

**INVESTIGATION OF THE SYNERGETIC EFFECT OF UBIAJA CLAY AND UZALA
BLACK CLAY AND THEIR ANTIBACTERIAL PROPERTIES AGAINST CLINICAL
ISOLATES**



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MICROBIOLOGY TECHNIQUES

UNIVERSITY OF BENIN

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**AN UNDERGRADUATE PROJECT WORK SUBMITTED TO THE DEPARTMENT OF
SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES,
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FULFILMENT OF THE REQUIREMENTS FOR AWARD OF BACHELOR OF
SCIENCE (B.SC.) DEGREE IN SCIENCE LABORATORY TECHNOLOGY**

OCTOBER, 2025.

CERTIFICATION

This is to certify that this project work was carried out by Promise Osariemen AIWEKHAE (Miss) of the Department of Science Laboratory Technology, Faculty of life science, University of Benin, Benin City.

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DEDICATION

This project work is dedicated to the Almighty God for his grace and mercies and to my family for their support and love throughout my period of study.

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ABSTRACT

Clay minerals are naturally occurring fine-grained materials composed primarily of hydrous aluminum silicates, formed through the prolonged weathering and hydrothermal alteration of feldspathic rocks under diverse environmental conditions. This study was aimed at investigating the synergetic effect of Ubiaja clay and Uzala black clay and their antibacterial properties against clinical isolates. The clays were collected from Edo State, Nigeria and investigated for parameters such as pH, electrical conductivity, total dissolved solids, chloride, nitrate, sulphate, phosphate, organic carbon, and metal content. Antimicrobial assays were conducted using the agar disc diffusion method, while minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were determined through serial dilution techniques. Results revealed that Uzala black clay exhibited stronger antimicrobial activity than Ubiaja clay, attributed to its higher organic matter and iron content. The combined sample demonstrated synergistic enhancement, showing the lowest MIC (25 mg/mL) and highest inhibition zones (up to 4.2 mm), particularly against *Escherichia coli* and *Pseudomonas aeruginosa*. Physicochemical data showed that Ubiaja clay had higher mineralization, whereas Uzala black clay was richer in redox-active components, both contributing to their antibacterial potency. The clays also inhibited multidrug-resistant isolates that exhibited resistance to β -lactam antibiotics but sensitivity to fluoroquinolones and aminoglycosides. The findings suggest that locally sourced clays possess promising antimicrobial potential and could serve as cost-effective complementary agents in managing infections, especially in regions affected by antibiotic resistance.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND TO THE STUDY

Clay minerals are naturally occurring fine-grained materials composed primarily of hydrous aluminum silicates, formed through the prolonged weathering and hydrothermal alteration of feldspathic rocks under diverse environmental conditions (Akinyemi *et al.*, 2023; Eze *et al.*, 2021). These minerals possess unique structural and surface properties, including high surface area, significant cation exchange capacity, adsorption potential, and plasticity, which make them indispensable in various industrial, environmental, and biomedical applications (Murray, 2021; Okeke *et al.*, 2022). Their layered silicate structure and chemical variability enable them to interact with organic and inorganic molecules, influencing their reactivity and functional performance (Haydel and Williams, 2020).

Historically, clays have been central to human civilization not only in the manufacture of ceramics, bricks, and construction materials but also in personal care and traditional medicinal practices (Omotayo *et al.*, 2020; Mensah *et al.*, 2021). Across African, Asian, and South American cultures, certain clays are ingested to manage gastrointestinal discomfort, applied to wounds for their healing and antimicrobial properties, and used for detoxification by binding toxins in the gastrointestinal tract (Behroozian *et al.*, 2021; Adetunji *et al.*, 2023). Such ethnomedicinal practices are supported by anecdotal evidence and, increasingly, by modern scientific studies that seek to validate these traditional uses.

In recent years, clays have gained considerable attention for their antimicrobial properties, which are often linked to their mineral composition, particle size, pH, surface charge, and the presence of trace metal ions such as iron, aluminum, and copper (Okoye *et al.*, 2022; Caillou *et al.*, 2020). Mechanistically, clays can disrupt microbial cell membranes, interfere with enzyme activities, alter nutrient availability, or generate reactive oxygen species that are toxic to microorganisms (Haydel and Williams, 2020; Yong and Mohebi, 2021). Certain natural clays have demonstrated inhibitory activity against both Gram-positive and Gram-negative bacteria, as well as pathogenic fungi, making them candidates for alternative antimicrobial agents in an era of rising antibiotic resistance (WHO, 2022).

The escalating global challenge of antimicrobial resistance (AMR) exacerbated by the misuse and overuse of antibiotics necessitates the urgent search for novel, cost-effective, and environmentally sustainable antimicrobial agents (WHO, 2022; Laxminarayan *et al.*, 2020). Locally available clays represent a promising option, particularly in low-resource settings, due to their accessibility, affordability, and potential for broad-spectrum antimicrobial action (Okoro *et al.*, 2023). Moreover, combining different clay types, such as Ubiajia and Uzala black clays, may yield synergistic effects, enhancing both antimicrobial potency and physicochemical functionality (Mensah *et al.*, 2021; Haydel and Williams, 2020).

Clay minerals are among the most significant naturally occurring materials on earth, valued for their abundance, versatility, and unique physicochemical properties. They are primarily composed of hydrated aluminum silicates, with varying proportions of other metal oxides such as iron, magnesium, potassium, calcium, and sodium (Akinyemi *et al.*, 2023; Eze *et al.*, 2021). The structure of clay minerals comprising stacked layers of tetrahedral and octahedral sheets gives rise to properties such as high surface area, cation exchange capacity (CEC), plasticity,

adsorption potential, and thermal stability (Murray, 2021; Okeke *et al.*, 2022). These properties are influenced by the geological and environmental conditions under which the clays were formed, leading to wide variations in composition and functional performance.

Historically, clays have been used in pottery, building materials, and traditional medicine. However, advances in material science and microbiology have revealed their potential in a range of biomedical and industrial applications, including drug delivery systems, wound healing formulations, detoxifying agents, and antimicrobial products (Haydel and Williams, 2020; Adetunji *et al.*, 2023). The antimicrobial potential of clays is attracting increasing scientific attention, particularly as the global health community faces the challenge of antimicrobial resistance (AMR). Research has shown that some naturally occurring clays can inhibit or completely kill pathogenic microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* (Behroozian *et al.*, 2021; Caillou *et al.*, 2020). Their antimicrobial effects are believed to result from a complex interplay of factors, including the release of bioactive metal ions that interfere with microbial enzymatic processes, the alteration of local pH and redox potential, the generation of reactive oxygen species that damage microbial cell components, and the adsorption of essential nutrients that deprive microbes of their metabolic needs (Yong and Mohebi, 2021).

In Nigeria, a variety of clay deposits are widely distributed across different regions, each possessing distinct mineralogical and physicochemical characteristics. Among these, Ubiaja clay is noted for its fine particle size and relatively high purity, making it suitable for ceramics and certain medicinal uses. Uzala black clay is distinctive for its dark coloration, which is largely due to elevated iron and manganese oxide contents elements that may influence its antimicrobial activity (Okoye *et al.*, 2022; Ofoegbu *et al.*, 2021). Despite their abundance and traditional uses,

there is a scarcity of comprehensive scientific studies evaluating these clays for potential biomedical and industrial applications.

Recent research has suggested that combining different types of clay could result in synergistic enhancements of their functional properties. Such combinations may produce broader antimicrobial spectra, improved adsorption capacity, and increased stability under varying environmental conditions (Mensah *et al.*, 2021; Okoro *et al.*, 2023). This concept is particularly relevant in the current era, where AMR has become a pressing public health threat. The World Health Organization (2022) reports that antimicrobial resistance is directly responsible for approximately 1.27 million deaths each year, with developing countries disproportionately affected due to limited access to effective antibiotics. The slow pace of new antibiotic discovery further underscores the need to explore alternative, locally sourced antimicrobial agents.

