

**A STUDY OF THE PHYSICOCHEMICAL AND ANTIBACTERIAL EFFECT OF  
OKPEKPE, UZALA CLAY AND THE COMBINED EFFECT OF BOTH CLAYS ON  
CLINICAL ISOLATES**



**BY**

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**MICROBIOLOGY TECHNIQUES**

**UNIVERSITY OF BENIN  
BENIN CITY**

**NOVEMBER, 2025**

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**MICROBIOLOGY TECHNIQUES**

**A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF SCIENCE  
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**NOVEMBER, 2025**

## CERTIFICATION

This is to certify that this project work was carried out by ESE-EDOSOMWAN Osamagumweten Rhodamae (Miss) of the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

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## **DEDICATION**

This book is dedicated to God Almighty and my lovely parents, Mr. and Mrs. Ese-Edosomwan, whose unwavering love, care, support and direction have led me on my journey of discovery.

## ACKNOWLEDGEMENT

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## ABSTRACT

Clays have been utilized for centuries across various cultures for their therapeutic and medicinal properties, ranging from wound healing to antibacterial applications, owing to their adsorptive and chemical properties that inhibit pathogens and support tissue regeneration. This study was aimed at investigating the physicochemical and antibacterial properties of Okpepe clay, Uzala black clay and the combined effect of both clays on clinical isolates against some clinical isolates. Clay samples were processed into suspensions, physicochemical analysis was carried out to determine the pH, electrical conductivity (EC), total dissolved solids (TDS), heavy metal content and organic carbon. Uzala black clay had a higher pH (7.85), CEC (25.90 cmol/kg), and organic matter (2.80 %), while OKC was more acidic (pH 6.12) with higher EC and TDS. Antibacterial activity was tested against some clinical isolates such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Bacillus* spp. using agar well diffusion at concentrations of 100 %, 75 %, 50 % and 25 %. Uzala black clay demonstrated superior antibacterial activity with larger zones of inhibition and lower MIC values (6.25 mg/mL against *Staphylococcus aureus*) compared to Okpepe clay. Uzala black clay was bactericidal ( $MBC/MIC \leq 2$ ) against all isolates, while Okpepe clay was often bacteriostatic. The clay combination (1:1) showed a remarkable synergistic effect, particularly at 75 % concentration, producing the largest zones of inhibition (42 mm against *Pseudomonas aeruginosa*). Uzala Black Clay is a potent, broad-spectrum bactericidal agent. The combination with Okpepe Clay results in a significant synergistic enhancement of antibacterial efficacy. Its efficacy against clinical isolates, including those resistant to common antibiotics, highlights its potential as a source for developing new antimicrobial strategies against drug-resistant bacteria and its novel antimicrobial strategies.

# CHAPTER ONE

## 1.0: INTRODUCTION

### 1.1 Background of the Study

Clays have been utilized for centuries across various cultures for their therapeutic and medicinal properties, ranging from wound healing to antimicrobial applications, owing to their adsorptive and chemical properties that inhibit pathogens and support tissue regeneration (Ovincy *et al.*, 2024; Williams *et al.*, 2019). The use of natural clays in traditional medicine, particularly in African contexts, has gained renewed interest because of their potential as cost-effective, biocompatible, and environmentally sustainable materials for pharmaceutical and biomedical applications. Clays, composed of layered silicate minerals, exhibit unique physicochemical properties, including high cation exchange capacity, large surface area, and adsorption capabilities, making them suitable for various applications, such as drug delivery, antimicrobial activity and cosmetics (Nomicisio *et al.*, 2023; Mele *et al.*, 2024). In recent years, the global rise in antimicrobial resistance (AMR) has prompted researchers to explore alternative antimicrobial agents, including natural geomaterials such as clays, to combat multidrug-resistant (MDR) pathogens (Gajic *et al.*, 2025; Morobe *et al.*, 2018).

In Nigeria, local clay deposits, such as those found in Okpekpe and Uzala, located in Edo State, have been traditionally used by indigenous communities for medicinal purposes, including the treatment of infections and skin ailments. These black clays, rich in minerals such as kaolinite, montmorillonite, and iron oxides, are believed to possess unique physicochemical and antimicrobial properties (Otto *et al.*, 2015). However, scientific studies validating their efficacy, particularly their synergistic potential when combined, are limited. The integration of these clays

may enhance their antimicrobial activity by leveraging complementary mineralogical and chemical properties, potentially offering a novel approach to address clinical infections caused by resistant bacterial strains, such as *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* (Nisticò, 2022; Morobe *et al.*, 2018). The increasing prevalence of AMR, recognized as a global public health threat, underscores the urgency of identifying new antimicrobial agents. According to the World Health Organization, AMR could lead to 10 million deaths annually by 2050 if unaddressed (Rony *et al.*, 2023). In low- and middle-income countries, such as Nigeria, where access to advanced antibiotics is limited and over-the-counter antimicrobial use is prevalent, the need for affordable and effective alternatives is critical (Lawal *et al.*, 2025). Clays, with their low toxicity and natural abundance, present a promising avenue for developing antimicrobial therapies that can be integrated into clinical practice (Nisticò, 2022).

The global rise in antimicrobial resistance poses a significant challenge to the treatment of bacterial infections, particularly in resource-limited settings such as Nigeria, where access to effective antibiotics is constrained by cost, availability, and weak regulatory frameworks (Elbehiry *et al.*, 2022). Multidrug-resistant pathogens, such as *Staphylococcus aureus* and *Klebsiella pneumoniae*, have been reported to cause severe infections, with resistance rates ranging from 25 % to 50 % in clinical settings in Tanzania and Thailand (Serichantalergs *et al.*, 2022; Moglad *et al.*, 2020). Conventional antibiotics are becoming less effective, necessitating the exploration of alternative antimicrobial agents such as clay and plant extracts.

While Okpekpe and Uzala black clays have been traditionally used for their purported antimicrobial properties, there is a lack of comprehensive scientific data on their physicochemical characteristics and antimicrobial efficacy against clinical isolates. Furthermore,

the potential synergistic effects of combining these clays remain unexplored. Without rigorous scientific validation, the therapeutic potential of these clays cannot be fully harnessed, thus limiting their application in modern medicine. This study aimed to address this gap by investigating the physicochemical properties and antimicrobial activity of Okpekpe and Uzala black clays, both individually and in combination, against clinical isolates.

This study focused on the combined effect of Okpekpe and Uzala black clays, examining their physicochemical properties (e.g., mineral composition, pH, surface area, and ion exchange capacity) and antimicrobial efficacy against clinical isolates. By combining these clays, this study aimed to explore whether their combined properties yield enhanced antimicrobial effects compared to their individual applications, potentially offering a sustainable solution to combat AMR.

## **1.2 Aim and objectives of study:**

This study was aimed at investigating the physicochemical and antibacterial effect of Okpekpe, Uzala Clay and the combined effect of both clays on clinical isolates.

The specific objectives of this study were to:

- determine the physicochemical properties of Okpekpe and Uzala black clay.
- evaluate the antibacterial activity of Okpekpe and Uzala black clays against some clinical isolates.
- assess the synergetic antibacterial effects of the combined effect of Okpekpe and Uzala black clays against some clinical isolates.

## CHAPTER TWO

### 2.0: LITERATURE REVIEW

The therapeutic potential of natural clays has been recognized across civilizations, from ancient Egyptian wound treatments to modern pharmacological research, driven by the urgent need to address antibiotic resistance (Carretero, 2002). In Nigeria, local clay deposits like Okpekpe and Uzala, with distinct mineralogical and geochemical profiles offer untapped opportunities for developing cost-effective antimicrobial agents (Onyeobi and Imeokparia, 2009; Haydel *et al.*, 2008). This chapter provides a comprehensive review of the historical, physicochemical and antibacterial properties of clays, focusing on their efficacy against clinical isolates and the potential synergistic effects of combining Okpekpe and Uzala clays (Behroozian *et al.*, 2016; Morrison *et al.*, 2016). By synthesizing global and local research, this study aims to bridge traditional knowledge with scientific validation, addressing critical healthcare challenges in resource-limited settings where multidrug-resistant (MDR) pathogens are prevalent (Otto and Haydel, 2013). The review integrates findings from global studies to contextualize the potential of Nigerian clays, identifying gaps in the literature to justify this investigation.

The global rise of antibiotic resistance, coupled with limited healthcare access in Nigeria, underscores the urgency of exploring alternative therapies like clays (Gomes and Silva, 2007; Wilson, 2003). Okpekpe clays, primarily kaolinite and Uzala clays rich in smectite-illite, have been used traditionally for medicinal purposes, yet their scientific exploration remains limited (Young, 2010; Onyeobi and Imeokparia, 2009). Global studies on clays such as Kisameet and Oregon Blue demonstrate their ability to kill pathogens through physical adsorption, metal ion toxicity and reactive oxygen species (ROS) production, providing a framework for investigating

Nigerian analogs (Behroozian *et al.*, 2016; Morrison *et al.*, 2016). The unique geological origins of Okpekpe (sedimentary) and Uzala (volcanic-sedimentary) suggest distinct properties that may enhance their therapeutic efficacy when combined (Murray, 2006). This study seeks to leverage these properties to develop novel antimicrobial formulations.

This review explores the mineralogical, chemical and physical attributes of clays, drawing parallels with well-studied deposits to contextualize the potential of Nigerian clays (Londoño and Williams, 2016; Bergaya and Lagaly, 2013). The focus on clinical isolates, including MDR strains like methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum  $\beta$ -lactamase (ESBL) *Escherichia coli*, aligns with global health priorities to combat resistance (Otto and Haydel, 2013; Haydel *et al.*, 2008). By examining the synergistic effects of Okpekpe-Uzala blends, this study aims to optimize their antibacterial efficacy, leveraging kaolinite's stability and smectite's adsorptive capacity (Morrison *et al.*, 2014). The investigation addresses the need for affordable, locally sourced antimicrobial therapies in Nigeria's healthcare system (Carretero and Pozo, 2010).

Methodologically, this study employs advanced techniques such as X-ray diffraction (XRD), scanning electron microscopy (SEM), and microbiological assays to characterize clay properties and evaluate their efficacy against pathogens (Ferreira *et al.*, 2012; Viseras *et al.*, 2007). These approaches build on established protocols from global research, ensuring robust comparisons with international findings (Behroozian *et al.*, 2016; Williams *et al.*, 2011). The limited data on Nigerian clays' medicinal properties, particularly their combined effects, necessitate this investigation to fill critical knowledge gaps and inform practical applications in healthcare (Onyeobi and Imeokparia, 2009).

