BACTERIOCIDAL EFFECT OF GARLIC (*Allium sativum* L.) EXTRACT ON *Pseudomonas aeruginosa* AND *Klebsiella pneumoniae* ISOLATES FROM WOUND SWABS IN UNIVERSITY OF BENIN TEACHING HOSPITAL.

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DEPARTMENT OF MEDICAL LABORATORY SCIENCE,

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A PROJECT WORK SUBMITTED TO THE
DEPARTMENT OF MEDICAL LABORATORY SCIENCE,
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SUPERVISED BY

DR. MRS) N.A OLISE

SEPTEMBER, 2025.

CERTIFICATION

This is to certify that this project work was carried out by **EHIOZUA NATHAN OSAEBHUE** with matriculation number **BMS2001158** in partial fulfillment of the requirements for the award of Bachelor of Medical Laboratory Science (BMLS) from the University of Benin, Benin City, Edo State, Nigeria.

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DEDICATION

This work is dedicated to my Heavenly Father who is the source of all knowledge and wisdom and to my wonderful parents for their unwavering love and support.

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ABSTRACT

Allium sativum (garlic) has long been recognized for its antimicrobial potential, yet the comparative efficacy of its ethanolic and aqueous extracts against clinically relevant pathogens requires further evaluation. This study assessed the antibacterial activity of ethanolic and aqueous garlic extracts against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* isolated from wound swabs. Garlic bulbs were authenticated, dried, pulverized, and subjected to maceration in ethanol and sterile water to obtain crude extracts with yields of 2.25% and 3.57%, respectively. Antimicrobial susceptibility was evaluated using ditch plate, cup plate, and agar dilution methods. Inhibition zone diameters (IZDs), minimum inhibitory concentrations (MICs), and minimum bactericidal concentrations (MBCs) were determined. Both extracts displayed concentration-dependent antibacterial activity, with ethanolic fractions showing greater potency than aqueous fractions. Ethanolic extract inhibited *K. pneumoniae* and *P. aeruginosa* at 100 mg/mL (IZD: 12–14 mm), while aqueous extract required 200 mg/mL for measurable inhibition (IZD: 11–13 mm). MIC values were 100 mg/mL for ethanolic and 200 mg/mL for aqueous extracts, whereas MBC was achieved only with ethanolic extract at 200 mg/mL. Statistical analysis confirmed that concentration significantly influenced antibacterial activity ($p = 0.000$), while differences between organisms or extract types were not statistically significant ($p = 0.292$). These findings suggest that garlic exhibits concentration-dependent antibacterial activity, with ethanolic extracts demonstrating superior efficacy.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

Wound infections remain a major cause of preventable morbidity, prolonged hospitalization, and treatment failure across healthcare systems, especially when chronicity and biofilm formation are involved (Schultz *et al*., 2017). Biofilms are now recognized as the dominant mode of microbial growth in chronic wounds; meta-analytic estimates suggest that approximately 78% of chronic, non-healing wounds harbor biofilms that perpetuate inflammation and delay tissue repair (Malone *et al*., 2017).

Within hospital environments, Gram-negative organisms have become increasingly prominent agents of wound and soft-tissue infections, with *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* frequently isolated from acute (e.g., surgical, burn, traumatic) and chronic wounds (Diban *et al*., 2023).

Pseudomonas aeruginosa is a motile, non-fermentative, Gram-negative bacillus, easily recognized by its rod-shaped morphology and distinctive blue-green pigment production. Belonging to the family *Pseudomonadaceae*, it possesses a comparatively large genome that confers remarkable adaptability. This versatility enables survival in diverse environments, synthesis of a wide range of virulence factors, and resistance to multiple classes of antibiotics (Sathe *et al.,* 2023).

The bacterium is widely distributed in soil and aquatic habitats, but it can also colonize humans without causing disease, having been detected on the skin, throat (~5%), and stool (~3%) of healthy individuals. Clinically, *P. aeruginosa* is a major

cause of hospital-acquired infections, including pneumonia, urinary tract infections (UTIs), wound infections, osteomyelitis, septic arthritis, and bloodstream infections (Sathe *et al*., 2023).