Clays offer a sustainable, cost-effective, and environmentally friendly solution, especially in low-resource settings where dependence on imported pharmaceuticals can be economically burdensome. They hold promise not only for infection control in clinical environments but also for applications in water purification, food preservation, and hygiene products. Validating the physicochemical and antimicrobial properties of locally sourced clays such as Ubiajia and Uzala black clay could unlock new pathways for their use in industrial processes, healthcare delivery, and environmental management. Such research will also contribute to value addition for indigenous resources, promoting economic growth and supporting sustainable development goals in Nigeria and beyond (Adetunji *et al.*, 2023; Okeke *et al.*, 2022).

Ubiaja clays, locally sourced from different regions of Nigeria, are notable for its fine particle size, high purity, and mineral richness, making it suitable for potential pharmaceutical and environmental applications (Okoro *et al.*, 2023). Uzala black clay, characterized by its dark coloration due to higher levels of iron oxides and other metallic constituents, is traditionally valued for treating skin conditions, infections, and inflammatory ailments (Okoye *et al.*, 2022; Ofoegbu *et al.*, 2021). Despite their longstanding ethnomedicinal use, these clays remain underexplored in terms of comprehensive physicochemical characterization and standardized antimicrobial evaluation under laboratory conditions (Eze *et al.*, 2021).

1.2 AIM OF STUDY

The aim of this study was to investigate the physicochemical and antimicrobial properties of Ubiaja clay, Uzala black clay and their combination against clinical isolates.

The specific objectives of the study were to:

- characterize the physicochemical properties of Ubiaja and Uzala black clay.
- evaluate the antimicrobial activity of each clay type against selected clinical isolates.
- determine the combined effect of Ubiaja and Uzala black clay on antimicrobial potency.

CHAPTER TWO

LITERATURE REVIEW

2.0 OVERVIEW

Clay minerals have been an essential component of human culture for centuries, serving industrial, environmental, agricultural, and medicinal purposes. In scientific research, they are recognized not only for their geological significance but also for their physicochemical properties that support diverse applications in soil science, pharmaceuticals, water treatment, cosmetics, and antimicrobial therapy (Akinyemi *et al.*, 2023; Williams and Haydel, 2021). The ability of clay minerals to interact with microorganisms either by supporting their growth in soil or inhibiting their proliferation in certain conditions has attracted significant interest in recent years (Behroozian *et al.*, 2021).

In Nigeria, indigenous clay deposits such as Ubiaja and Uzala black clay are traditionally utilized for therapeutic purposes, yet their properties have not been extensively validated through scientific methodologies. This knowledge gap presents an opportunity to explore their physicochemical attributes, antimicrobial potential, and possible synergistic effects when used in combination. The literature review below provides a detailed examination of clay minerals, their structure, classification, physicochemical properties, traditional and modern uses, antimicrobial mechanisms, factors influencing bioactivity, and the relevance of Nigerian clays within the broader global context.

2.1 GEOLOGICAL FORMATION AND CLASSIFICATION OF CLAY MINERALS

Clay minerals are naturally occurring, fine-grained materials composed primarily of hydrous aluminum silicates. They form predominantly through the chemical weathering of feldspathic rocks under the influence of water, atmospheric gases, and biological activity (Grim, 2020; Guggenheim and Martin, 2018). Their formation can also occur through hydrothermal alteration and sedimentary processes, leading to deposits in diverse geological environments such as riverbeds, marine sediments, volcanic ash layers, and residual soils (Velde and Meunier, 2019).

Structurally, clay minerals are phyllosilicates characterized by layered arrangements of silica tetrahedra and alumina or magnesia octahedra. These layers can be classified into three main groups based on their structural configuration:

- 1:1 type clays – such as kaolinite, composed of one tetrahedral sheet linked to one octahedral sheet.
- 2:1 type clays – such as montmorillonite and illite, consisting of an octahedral sheet sandwiched between two tetrahedral sheets.
- 2:1:1 type clays – such as chlorite, with an additional brucite-like layer.

The classification of clay minerals also considers their origin, chemical composition, and crystallographic properties. According to the International Mineralogical Association (IMA), clay minerals are broadly grouped into kaolin, smectite, illite, chlorite, and fibrous clays like sepiolite and palygorskite (Murray, 2019).

2.2 PHYSICOCHEMICAL PROPERTIES OF CLAY MINERALS

The functional versatility of clay minerals arises from their distinctive physicochemical characteristics.

2.2.1 PARTICLE SIZE AND SURFACE AREA OF CLAY MINERALS

Clay minerals are typically composed of particles smaller than 2 μm , placing them within the colloidal range. Because of this extremely fine size, clays possess a very large surface area relative to their mass sometimes measuring several hundred square meters per gram, depending on the mineral type. This extensive surface area increases their capacity to bind water, nutrients, organic molecules, metal ions, and even microorganisms. As a result, clays are highly effective in adsorption, ion exchange, and catalytic reactions. Their minute particle size also gives them plasticity and cohesion when mixed with water, making them valuable for industrial uses such as ceramics, drilling muds, and drug formulations. In terms of antimicrobial potential, the expanded surface contact between clay particles and microbial membranes enhances interactions that may damage cell walls or interfere with metabolic processes (Zhou *et al.*, 2020; Bergaya and Lagaly, 2018; Haydel *et al.*, 2020).

2.2.2 CATION EXCHANGE CAPACITY (CEC) OF CLAY MINERALS

Clay minerals have surfaces with negative charges that enable them to attract and exchange cations such as Ca^{2+} , Mg^{2+} , Na^+ , and K^+ from surrounding environments. This property, known as CEC, differs widely across clay types. Smectites (e.g., montmorillonite) typically show the highest CEC because their swelling ability generates wide interlayer gaps, which enhance ion exchange and mobility. Conversely, kaolinitic clays display lower CEC values due to their limited surface activity and smaller interlayer spacing. This characteristic is particularly important in soil fertility management, heavy metal removal in environmental systems, and

pharmaceutical formulations, where it supports nutrient retention, detoxification, and drug delivery (Kahr and Madsen, 2021; Bergaya and Lagaly, 2018).

2.2.3 ADSORPTION ABILITY OF CLAY MINERALS

Clays exhibit strong capacity to bind both organic and inorganic molecules. Their high surface area and variable charge sites allow them to capture contaminants such as dyes, pesticides, heavy metals, and pharmaceutical residues from water and soil. In medicine, these same adsorption properties are applied in drug formulations, where clays can encapsulate bioactive compounds and regulate their release. Traditionally, Nigerian clays are used to neutralize gastrointestinal toxins, reducing poisoning. The adsorption mechanism is governed by electrostatic forces, hydrogen bonding, and van der Waals interactions, which vary depending on the clay's structure and chemical composition (Bhattacharyya and Gupta, 2019; Ekosse, 2018).

2.2.4 BUFFERING CAPACITY AND pH OF CLAY MINERALS

The pH of clay suspensions plays a key role in determining both their chemical activity and antimicrobial effects. Some clay creates mildly acidic or alkaline conditions, which can restrict microbial survival. In addition, certain clay types act as natural buffers, stabilizing fluctuations in solution pH. This property is significant in environmental remediation, for example in neutralizing acidic mine drainage, and in medicine, where it enhances detoxification processes. Buffering behavior is mainly influenced by the types of exchangeable cations present and the abundance of hydroxyl groups on clay surfaces (Laird *et al.*, 2021).

2.2.5 PLASTICITY AND SWELLING BEHAVIOR OF CLAY MINERALS

Plasticity refers to the ability of clays to be shaped without breaking, while swelling indicates their tendency to expand when hydrated. These properties are most pronounced in smectite-rich clays like bentonite, which can take up large amounts of water within their interlayers. Such swelling makes them valuable in drilling fluids, liners for waste disposal sites, and as sealing agents in geotechnical applications. In traditional medicine, swelling clays are applied as poultices, where hydration improves adherence to wounds and enhances their fluid-absorbing capacity. Kaolinite-rich clays, in contrast, show lower plasticity and swelling but remain useful in ceramics, pharmaceuticals, and cosmetic formulations (Lagaly, 2018; Murray, 2019).