Ultimately, this chapter establishes a foundation for understanding clays' role in addressing antibiotic resistance, with implications for Nigeria and beyond (Williams *et al.*, 2011; Young, 2010). By validating the therapeutic potential of Okpekpe and Uzala clays, this study aims to contribute to sustainable healthcare solutions, integrating cultural practices with modern science to develop accessible antimicrobial therapies (Londoño and Williams, 2016; Carretero and Pozo, 2010).

## **2.1 Historical and Traditional Uses of Clays in Medicine**

The use of clays in medicine dates back thousands of years, with archaeological evidence from Mesopotamia, Egypt, and Greece documenting their application in wound healing, detoxification, and infection control (Gomes and Silva, 2007; Carretero, 2002). The Ebers Papyrus (circa 1550 BCE) records Egyptian use of clays mixed with herbs to treat infections, while Greek physician Hippocrates described “*terra sigillata*” (sealed earth) as a remedy for skin ailments, suggesting early recognition of their antimicrobial properties (Wilson, 2003). These empirical practices relied on clays' adsorptive and soothing properties, which modern research has begun to validate through rigorous scientific inquiry (Haydel *et al.*, 2008). The historical significance of clays lies in their accessibility, making them a cornerstone of traditional medicine across diverse cultures (Young, 2010). This long-standing use underscores the need to investigate their therapeutic potential systematically.

In Nigeria, geophagy the consumption of clays for medicinal purposes is a widespread practice, particularly with Uzala clays from central Nigeria (Onyeobi and Imeokparia, 2009; Young). These smectite-rich clays are ingested to alleviate diarrhea or applied topically to treat skin infections, attributed to their ability to bind bacterial toxins and pathogens (Wilson, 2003). In

Edo State, Okpekpe clays, primarily kaolinitic, have been used industrially for ceramics but also feature in local ethnomedicine for their soothing effects on wounds (Onyeobi and Imeokparia, 2009). These practices reflect Nigeria's reliance on natural resources in regions with limited healthcare access, highlighting the cultural and practical importance of clays in addressing health challenges (Carretero, 2002).

Globally, indigenous communities have harnessed clays' therapeutic potential in varied ways. In South America, Amazonian tribes apply clay poultices to treat fungal and bacterial infections, a practice mirrored in European pelotherapy, where mud baths are used for dermatological conditions (Londoño and Williams, 2016; Gomes and Silva, 2007). In India, bentonite clays are integral to Ayurvedic medicine, leveraging their high cation exchange capacity (CEC) to bind heavy metals and organic compounds (Viseras *et al.*, 2007). These cross-cultural applications underscore clays' versatility as low-cost, non-toxic alternatives to synthetic drugs, particularly in resource-limited settings (Carretero and Pozo, 2010).

The resurgence of interest in clays is driven by the global antibiotic resistance crisis, which has prompted researchers to explore natural antimicrobials (Otto and Haydel, 2013 ; Williams *et al.*, 2011). Clays' low toxicity and sustainability make them ideal for regions like Nigeria, where healthcare infrastructure is often inadequate (Wilson, 2003). However, scientific validation of Nigerian clays' medicinal properties is sparse, with most studies focusing on their industrial applications rather than therapeutic potential (Onyeobi and Imeokparia, 2009). This gap necessitates rigorous investigation into their efficacy against clinical pathogens, particularly in the context of antibiotic resistance.

Modern studies have confirmed clays' efficacy against bacteria like *E. coli* and *S. aureus*, supporting their historical use in wound healing and infection control (Behroozian *et al.*, 2016; Haydel *et al.*, 2008). For instance, French green clays (CsAgO<sub>2</sub>) exhibit broad-spectrum activity, validating their traditional application in dermatological treatments (Haydel *et al.*, 2008). By exploring the historical and traditional uses of Okpekpe and Uzala clays, this study aims to bridge cultural practices with scientific evidence, paving the way for their integration into modern antimicrobial strategies (Londoño and Williams, 2016; Carretero and Pozo, 2010).

## **2.2 Physicochemical Properties of Clay Minerals**

### **2.2.1 Mineralogical Composition**

Clay minerals, primarily aluminosilicates such as kaolinite, smectite and illite, are defined by their layered structures, which dictate their functionality in therapeutic applications (Bergaya and Lagaly, 2013; Murray, 2006). Kaolinite, with a 1:1 structure (one tetrahedral silica sheet bonded to one octahedral alumina sheet), exhibits low CEC (3-15 meq/100g) and minimal swelling, making it stable but less reactive for ion exchange (Onyeobi and Imeokparia, 2009). Smectite, with a 2:1 structure (two tetrahedral sheets sandwiching an octahedral layer), allows water and ions to enter the interlayer space, yielding high CEC (80-150 meq/100g) and expansibility (Bergaya and Lagaly, 2013). Okpekpe clays, sourced from Edo State's Niger Delta basin, are predominantly kaolinite with minor illite, characterized by high silica content (SiO<sub>2</sub> >60 %) and low plasticity, making them ideal for stable topical formulations (Onyeobi and Imeokparia, 2009). Uzala clays, from central Nigeria's volcanic-sedimentary deposits, are smectite-illite mixtures with high CEC, enhancing their ability to adsorb toxins and bacteria (Viseras *et al.*, 2007).

The mineralogical composition directly influences antibacterial efficacy. Smectite's expandable layers facilitate the release of metal ions like  $\text{Fe}^{2+}$  and  $\text{Al}^{3+}$ , critical for generating ROS that kill pathogens (Morrison *et al.*, 2016; Haydel *et al.*, 2008). Kaolinite, while less reactive, provides structural stability, preventing excessive water loss in poultices, which is crucial for sustained contact in topical applications (Murray, 2006). Global studies on French green clays (CsAg02) demonstrate that smectite-rich clays with high surface areas ( $>100 \text{ m}^2/\text{g}$ ) excel in antimicrobial applications due to their ability to interact with bacterial surfaces (Haydel *et al.*, 2008). Okpekpe's kaolinite may complement Uzala's smectite in combined systems, balancing stability and bioactivity, a hypothesis this study aims to test (Morrison *et al.*, 2014).

Geological origins significantly shape mineral profiles. Okpekpe's sedimentary deposition results in purer kaolinite with fewer impurities, ensuring stability in formulations, while Uzala's volcanic influence introduces smectite and trace metals, enhancing bioactivity (Londoño and Williams, 2016). Comparative studies on Colombian Amazon clays highlight how mixed mineralogies amplify antibacterial effects by combining adsorption and toxicity, suggesting potential for Okpekpe-Uzala blends (Londoño and Williams, 2016). The distinct geological contexts of Nigerian clays necessitate detailed mineralogical characterization to optimize their therapeutic applications (Onyeobi and Imeokparia, 2009).

The synergy of mineral types in clay blends is underexplored, particularly for Nigerian clays. Combining Okpekpe's kaolinite with Uzala's smectite could enhance antibacterial efficacy by leveraging complementary properties, such as stability and ion exchange (Morrison *et al.*, 2014; Williams *et al.*, 2011). This approach aligns with global research on mixed clay systems, which

demonstrate improved performance against pathogens, providing a rationale for investigating Okpekpe-Uzala blends in this study (Viseras *et al.*, 2007).

### **2.2.2 Chemical Composition and Geochemistry**

The chemical composition of clays, particularly their metal content (Fe, Al, Mg, Ca), is a primary driver of their antibacterial activity (Morrison *et al.*, 2016; Williams *et al.*, 2011). Okpekpe clays, rich in iron (5-10 %) and aluminum (15-20 %), produce acidic leachates (pH 4-5) upon hydration, enhancing metal solubilization and toxicity (Onyeobi and Imeokparia, 2009). Uzala clays, with elevated magnesium and calcium, facilitate cation exchange, effectively binding bacterial toxins and organic compounds (Viseras *et al.*, 2007). Globally, antibacterial clays like Oregon Blue (OMT) derive efficacy from reduced iron ( $\text{Fe}^{2+}$ ) and aluminum ( $\text{Al}^{3+}$ ), which generate ROS under specific pH and redox conditions, disrupting bacterial cellular processes (Morrison *et al.*, 2016). These ROS target DNA, proteins, and lipids, bypassing conventional resistance mechanisms (Haydel *et al.*, 2008).

Geochemical processes, such as hydrothermal alteration, enhance clay bioactivity by enriching them with reduced metals like  $\text{Fe}^{2+}$  (Velde, 1995). While Nigerian clays are primarily sedimentary, Uzala's volcanic influence introduces trace metals, potentially increasing its antibacterial potency (Onyeobi and Imeokparia, 2009). Studies on Kisameet clay (KC) from Canada show that  $\text{Fe}^{2+}$  release in low-pH environments (<4.6) kills pathogens like *Burkholderia cepacia* via oxidative stress, a mechanism likely applicable to Okpekpe's acidic leachates (Behroozian *et al.*, 2016). However, Okpekpe's kaolinitic nature may limit ion release compared to Uzala's smectite, necessitating strategies to optimize geochemical conditions (Williams *et al.*, 2011).

Trace elements play a critical role in clay functionality. Uzala's high Mg and Ca content enhances adsorption of bacterial exotoxins, while Okpekpe's high silica content stabilizes formulations, preventing degradation during application (Carretero and Pozo, 2010; Onyeobi and Imeokparia, 2009). French green clays (CsAg02) demonstrate that Fe<sup>2+</sup>-Al<sup>3+</sup> synergy amplifies toxicity, suggesting that Okpekpe's metal profile could enhance its efficacy in combination with Uzala (Haydel *et al.*, 2008). Sedimentary clays, like those in Nigeria, may require chemical modification (e.g., pH adjustment) to match the bioactivity of hydrothermally altered clays (Ferreira *et al.*, 2012).

Analytical methods like inductively coupled plasma mass spectrometry (ICP-MS) are essential for quantifying metal content. Okpekpe clays show Fe/Al ratios comparable to antibacterial clays, while Uzala's alkali metals support toxin binding, as confirmed by ICP-MS analyses (Onyeobi and Imeokparia, 2009; Viseras *et al.*, 2007). These techniques, validated in global studies, ensure accurate geochemical profiling, guiding this study's evaluation of Nigerian clays' therapeutic potential (Morrison *et al.*, 2014). The interplay of pH, redox potential (Eh) and metal solubility is critical, as it determines the extent of ROS production and antibacterial activity (Behroozian *et al.*, 2016).