Its pathogenicity is driven by quorum-sensing networks that regulate virulence determinants (e.g., elastases, exotoxins, rhamnolipids) and promote robust biofilm formation on wound surfaces. Biofilm architecture reduces antibiotic penetration and sustains phenotypic heterogeneity (including persistent and slow growing cells), translating clinically into recalcitrant infections that are difficult to eradicate (Attinger and Wolcott, 2012).

Klebsiella pneumoniae is a fermenting, encapsulated Gram-negative bacillus and a leading cause of healthcare-associated infections, including wound and soft-tissue infections (Wyres *et al*., 2020). First described in 1882, it is ubiquitously found in soil, water, animals, and hospital environments. While it often colonizes humans without harm, it can cause severe infections, particularly among neonates, elderly, and immunocompromised patients. Hypervirulent strains are of special concern due to their ability to disseminate within the body, resulting in significant morbidity and mortality (Bengoechea and Pessoa, 2018).

Rising antimicrobial resistance has made *K. pneumoniae* a global health threat. Many isolates are resistant to multiple drug classes, including carbapenems, leaving very limited treatment options. In addition, the bacterium serves as a reservoir of resistance genes transferable to other pathogens. Alarmingly, recent reports describe multidrug-resistant strains that also carry hypervirulence traits, posing risks even to otherwise healthy individuals. These developments highlight the urgent need for deeper

understanding of its pathogenesis and for improved strategies in prevention and treatment (Bengoechea and Pessoa, 2018).

Its prominent polysaccharide capsule, together with adhesins and strong biofilm-forming ability, enhances persistence on both biotic and abiotic surfaces, including inflamed wound beds and medical devices. In clinical series of surgical, burn, and diabetic wounds, *K. pneumoniae* is frequently isolated either as a single pathogen or in polymicrobial associations, often alongside *P. aeruginosa* and *Staphylococcus aureus*, reinforcing its role in complex wound ecology (Oladeinde *et al*., 2013).

Plant-derived compounds remain a vital frontier in antimicrobial discovery, with *Allium sativum* being among the best studied. When fresh garlic is crushed, the enzyme alliinase converts the precursor alliin into allicin (diallyl thiosulfate), a highly reactive organosulfur compound responsible for much of garlic's antimicrobial activity. Allicin and related compounds (ajoene, diallyl polysulfides) exert antibacterial effects by reacting with thiol groups in microbial enzymes and structural proteins, thereby disrupting essential redox-regulated pathways (Ankri and Mirelman, 1999).

Allium sativum demonstrates potent bactericidal, antibiofilm, and anti-quorum-sensing activities against both Gram-positive and Gram-negative bacteria, including multidrug-resistant strains. Notably, garlic extracts often act synergistically with conventional antibiotics, enhancing their efficacy against resistant pathogens. Given the global escalation of antimicrobial resistance and the pressing demand for affordable, accessible adjuncts to conventional therapy, particularly in resource-limited settings garlic represents a promising candidate for in-vitro evaluation and potential therapeutic development (Bhatwalkar *et al*., 2021).

1.2 Statement of the Problem

Wound infections remain a pressing challenge in clinical practice, not only because they delay healing and increase morbidity but also because they now serve as reservoirs of antimicrobial resistance (AMR). Globally, the spread of resistant Gram-negative pathogens has placed wound care at the frontline of the AMR crisis, with *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* identified as two of the most problematic culprits (Monk *et al*., 2024). These organisms not only resist most first-line antibiotics but also form biofilms that further shield them from therapeutic intervention.

In Nigeria, the picture is even more alarming. Recent clinical reports have shown that over 90% of *K. pneumoniae* isolates from tertiary hospitals are multidrug-resistant, with significant carbapenem resistance, a class of drugs once reserved as last-resort therapy (Akintoyese *et al.*, 2025). Similarly, *P. aeruginosa* isolates continue to demonstrate resistance to cephalosporins and aminoglycosides, limiting available treatment options (Shah *et al.*, 2025). Within West Africa, and specifically in Edo State, wound infection studies confirm that resistant *Pseudomonas* and *Klebsiella* strains are commonly recovered from patients, highlighting both the therapeutic challenge and the urgent need for locally relevant alternatives (De Sousa, 2025).