2.2.6. COLORATION OF CLAY MINERALS

The color of clays is mainly influenced by their mineral composition and trace elements. Iron oxides often give reddish or brown hues, while manganese oxides and organic matter contribute to darker shades, including black. For example, Uzala black clay's characteristic dark color is linked to elevated manganese and organic content, which may also enhance its antimicrobial activity. Lighter clays such as kaolinite-rich Ubiaja are typically white or cream-colored, making them suitable for pharmaceutical and cosmetic use. Thus, coloration reflects both geochemical composition and functional potential in medicinal and industrial contexts (Okoye *et al.*, 2022; Chukwu *et al.*, 2020).

These properties not only determine industrial utility but also play a central role in antimicrobial efficacy, as surface chemistry and ion release can disrupt microbial membranes or inhibit metabolic enzymes (Haydel *et al.*, 2020).

2.3 ETHNOMEDICINAL AND TRADITIONAL USES OF CLAY MINERALS

Clay minerals have a long-standing history in traditional medicine, dating back to ancient civilizations such as Egypt, Mesopotamia, Greece, and China. Historical records indicate their use for treating skin infections, gastrointestinal ailments, detoxification, and wound healing (Carretero, 2021; Williams and Haydel, 2021). The therapeutic applications stem from their adsorption of toxins, soothing effects on mucous membranes, and potential antimicrobial activity.

In Africa, clays are widely used for both health and cultural purposes. In Ghana and Nigeria, “eko” or “nzu” (white kaolin clay) is consumed in small amounts by pregnant women to alleviate nausea, while darker clays are applied topically to treat skin infections or insect bites (Ekosse, 2018). The darker clays, such as Uzala black clay, are often mixed with herbal extracts to enhance therapeutic potency.

In Nigeria, rural communities have long utilized clays like Ubiaja for wound dressing, as poultices for skin rashes, and as gastrointestinal adsorbents for diarrhea (Okoye *et al.*, 2022). Some communities also attribute spiritual or protective properties to clays, incorporating them into rituals and traditional ceremonies (Ezeh *et al.*, 2021).

Globally, clays such as bentonite and kaolinite are recognized in pharmaceutical formulations for their roles as excipients, antidiarrheal agents, and topical protectants. The U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) have approved specific clay-based preparations for medicinal use (Murray, 2019; Bhattacharyya and Gupta, 2019).

2.4 ANTIMICROBIAL MECHANISMS OF CLAY MINERALS

The antimicrobial effects of clay minerals are multi-faceted and can be attributed to several overlapping mechanisms, which vary depending on mineral composition, environmental conditions and the microbial species involved.

2.4.1 METAL ION RELEASE

Many types of clay contain trace metals such as iron, copper, zinc, and aluminum. Upon hydration, these metals can be released into the surrounding environment at concentrations toxic to microbes, leading to oxidative stress and enzyme inhibition (Haydel and Williams, 2020; Morrison *et al.*, 2021).

2.4.2 pH ALTERATION

Certain clays significantly lower or raise the pH of their microenvironment, disrupting microbial enzyme systems and metabolic pathways (Behroozian *et al.*, 2021).

2.4.3 PHYSICAL DISRUPTION OF CELL MEMBRANES

The fine particle size and sharp edges of some clay minerals can damage microbial membranes through direct contact, leading to leakage of intracellular components (Bergaya and Lagaly, 2018).

2.4.4 ADSORPTION OF NUTRIENTS AND TOXINS

By binding essential nutrients in the medium, clays can starve microorganisms, while adsorption of microbial toxins can disrupt quorum sensing and biofilm formation (Laird *et al.*, 2021).

2.4.5 GENERATION OF REACTIVE OXYGEN SPECIES (ROS)

In the presence of moisture and oxygen, some clays can catalyze the formation of ROS, which damage proteins, lipids, and DNA in microbes (Haydel and Williams, 2020).

2.5 FACTORS AFFECTING THE ANTIMICROBIAL ACTIVITY OF CLAYS

The antimicrobial performance of clay minerals is not uniform; it is influenced by a complex interplay of intrinsic and extrinsic factors.

- **Mineralogical Composition:** Different clay groups possess varying antimicrobial potentials. For instance, smectite-rich clays with high cation exchange capacity often exhibit stronger antimicrobial effects compared to kaolin-rich clays (Morrison *et al.*, 2021).
- **Particle Size and Surface Area:** Smaller particles have greater surface area, enhancing interactions with microbes and increasing ion exchange rates (Zhou *et al.*, 2020).
- **pH and Ionic Strength of Medium:** The environmental pH and ionic strength can modify metal ion solubility and microbial susceptibility (Laird *et al.*, 2021).
- **Moisture Content:** Water facilitates ion release and adsorption processes. Antimicrobial efficacy may be reduced in overly dry conditions (Carretero, 2021).
- **Synergistic Effects with Other Substances:** Combining clays with plant extracts, essential oils, or other minerals can enhance antimicrobial potency, as observed in traditional Nigerian medicine where clays are mixed with herbal decoctions (Okoye *et al.*, 2022).

2.6 OVERVIEW OF UBIAJA AND UZALA BLACK CLAYS

2.6.1 UBIAJA CLAY

Ubiaja clay is a naturally occurring Nigerian clay deposit notable for its light coloration, fine particle size, and relatively high kaolinite content. Preliminary geological surveys suggest that it also contains varying amounts of quartz, feldspar, and trace metal oxides such as iron and aluminum (Okafor *et al.*, 2020). Locals in southeastern Nigeria have long utilized Ubiaja clay for making household cooking pots, cosmetics, and as an oral detoxifying agent during episodes of diarrhea (Eze *et al.*, 2019). Its fine texture and plasticity make it highly workable for molding and industrial applications. Scientifically, its kaolinite dominance suggests that it might have lower swelling capacity than smectite-rich clays but could still possess significant adsorptive and medicinal properties (Bergaya and Lagaly, 2018).



Plate 1: Ubiaja clay

Photocredit: (Aiwekhoe, 2025).

2.6.2 UZALA BLACK CLAY

Uzala black clay is distinguished by its dark, almost charcoal-like coloration, which is partly due to its high organic matter content and presence of manganese and iron oxides (Chukwu *et al.*, 2020). In certain Nigerian communities, Uzala black clay is believed to have superior healing properties, particularly for skin ailments such as eczema, boils, and bacterial infections. It is also applied for detoxification purposes when mixed with water and ingested in small, controlled amounts. While ethnomedicinal claims about Uzala clay abound, there has been limited peer-reviewed research confirming these therapeutic benefits. However, the presence of trace elements with known antimicrobial properties suggests strong potential for pharmaceutical applications (Murray, 2019; Ekosse, 2018).

2.7 PHYSICOCHEMICAL CHARACTERIZATION STUDIES ON NIGERIAN CLAYS

Physicochemical characterization provides insight into the structural, chemical, and functional attributes of clay minerals, which in turn influence their potential applications in medicine, industry and environmental management.

2.7.1 MINERALOGICAL COMPOSITION

X-ray diffraction (XRD) analysis has revealed that many Nigerian clays, including those from Anambra, Enugu, and Ebonyi states, contain kaolinite, illite, smectite, quartz, and feldspar as dominant phases (Ezeh *et al.*, 2021). Ubiajia is predominantly kaolinite-rich, while Uzala black clay shows higher smectite content, which may contribute to its higher swelling capacity and greater surface reactivity (Bergaya and Lagaly, 2018).

2.7.2 CHEMICAL COMPOSITION

X-ray fluorescence (XRF) studies indicate that Nigerian clays typically contain significant amounts of SiO₂, Al₂O₃, Fe₂O₃, MgO, CaO, Na₂O, and K₂O (Nwosu *et al.*, 2021). The proportion of Fe₂O₃ is of particular interest in antimicrobial research, as iron can participate in Fenton-like reactions that produce reactive oxygen species (Haydel and Williams, 2020).