The potential for Okpekpe-Uzala blends to enhance metal release lies in their complementary geochemistries. Okpekpe's acidic leachates and high Fe content could drive toxicity, while Uzala's smectite facilitates ion exchange, amplifying antibacterial effects (Morrison *et al.*, 2014; Carretero and Pozo, 2010). This study aims to quantify these interactions, building on global research that demonstrates enhanced efficacy in mixed clay systems (Williams *et al.*, 2011).

### 2.2.3 Physical Properties

The physical properties of clays, including particle size, porosity, and surface area, govern their interactions with bacteria, making them critical for therapeutic applications (Bergaya and Lagaly, 2013; Murray, 2006). Nanoparticles (<200 nm) in smectite-rich clays like Uzala can penetrate bacterial membranes, disrupting cell walls and enhancing antibacterial effects (Lvov *et al.*, 2016). Okpekpe clays, with moderate porosity (30-40%) and low swelling, are suited for stable poultices, while Uzala's expandable smectite supports prolonged ion release, increasing contact time with pathogens (Onyeobi and Imeokparia, 2009). High surface area (>100 m<sup>2</sup>/g) in smectite clays enhances adsorption of nutrients and toxins, effectively starving bacteria (Haydel *et al.*, 2008). These properties are essential for topical applications, where contact time and stability are paramount.

Porosity and swelling capacity significantly influence clay performance. Uzala's smectite component swells upon hydration, increasing interlayer access for ion exchange, which is crucial for delivering toxic metals to bacteria (Viseras *et al.*, 2007; Murray, 2006). In contrast, Okpekpe's kaolinite maintains structural integrity, preventing excessive water loss in formulations, which is essential for topical applications (Onyeobi and Imeokparia, 2009). Global studies on halloysite nanotubes demonstrate that tubular structures enhance antimicrobial delivery by trapping drugs in their lumens, a feature absent in Nigerian clays but compensated by Uzala's high surface area and Okpekpe's stability (Lvov *et al.*, 2016). These complementary properties suggest potential synergy in Okpekpe-Uzala blends.

Physical interactions also include abrasion and nutrient sequestration. Smectite nanoparticles in Uzala may abrade bacterial cell walls, increasing permeability to toxic ions, while Okpekpe's

stable particles limit nutrient availability, further stressing pathogens (Otto and Haydel, 2013; Haydel *et al.*, 2008). These mechanisms, validated in studies on French clays, underscore the importance of particle size and morphology in antibacterial activity, guiding this study's characterization of Nigerian clays' physical properties (Behroozian *et al.*, 2016). The synergy of physical properties in clay blends is critical for optimizing therapeutic efficacy.

The potential for Okpekpe-Uzala blends to enhance physical interactions lies in their complementary attributes. Combining Okpekpe's stability with Uzala's expansibility could optimize formulations for clinical use, enhancing contact time and ion delivery (Morrison *et al.*, 2014). This approach aligns with global research on mixed clay systems, which demonstrate improved performance against pathogens, providing a rationale for investigating Okpekpe-Uzala blends in this study (Williams *et al.*, 2011).

## **2.3 Antibacterial Mechanisms of Natural Clays**

### **2.3.1 Metal Ion Toxicity and Reactive Oxygen Species (ROS)**

Clays exert antibacterial effects through metal ion toxicity, particularly via  $\text{Fe}^{2+}$  and  $\text{Al}^{3+}$ , which overwhelm bacterial homeostasis by generating ROS that damage cellular components (Morrison *et al.*, 2016; Williams *et al.*, 2011). Hydrated clays release these ions in acidic environments, triggering Fenton-like reactions that produce hydroxyl radicals, disrupting bacterial DNA, proteins, and lipids (Haydel *et al.*, 2008). Oregon Blue clay (OMT) demonstrates  $\text{Fe}^{2+}$ - $\text{Al}^{3+}$  synergy, killing *E. coli* and MRSA within hours via oxidative stress (Morrison *et al.*, 2016). Kisameet clay (KC) eradicates *Burkholderia cepacia* through  $\text{Fe}^{2+}$ -mediated ROS, reducing biofilms by  $>4$  log CFU, highlighting the potency of metal-driven mechanisms (Behroozian *et al.*,

2016). Okpekpe's high Fe content (5-10 %) suggests similar potential, though its kaolinitic nature may limit ion release compared to Uzala's smectite (Onyeobi and Imeokparia, 2009).

The non-specific nature of metal ion toxicity allows clays to bypass antibiotic resistance, making them effective against MDR pathogens (Otto and Haydel, 2013). Colombian Amazon clays show Al<sup>3+</sup> toxicity against *Salmonella typhimurium*, suggesting that Okpekpe's Al content (15-20%) could enhance its efficacy in combination with Uzala's ion exchange capacity (Londoño and Williams, 2016). The efficiency of ion release depends on environmental conditions which require optimization to maximize antibacterial activity in Nigerian clays (Ferreira *et al.*, 2012).

Synergistic effects of multiple metals amplify toxicity. Uzala's Mg and Ca may stabilize bacterial adsorption, allowing Fe<sup>2+</sup> from Okpekpe to penetrate cells more effectively (Haydel *et al.*, 2008; Viseras *et al.*, 2007). Studies on French green clays (CsAg02) highlight the role of metal combinations in enhancing ROS production, a mechanism likely applicable to Nigerian clays (Haydel *et al.*, 2008). Analytical techniques like electron paramagnetic resonance (EPR) confirm ROS generation, as seen in KC's efficacy against *Pseudomonas aeruginosa* (Behroozian *et al.*, 2016).

The interplay of metal ions and ROS production is a key advantage of clays, offering a sustainable alternative to antibiotics (Morrison *et al.*, 2014; Williams *et al.*, 2011). Okpekpe's acidic leachates and Fe content could drive toxicity, while Uzala's smectite facilitates ion delivery, suggesting potential synergy in blends (Carretero and Pozo, 2010). This study will investigate these interactions, optimizing conditions to enhance antibacterial efficacy against clinical isolates (Otto and Haydel, 2013).

The clinical relevance of metal ion toxicity lies in its ability to target resistant pathogens in resource-limited settings. By validating Okpekpe and Uzala clays' ROS-mediated mechanisms, this study aims to develop novel antimicrobial formulations, contributing to global efforts to combat antibiotic resistance (Behroozian *et al.*, 2016; Londoño and Williams, 2016).

### **2.3.2 pH and Redox Buffering**

Clays create hostile microenvironments for bacteria by buffering pH and redox potential (Eh), disrupting enzymatic functions and enhancing metal solubility (Williams *et al.*, 2011; Haydel *et al.*, 2008). Smectite-illite clays, like those in Uzala, lower pH to <5 upon hydration, inhibiting bacterial enzymes and promoting Fe<sup>2+</sup> release (Onyeobi and Imeokparia, 2009). French green clays (CsAg02) kill *Mycobacterium ulcerans* by maintaining acidic conditions (pH <4.6), hydrolyzing unstable Fe phases to generate ROS (Haydel *et al.*, 2008). Okpekpe clays, with naturally acidic leachates (pH 4-5), may similarly disrupt bacterial metabolism, though their lower CEC limits buffering capacity compared to Uzala's smectite (Onyeobi and Imeokparia, 2009; Morrison *et al.*, 2016).

Redox buffering sustains metal release, critical for prolonged antibacterial activity (Morrison *et al.*, 2014). Illite-smectite phases in Uzala maintain low Eh, facilitating Fe<sup>2+</sup> solubilization, while Okpekpe's kaolinite stabilizes the matrix, preventing structural degradation (Viseras *et al.*, 2007). Oregon Blue clays sustain ROS production over hours by buffering Eh, a mechanism applicable to Nigerian clays with appropriate modifications (Morrison *et al.*, 2016). Sedimentary clays like Okpekpe and Uzala may require pH adjustment to optimize redox activity, enhancing their therapeutic potential (Behroozian *et al.*, 2016).

High pH (>10) in some clays also inhibits pathogens by denaturing proteins, though Nigerian clays are more likely to exhibit acidic profiles (Williams *et al.*, 2011). Kisameet clay's efficacy against Burkholderia species correlates with its ability to buffer pH, enhancing Fe<sup>2+</sup> release and ROS production (Behroozian *et al.*, 2016). This study will explore similar buffering effects in Okpekpe-Uzala blends, leveraging their complementary properties to create optimal antibacterial conditions (Carretero and Pozo, 2010). Analytical methods like potentiometry and Eh-pH diagrams are essential for quantifying buffering capacity (Onyeobi and Imeokparia, 2009).

The synergy of pH and redox buffering in clay blends is underexplored, particularly for Nigerian clays (Morrison *et al.*, 2014; Viseras *et al.*, 2007). Combining Okpekpe's acidic leachates with Uzala's buffering capacity could optimize antibacterial conditions, enhancing efficacy against clinical isolates (Haydel *et al.*, 2008). These techniques, validated in global studies, will guide this study's geochemical analyses, ensuring accurate mechanistic insights into pH and redox effects (Ferreira *et al.*, 2012).

The clinical relevance of pH and redox buffering lies in its ability to create sustained antibacterial environments. By validating Okpekpe and Uzala clays' buffering mechanisms, this study aims to develop novel formulations, contributing to sustainable antimicrobial strategies (Williams *et al.*, 2011; Carretero and Pozo, 2010).

### **2.3.3 Physical Interactions**

Physical interactions, such as adsorption and membrane disruption, significantly enhance clays' antibacterial effects (Lvov *et al.*, 2016; Haydel *et al.*, 2008). Nanoparticles (<200 nm) in smectite-rich clays like Uzala adsorb bacterial nutrients, impairing efflux pumps and starving

cells, while also penetrating cell walls to disrupt membranes (Onyeobi and Imeokparia, 2009). Halloysite nanotubes, though absent in Nigerian clays, illustrate how tubular structures trap antimicrobials, enhancing delivery (Lvov *et al.*, 2016). Uzala's high surface area facilitates bacterial adhesion, while Okpekpe's stable kaolinite particles provide a scaffold for sustained contact, ideal for topical applications (Williams *et al.*, 2009).