The persistence of these infections not only prolongs hospital stays and increases healthcare costs but also translates to higher risks of sepsis, amputation, and mortality. Current antibiotic-centered strategies are failing, and the reality is stark in the sense that, without adjunctive or alternative measures, wound care outcomes in resource-limited settings will continue to deteriorate.

Plant derived antimicrobials provide one such avenue of hope. *Allium sativum*, through its bioactive compound allicin, has been shown to exert broad-spectrum antimicrobial effects, including activity against multidrug resistant Gram-negative bacteria (Magryś *et al*., 2021). More importantly, synergistic studies suggest that garlic extracts may enhance the efficacy of conventional antibiotics, potentially restoring activity against resistant pathogens (Ismail *et al*.,2020).

Thus, the problem is this; resistant *K. pneumoniae* and *P. aeruginosa* continue to undermine wound care in Nigeria, while therapeutic pipelines remain nearly exhausted. Unless alternative approaches such as plant-based antimicrobials are rigorously evaluated, clinicians will be left increasingly powerless against infections that were once easily treatable. This study, therefore, addresses a critical gap by investigating the bactericidal effects of garlic extract on these resistant pathogens, with the ultimate aim of informing affordable and locally adaptable adjuncts for wound management.

1.3 Justification of the Study

The persistence of wound infections caused by *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* poses a critical challenge in clinical practice, particularly in regions like Nigeria where healthcare resources are limited. Rising rates of multidrug resistance in these pathogens have left clinicians with fewer effective therapeutic options, thereby prolonging hospital stays, increasing treatment costs, and heightening mortality risk (Monk *et al*., 2024). Moreover, the biofilm-forming capabilities of these organisms further reduce antibiotic efficacy, complicating wound healing and necessitating alternative or adjunct strategies. Given these realities, there is an urgent

need to investigate accessible and cost-effective solutions that can complement existing therapies and address the gaps in current antimicrobial management.

Allium sativum, a widely available medicinal plant, presents a compelling candidate in this regard. Its bioactive compounds, particularly allicin and related organosulfur derivatives, have demonstrated broad-spectrum antibacterial, antibiofilm, and anti-quorum-sensing activity against both Gram-positive and Gram-negative organisms, including resistant strains (Magryś *et al*., 2021). Evidence also suggests synergistic interactions between garlic extracts and conventional antibiotics, enhancing the overall effectiveness of treatment regimens while potentially reducing drug resistance development. Considering the dual challenges of rising resistance and limited access to advanced therapeutics in resource-constrained settings, investigating the antimicrobial potential of garlic in wound infections is both scientifically justified and of significant public health relevance.

1.4 Aim of the Study

The study aimed to evaluate the antimicrobial activities of *Allium sativum* extracts against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* isolated from wound infections from the University of Benin Teaching Hospital, in Benin City.

1.5 Specific Objectives

This study seeks to:

1. determine the Inhibition Zone Diameter (IZD) of the extracts against the selected wound bacterial isolates.

2. determine the Minimum Inhibitory Concentration (MIC) of the extracts against the selected wound bacterial isolates.

3. determine the Minimum Bactericidal Concentration (MBC) of the extracts against the selected wound bacterial isolates.

4. compare the antimicrobial activity of ethanolic and aqueous extracts from *Allium sativum Linn. w*ith standard antibiotics commonly used to treat wound infections.

5. compare the efficacy of garlic extracts with conventional antibiotics to explore possible synergistic effects

1.6 Research Questions

1. How do the Inhibition Zone Diameters produced by the ethanolic and aqueous garlic extracts compare with those produced by standard antibiotics commonly used for treating wound infections?

2. Is there a significant difference in the Inhibition Zone Diameters produced by the aqueous and ethanolic garlic extracts against the selected wound pathogens?