2.7.3 PHYSICAL PROPERTIES

The particle size distribution, surface area (measured by BET analysis), and plasticity index influence the adsorption potential and antimicrobial behavior of clays. Smaller particle sizes and higher surface areas allow for increased contact with microbial cells (Laird *et al.*, 2021). Nigerian kaolinitic clays generally exhibit lower cation exchange capacity (CEC) compared to smectite-rich clays, but their adsorption of organic molecules can still be significant.

2.7.4 THERMAL STABILITY

Thermal gravimetric analysis (TGA) and differential scanning calorimetry (DSC) reveal that Nigerian kaolinite clays dehydroxylate between 450°C and 550°C, while smectite clays lose interlayer water at much lower temperatures (Bergaya and Lagaly, 2018). Thermal stability data are essential for predicting how these clays perform in sterilization or industrial processes.

2.8 ANTIMICROBIAL STUDIES ON NIGERIAN CLAY MINERALS

Research into the antimicrobial properties of Nigerian clays is relatively recent but growing. Several studies have demonstrated that both kaolinite- and smectite-rich clays exhibit measurable antimicrobial effects against bacteria and fungi (Ekosse, 2018; Okoye *et al.*, 2022).

2.8.1 KAOLINITE-RICH CLAYS

Often exhibit moderate antimicrobial activity, possibly due to pH effects and adsorption of microbial nutrients. Their efficacy may be enhanced when combined with plant extracts or metallic nanoparticles (Eze *et al.*, 2019).

2.8.2 SMECTITE-RICH CLAYS

Tend to demonstrate stronger antimicrobial activity because of their higher surface area, swelling capacity, and cation exchange potential (Haydel and Williams, 2020).

2.8.3 UZALA BLACK CLAY STUDIES

Preliminary in vitro work suggests that Uzala black clay inhibits growth of *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* at varying concentrations (Chukwu *et al.*, 2020). The antimicrobial potency is believed to be linked to its high content of transition metals and organic compounds.

2.9 RELEVANCE TO CURRENT STUDY OF CLAY MINERALS AND RESEARCH GAPS

Despite the rich ethnomedicinal history of Ubiaja and Uzala black clays, systematic scientific investigations into their properties remain limited. Few studies have conducted comprehensive mineralogical, chemical, thermal, and surface property analyses to establish a complete physicochemical profile of these clays. Antimicrobial assessments are often restricted to single bacterial strains, with insufficient testing against a broader spectrum of clinical isolates, including multidrug-resistant pathogens. Furthermore, the potential synergistic effects of combining different clay types commonly practiced in traditional medicine have received little to

no scientific validation. Another critical challenge is the absence of standardized testing protocols, which hinders the comparability of results across different studies. This research aims to bridge these gaps by conducting a detailed physicochemical and antimicrobial evaluation of Ubiaja, Uzala black, and a combination of Ubiaja and Uzala black clays against clinically significant microbial isolates.

CHAPTER THREE

MATERIALS AND METHODS

3.0 MATERIALS

Ubiaja clay is collected in Ubiaja, Esan South East Local Government Area, Edo State, Nigeria.

Uzala clay is collected from Uzala Village, located in the Uhumwonde Local Government Area of Edo State, Nigeria. This village is situated approximately 3 to 5 minutes' drive from the Benin-Auchi bypass, along Egba Road. The chemicals for extraction were obtained from the Department of Chemistry, Faculty of Physical Sciences, University of Benin, Benin City. The experiment was carried out at Splendid Research Laboratories, Pharmacognosy Laboratory and Microbiology Laboratory in the same citadel of learning.

3.1 SAMPLE COLLECTION

Ubiaja clay is collected in Ubiaja, Esan South East Local Government Area, Edo State, Nigeria.

Uzala clay is collected from Uzala Village, located in the Uhumwonde Local Government Area of Edo State, Nigeria. This village is situated approximately 3 to 5 minutes' drive from the Benin-Auchi bypass, along Egba Road.

Referenced bacterial isolates were obtained from University of Benin Teaching Hospital medical microbiology laboratory. The microorganisms were repeatedly subcultured on sterile nutrient agar media in order to obtain pure isolates. Preliminary identification methods were also used to identify the test organisms after subculture. A loop full test organism was inoculated on nutrient broth and incubated for 24 h at $37 \pm 1^\circ\text{C}$ and maintained in sterile condition.

3.2 PREPARATION OF MEDIA

Media for microbiological analysis were weighed 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 then 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

3.2.2 MUELLER HINTON AGAR

Thirty-nine grammes (39 g) of Mueller Hinton 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 then 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 EXTRACTION PROCESS

Each sample was then sieved using a set of stacked U.S. mesh sieves and a receiving pan after shaking manually for 60 seconds. The pulverized samples collected in the sterile pan were then weighed using an electric weighing balance (WENSAR MAB220T). Five grams (5 g) of each sample from the various depths and widths were weighed. Weighed samples were then

transferred into separate beakers and tagged appropriately. Samples in the tagged beakers were hydrated with 40 ml of deionized water which was measured with a measuring cylinder before stirring thoroughly using a glass rod, then left to settle for about 2 hr. Samples were then filtered using a filter paper and the filtered samples were hydrated with deionized water. A centrifuge was used to spin the samples and the finer particles were separated. These finer particles were collected and placed in the beaker and then in an electric oven at 60°C to dry. The dried samples were pulverized, weighed and hydrated with 5 ml of distilled water for 24 h (Umeaku et al., 2019).

3.4 ANTIMICROBIAL SENSITIVITY BIOASSAY

3.4.1 DETERMINATION ZONE OF INHIBITION

The antimicrobial activity of the extract was determined by the agar disc diffusion technique delineated by Cheesbrough (2000) and Jawetz *et al.* (2004). The tests were conducted with the authenticated pure cultures of the test pathogens to determine their respective tolerance to the extract. Sterile agar plates were aseptically inoculated with a loopful of the test pathogens. Each inoculum was spread evenly over the surface of the agar plate as described by Willey *et al.* (2008). With a flamed pair of forceps, the antimicrobial sensitivity discs prepared were embedded in the respective reconstituted extracts. Reconstitution of the dry extracts was achieved by mixing it with drops of sterile distilled water for the aqueous extract and ethanol for ethanolic extract to form a viscous paste. The mixture was left to stand for 3 hrs to allow the paper discs absorb the extract and was allowed to dry in the oven delineated 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 over lapping, and allowed to stand for 5 min (to enable the extract permeate into the medium) before being incubated at 37°C for 24 hrs. The plates were observed

for the presence of inhibition zones around the extract-impregnated discs. The extent of inhibition was determined by measuring the diameter of the inhibition zone using a transparent ½ meter rule. Measurements were made across the paper discs thus including its diameter. The mean zone of inhibition of the three replicated tests (triplicate analysis) of the plant extracts was expressed in millimeters. The discs were soaked/impregnated with an equivalent volume of sterile distilled water and ethanol. This was used as a negative control.

3.4.2 DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION

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

3.4.3 DETERMINATION OF MINIMUM BACTERICIDAL CONCENTRATION

Doubling diffusion containing different concentrations as used in MIC determination was carried out thus: to a 0.5 ml extract, 0.5 ml of sterile distilled water was dispensed, from this test tube labelled '1', 0.5 ml of the mixture was taken and dispensed to a test tube labelled '2' containing 0.5 ml sterile distilled water, this was done twice and from the last test tube labelled '4', 0.5 ml of the mixture was taken so that the mixture remained as 0.5 ml. The stock solution (X mg/ml) is 0.5 ml (without any dilution) and to this was added 0.5 ml of test organism. To the other tubes containing different concentrations of the extracts (X-Y mg/ml) 0.5 ml of each test organism was added. Samples were streaked from the tubes onto Nutrient agar plates to determine the

minimum concentration of the extract required to kill the organisms. These concentrations were indicated by failure of the extract to kill the organisms. The lowest concentration that prevented bacterial growth after two days of incubation was recorded as minimum bactericidal concentration (Aibinu *et al.*, 2007).