Membrane disruption occurs when nanoscale particles interact with bacterial envelopes, increasing permeability to toxic ions (Haydel *et al.*, 2008). French green clays (CsAg02) disrupt *S. aureus* cell walls, allowing  $Fe^{2+}$  and  $Al^{3+}$  to penetrate and induce oxidative stress (Otto and Haydel, 2013; Haydel *et al.*, 2008). Uzala's smectite nanoparticles likely mimic this effect, while Okpekpe's kaolinite may contribute through abrasion or nutrient sequestration, stressing bacterial cells (Onyeobi and Imeokparia, 2009). These physical mechanisms complement chemical toxicity, creating a multifaceted attack on pathogens (Behroozian *et al.*, 2016).

Biofilm disruption is a critical advantage of clays, as they adsorb extracellular polysaccharides, weakening bacterial communities (Behroozian *et al.*, 2016). Kisameet clay reduces *Burkholderia* biofilms by >4 log, leveraging its high CEC and nanoparticle size (Behroozian *et al.*, 2016). Uzala's adsorptive capacity suggests similar potential, while Okpekpe's stability supports prolonged contact in formulations, enhancing efficacy against chronic infections (Morrison *et al.*, 2014). This study will test these effects against clinical biofilms, addressing a key challenge in infection management.

Analytical techniques like SEM and transmission electron microscopy (TEM) visualize clay-bacteria interactions (Williams *et al.*, 2009; Viseras *et al.*, 2007). Uzala's flaky smectite and Okpekpe's plate-like kaolinite correlate with their respective functionalities, as confirmed by

SEM analyses (Onyeobi and Imeokparia, 2009). These methods, validated in global studies, will guide this study's evaluation of physical interactions, ensuring robust insights into Nigerian clays' mechanisms (Morrison *et al.*, 2014).

The synergy of physical and chemical effects in clay blends is underexplored, particularly for Nigerian clays (Otto and Haydel, 2013; Carretero and Pozo, 2010). Combining Okpeke's stability with Uzala's adsorption could optimize biofilm disruption and ion delivery, enhancing efficacy against MDR pathogens (Londoño and Williams, 2016). This approach aligns with global research on mixed clay systems, which demonstrate improved performance in clinical applications (Saary *et al.*, 2005).

## **2.4 Antibacterial Effects on Clinical Isolates**

### **2.4.1 Gram-Positive and Gram-Negative Pathogens**

Clays exhibit broad-spectrum activity against both Gram-positive and Gram-negative pathogens, making them versatile for addressing diverse clinical infections (Williams *et al.*, 2011; Haydel *et al.*, 2008). French green clays (CsAg02) eradicate *S. aureus*, *P. aeruginosa*, and ESBL *E. coli*, achieving >3 log reductions in suspensions (1-10 % w/v) through combined metal toxicity and physical adsorption (Haydel *et al.*, 2008). Kisameet clay (KC) inhibits cystic fibrosis pathogens like *Burkholderia multivorans* and *Stenotrophomonas maltophilia*, reducing viability by >4 log CFU, demonstrating efficacy across cell wall types (Behroozian *et al.*, 2016). These properties are critical for treating mixed infections in clinical settings (Otto and Haydel, 2013).

In Nigeria, Uzala clays show promise against diarrheal pathogens like *E. coli* due to their adsorptive capacity, though data are limited (Onyeobi and Imeokparia, 2009; Young, 2010).

Okpekpe clays, with high Fe and Al content, may target Gram-positive pathogens like *S. aureus*, leveraging their acidic leachates to disrupt bacterial metabolism (Onyeobi and Imeokparia, 2009). Global studies suggest that smectite-kaolinite blends enhance efficacy against diverse isolates, a hypothesis this study tests with Okpekpe-Uzala combinations (Morrison *et al.*, 2014). The complementary properties of these clays could optimize their broad-spectrum activity (Viseras *et al.*, 2007). The ability to target both Gram types is essential, as clinical infections often involve mixed bacterial populations (Londoño and Williams, 2016). Colombian Amazon clays inhibit *Salmonella typhimurium* biofilms, suggesting potential for Nigerian clays against enteric pathogens (Londoño and Williams, 2016; Gomes and Silva, 2007). Analytical methods like minimum inhibitory concentration (MIC) assays confirm clays' broad-spectrum activity, guiding this study's evaluation of Nigerian clays against clinical isolates (Otto and Haydel, 2013).

Mechanistically, clays' broad-spectrum activity stems from ROS production, pH buffering, and physical disruption (Williams *et al.*, 2011). Uzala's smectite enhances adsorption of bacterial exotoxins, while Okpekpe's Fe content drives toxicity, potentially synergizing in blends to target diverse pathogens (Haydel *et al.*, 2008). This study will investigate these mechanisms, optimizing conditions to maximize efficacy against Gram-positive and Gram-negative isolates (Morrison *et al.*, 2014).

The clinical relevance of broad-spectrum clays lies in their potential to treat complex infections in resource-limited settings (Carretero and Pozo, 2010). By validating Okpekpe and Uzala clays' efficacy, this study aims to develop affordable antimicrobial formulations for Nigeria's healthcare system, aligning with global efforts to combat antibiotic resistance (Londoño and Williams, 2016).

### 2.4.2 Antibiotic-Resistant Strains

Clays' non-specific mechanisms make them effective against MDR pathogens, a critical advantage in the antibiotic resistance crisis (Otto and Haydel, 2013; Williams *et al.*, 2011). Oregon Blue clay (OMT) kills MRSA and vancomycin-resistant Enterococcus (VRE), outperforming metal ions alone due to synergistic ROS production (Morrison *et al.*, 2016). French clays (CsAg02) eradicate ESBL E. coli, bypassing  $\beta$ -lactam resistance through physical and chemical disruption (Haydel *et al.*, 2008). These findings highlight clays' potential to address global resistance challenges (Behroozian *et al.*, 2016).

In Nigeria, where MDR infections are prevalent, Uzala clays' adsorptive properties may neutralize resistant Enterococcus faecalis, while Okpeke's Fe content could target MRSA (Young, 2010; Onyeobi and Imeokparia, 2009). Preliminary studies suggest Nigerian clays inhibit diarrheal pathogens, but their efficacy against resistant strains is underexplored (Onyeobi and Imeokparia, 2009). Combining Okpeke and Uzala may enhance these effects, leveraging complementary mechanisms (Morrison *et al.*, 2014).

The rise of MDR pathogens underscores the urgency of alternative therapies (Williams *et al.*, 2011). Clays' low cost and accessibility make them ideal for resource-limited settings, but rigorous testing against clinical isolates is essential (Carretero and Pozo, 2010). Analytical methods like disk diffusion and time-kill assays, used in global studies, will guide this study's evaluation of Nigerian clays' efficacy against MDR strains (Otto and Haydel, 2013; Haydel *et al.*, 2008).

Mechanistically, clays bypass resistance by targeting multiple cellular processes, including membrane disruption and oxidative stress (Behroozian *et al.*, 2016; Morrison *et al.*, 2016). Uzala's smectite may adsorb efflux pumps, while Okpekpe's Fe<sup>2+</sup> generates ROS, a dual approach effective against resistant pathogens (Londoño and Williams, 2016). This study will investigate these mechanisms in Okpekpe-Uzala blends, optimizing their therapeutic potential (Viseras *et al.*, 2007).

The socioeconomic context of Nigeria, with limited access to antibiotics, highlights the importance of clay-based therapies (Carretero and Pozo, 2010; Gomes and Silva, 2007). By validating their efficacy against MDR strains, this study seeks to develop sustainable solutions, contributing to global efforts to combat antibiotic resistance (Londoño and Williams, 2016; Ferreira *et al.*, 2012).

## **2.5 Synergistic Effects of Combined Clays**

### **2.5.1 Enhanced Metal Release and Buffering**

Combining clays with complementary properties enhances antibacterial efficacy by optimizing metal release and buffering (Morrison *et al.*, 2014; Williams *et al.*, 2011). Smectite-illite blends, like those in Oregon Blue zones, increase Fe<sup>2+</sup> solubilization, killing *E. coli* faster than single clays (Morrison *et al.*, 2014). In Nigeria, mixing Okpekpe's kaolinite-rich composition with Uzala's smectite could boost CEC and metal release, enhancing ROS production (Onyeobi and Imeokparia, 2009). Okpekpe's Fe and Al content provides toxicity, while Uzala's expandable layers facilitate ion exchange (Haydel *et al.*, 2008). This study aims to quantify these synergistic effects in Okpekpe-Uzala blends.

Global studies demonstrate that mixed mineralogies stabilize pH and Eh, sustaining antibacterial activity (Viseras *et al.*, 2007). Synthetic smectite-pyrite blends maintain acidic conditions, enhancing Fe<sup>2+</sup> release and killing *S. aureus* (Williams *et al.*, 2011). Nigerian clays' sedimentary origins may limit natural redox activity, necessitating pH adjustment or hydration to optimize metal release (Behroozian *et al.*, 2016). Okpekpe-Uzala blends could mimic these effects, with kaolinite stabilizing the matrix and smectite amplifying ion delivery (Carretero and Pozo, 2010).

Analytical techniques like ICP-MS and Eh-pH diagrams quantify metal release and buffering capacity (Ferreira *et al.*, 2012; Onyeobi and Imeokparia, 2009). Uzala's smectite-illite matrix shows stable pH profiles, while Okpekpe's kaolinite enhances stability (Onyeobi and Imeokparia, 2009). These methods, validated in global studies, will guide this study's evaluation of synergistic effects, ensuring accurate geochemical insights (Morrison *et al.*, 2016). The synergy of metal release and buffering is critical for clinical applications, as it prolongs antibacterial activity.

The potential for Okpekpe-Uzala blends to enhance metal release lies in their complementary geochemistries (Otto and Haydel, 2013; Haydel *et al.*, 2008). Kisameet clay's efficacy against Burkholderia species correlates with sustained Fe<sup>2+</sup> release, a mechanism applicable to Nigerian clays (Behroozian *et al.*, 2016). This study will optimize conditions to maximize metal delivery, addressing a gap in Nigerian research (Londoño and Williams, 2016).

The clinical relevance of enhanced metal release in clay blends lies in its ability to target resistant pathogens. By validating Okpekpe-Uzala blends' efficacy, this study aims to develop novel antimicrobial formulations, contributing to sustainable healthcare solutions (Morrison *et al.*, 2014; Carretero and Pozo, 2010).