3.What are the Minimum Inhibitory Concentrations (MICs) of the ethanolic and aqueous extracts of *Allium sativum* Linn. against the selected wound bacterial isolates?

4. What are the Minimum Bactericidal Concentrations (MBCs) of the ethanolic and aqueous extracts of *Allium sativum* Linn. against the selected wound bacterial isolates?

5. Is there a significant difference between the antimicrobial effectiveness of aqueous and ethanolic garlic extracts against the selected wound pathogens?

1.7 Research Hypotheses

1.7.1 Null Hypotheses (H_0)

1. H_{01}: The Inhibition Zone Diameters produced by the ethanolic and aqueous garlic extracts do not significantly differ from those produced by standard antibiotics used for treating wound infections.

2. H_{02}: There is no significant difference in the Inhibition Zone Diameters produced by the aqueous and ethanolic garlic extracts against the selected wound pathogens.

3. H_{03}: There is no significant difference in the Minimum Inhibitory Concentration of the ethanolic and aqueous *Allium sativum* extracts against the selected wound bacterial isolates.

4. H_{04}: There is no significant difference in the Minimum Bactericidal Concentration of the ethanolic and aqueous *Allium sativum* extracts against the selected wound bacterial isolates.

5. H_{05}: There is no significant difference in the overall antimicrobial effectiveness of aqueous and ethanolic garlic extracts against the selected wound pathogens.

1.7.2 Alternative Hypotheses (H_1)

1. H_{11}: The Inhibition Zone Diameters produced by the ethanolic and aqueous garlic extracts significantly differ from those produced by standard antibiotics used for treating wound infections.

2. H_{12}: There is a significant difference in the Inhibition Zone Diameters produced by the aqueous and ethanolic garlic extracts against the selected wound pathogens.

3. H_{13}: There is a significant difference in the Minimum Inhibitory Concentration of the ethanolic and aqueous *Allium sativum* extracts against the selected wound bacterial isolates.

4. H_{14}: There is a significant difference in the Minimum Bactericidal Concentration of the ethanolic and aqueous *Allium sativum* extracts against the selected wound bacterial isolates.

5. H_{15}: There is a significant difference in the overall antimicrobial effectiveness of aqueous and ethanolic garlic extracts against the selected wound pathogens**.**

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1: Concept of Wound Infection

Wound infection is a pathological condition that arises when microorganisms breach the skin or underlying tissue and provoke a host immune response sufficient to impair the natural process of repair. The concept is broader than the mere presence of bacteria, as many wounds are colonized by microorganisms without clinical consequences. An infection is established when microbial load and virulence exceed the host's ability to contain them, thereby delaying healing and potentially causing systemic complications (Swanson *et al.*,2022).

Historically, the understanding of wound infection has evolved significantly. In earlier centuries, the presence of pus was considered a positive sign of healing, encapsulated in the term *pus bonum et laudabile*. However, with the development of antiseptic principles by Ignaz Semmelweis and Joseph Lister, it became clear that wound suppuration was instead a sign of microbial invasion and poor asepsis (Singhal and Kaur, 2023). Current scientific consensus describes wound infection as part of a continuum that progresses from contamination to systemic spread. Contamination refers to the mere presence of microorganisms in a wound without active proliferation or host reaction. Colonization involves microbial growth but does not yet impair healing or elicit inflammation. Infection becomes clinically significant when microbial activity produces host responses such as erythema, swelling, heat, and purulent discharge. At its most advanced, microorganisms may spread beyond wound margins, resulting in systemic infection and sepsis (Swanson *et al*.,2022).

Wound infection arises from both endogenous and exogenous microbial sources. Most surgical wound infections originate from endogenous flora residing on the patient's skin, mucosa, or hollow viscera, with *Staphylococcus aureus* especially common in many procedures. Exogenous organisms are introduced from the clinical environment via airborne spread, contaminated instruments or materials, or healthcare personnel, particularly when asepsis breaks down (Zabaglo *et al.*, 2024). Susceptibility to wound infection increases with host factors such as poorly controlled diabetes, malnutrition or obesity, conditions that impair tissue perfusion (e.g., peripheral arterial disease), immunosuppression, and corticosteroid therapy. Global guidelines similarly emphasize optimization of modifiable risks (e.g., glycemic control, nutrition, smoking cessation) to reduce infection risk (WHO, 2018).