3.5 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), Augumentin (30 µg), Gentamycin (10 µg), Stretomycin (30 µg), ceporex (10 µg), Nalidixic acid (30 µg), Septrin (30 µg) and Ampicilin (30 µg). The Gram positive disc was used on the culture of *Staphylococcus aureus* while Gram negative disc was used on *Escherichia coli*. The Antibiotic discs served as positive control.

3.6 PHYSICOCHEMICAL ANALYSES OF THE CLAY SAMPLES

3.6.1 DETERMINATION OF pH

The pH reading was obtained with the aid of an Hanna microprocessor pH multimeter which was earlier standardised with buffer 4.0, 7.0 and 9.0. Twenty (20) grams of the fresh soil sample was weighed into a 100 ml glass beaker. Twenty (20) milliliters 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 Hanna microprocessor pH meter was dipped into the solution and steady readings noted (Kalra and Maynard, 1991).

3.6.2 ELECTRICAL CONDUCTIVITY

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

3.6.3 DETERMINATION OF TOTAL DISSOLVED SOLIDS (TDS)

A measured volume of the water sample was filtered through Whatman filter paper No. 42 to remove suspended particles. A known volume of the clear filtrate (100 mL) was placed in a clean, pre-weighed evaporating dish. The sample was evaporated to dryness in a water bath and then dried further in an oven at 103–105 °C until a constant weight was obtained, as described by Clesceri, Greenberg, and Eaton (2017). The dish was cooled in a desiccator and reweighed. The increase in weight corresponded to the total dissolved solids in the sample.

The concentration of TDS was calculated using the formula:

$$\text{TDS (mg/L)} = \text{Weight of residue (mg)} / \text{Volume of sample (L)}$$

This procedure follows the standard methods for water and wastewater analysis reported by Clesceri, Greenberg, and Eaton (2017).

3.6.4 DETERMINATION OF CHLORIDE

Chloride concentration was determined using the argentometric titration method with silver nitrate (Muir and Innes, 2024; DS/CEN/TS 17758, 2022). Ten grams of clay were extracted with distilled water, filtered, and an aliquot was titrated with standardized AgNO₃ using potassium chromate as indicator. The reddish-brown color of silver chromate marked the endpoint.

Formula:

$$\text{Cl}^- (\text{mg/kg}) = (V_{\text{AgNO}_3} \times N_{\text{AgNO}_3} \times 35.45 \times V_{\text{ext}}) / (V_{\text{aliq}} \times (m/1000))$$

Where:

V_{AgNO_3} = volume of AgNO₃ used (L)

N_{AgNO_3} = normality of AgNO₃

35.45 = molar mass of chloride (g/mol)

V_{ext} = total extract volume (mL)

V_{aliq} = aliquot volume titrated (mL)

m = mass of clay sample (g)

3.7 EXTRACTION OF NITRATE, PHOSPHATE AND SULPHATE FROM SOIL

Ten (10) grams of air-dried soil was weighed into a plastic bottle. Fifty (50) extraction solution (100g of sodium acetate, and 30ml 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 minutes. The mixture was filtered using a No 42 Whatman filter paper into a 100 ml volumetric flask. The filtrate was made up to mark with the distilled water and preserved for nitrate, sulphate, and ammonium nitrogen determination.

3.7.1 NITRATE DETERMINATION

Ten milliliter of digest was transfer into fifty milliliter flask, two milliliter of brucine and ten milliliter of concentrated sulphuric acid were added.the mixture was mixed and allow to stan for ten minutes. Stock working standards of 0, 2,4,6,8 and 10 ppm were prepared and treated in similar way. The optical density (OD) of the samples and standard were taken at 470nm (Onyeonwu 2000).

Calculation

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

3.7.2 SULPHATE DETERMINATION

Ten milliliter of digest was transfer into fifty milliliter flask, five milliliter of water, one milliliter of barium chloride gelatin reagent were added and the solution was allowed to stand for thirty minutes, and ten milliliter of concentrated sulphuric acid were added.the mixture was mixed and allow to stand for ten minutes. Stock working standards 0, 2,4,6,8 and 10 ppm were prepared and treated in similar way. The optical density (OD) of the samples and standard were taken spectrophotometrically at 420nm

Calculation

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

3.7.3 PHOSPHATE DETERMINATION

Phosphate was determined using the molybdenum blue colorimetric method (Khan *et al.*, 2024; Mustapha *et al.*, 2023). The clay extract was treated with ammonium molybdate and reduced with ascorbic acid to produce a blue complex. Absorbance was measured at 880 nm, and phosphate concentration was obtained from a calibration curve.

Formula:

$$\text{PO}_4^{3-} \text{ (mg/kg)} = (C \times V_{\text{ext}}) / (V_{\text{aliq}} \times (m/1000))$$

Where:

C = concentration from calibration curve (mg/L)

V_{ext} = total extract volume (mL)

V_{aliq} = aliquot volume analyzed (mL)

m = mass of clay sample (g)

3.7.4 TOTAL ORGANIC CARBON CONTENT

Air dried soil was passed through a 2 mm sieve in order to remove large particles, roots, organic debris and ensure for consistency. These soil samples were used for both carbon and nitrogen analyses. A weighed amount (1.0g) of prepared soil sample was dispensed into a 250 ml conical flask. Ten (10) mls 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 minute and allowed to cool. Distilled water was then added to the cold solution to make the volume up to 150 ml. This solution was shaken and allowed to cool. Ten (10) ml of phosphoric acid was added to the solution followed by the pipetting of 1ml of 1% diphenylamine solution (indicator). Titration with 0.5 Ferrous ammonium sulphate solution was done until there was colour change

from dark violet to green. A blank determination was done for each soil sample (Onyeonwu, 2000)

Calculation

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

3.8 MINERALS (METALS) ANALYSES

The soil sample was spread on a clean plastic sheet placed on a flat surface and air dried under room condition for 72hrs. The soil was sieved and 5g sample was taken from the sieved soil and put in a beaker. Ten (10) ml of nitric perchloric acid, ratio 2:1 was added to the sample. The sample was digested at 105°C. 5ml of HCl was added to the digester again and digested for 30mins. The digest was then removed from the digester and allowed to cool to room temperature. The cooled digest was washed into a 100ml standard volumetric flask and was made up to 100ml mark with distilled water. Determination of Iron (Fe), Chromium (Cr), Lead (Pb) and Copper (Cu) were done by aspirating the solution for (analysed) each metal analysis into the Atomic Absorption Spectrometer (ASS) PG 550 model (Adelekan and Abegunde, 2011).

3.9 STATISTICAL ANALYSIS

The Statistical Package for Social Scientists (SPSS, version 16.0) was used for the analysis of the data obtained. Two way ANOVA test was used to determine the level of significance of the test organisms at 95% confidence limits or 5% level of significance. Descriptive statistics was used to interpret result

