-Zn nanoparticles (V1, V2, and V3) exhibited measurable inhibitory effects against *K.pneumoniae* UPCC 1382 (ATCC13883), while the malunggay

extract alone produced no observable zone of inhibition. The findings indicate that the antimicrobial activity observed is primarily attributable to the Cu-Zn nanoparticles rather than the plant extract itself.
Among the tested formulations, V3 (1:3 ratio of malunggay extract to metal salt solution) produced the largest zones of inhibition (27, 26, and 26 mm) and the highest antimicrobial index (AI = 1.6). In contrast, V1 (1:1) and V2 (3:1) showed smaller and relatively similar clearing zones (~19–21 mm) with identical antimicrobial indices (AI = 1.0). When compared with the positive control, chloramphenicol (30 µg), V3 displayed a comparable zone of inhibition (~26–27 mm).

H. Data Analysis

Analysis of Variance (ANOVA) or F-test was employed to determine the statistically significant difference in the antimicrobial index between the experimental setups and controlled groups.

Table 5.
ANOVA Table

Source of Variation	SS	df	MS	F	P-value
Between Groups	1441.333		360.3333	1801.6683	3.064e-14
Within Groups	2	10	0.2		
Total	1443.333	14	103.0952		

The null hypothesis, which posited no difference in antibacterial activity across treatment groups, was rejected based on the one-way ANOVA results, $F(4, 10) = 1801.67$, $p < .001$. The obtained p-value (3.06×10^{-14}) represents the probability of committing a Type I error—incorrectly rejecting a true null hypothesis. Given that this probability is infinitesimally small (approximately 3.1×10^{-12} %), the evidence strongly supports the alternative hypothesis that at least some group means differ significantly. The test statistic ($F = 1801.67$) substantially exceeded the critical value (3.478) for the 95% region of acceptance, further corroborating the statistical significance of the observed differences. In practical terms, the synthesis ratio significantly influences the antimicrobial potency of the nanoparticles, with certain formulations demonstrating markedly superior activity.
Beyond statistical significance, the effect size metrics revealed substantial practical significance. The observed effect size was large ($f = 26.85$), indicating that the magnitude of differences between group averages is considerable. Furthermore, eta squared ($\eta^2 = .99$) demonstrated that treatment condition accounts for approximately 99.9% of the variance in antibacterial activity—an exceptionally high proportion analogous to an R^2 value in regression analysis. This finding indicates that the synthesis parameters are the primary determinants of nanoparticle bioactivity, with minimal variance attributable to random error or unmeasured factors. Such a robust effect size reinforces the reliability of the findings and supports the systematic optimization of green synthesis protocols for maximum therapeutic efficacy.
Tukey HSD/Tukey Kramer was used as a Post-Hoc Analysis of the results of Analysis of Variance (ANOVA) to compare the five groups from each other, all the experimental, positive, and negative controls groups.

Table 6.
Tukey HSD results

Pair	Difference	SE	Q	Lower CI	Upper CI	Crit. Mean	p-value
x1-x2	0.6667	0.2582	2.582	-0.5351	1.8684	1.2017	0.4112
x1-x3	6	0.2582	23.2379	4.7983	7.2017	1.2017	1.21e-7
x1-x4	20.3333	0.2582	78.7507	19.1316	21.5351	1.2017	6.881e-13
x1-x5	6.6667	0.2582	25.8199	5.4649	7.8684	1.2017	4.465e-8
x2-x3	6.6667	0.2582	25.8199	5.4649	7.8684	1.2017	4.465e-8
x2-x4	19.6667	0.2582	76.1687	18.4649	20.8684	1.2017	1.171e-12
x2-x5	7.3333	0.2582	28.4019	6.1316	8.5351	1.2017	1.636e-8
x3-x4	26.3333	0.2582	101.9886	25.1316	27.5351	1.2017	3.331e-14
x3-x5	0.6667	0.2582	2.582	-0.5351	1.8684	1.2017	0.4112
x4-x5	27	0.2582	104.5706	25.7983	28.2017	1.2017	3.242e-14