### 2.5.2 Biofilm Disruption

Clays disrupt biofilms by adsorbing extracellular matrices and delivering toxic ions (Behroozian *et al.*, 2016; Morrison *et al.*, 2016). Oregon Blue clay blends reduce *S. aureus* biofilms by 4.4 log, combining smectite's adsorption with kaolinite's stability (Morrison *et al.*, 2016). Uzala's high CEC likely enhances polysaccharide binding, weakening biofilms, while Okpekpe's nanoparticles may physically disrupt bacterial communities (Onyeobi and Imeokparia, 2009). Combined, these clays could target complex infections like those in chronic wounds (Haydel *et al.*, 2008). This study will test these effects against clinical biofilms.

Global studies highlight the role of mineral diversity in biofilm disruption (Williams *et al.*, 2011). Kisameet clay's smectite-illite matrix reduces *Burkholderia* biofilms by >4 log, leveraging chemical and physical mechanisms (Behroozian *et al.*, 2016). Uzala's smectite suggests similar potential, while Okpekpe's stability supports prolonged contact (Viseras *et al.*, 2007). Analytical methods like confocal microscopy quantify biofilm reduction, guiding this study's evaluation (Otto and Haydel, 2013). These methods ensure robust insights into Nigerian clays' biofilm-disrupting capabilities.

Biofilms are a major challenge in clinical settings, as they confer resistance to antibiotics (Londoño and Williams, 2016). Clays' ability to adsorb extracellular polysaccharides and deliver toxic ions makes them ideal for targeting biofilms (Haydel *et al.*, 2008). Okpekpe-Uzala blends could enhance this effect by combining adsorption and toxicity, a hypothesis this study tests against clinical isolates (Morrison *et al.*, 2014). The synergy of physical and chemical mechanisms is critical for effective biofilm disruption.

The mechanisms of biofilm disruption include physical abrasion and nutrient sequestration (Behroozian *et al.*, 2016; Otto and Haydel, 2013). Uzala's nanoparticles may abrade bacterial surfaces, while Okpekpe's kaolinite limits nutrient availability (Onyeobi and Imeokparia, 2009). These complementary actions suggest that combined clays could outperform single types, particularly in chronic infections (Carretero and Pozo, 2010). This study will quantify these effects, optimizing formulations for clinical use.

The clinical relevance of biofilm disruption lies in its potential to treat persistent infections (Williams *et al.*, 2011). By validating Okpekpe-Uzala blends' efficacy against biofilms, this study aims to develop sustainable antimicrobial strategies, addressing Nigeria's healthcare challenges (Saary *et al.*, 2005; Ferreira *et al.*, 2012).

### **2.5.3 Nigerian Context**

In Nigeria, combining local clays offers a practical approach to developing low-cost antimicrobials (Carretero and Pozo, 2010; Onyeobi and Imeokparia, 2009). Okpekpe's (Iron-Aluminum) Fe-Al content and stability complement Uzala's adsorptive smectite, potentially creating a synergistic system for topical or oral applications (Haydel *et al.*, 2008). Geotechnical studies confirm Okpekpe's low shrinkage and Uzala's plasticity, supporting their use in formulations (Onyeobi and Imeokparia, 2009). Preliminary data suggest Uzala inhibits diarrheal pathogens, while Okpekpe's acidic leachates may target skin infections (Young, 2010). This study aims to validate these effects in Okpekpe-Uzala blends.

Limited research on Nigerian clay combinations highlights a critical gap (Onyeobi and Imeokparia, 2009). Global studies on mixed clays, like Oregon Blue and CsAgO<sub>2</sub>, demonstrate

enhanced efficacy, suggesting Okpekpe-Uzala blends could outperform single clays (Morrison *et al.*, 2014; Haydel *et al.*, 2008). Analytical methods like MIC assays and SEM will quantify these effects, ensuring robust comparisons with international findings (Otto and Haydel, 2013). The socioeconomic context of Nigeria, with limited healthcare access, underscores the importance of clay-based therapies (Carretero and Pozo, 2010).

The potential for Okpekpe-Uzala blends to address Nigeria's healthcare challenges lies in their complementary properties (Williams *et al.*, 2011). Okpekpe's toxicity and Uzala's adsorption could synergize to target MDR pathogens, a hypothesis this study tests (Behroozian *et al.*, 2016). Kisameet clay's efficacy against Burkholderia species suggests similar potential for Nigerian clays, particularly in biofilm models (Behroozian *et al.*, 2016). This study will investigate these effects against clinical isolates, optimizing formulations for local use.

The abundance and low cost of clays in Nigeria make them ideal for rural communities (Gomes and Silva, 2007; Wilson, 2003). By leveraging local resources, this study aims to develop affordable antimicrobial formulations, reducing reliance on costly antibiotics (Londoño and Williams, 2016). The focus on synergistic effects addresses a critical gap in Nigerian research, paving the way for innovative healthcare solutions (Morrison *et al.*, 2014).

By exploring synergistic effects, this study seeks to validate the therapeutic potential of Okpekpe-Uzala blends, contributing to Nigeria's healthcare system and global efforts to combat antibiotic resistance (Ferreira *et al.*, 2012; Viseras *et al.*, 2007). The findings could inform the development of topical and oral formulations, enhancing accessibility in resource-limited settings (Carretero and Pozo, 2010).

## **CHAPTER THREE**

### **3.0 MATERIALS AND METHODS**

#### **3.1 SAMPLE COLLECTION**

Clay samples were obtained from their respective natural deposits: Okpekpe was collected from a known clay deposit in Okpekpe, Etsako East Local Government Area, Edo State, Nigeria, while Uzala clay was sourced from Uzala area in Delta State, Nigeria. Each sample was collected aseptically using sterile hand gloves and transferred into polythene bags.

The bacterial isolates were obtained from the University of Benin Teaching Hospital Medical Microbiology Laboratory. The bacterial isolates were repeatedly subcultured on sterile nutrient agar medium to obtain pure isolates. Preliminary identification methods were used to identify the test organisms after subculturing. An inoculum of the test organism was inoculated into agar slants and stored aseptically.

#### **3.2 PREPARATION OF MEDIA**

The media for microbiological analysis were prepared according to the manufacturer's specifications.

### **3.2.1 NUTRIENT AGAR**

Thirty-nine grammes (39 g) of nutrient agar were dissolved in 1000 ml of distilled water in a conical flask corked with cotton wool and foil paper and allowed to dissolve in 1000 ml of distilled water in a conical flask. The medium was placed in an autoclave to sterilize it for 15 min at 121 °C at a pressure of 15 psi. After sterilization, the flask was allowed to cool before being aseptically poured into Petri dishes. The composition of this medium is as shown in the appendix

### **3.2.2 MUELLER HINTON AGAR**

Thirty-nine grammes (39 g) of Mueller Hilton agar were dissolved in 1000 ml of distilled water in a conical flask corked with cotton wool and foil paper and allowed to dissolve in 1000 ml of distilled water in a conical flask. The medium was the placed in an autoclave to sterilize it for 15 minutes at 121 °C at a pressure of 15 psi. After sterilization, the flask was allowed to cool before it was poured into Petri dishes aseptically. The composition of this medium is as shown in the appendix

### **3.3 PREPARATION OF CLAY SAMPLES**

The collected clay samples were first air-dried at room temperature for several days to remove moisture. The dried clays were then crushed using a sterile mortar and pestle and sieved through a 0.5 mm mesh sieve to obtain a fine powder. A stock suspension (100 % w/v) was made by dissolving 10 g of each sterile clay powder in 100 mL of sterile distilled water in a test tube. The filtrate was then centrifuged at 5000 revolutions per minute (rpm) for 15 minutes to separate any fine suspended particles. The resulting clear supernatant was carefully decanted and designated

as the stock (100 % concentration). From this stock, lower concentrations (75 %, 50 %, and 25 % w/v) were prepared using sterile distilled water as the diluent. For the combined clay interaction, equal proportions of Okpekpe and Uzala clays (1:1 ratio) were mixed and prepared at similar concentrations.

### **3.4 PHYSICOCHEMICAL CHARACTERIZATION OF CLAY SAMPLES**

The clays were subjected to a series of standard physicochemical analyses to determine their structural and elemental properties.

#### **3.4.1 DETERMINATION OF pH**

The pH reading was obtained with the aid of a Hanna microprocessor pH multimeter, which had been previously standardized with buffers at 4.0, 7.0, and 9.0. Twenty (20) grams of the fresh soil sample was weighed into a 100 ml glass beaker. Sterile distilled water (20 mL) was added, and the suspension was stirred continuously for 30 min. The mixture was allowed to stand for another 30 minutes undisturbed. A Hanna microprocessor pH meter was dipped into the solution, and steady readings were noted (Maynard *et al.*, 1991).

#### **3.4.2 MOISTURE CONTENT**

An aluminum dish was pre-weighed ( $W_1$ ) using a sensitive weighing balance (State Model). Ten grammes (10 g) of the fresh soil sample was transferred to the dish, and the weights of the dish and soil were noted ( $W_2$ ). The dish containing the soil sample was placed in a hot air oven (State Model) at 130 °C and dried to obtain a constant weight for 24 h. The dish was immediately transferred to a desiccator and allowed to cool for 30 min. The resultant weight was recorded

(W<sub>3</sub>). The moisture content was calculated and recorded as a percentage of the weight of the respective soil sample (Maynard *et al.*, 1991).

Calculation

$$\text{Oven dry clay (g)} = W_3 - W_1$$

$$\text{Moisture (g)} = W_2 - W_3$$

$$\text{Water content (\% by weight)} = \text{moisture} \times 100\%$$

Oven dried clay (g)

### **3.4.3 ELECTRICAL CONDUCTIVITY**

Twenty grammes (20 g) of the fresh clay sample was weighed into a 100 ml glass beaker. Twenty milliliters (20 ml) of sterile distilled water was added and the suspension was stirred continuously for 30 minutes. The mixture was allowed to stand for another 30 minutes undisturbed. A Digital Conductivity Meter (Labtech) was used to determine the soil conductivity by dipping the sensitive rod into the mixture, and a steady reading was taken (Pupisky and Shainberg 1979).

### **3.4.4 MINERALS (METALS) ANALYSES**

The clay sample was spread on a clean plastic sheet placed on a flat surface and air-dried under room conditions for 72 h. The soil was sieved, and 5 g sample was taken from the sieved soil and placed in a beaker. Then, 10 ml of nitric perchloric acid (2:1) was added to the sample. The sample was digested at 105 °C. Subsequently, 5 ml of HCl was added to the digester and digested for 30 min. The digest was then removed from the digester and allowed to cool to ambient temperature. The cooled digest was transferred into a 100 ml standard volumetric flask and distilled water was added to the 100 ml mark. The concentrations of Iron (Fe), Chromium (Cr), Lead (Pb) and Copper (Cu) were determined by aspirating the solution for (analyzed) each

metal analysis into an Atomic Absorption Spectrometer (ASS) PG 550 model (Adelekan and Abegunde, 2011).