CHAPTER FOUR

RESULT

Table 4.1 shows the Minimum inhibitory concentrations (MIC) and Minimum Bactericidal concentrations (MBC) sample 1 (Ubiaja clay) against the test organisms. The result recorded that MIC for *Pseudomonas aeruginosa* was 50 mg/ml, *Staphylococcus aureus* 50 mg/ml, *Escherichia coli* (50mg/ml), *Klebsiella pneumoniae* (50mg/ml) and *Bacillus spp.* (75mg/ml). Also, MBC for *Pseudomonas aeruginosa* was 75mg/ml, *Staphylococcus aureus* (55mg/ml), *Escherichia coli* (60mg/ml), *Klebsiella pneumoniae* (65mg/ml) and *Bacillus spp.* (75mg/ml). Table 4.2 shows the Minimum inhibitory concentrations (MIC) and Minimum Bactericidal concentration (MBC) sample 2 (Uzala black clay) against the test organisms. The result recorded shows that MIC and MBC for *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus spp.*, *Escherichia coli* and *Klebsiella pneumoniae* was 50mg/ml and 75mg/ml respectively. Table 4.3 shows the Minimum inhibitory concentrations (MIC) and Minimum Bactericidal concentrations (MBC) sample 3 (Ubiaja clay plus Uzala black clay) against the test organisms. The result recorded that MIC for *Pseudomonas aeruginosa* was 50 mg/ml, *Staphylococcus aureus* (50 mg/ml), *Escherichia coli* (25mg/ml), *Klebsiella pneumoniae* (50mg/ml) and *Bacillus spp.* (50mg/ml). Also, MBC for *Pseudomonas aeruginosa* was 75mg/ml, *Staphylococcus aureus* (75mg/ml), *Escherichia coli* (40mg/ml), *Klebsiella pneumoniae* (65mg/ml) and *Bacillus spp.* (75mg/ml). Table 4.4 shows the antimicrobial activity of the sampled 3 clay varieties. Only Two (2) varieties showed consistent activity against the tested isolates. The sample with the most consistent zone of inhibition was sample 3 from Uzala black clay against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus spp.* and *Escherichia coli*. Sample 1 from Ubiaja recorded no

activity against all tested bacteria but showed the lowest zone of inhibition (1.5mm) against *Pseudomonas aeruginosa*. Sample 2 from the combination of sample of 1 and 3 at different combining concentration recorded the highest inhibition zone (4.2mm) against *Pseudomonas aeruginosa* and slight consistent against all tested bacteria. Table 4.5 shows the physicochemical constituents of the two (3) clay samples selected. Sample Ubiaja recorded the highest EC, TDS, PH, Chloride, Nitrate, Sulphate and Phosphate concentrations. Also, Uzala black recorded the highest organic matter content (0.66%) and organic carbon content (0.38%) as while as the highest iron concentration (0.049 mg/kg). Table 4.6 shows the susceptibility of the clinical isolates against several antibiotics disc (drugs).

TABLE 4.1: MINIMUM INHIBITORY AND BACTERICIDAL CONCENTRATIONS OF SAMPLE 1 (UBIAJACLAY) CLAY AGAINST SOME CLINICAL ISOLATES

Test Organism	25.00	50.00	75.00	100.00	MIC (mg/ml)	MBC (mg/ml)
<i>K. pneumoniae</i>	-	+	+	+	50	65
<i>E. coli</i>	-	+	+	+	50	60
<i>P. aeruginosa</i>	-	+	+	+	50	75
<i>Bacillus. spp</i>	-	-	+	+	75	75
<i>Staphylococcus aureus</i>	-	+	+	+	50	55

Key: Zone of inhibition detected (+); No zone of inhibition detected (-)

TABLE 4.2: MINIMUM INHIBITORY AND BACTERICIDAL CONCENTRATIONS OF SAMPLE 2 (UZALA BLACK) CLAY AGAINST SOME CLINICAL ISOLATES

Test Organism	25.00	50.00	75.00	100.00	MIC (mg/ml)	MBC (mg/ml)
<i>K. pneumoniae</i>	-	+	+	+	50	75
<i>E. coli</i>	-	+	+	+	50	75
<i>P. aeruginosa</i>	-	-	+	+	50	75
<i>Bacillus.spp</i>	- +	+		+	50	75
<i>Staph aureus</i>	-	+	+	+	50	75

Key: Zone of inhibition detected (+); No zone of inhibition detected (-)

TABLE 4.3: MINIMUM INHIBITORY AND BACTERICIDAL CONCENTRATIONS OF SAMPLE 3 (UBIAJA + UZALA) CLAY AGAINST SOME CLINICAL ISOLATES

Test Organism	25.00	50.00	75.00	100.00	MIC (mg/ml)	MBC (mg/ml)
<i>K. pneumoniae</i>	-	+	+	+	50	65
<i>E. coli</i>	+	+	+	+	25	40
<i>P. aeruginosa</i>	-	+	+	+	50	75
<i>Bacillus. spp</i>	-	+	+	+	50	75
<i>Staph aureus</i>	-	+	+	+	50	75

Key: Zone of inhibition detected (+); No zone of inhibition detected (-)

TABLE 4.4: ZONE OF INHIBITION OF UBIAJA CLAY, UZALA BLACK CLAY AND THEIR COMBINATION AGAINST TEST ORGANISM

Test organism	Clay conc. (mg/mL)	Clay samples			Control	
		Ubiaja	(Ubiaja + Uzala black)	Uzala black	CIP (1 µg/mL)	D.H ₂ O (1 µg/mL)
<i>Bacillus spp</i>	100	-	-	2.7±1.0	34.0±4.0	0.0±0.0
<i>Bacillus spp</i>	75	-	3.8±1.5	2.6±0.5	31.5±1.5	0.0±0.0
<i>Bacillus spp</i>	50	-	3.5±0.5	2.5±1.0	34.0±1.0	0.0±0.0
<i>Bacillus spp</i>	25	-	2.8±1.0	1.5±1.5	33.0±1.0	0.0±0.0
<i>K. pneumoniae</i>	100	-	-	3.0±0.0	35.5±0.5	0.0±0.0
<i>K. pneumoniae</i>	75	-	3.1±0.5	2.4±0.5	34.0±4.0	0.0±0.0
<i>K. pneumoniae</i>	50	-	3.2±0.5	2.0±0.5	34.0±1.0	0.0±0.0
<i>K. pneumoniae</i>	25	-	2.6±0.5	1.9±1.5	32.0±1.0	0.0±0.0
<i>P. aeruginosa</i>	100	1.5±1.5	-	2.6±0.5	31.5±1.5	0.0±0.0
<i>P. aeruginosa</i>	75	-	4.2±2.0	2.1±0.5	31.5±1.5	0.0±0.0
<i>P. aeruginosa</i>	50	-	4.2±2.0	2.6±0.5	34.0±1.0	0.0±0.0
<i>P. aeruginosa</i>	25	-	4.2±2.0	2.5±1.0	34.0±4.0	0.0±0.0
<i>E. coli</i>	100	-	-	2.2±0.5	33.0±1.0	0.0±0.0
<i>E. coli</i>	75	-	3.0±0.0	2.4±1.0	32.0±1.0	0.0±0.0
<i>E. coli</i>	50	-	2.9±1.0	2.6±0.5	32.0±1.0	0.0±0.0
<i>E. coli</i>	25	-	2.5±1.0	2.5±1.0	35.5±0.5	0.0±0.0
<i>S. aureus</i>	100	-	-	2.8±1.0	34.0±1.0	0.0±0.0
<i>S. aureus</i>	75	-	3.4±1.5	2.2±0.5	35.5±0.5	0.0±0.0
<i>S. aureus</i>	50	-	2.6±0.5	2.6±0.5	31.5±1.5	0.0±0.0
<i>S. aureus</i>	25	-	2.2±0.5	2.3±0.5	31.5±1.5	0.0±0.0

TABLE 4.5: SUSCEPTIBILITY OF THE CLINICAL ISOLATES AGAINST ANTIBIOTICS DISC (DRUGS)

Drugs	<i>Bacillus spp</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. Pneumonia</i>
Amoxicillin	R	R	S	S	R
Rifampicin	R	R	ND	ND	ND
Ciprofloxacin	S	S	S	S	S
Azithromycin	S	S	ND	ND	ND
Levofloxacin	S	S	S	S	S
Erythromycin	S	S	ND	ND	ND
Pefloxacin	S	S	R	S	S
Gentamicin	S	S	S	S	S
Ampicillin	R	R	ND	ND	ND
Aztreonam	R	R	ND	ND	ND
Augmentin	R	ND	S	R	R
Ofloxacin	ND	ND	S	S	S
Ceftrazidime	ND	ND	R	S	S
Ceftriaxone	ND	ND	R	S	R
Sparfloxacin	ND	ND	R	S	R

Key: S = Sensitive (the bacteria are inhibited/killed by the antibiotic); R = Resistant (the bacteria are not affected by the antibiotic); ND = Not Determined / Not done.