Table 7.
Tukey HSD results

Group	**x2**	**x3**	**x4**	**x5**
x1	0.67	6	20.33	6.67
x2	0	6.67	19.67	7.33
x3	6.67	0	26.33	0.67

x4	19.67	26.33	0	27

Tukey's Honestly Significant Difference (HSD) post-hoc analysis identified the specific pairwise differences driving the overall significant effect. Significant differences were observed between the 1:1 ratio and the 3:1 ratio (x1-x3), the 1:1 ratio and chloramphenicol (x1-x5), the control and 3:1 ratio (x2-x3), the control and chloramphenicol (x2-x5), the 1:3 and 3:1 ratios (x3-x4), and the 3:1 ratio and chloramphenicol (x4-x5). Notably, the 3:1 ratio group exhibited substantially higher antibacterial activity compared to all other formulations, including the conventional antibiotic control. This finding suggests that optimal plant extract-to-metal precursor ratios enhance capping and stabilizing effects, thereby improving nanoparticle bioactivity beyond that of standard antibiotics (Rajeshkumar & Bharath, 2017). Conversely, the absence of significant differences between certain pairs (e.g., x1-x2, x3-x5) indicates that at suboptimal ratios, nanoparticle activity may not exceed that of the phytochemical constituents alone.

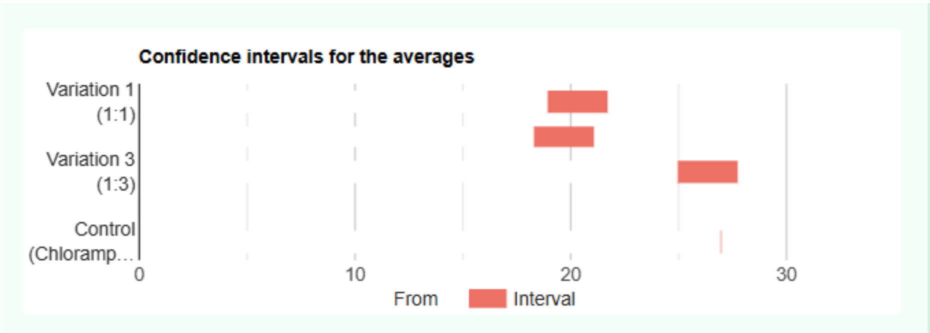


Figure 12. Confidence Intervals for the Averages of Each Variable

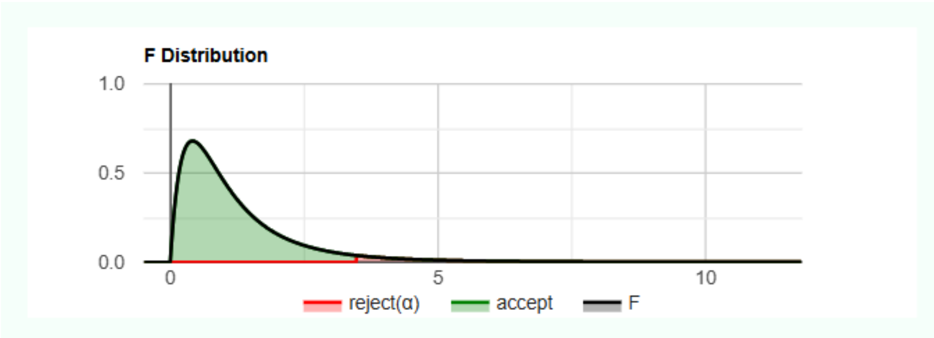


Figure 13. F-distribution

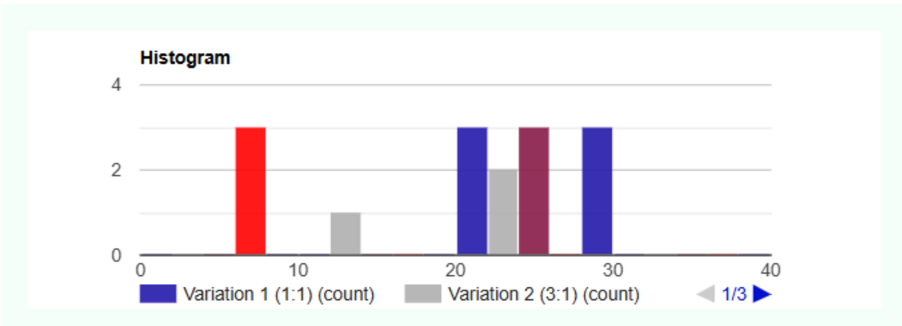


Figure 14. Histogram of Each Variabl

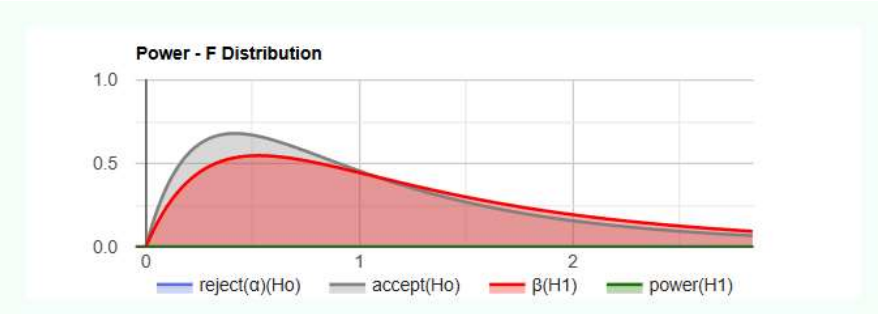


Figure 15. Power-F Distribution

The validity of these parametric findings rests on satisfactory assumption checks. Levene's test confirmed homogeneity of variances across groups ($p = .737$), indicating that the population variances can be considered equal. This equality of variances, combined with similar group sizes (ratio = 1:1), renders the ANOVA robust to violations of the homogeneity assumption. Additionally, the Shapiro-Wilk test supported the normality assumption, with all groups assumed to be normally distributed.