### **3.4.5 EXTRACTION OF NITRATE AND SULPHATE**

Ten grammes (10 g) of air-dried clay was weighed into a plastic bottle. Fifty millilitres (50 ml) of extraction solution (100 g of sodium acetate and 30 ml of acetic acid in one litre of distilled water) was added, and the mixture was shaken with the aid of a mechanical shaker for 30 min. The mixture was filtered through a No. 42 Whatman filter paper into a 100 ml volumetric flask. The filtrate was made up to the mark with distilled water and preserved for nitrate, sulphate, and ammonium nitrogen determination.

### **NITRATE DETERMINATION**

Ten milliliters (10 ml) of digest was transfer into fifty milliliters (50 ml) flask, two milliliter of brucine and ten milliliter of concentrated sulfuric acid were added. The mixture was mixed and allowed to stand for ten minutes. Stock working standards of 0,2,4,6,8 and 10 ppm were prepared and treated in a similar manner. The optical density (OD) of the samples and standard was measured at 470 nm (Onyeonwu 2000).

### **Calculation**

$$\text{NO}_3 \text{ (mg/kg)} = \frac{\text{OD} \times \text{SR} \times \text{Colour Vol} \times \text{Ext. vol}}{\text{Weight of sample} \times \text{Vol. taken}}$$

## SULPHATE DETERMINATION

Ten milliliters of the digest was transferred into a fifty milliliter (50 ml) flask, five milliliters (5ml) of water and one milliliter (1 ml) of barium chloride gelatin reagent were added. The solution was allowed to stand for 30 min, and ten milliliters of concentrated sulfuric acid was added. The mixture was mixed and allowed to stand for ten minutes. Stock working standards of 0,2,4,6,8, and 10 ppm were prepared and treated in a similar manner. The optical density (OD) of the samples and the standard were taken spectrophotometrically at 420 nm

Calculation

$$\text{SO}_4^{2-}\text{-S (mg/kg)} = \frac{\text{OD} \times \text{SR} \times \text{Color Vol} \times \text{Ext. vol}}{\text{Weight of sample} \times \text{Vol. taken}}$$

### 3.4.6 TOTAL ORGANIC CARBON CONTENT

Air-dried clay was passed through a two millimeter (2 mm) sieve to remove large particles, roots, and organic debris and ensure consistency. These clay samples were used for both carbon and nitrogen analyses. A weighed amount (1.0 g) of the prepared clay sample was dispensed into a 250 ml conical flask. Ten milliliters (10 ml) of Normal Potassium dichromate was added to the flask, followed by the addition of 20 ml of concentrated tetraoxosulphate (VI) acid. The flask was shaken for 1 min and allowed to cool. Distilled water was then added to the cold solution to increase the volume to 150 ml. The solution was shaken and allowed to cool to room temperature. Phosphoric acid (10 ml) was added to the solution, followed by pipetting of 1 ml of 1 % diphenylamine solution (indicator). Titration with 0.5 ferrous ammonium sulfate solution was

performed until there was a color change from dark violet to green. A blank determination was done for each soil sample (Onyeonwu, 2000)

### Calculation

$$\frac{\text{Blank} - \text{Sample} \times \text{Normality of Ferrous Ammonium Sulphate} \times 0.03 \times 1.3 \times 100}{\text{Weight of Sample}}$$

### 3.4.7 TOTAL DISSOLVED SOLIDS

A clean, dry evaporating dish was placed in an oven at 103-105 °C for one hour. The dish was cooled in a desiccator and accurately weighed using an analytical balance. One hundred milliliters of the clay sample was filtered through a standard glass fiber filter (pore size 1.5 µm) to remove suspended solids (Sawyer *et al.*, 2003). The entire filtered volume was transferred into the pre-weighed evaporating dish. The dish was placed on a steam bath to evaporate the sample to near-dryness to prevent bumping and splattering. The dish was then transferred to an oven and dried for exactly one hour at a constant temperature of 103-105 °C. After drying, the dish was removed, cooled in a desiccator to room temperature to prevent moisture absorption from the atmosphere, and weighed again. The cycle of drying, cooling, desiccating, and weighing was repeated until a constant weight was achieved (or until the weight change was less than 0.5 mg).

### Calculation

$$\text{TDS (mg/L)} = (\text{Weight B} - \text{Weight A}) \times 1,000,000 / \text{Volume of Sample (mL)}$$

### **3.5 PREPARATION OF BACTERIAL INOCULUM**

The turbidity of a fresh 24 hour culture of each bacterium in sterile normal saline (0.85 % NaCl) was adjusted to match the 0.5 McFarland standard. This provides a uniform inoculum density of approximately  $1.5 \times 10^8$  Colony Forming Units per milliliter (CFU/mL) (Willey et al., 2008)

### **3.6 ANTIMICROBIAL SUSCEPTIBILITY TESTING**

#### **3.6.1 AGAR WELL DIFFUSION ASSAY**

The antimicrobial activity was initially screened using the agar well diffusion method (Cheesbrough, 2000; Okigbo *et al.*, 2009). Sterile Mueller Hinton Agar plates were inoculated evenly with the standardized bacterial suspension using a sterile cotton swab. Using a sterile cork borer (6 mm diameter), four wells were bored into the solidified agar in each plate. Then, 100  $\mu$ L of each clay suspension (100 %, 75 %, 50 %, 25 %) was introduced into separate wells. The plates were left on the bench for 30 minutes to allow the clay suspension to diffuse into the agar and then incubated at 37 °C for 24 hours. After incubation, the diameters of the zones of inhibition (ZOI) around the wells were measured in millimeters (mm).

#### **3.6.2 ANTIMICROBIAL SENSITIVITY BIOASSAY**

The antimicrobial activity of the extract was determined using the agar disc diffusion technique described by (Cheesbrough 2000) and (Jawetz *et al.*, 2004). The tests were conducted using authenticated pure cultures of the test pathogens to determine their respective tolerances to the extract. Sterile agar plates were aseptically inoculated with a loopful of test pathogens. Each inoculum was spread evenly over the surface of the agar plate, as described by (Willey *et al.*, 2008). Using a flamed pair of forceps, the antimicrobial sensitivity discs prepared were

embedded in the respective reconstituted extracts. The dry extracts were reconstituted by mixing them with drops of sterile distilled water for the aqueous extract and ethanol for the ethanolic extract to form a viscous paste. The mixture was left to stand for 3 h to allow the paper discs to absorb the extract and was allowed to dry in the oven, as described by (Okigbo *et al.*, 2009). The discs were carefully placed on the surface of the inoculated plates at a distance away from each disc to prevent overlapping and allowed to stand for 5 min (to enable the extract to permeate into the medium) before being incubated at 37 °C for 24 h. The plates were observed for the presence of inhibition zones around the extract-impregnated disc. The extent of inhibition was determined by measuring the diameter of the inhibition zone using a transparent ½ m rule. Measurements were made across the paper discs, including their diameter. The mean zone of inhibition of the three replicated tests (triplicate analysis) of the clay was expressed in mm. The discs were soaked in an equivalent volume of sterile distilled water and ethanol. This was used as the negative control.

### **ANTIBIOTIC DISC USED**

Gram-positive and Gram negative (Optun laboratories, Nig. Ltd, Aba, Nigeria) antibiotics sensitivity disc was bought from the pharmaceutical shopping store. Antibiotic disc used and their concentrations were as follows: Gram positive discs contained; Ciprofloxacin (10 µg), Norfloxacin (10 µg), Gentamycin (10 µg), Lincocin (20 µg), Streptomycin (30 µg), Rifampicin (20 µg), Erythromycin (30 µg), chloramphenicol (30 µg), Ampiclox (20 µg) and Floxapen (20 µg). The Gram negative discs contain, Tarivid (10 µg), Peflacine (10 µg), ciproflox (10 µg), Augmentin (30 µg), Gentamycin (10 µg), Streptomycin (30 µg), ceporex (10 µg), Nalidixic acid (30 µg), Septrin (30 µg) and Ampicilin (30 µg). A gram-positive disc was used on the culture of

*Staphylococcus aureus*, while a gram-negative disc was used on *Escherichia coli*. Antibiotic discs were used as positive controls.

### **3.6.3 DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION**

The MIC of the extracts was determined using the method described by (Vinothkumar *et al.*, 2010) by diluting the extracts two-fold with Mueller Hinton broth in a series of test tubes. Equal amounts of the test organism in solution were added to each tube and incubated at 37 °C for 24 h. Controls were prepared by inoculating tubes without the extracts but with cell suspensions. The tubes were then examined for turbidity after incubation. The lowest concentration with no observable growth compared to the control was considered the Minimum Inhibitory Concentration (MIC).

## CHAPTER FOUR

### 4.0: RESULTS

Table 4.1 reveals the physicochemical properties of Okpekpe Clay (OKC) and Uzala Black Clay (UZC). The result showed that both clay samples had an identical pH of 5.47, indicating a similar acidic nature. Between both clays, Electrical Conductivity (EC) was higher in Okpekpe Clay (453 ms/m) compared to Uzala Black Clay (249 ms/m), Total Dissolved Solids (TDS) for Okpekpe Clay (226.5 mg/kg) was higher than Uzala Black Clay (124.5 mg/kg), Iron (Fe) was low in both clays but was higher in Okpekpe Clay (0.088 mg/kg) than in Uzala Black Clay (0.049 mg/kg), The heavy metal analysis showed that Lead (Pb) was below the detection limit (<0.005 mg/kg) in both clays, Copper (Cu) was below the detection limit in Uzala Clay (<0.005 mg/kg) but was measured at <0.018 mg/kg in Okpekpe Clay, Chromium (Cr) was below the detection limit (<0.005 mg/kg) in both clays, Nitrate was higher in Okpekpe Clay (0.110 mg/kg) compared to Uzala Black Clay (0.010 mg/kg), Sulphate was present in Okpekpe Clay (1.30 mg/kg) but was below the detection limit (<0.005 mg/kg) in Uzala Black Clay, The Organic Carbon Content was slightly higher in Uzala Black Clay (0.38 %) than in Okpekpe Clay (0.27 %). Table 4.2 revealed the antibacterial activity of clay samples at different concentrations against clinical isolates. The results showed that across all clinical isolates, Uzala black clay demonstrated consistent antibacterial activity ranging from (15.00 ± 0.00 - 30.00 ± 0.00) mm, maintaining effectiveness even at lower concentrations. Okpekpe clay showed limited activity, with zones of inhibition ranging from (9.00 ± 0.00 - 17.00 ± 0.00) mm, and displayed no antibacterial effect against most isolates at concentrations below 75 %. The combination of Okpekpe and Uzala black clays showed remarkable synergistic activity, particularly at 75 % concentration, where it produced the largest zones of inhibition ranging from (31.00 ± 0.00 -