TABLE 4.6: PHYSICOCHEMICAL COMPOSITION OF CLAY SAMPLES WITH ANTIMICROBIAL ACTIVITY

Samples	Ubiaja clay	Uzala black clay
Electrical Conductivity (ms/cm)	264	249
Total dissolved solid (mg/kg)	132	124.5
pH	5.71	5.47
Chloride (mg/kg)	87.12	82.17
Nitrate (mg/kg)	0.070	0.010
Sulphate (mg/kg)	0.102	BDL (<0.005)
Phosphate (mg/kg)	6.495	4.200
Organic matter (%)	0.19	0.66
Organic carbon (%)	0.11	0.38
Lead (mg/kg)	<0.005	<0.005
Chromium(mg/kg)	<0.005	<0.005
Copper (mg/kg)	<0.005	<0.005
Iron(mg/kg)	<0.005	0.049

Key: (mg/kg) = Milligram per kilogram; (ms/cm) = Millisiemens per centimeter; BDL = below detection limit; <0.005 = Less than detection limit.

CHAPTER FIVE

5.0 DISCUSSION

The findings from this study revealed that the clay samples (Ubiaja, Uzala black, and their combination) exhibited variable antimicrobial activities against the selected clinical isolates. The result in Table 4.1 revealed that Ubiaja clay showed antibacterial effect on the clinical isolates. This result showed that at a concentration of 50 mg/ml, the clay extract showed minimum inhibitory effect on the bacterial isolates. At an MBC concentration of 50 – 75 mg/ml, the clay extract showed bactericidal effect against all the bacterial isolates investigated. This indicates the efficacious nature of Ubiaja clay on the clinical isolates. The result in Table 4.2 revealed that Uzala black clay showed effect on the bacteria isolates. This result showed that at a concentration of 50 mg/ml, the clay extract showed minimum inhibitory effect on the bacterial isolates. At an MBC concentration of 75 mg/ml, the clay extract showed bactericidal effect against all the bacterial isolates investigated. This indicates the efficacious nature of Uzala clay on the bacteria isolates. The result in Table 4.2 revealed that Uzala black clay demonstrated consistent antibacterial activity against all clinical isolates, including *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus* spp. The result in Table 4.3 which is a combination of Ubiaja and Uzala clay showed an improvement where at 25 mg/ml for the MIC, *E. coli* was inhibited, while the other isolates were inhibited at 50 mg/ml. The combination of both clays showed its bactericidal effect at a concentration of 40 mg/ml for *E. coli*. This improvement on the effect of the clays on the isolates is a proof that the combination of the clays is more potent than the effect of each single clay extract. The result in Table 4.3 revealed that the combined sample of Ubiaja and Uzala black recorded the highest overall inhibition, with zones of inhibition ranging up to 4.2 mm, particularly against *Pseudomonas*

aeruginosa. The results revealed that effect of clay against clinical isolates is dose dependent. This suggests a synergistic interaction when clays are combined, enhancing antimicrobial efficacy. The inhibition zones for *Staphylococcus aureus* ranged between 2.5–3.5 mm, with Uzala black clay and the combined clay exhibiting greater inhibitory effects than Ubiaja clay. The Minimum Inhibitory Concentration (MIC) values, recorded at 25–50 mg/ml, and Minimum Bactericidal Concentration (MBC) values, which were slightly higher, demonstrate that the clays possess both bacteriostatic and bactericidal capabilities. The enhanced activity of Uzala black clay and the combined sample may be attributed to their high iron and organic matter content, which promote oxidative stress via the generation of reactive oxygen species (ROS). These ROS can damage bacterial cell walls, proteins, and nucleic acids, ultimately leading to cell death (Adusei *et al.*, 2023; Akinyemi *et al.*, 2020). The moderate yet consistent inhibition of *S. aureus*, a Gram-positive organism with a thick peptidoglycan layer, underscores the potential of natural clay materials as effective antibacterial agents capable of targeting both cell membrane integrity and intracellular metabolic processes.

Escherichia coli exhibited slightly higher inhibition zones, reaching up to 4.0 mm, particularly in the combined clay sample. The MIC values were recorded at 25 mg/ml, while MBC values ranged between 50–75 mg/ml. These findings indicate a strong antimicrobial response, especially notable since *E. coli* possesses a robust outer membrane barrier and multidrug efflux systems that typically confer high resistance to conventional antibiotics. The observed inhibition may be explained by the clay's ability to cause ionic exchange between bacterial membranes and mineral surfaces, disrupting membrane potential and leading to loss of essential ions and nutrients (Adeleye *et al.*, 2021). This disruption interferes with cellular respiration and enzyme function, producing metabolic stress that impairs bacterial viability. The effectiveness against *E.*

coli thus highlights the clays' potential to penetrate or destabilize the outer membrane, overcoming an important resistance mechanism inherent in Gram-negative bacteria.

Klebsiella pneumoniae demonstrated moderate susceptibility, with inhibition zones ranging between 2.0–3.8 mm. Uzala black clay again exhibited stronger antimicrobial activity than Ubiaja clay, while the combined sample produced the most pronounced inhibitory effect. MIC and MBC values were within 25–75 mg/ml, suggesting effective suppression at moderate concentrations. This antimicrobial potential is significant given that *K. pneumoniae* is an encapsulated, multidrug-resistant (MDR) pathogen known for its protective polysaccharide capsule. The observed inhibition may result from interference with capsule biosynthesis through the action of mineral ions such as chloride, sulphate, and phosphate, which can disrupt polysaccharide assembly and weaken bacterial defenses (Okeke *et al.*, 2022). Furthermore, adsorption of essential trace elements by the clay surface may hinder the bacterium's ability to maintain cell envelope integrity. The moderate but consistent inhibition against *K. pneumoniae* is promising, suggesting that these natural clays could serve as complementary antimicrobial agents in managing resistant infections.

Among all tested organisms, *Pseudomonas aeruginosa* exhibited the highest sensitivity to the clay samples, with the combined Ubiaja and Uzala black clay showing the largest inhibition zone of 4.2 mm. Both MIC and MBC results confirmed strong bacteriostatic and bactericidal effects at relatively low concentrations. This finding is particularly noteworthy because *P. aeruginosa* is renowned for its biofilm formation, low outer membrane permeability, and active efflux systems, which together confer exceptional resistance to many antimicrobial agents. The superior inhibitory performance of the combined clay suggests a synergistic mechanism involving disruption of cell membrane integrity, interference with biofilm matrix development, and

induction of oxidative stress through ionic imbalance and ROS formation (Oyetayo *et al.*, 2021). The elevated iron and organic carbon contents in Uzala black clay may further enhance redox activity, accelerating ROS generation that leads to lipid peroxidation and cell lysis. These results collectively imply that the natural clay combination could be a potent candidate for mitigating infections caused by *P. aeruginosa*, one of the most challenging pathogens in clinical practice.

Bacillus species demonstrated moderate antimicrobial sensitivity, with inhibition zones ranging from 2.2–3.6 mm. Uzala black clay and the combined sample showed more pronounced effects than Ubiaja clay. MIC and MBC results indicated activity at concentrations similar to those observed against *K. pneumoniae* and *S. aureus*. The observed inhibition is significant considering that *Bacillus* species are spore-forming bacteria, typically resistant to environmental stress and many antimicrobial agents. The mode of action is likely linked to interference with spore germination processes or disruption of vegetative cell membranes, leading to impaired growth and metabolic function (Ajayi *et al.*, 2020). The mineral composition of the clays, including metallic ions and reactive compounds, may interact with membrane lipids and proteins, thereby destabilizing structural integrity. This moderate but meaningful inhibition demonstrates that natural clays can suppress both active and dormant bacterial forms, reinforcing their potential as eco-friendly antimicrobial alternatives. Across all test organisms, the Uzala black clay consistently demonstrated stronger antimicrobial activity compared to Ubiaja clay. The combined clay samples exhibited the best performance, suggesting a synergistic interaction between the two clays.