Local wound and environmental factors also elevate risk: the presence of necrotic (devitalized) tissue and foreign material (e.g., suture or dressing fragments) provide foci for microbial proliferation and should be removed through appropriate debridement (Swanson *et al.*, 2022). In practice, additional procedure and care related risks include contamination of the site/equipment/personnel, inadequate antibiotic prophylaxis, prolonged operative time, hypothermia, hematoma/seroma, and suboptimal wound hygiene,each of which increases infection likelihood (Zabaglo *et al.*, 2024).

The microbial spectrum of wound infection is diverse. *Staphylococcus aureus*, including methicillin-resistant strains (MRSA), remains the leading pathogen worldwide. Other significant organisms include *Pseudomonas aeruginosa*, *Escherichia coli*, and anaerobes in contaminated or deep tissue wounds (Kallstrom, 2014).

2.2 Common Pathogens Implicated in Wound Infections

In Nigeria, wound infections are often polymicrobial, with *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Enterobacteriaceae* being the most frequently isolated pathogens. A study in Abuja found *S. aureus* (31.4%)*, P. aeruginosa* (21.4%), and *E. coli* (12.9%) as leading causes of SSIs (Olowo-Okere *et al*.,2019). Diabetic foot ulcer (DFU) infections in Nigeria are usually polymicrobial; *S. aureus* and *Pseudomonas* dominate, with resistant strains (MRSA, ESBL-producers) commonly detected (Ugwu *et al*., 2019). In Kano, wound swab analysis revealed high prevalence of Gram-negative organisms (59%), especially *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Aliyu *et al.*, 2023). Burn wound infections in Lagos showed *Pseudomonas spp*. (45%) as the leading pathogen, followed by *Staphylococcus aureus* (27%) (Fadeyibi *et al*., 2012). Anaerobic bacteria, though less frequently reported, also contribute to chronic wound infections in Nigerian hospitals.

2.3 Overview of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative, opportunistic pathogen widely implicated in wound infections, particularly burns, surgical sites, and chronic ulcers (Turner *et al.*, 2014). It thrives in moist environments, including hospital sinks, dressings, and catheters, making it a major cause of healthcare-associated infections (HAIs) (Volling *et al*., 2024). This pathogen possesses intrinsic resistance to many antibiotics due to efflux pumps, low outer membrane permeability, and biofilm formation, which complicates treatment (Thomaz *et al*., 2020). In wound infections, *P. aeruginosa* is particularly associated with delayed healing, tissue necrosis, and higher risk of sepsis (Malone *et al.*, 2017). Its virulence is mediated by toxins such as

elastases, exotoxin A, and pyocyanin, which disrupt host immune response and damage tissues (Malone *et al.*, 2017).

2.4 Pathogenicity of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an opportunistic Gram-negative pathogen with multiple virulence systems that make it a leading cause of difficult to treat wound infections (Qin *et al.,* 2022). A major pathogenic trait is biofilm formation, in which communities of bacteria embed in an extracellular matrix on wound surfaces and medical devices; biofilms protect bacteria from immune clearance and greatly reduce antibiotic susceptibility, promoting chronic infection (Thi *et al.*, 2020). The organism secretes an array of exotoxins and degradative enzymes including Exotoxin A (inhibits host protein synthesis), elastases, proteases and pyocyanin that damage host tissues and impair immune responses (Qin *et al.*, 2022). Its ability to persist in moist hospital environments (sinks, plumbing, wound dressings and catheters) and form biofilms facilitates transmission and healthcare-associated outbreaks (Thi *et al*., 2020). Because of its virulence and drug-resistance profile, *P. aeruginosa* is highlighted on global priority lists for antibiotic development and surveillance (Division, 2024).