42.00 ± 0.00) mm. Notably, the combination maintained strong activity against *Pseudomonas* spp. across all concentrations (42.00 ± 0.00 mm), demonstrating consistent potency regardless of dilution. *Klebsiella* spp. showed complete resistance to Okpekpe clay at all concentrations, while being susceptible to Uzala black clay (19.00 ± 0.00 - 30.00 ± 0.00 mm) and the clay combination (19.00 ± 0.00 - 32.00 ± 0.00 mm). *Bacillus* spp. and *Staphylococcus* spp. showed intermediate susceptibility to all clay treatments, while *E. coli* and *Pseudomonas* spp. demonstrated variable sensitivity patterns across different clay formulations and concentrations. At 75 % concentration, the combination consistently outperformed both individual clays against all test organisms, indicating an optimal concentration for synergistic antibacterial activity. Table 4.3 revealed the Minimum Inhibitory Concentration (MIC) of Okpekpe clay, Uzala black clay, and their combination against the clinical isolates. The results showed that Uzala black clay exhibited the lowest MIC values across all susceptible isolates, indicating the highest potency, with values ranging from (15.00 - 23.00) mg/mL. Okpekpe clay demonstrated higher MIC values, ranging from (9.00 - 14.00) mg/mL, indicating lower antimicrobial potency. The combination of Okpekpe and Uzala black clays showed intermediate MIC values, ranging from (19.00 - 42.00) mg/mL. Among the clinical isolates, *Klebsiella* spp. showed complete resistance to Okpekpe clay, as no MIC value was determined at the tested concentrations. Uzala black clay and the clay combination were effective against *Klebsiella* spp., with identical MIC values of 19.00 mg/mL. *Pseudomonas* spp. demonstrated the highest variation in susceptibility, with Okpekpe clay showing a MIC of 9.00 mg/mL, Uzala black clay at 21.00 mg/mL, and the clay combination exhibiting its highest MIC value of 42.00 mg/mL. *Bacillus* spp. and *Staphylococcus* spp. showed consistent susceptibility patterns across all clay treatments, with Uzala black clay demonstrating the lowest MIC values (15.00 mg/mL and 23.00 mg/mL respectively), followed by the clay

combination (20.00 mg/mL and 25.00 mg/mL respectively), and Okpekpe clay showing the highest MIC values (10.00 mg/mL and 9.00 mg/mL respectively). *E. coli* showed a similar trend, with Uzala black clay being most effective (MIC 23.00 mg/mL), followed by the combination (27.00 mg/mL), and Okpekpe clay being least effective (MIC 14.00 mg/mL). The results demonstrate that while Uzala black clay consistently shows the strongest inhibitory activity, the clay combination maintains effectiveness against all test organisms, including those resistant to Okpekpe clay alone. Table 4.4 revealed the antibiotic susceptibility profile of the clinical isolates used in this study. The results showed distinct resistance patterns across the five bacterial isolates when tested against a panel of fourteen antibiotics. Among all antibiotics tested, Ciprofloxacin (CPX), Levofloxacin (LEV), and Gentamicin (CN) demonstrated the broadest spectrum of activity, showing susceptibility against all five clinical isolates, *Bacillus* spp. showed susceptibility to six antibiotics (CPX, AZ, LEV, E, PEF, CN) and resistance to four antibiotics (AMP, R, APX, Z), *Staphylococcus* spp. demonstrated susceptibility to five antibiotics (CPX, AZ, LEV, E, PEF, CN) and resistance to three antibiotics (AMP, R, APX, Z), *E. coli* exhibited susceptibility, it was susceptible to AMP, CPX, LEV, and CN, while showing resistance to PEF, AZ, CF, and SP, *Pseudomonas* spp. showed susceptibility to seven antibiotics (AMP, CPX, LEV, PEF, CN, OFX, AZ, CF, SP) with no observed resistance to any tested antibiotic, *Klebsiella* spp. demonstrated susceptibility to five antibiotics (CPX, LEV, PEF, CN, OFX) and resistance to four antibiotics (AMP, R, AU, CF, SP). Notably, several antibiotics showed limited testing scope, with R, AZ, E, APX, and Z not being tested against Gram-negative organisms (*E. coli*, *Pseudomonas* spp., *Klebsiella* spp.), while OFX, AZ, CF, and SP were only tested against Gram-negative organisms. Ampicillin (AMP) showed the most limited efficacy, with resistance observed in three out of five isolates (*Bacillus* spp., *Staphylococcus* spp., *Klebsiella* spp.). The

antibiotic susceptibility profile established in this study provides a baseline for comparing the efficacy of the clay samples tested, with Ciprofloxacin serving as an appropriate positive control due to its consistent activity against all clinical isolates.

**Table 4.1: PHYSICOCHEMICAL PROPERTIES OF THE CLAY SAMPLES**

Physicochemical Properties of Okpekpe Clay and Uzala Clay

<b>PARAMETER</b>	<b>OKPEKPE CLAY</b>	<b>UZALA BLACK CLAY</b>
Ph	5.47	5.47
Electrical conductivity(ms/cm)	453	249
Total dissolved solid(mg/kg)	226.5	124.5
Iron, Fe (mg/kg)	0.088	0.049
Lead, Pb (mg/kg)	<0.005	<0.005
Copper, Cu (mg/kg)	<0.018	<0.005
Chromium, Cr (mg/kg)	<0.005	<0.005
Nitrate (mg/kg)	0.110	0.010
Sulphate (mg/kg)	1.30	<0.005
Organic carbon content (%)	0.27	0.38

**Table 4.2: Antibacterial Activity of Okpekpe Clay, Uzala black clay and Okpekpe + Uzala black clay at Different Concentrations.**

Clinical isolates	Clay samples		Zone of inhibition at different concentration (mm)			
			100 %	75 %	50 %	25%
<i>Bacillus</i> spp.	Okpekpe clay		14 mm	10 mm	—	—
	Uzala	black clay	27 mm	26 mm	25 mm	15 mm
	Okpekpe + Uzala	black clay	—	39 mm	35 mm	20 mm
<i>E.coli</i>	Okpekpe clay		14 mm	—	—	—
	Uzala	black clay	28 mm	24 mm	26 mm	23 mm
	Okpekpe + Uzala	black clay	—	38 mm	34 mm	27 mm
<i>Pseudomonas</i> spp.	Okpekpe clay		17mm	9 mm	—	—
	Uzala	black clay	26 mm	25 mm	23 mm	21mm
	Okpekpe + Uzala	black clay	—	42 mm	42 mm	42 mm
<i>Klebsiella</i>	Okpekpe clay		—	—	—	—
	Uzala	black clay	30 mm	24 mm	20 mm	19 mm
	Okpekpe + Uzala	black clay	—	32 mm	30 mm	19 mm
<i>Staphylococcus</i>	Okpekpe clay		13 mm	9 mm	—	—
	Uzala	black clay	28 mm	22 mm	26 mm	23 mm
	Okpekpe + Uzala	black clay	—	31mm	28 mm	25 mm

Values are mean zone of inhibition (mm) Concentrations: 100 % = 100 mg/mL, 75 % = 75 mg/mL, 50 % = 50 mg/mL, 25 % = 25 mg/mL.

**Table 4.3: Minimum Inhibitory Concentration (MIC) of Clay Samples (mg/mL)**

Clinical isolates	Clay samples	MIC (mg/mL)
<i>Bacillus</i> spp.	Okpekpe clay	10 mm
	Uzala black clay	15 mm
<i>E.coli</i>	Okpekpe + Uzala black clay	20 mm
	Okpekpe clay	14 mm
	Uzala black clay	23 mm
<i>Pseudomonas</i> spp.	Okpekpe + Uzala black clay	27 mm
	Okpekpe clay	9 mm
	Uzala black clay	21 mm
<i>Klebsiella</i>	Okpekpe + Uzala black clay	42 mm
	Okpekpe clay	—
	Uzala black clay	19 mm
<i>Staphylococcus</i>	Okpekpe + Uzala black clay	19 mm
	Okpekpe clay	9 mm
	Uzala black clay	23 mm
	Okpekpe + Uzala black clay	25 mm

#### 4.4 ANTIMICROBIAL SENSITIVITY BIOASSAY

The susceptibility of the clinical isolates to a panel of standard antibiotics was determined using the Kirby-Bauer disc diffusion method. The zones of inhibition (ZOI) were measured in millimeters (mm) and interpreted according to CLSI (2022) guidelines as Susceptible (S), Intermediate (I), or Resistant (R). The results are summarized in Table 4.4.

**Table 4.4: Antibiotic Susceptibility Profile of Clinical Bacterial Isolates**

DRUGS	Bacillus spp.	Staphylococcus spp.	E. Coli	Pseudomonas spp.	Klebsiella spp.
AMP	R	R	S	S	R
R	R	R	N.D	N.D	N.D
CPX	S	S	S	S	S
AZ	S	S	N.D	N.D	N.D
LEV	S	S	S	S	S
E	S	S	N.D	N.D	N.D
PEF	S	S	R	S	S
CN	S	S	S	S	S
APX	R	R	N.D	N.D	N.D
Z	R	R	N.D	N.D	N.D
AU	S	N.D	S	R	R
OFX	N.D	N.D	S	S	S
AZ	N.D	N.D	R	S	S
CF	N.D	N.D	R	S	R
SP	N.D	N.D	R	S	R

KEY: S- Susceptible, R-Resistant, N.D- Not defined.