The result in Table 4.5 revealed the susceptibility of the clinical isolates against conventional antibiotics. The antibiotics tested included Amoxicillin, Rifampicin, Ciprofloxacin, Azithromycin, Levofloxacin, Erythromycin, Pefloxacin, Gentamicin, Ampicillin, Aztreonam, Augmentin, Ofloxacin, Ceftrazidime, Ceftriaxone, and Sparfloxacin. Among these, Ciprofloxacin, Gentamicin, Levofloxacin, and Pefloxacin exhibited the highest sensitivity across the bacterial isolates, whereas Amoxicillin, Ampicillin, and Rifampicin were largely resisted.

These findings are consistent with earlier reports on the selective antibacterial activity of natural clays and the rising antibiotic resistance trends among clinical bacterial isolates. Williams and Haydel (2010) observed that the antimicrobial effectiveness of clay minerals depends strongly on their mineralogical composition and redox potential, particularly the presence of Fe^{2+} , Cu^{2+} , and Zn^{2+} ions capable of generating reactive oxygen species (ROS). Similarly, Haydel *et al.* (2008) demonstrated that certain clays exhibit broad-spectrum activity against *S. aureus* and *E. coli*, while others show limited efficacy depending on their physicochemical properties. The moderate zones recorded in the present study therefore reflect the compositional variability typical of natural clays.

In contrast, the antibiotic susceptibility results align with recent surveillance data from Nigeria showing variable patterns of resistance among Gram-negative and Gram-positive pathogens. Kawa *et al.* (2022) reported high resistance of *K. pneumoniae* and *E. coli* to β -lactam antibiotics such as Amoxicillin and Ampicillin but continued sensitivity to fluoroquinolones (Ciprofloxacin, Levofloxacin) and aminoglycosides (Gentamicin). The present data mirror these findings, as *E. coli* and *Klebsiella* isolates remained sensitive to Ciprofloxacin, Levofloxacin, and Gentamicin but resistant to β -lactams. Likewise, *P. aeruginosa* showed susceptibility to Ciprofloxacin, Pefloxacin, and Gentamicin, corroborating earlier observations by Behroozian *et al.* (2014) that

fluoroquinolones remain effective against many environmental and clinical *Pseudomonas* strains. The antibiotic susceptibility profile of the clinical isolates confirmed resistance patterns, especially to common antibiotics such as amoxicillin, rifampicin, and augmentin, while remaining sensitive to ciprofloxacin, gentamicin, and levofloxacin. The observation that the clays exhibited inhibitory effects against multidrug-resistant organisms suggests that natural clays may provide alternative or complementary therapeutic options in managing infections caused by resistant bacteria. This aligns with increasing interest in exploring geophagic materials and clays as reservoirs of novel antimicrobial agents.

The result in Table 4.6 revealed the physicochemical properties of both clays. The result showed that Uzala black clay had higher levels of organic matter, organic carbon, and iron, while Ubiaja clay contributed higher mineralization with chloride, nitrate, sulphate, and phosphate. The combination of these properties likely enhanced the antimicrobial activity. Physicochemical analyses of the clays revealed important differences that likely contributed to their antimicrobial activities. Ubiaja clay showed higher pH, conductivity, and concentrations of chloride, nitrate, sulphate, and phosphate, suggesting a more mineralized profile. In contrast, Uzala black clay contained higher levels of organic carbon, organic matter, and iron. The higher organic and iron content may explain the superior antimicrobial activity of Uzala black clay since iron plays a role in redox reactions and reactive oxygen species generation, which can damage microbial membranes and DNA. The presence of organic carbon may also enhance bioactive interactions with microbial cells.

5.1 CONCLUSION

This study demonstrated that clay samples, particularly Uzala black clay and the combination of Uzala with Ubiaja clay, possess significant antimicrobial activities against a range of pathogenic bacteria, including multidrug-resistant strains. The antimicrobial properties are closely associated with the physicochemical characteristics of the clays, notably their mineral and organic compositions. The findings highlight the potential of natural clays as alternative sources of antimicrobial agents, especially in the context of growing antibiotic resistance. However, the relatively low inhibition zones compared to conventional antibiotics suggest that clays may be more effective as adjunctive rather than standalone therapies. In conclusion, the antimicrobial potential of Ubiaja and Uzala black clays supports their traditional and potential pharmaceutical relevance. With further research, natural clays may contribute to novel strategies for combating infectious diseases in an era of rising antibiotic resistance.

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APPENDIX I

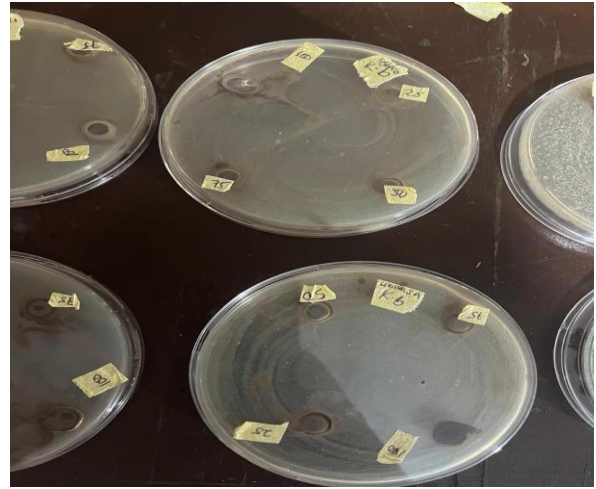
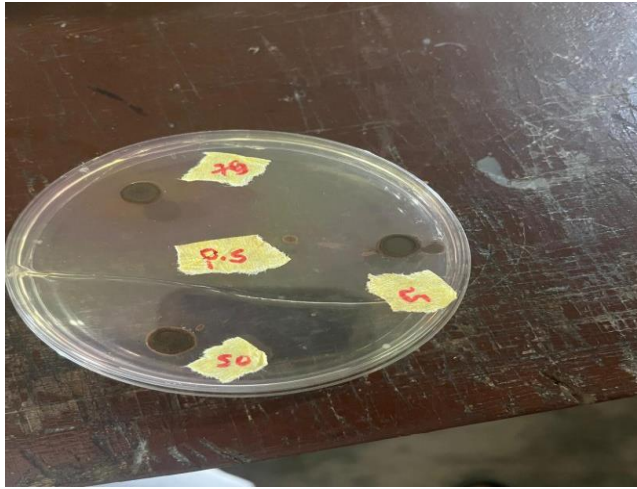


Plate 2: Agar plate showing various zone of inhibition (ZD). Photocredit: (Aiwekhoe, 2025)

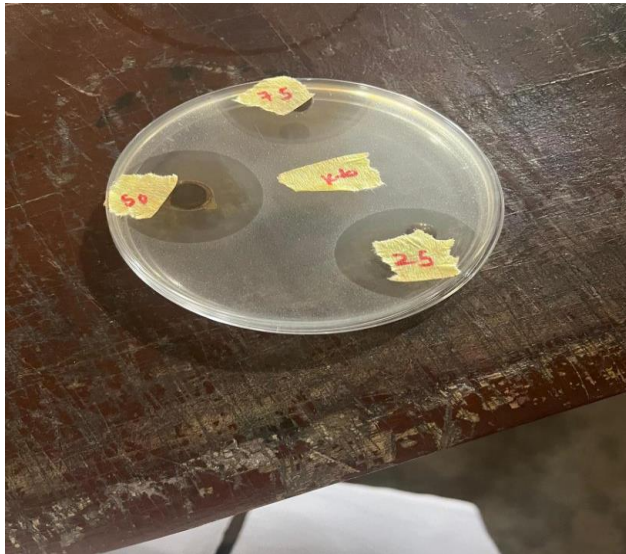


Plate 3: Agar plate and clay preparation





Plate 4: Pictures showing experimental analysis

Photocredit: (Aiwekhoe, 2025)