However, two methodological considerations warrant acknowledgment. First, the a priori statistical power was low (0.083), reflecting the small sample size. Despite this limitation, the effect size was sufficiently large to achieve statistical significance, suggesting that the true differences between groups are substantial. Second, the software generated a recommendation to consider the Kruskal-Wallis non-parametric test. While this suggestion reflects conservative statistical practice, the satisfaction of parametric assumptions (normality and homogeneity of variances) supports the validity of the ANOVA results. Nevertheless, future studies should employ larger sample sizes to enhance statistical power and confirm these findings through both parametric and non-parametric approaches.

Table 8. Results

Hypothesis			
H_0 : The green-synthesized Cu–Zn nanoparticles exhibit no morphological features associated with inhibition and does not demonstrate antibacterial effect against *Klebsiella pneumoniae* UPCC 1382 (ATCC 13883). **H_A :** The green-synthesized Cu–Zn nanoparticles exhibit morphological features associated with inhibition and demonstrate antibacterial effect against *Klebsiella pneumoniae* UPCC 1382 (ATCC 13883).			
Test Statistic	**Alpha**	**P-value**	**Decision**
One-Way ANOVA	0.05	3.064×10^{-14}	Reject the Null Hypothesis

SUMMARY, CONCLUSION, & RECOMMENDATION

In this chapter, the major discoveries from the research are summarized, leading to drawn conclusions and suggestions for further research. The research team synthesized Cu-Zn nanoparticles using an ecological synthesis method and characterizing methods to measure their antibacterial properties. The chapter describes how varying the ratio of metal precursors to extracts resulted in different shapes and materials of nanoparticles and how the shapes/materials of the various nanoparticles affect their ability to fight against K. pneumoniae bacterial infection. This chapter examines how these results will impact research, in addition to providing guidance for future developments in green nanotechnology and antimicrobials, using meta-analysis input from related studies.