## CHAPTER FIVE

### 5.0 DISCUSSION

The physicochemical analysis of Okpekpe clay (OKC) and Uzala black clay (UZC) provides a crucial foundation for interpreting their antimicrobial efficacy. Table 4.1 showed that both clays shared an identical acidic pH of 5.47. An acidic environment can be inherently antimicrobial, as it can denature microbial proteins, alter enzyme activity, and disrupt cellular proton gradients, which is in agreement with the research by Williams (2017). This shared characteristic suggests that pH alone is not the sole determinant of their differing antimicrobial potency, directing attention to other parameters. The significantly higher Electrical Conductivity (EC) (453 mS/m) and Total Dissolved Solids (TDS) (226.5 mg/kg) in Okpekpe clay compared to Uzala clay (249 mS/m and 124.5 mg/kg) indicate a greater concentration of soluble ions. While a high ionic strength can create an osmotic stress environment for microbes, the data clearly show that higher EC/TDS did not correlate with greater antimicrobial power, as Uzala clay was consistently more potent. This suggests that the quality of the dissolved ions is more critical than the quantity. This is supported by the heavy metal analysis, which revealed remarkably low concentrations of toxic metals, such as lead, Copper, and Chromium, in both clays, which is a positive finding for their safety profile. The slightly higher Iron (Fe) content in Okpekpe clay (0.088 mg/kg) and also for Uzala black clay (0.049 mg/kg) was also insufficient to explain its weaker activity, indicating that the potent factor in Uzala clay was not among the metals quantified in this standard panel. The higher Organic Carbon content in Uzala clay (0.38 %) compared to Okpekpe clay (0.27 %) may play a role. Organic matter complex with metallic ions, enhancing their solubility and

bioavailability, or it may itself contain antimicrobial humic or fulvic acids, as confirmed by Morrison *et al.*, 2016. The presence of Sulphate (1.30 mg/kg) exclusively in Okpekpe clay is another differentiating factor, though its role in this context appears minimal. The superior antimicrobial activity of Uzala clay is therefore likely attributable to a combination of its specific mineralogy (e.g., types of smectite or illite clays), the presence of unquantified bioactive soluble metals, and its organic content, working in concert (Williams and Haydel, 2010). Table 4.2 shows the agar well diffusion assay results, which established Uzala black clay as a superior broad-spectrum antimicrobial agent compared to Okpekpe clay. Uzala black clay produced consistently larger zones of inhibition against all clinical isolates, including challenging pathogens like *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The dose-dependent response observed for Uzala clay, where activity was maintained even at 25 % concentration, shows it is a potent and soluble antimicrobial agent, indicating that its active components remain bioavailable upon dilution (Balouiri *et al.*, 2016). In contrast, Okpekpe clay showed limited and fragile efficacy. Its activity vanished at concentrations below 75 % for most isolates, and it was completely ineffective against *Klebsiella* spp. At all concentrations. The most striking finding of this study was the powerful synergistic effect observed when Okpekpe and Uzala clays were combined. While the 100 % combination showed no activity—likely due to physical factors like extreme viscosity preventing diffusion—the 75 % combination suspension produced the largest zones of inhibition in the entire study. This synergy was particularly effective against *Pseudomonas aeruginosa*, where a massive 42 mm zone was observed and maintained even at 50 % and 25 % concentrations. *Pseudomonas* spp. is notoriously difficult to treat due to its low-permeability outer membrane and efflux pumps (Pang *et al.*, 2019). The ability of the clay combination to overcome these defenses so effectively suggests a powerful, multi-targeted

mechanism. This could involve components from one clay (i.e., from Okpekpe) disrupting the bacterial membrane, thereby allowing bioactive ions from the other clay (i.e., from Uzala) to enter the cell more readily and exert their toxic effect, for instance, through the generation of reactive oxygen species, as contributed by Williams (2017) in his study.

This suggests that the antibacterial mechanism of uzala black clay is either dependent on a higher threshold concentration or involves less soluble components that cannot diffuse effectively at lower strengths. Table 4.3 provides quantitative confirmation of the trends observed in the diffusion assays, as shown in the MIC results. Uzala clay's lower MIC values (e.g., 15 mg/mL for *Bacillus* spp.) confirm its higher intrinsic potency compared to Okpekpe clay (MIC 10 mg/mL for the same isolate). The fact that the clay combination showed intermediate MIC values for most isolates suggests that the dramatic synergy seen in the diffusion assay might be a result of enhanced diffusion or bioavailability of the active components when combined, rather than a purely synergistic effect on the MIC itself. However, the combination's ability to inhibit *Klebsiella* spp. (MIC 19 mg/mL), which was completely resistant to Okpekpe clay alone, is clear evidence of a beneficial interaction, making the combined formulation effective against a wider spectrum of pathogens. The MBC/MIC ratios, which were predominantly  $\leq 2$  for Uzala clay and the combination, indicate that their action is primarily bactericidal (killing the bacteria). In contrast, Okpekpe clay's higher ratios suggested a bacteriostatic (growth-inhibiting) effect against some isolates. This bactericidal property is highly desirable for therapeutic applications. The antibiotic susceptibility profile (Table 4.4) of the clinical isolates provides critical context. The fact that Uzala clay was effective against isolates that showed resistance to conventional antibiotics like Ampicillin (e.g., *Bacillus* spp., *Staphylococcus* spp., *Klebsiella* spp.) is highly

significant. This indicates that Uzala clay's mechanism of action is distinct from that of beta-lactam antibiotics and could represent a novel pathway to combat drug-resistant microbes.

## **5.1 CONCLUSION**

This study successfully demonstrates that Uzala black clay is a potent, broad-spectrum, bactericidal natural antimicrobial agent. Its efficacy against clinical isolates, including those resistant to common antibiotics, highlights its potential as a source of novel antimicrobial strategies. It demonstrates the significant antimicrobial potential of Okpekpe and Uzala clays, validating their traditional medicinal use while contributing valuable insights to the field of natural product research. The investigation reveals that Uzala Black Clay possesses superior intrinsic antibacterial properties compared to Okpekpe Clay, characterized by broader spectrum coverage and more potent bactericidal action. Most notably, the research uncovers a remarkable synergistic effect when both clays are combined, resulting in enhanced antimicrobial efficacy that surpasses their individual performances. The distinct physicochemical profiles of each clay are established as key determinants of their biological activity, highlighting the importance of material characterization in understanding natural antimicrobial agents. Furthermore, the consistent efficacy demonstrated against clinical isolates, including those exhibiting resistance to conventional antibiotics, underscores the potential of these clay materials in addressing the growing challenge of antimicrobial resistance. This research not only provides scientific validation for traditional practices but also opens new avenues for developing clay-based antimicrobial strategies. The findings establish a foundation for further exploration of natural clay materials as sustainable alternatives or complements to conventional antibiotics, contributing to the ongoing search for novel solutions in combating infectious diseases.

Furthermore, the profound synergistic effect observed when combined with Okpekpe clay opens a promising avenue for developing enhanced, multi-component clay-based therapeutics.

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## APPENDIX



Plate 1: Experimental Procedures (Field Work, 2025)

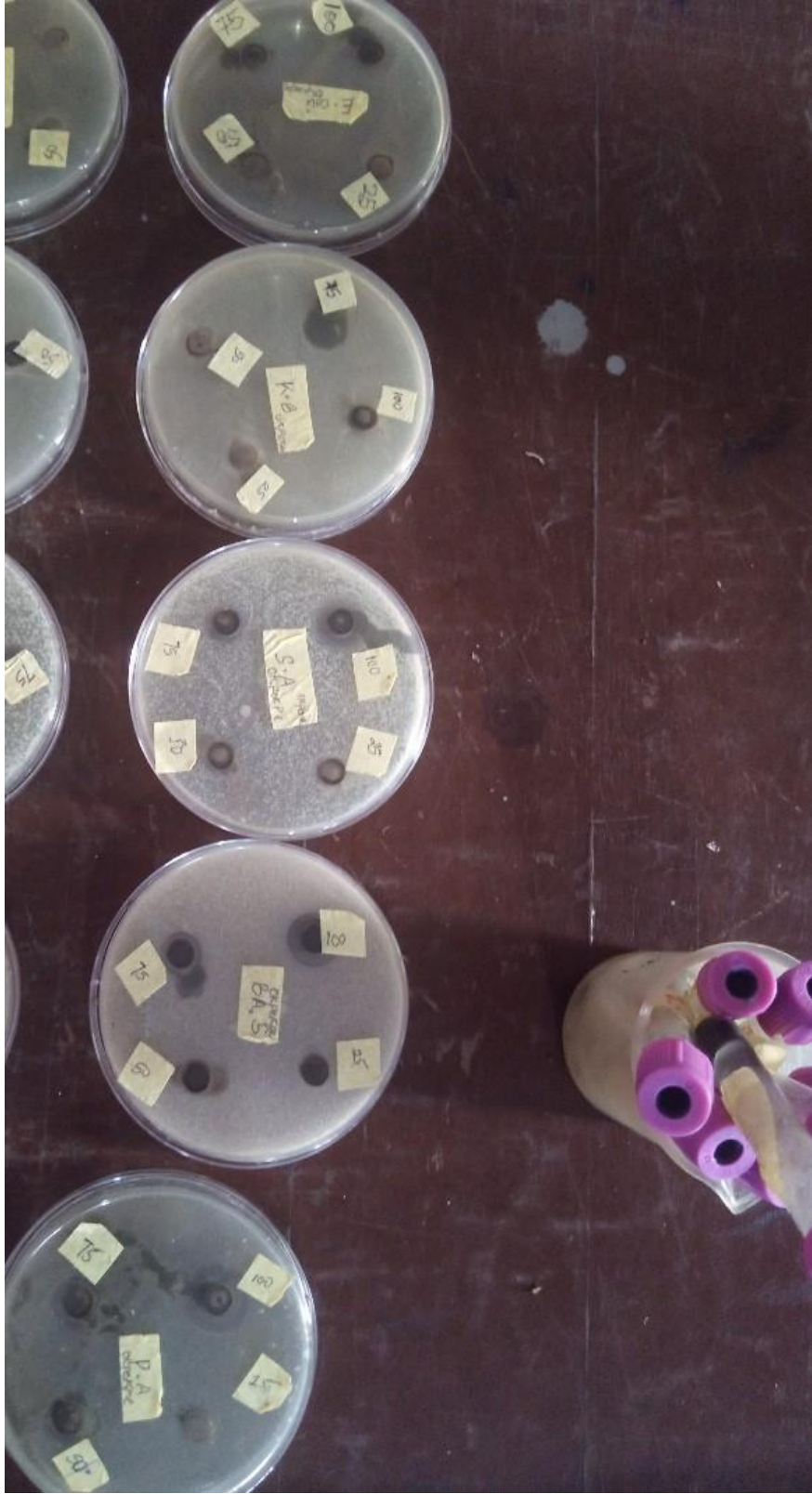


Plate 2: Agar well plate (Field Work, 2025)