V. Conclusions and Recommendations

A. Summary of Findings

1. SEM results indicated that Cu-Zn nano-particles produced using a green synthesis technique had different morphologies and sizes depending on which of three different ratios (1:1; 3:1; and 1:3) were employed. It was also observed that these particles displayed a high degree of aggregate formation and had the appearance of numerous clusters or large, surface mass formations rather than individual, independent items.
2. The elemental analysis performed by EDX confirmed that the incorporation of both Copper and Zinc into each of the nanoparticles was successful for all assayed particles. The elemental peaks of the Copper and Zinc in the EDX data confirmed that both metals have been chemically reduced and physically stabilized in all of the manufactured nanosized structures.
3. Heating at 80 °C resulted in each of the three ratios having very different physical characteristics. The 3:1 metal precursor/extract ratio produced a compound that was denser and darker in color than the other two ratios. The compounds prepared using the 1:1 and 1:3 ratios had lighter colors and were less compact than the 3:1 ratio. The difference in these compounds demonstrates that synthesis ratio affects how the nanoparticles are formed and their structure.
4. Overall, the meta-analysis of 44 selected studies on green-synthesized copper (Cu), zinc (Zn), and copper and zinc (Cu-Zn) nanoparticles providing a strong overall antibacterial effect ($I^2 = 72.87\%$ - a substantial amount of heterogeneity was demonstrated across the studies, but all of the effects' direction of effects for nanoparticle treatments were positive), indicates that the variance among studies can be attributed to differences in synthesis methods, nanoparticle concentrations, bacterial strains tested, and method of measurement. The pooled results showed evidence of antimicrobial potential for green-synthesized nanoparticles despite the overall variability among individual studies.
5. Testing the antibacterial properties of three different treatments to kill Klebsiella pneumoniae UPCC 1382 (ATCC 13883) revealed significant differences in the efficacy of each treatment. One-way ANOVA analysis ($F(4,10) = 1801.67$, $p < .001$) indicated that the null hypothesis can be rejected, showing that the synthesis ratio of the treatment does significantly impact the antibacterial activity of the treatment.
6. The post hoc Tukey HSD test showed that the best formulation was 3:1 ratio of metal precursor to extract, with significantly greater antibacterial activity than any of the other ratios of nanoparticles and controls. Some lower ratios were not significantly different from negative controls, and this suggests that sub-optimal conditions during synthesis may limit the biological activity of the nanoparticles produced.

B. Conclusion

The study results demonstrate that green-synthesized Cu-Zn nanoparticles serve as an effective replacement for standard antibiotics against multidrug-resistant Klebsiella pneumoniae UPCC 1382 (ATCC 13883). The study achieved its goals through the creation of Cu-Zn nanoparticles from Malunggay

(Moringa oleifera) leaf extract which scientists studied to determine their antibacterial properties at various metal precursors to plant extract ratios through size and morphological and elemental analysis. The study established that the antibacterial effectiveness of nanoparticles depends on the metal precursor to Malunggay extract ratio which showed peak antibacterial performance at a 3:1 ratio because optimization of synthesis parameters must happen before scientists can reach maximum bioactivity. The process of plant-mediated synthesis enables scientists to create stable nanosized copper and zinc structures which demonstrate that environmentally friendly methods can produce nanoparticles with strong antimicrobial properties. The meta-analysis showed that green-synthesized Cu Zn and Cu Zn nanoparticles produced positive antibacterial results in all studies despite their different research methods which confirmed their effectiveness in various settings. The study results demonstrate that plant-based bimetallic nanoparticles effectively combat drug-resistant bacterial strains through their sustainable process which enables the development of new antimicrobial solutions. The study establishes a foundation for future research on product development and medical applications while promoting the creation of sustainable nanotechnology to address increasing antimicrobial resistance.

C. Recommendations

The researchers established their recommendations based on the findings and conclusions which their study about green synthesis and characterization and antibacterial properties of Cu-Zn Nanoparticles reached:

1. The researchers should study all native plants which grow in their area to discover which ones can produce Cu-Zn Nanoparticles through environmental-friendly methods.
2. The researchers need to improve synthesis parameters through their upcoming experiments which will test different metal precursor-to-extract ratios and reaction temperature and duration to achieve better nanoparticle stability and uniformity and bioactivity results that SEM and ImageJ analyses showed.
3. The researchers should use UV-Vis and FTIR techniques to achieve a more complete understanding of how nanoparticles form through their various processes and functional groups.
4. The researchers should enhance their nanoparticle analysis through advanced techniques which use higher-resolution systems to provide them with better imaging capabilities and structural information.
5. The researchers need to assess how effective the synthesized Cu-Zn Nanoparticles kill Klebsiella pneumoniae UPCC 1382 (ATCC 13883) because their study did not include this assessment.
6. The researchers need to conduct cytotoxicity and biocompatibility assessments to evaluate whether the nanoparticles can be used safely in medical applications and environmental settings.

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