Pathogenic factor	Features and biological role	Therapeutic intervention	Vaccine availability
Proteases	*P. aeruginosa* secreted proteases include elastase A, elastase B, large protease, protease IV, alkaline protease, Pseudomonas small protease, MucD, and *P. aeruginosa* aminopeptidase. They exhibit high proteolytic enzyme activity that damages host tissues by degrading proteins.	Protease inhibitors	Preclinical[369,392,408]
Toxins	*P. aeruginosa* produces a variety of extracellular toxins, including pigments, phytotoxic factors, hydrocyanic acid, phospholipase, protein convertase, enterotoxin, exotoxin, and mucus. These exotoxins can cause leukopenia, acidosis, liver necrosis, pulmonary edema, circulatory failure, renal tubular necrosis and bleeding, and many other serious damages.	Bacteriophages	Preclinical[410,415,436]
LPS	LPS is an integral component of cell envelope. It is the major virulence factor of *P. aeruginosa* and can be recognized by host pattern-recognition receptors to initiate inflammation and immunity response.	Antibody	Phase III[380,389,425]
Pili and fimbriae	Pili and fimbriae are the major adherence factors. They contribute to the adherence and motility of *P. aeruginosa* in host.	Phages[380,422,424]	None
Flagella	The main protein component of flagella is flagellin. Flagella provide motility and chemotaxis toward specific substrates and provide the ligand for clearance by phagocytic cells.	Bacteriophages	Phase III[366,393,410]
Leukocidin (cytotoxin)	They are secreted by the typical secretion system (e.g., ExoU secreted by Type III secretion system) and are the main cytotoxin targeting lymphocytes and neutrophils.	Natriuretic peptides[376,380]	None
Siderophores	There are two siderophores produced by *P. aeruginosa*: pyoverdine (formerly called fluorescein) and pyochelin. In addition to the iron needs, siderophores can support other virulence factors production by transferring iron, such as biofilms and toxic themselves.	Antibiotic-siderophore[387]	None
Urease	Urease enzyme is a virulence factor (limited extent) of *P. aeruginosa*. It can hydrolyze urea to produce ammonia and carbon dioxide (CO_2). It is associated with urinary tract infection.[378,427]	None	None
Outer membrane proteins	The outer membrane contains a large number of outer membrane proteins. These protein members are involved in the transportation of amino acids and peptides, the absorption of antibiotics, and the transportation of carbon sources. They are essential for bacterial adherence, virulence secretion, and host recognition.	Potential receptor for the internalization of host	Phase I[372,456,457]

Figure 2.1 The major pathogenesis factors of *P. aeruginosa* and therapeutics (Qin *et al*., 2022).

2.5 Resistance Mechanisms in *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is notoriously difficult to treat due to its multifaceted resistance mechanisms; intrinsic, acquired, and adaptive.

Intrinsic Resistance: This pathogen's natural defenses stem from its low outer membrane permeability, expression of Mex-type RND efflux pumps, and baseline production of AmpC β-lactamase, all reducing the efficacy of many antibiotics from the outset (Elfadadny *et al.,* 2024).

Acquired Resistance: *P. aeruginosa* frequently gains resistance through horizontal gene transfer or mutations, leading to carbapenemase production (e.g., VIM, IMP) and extended spectrum β-lactamases that render key antibiotics ineffective (Giovagnorio *et al.*, 2023).

Adaptive Resistance: Upon antibiotic stress or environmental changes, the bacterium often transitions into biofilm states or forms small colony variants, both of which enhance survival by reducing susceptibility to antimicrobials (Haidar *et al.*, 2024)

Multi-layered Interaction: These three resistance modes,which are:intrinsic, acquired, and adaptive often act to create a robust and layered defense system that enables *P. aeruginosa* to withstand multiple antibiotic classes(Langendonk *et al.*, 2021).

2.6 Overview of *Klebsiella pneumoniae*

Klebsiella pneumoniae is a Gram-negative, encapsulated bacterium commonly responsible for healthcare associated infections, including pneumonia, urinary tract infections, bloodstream infections, wound and surgical site infections, and meningitis. It is a significant opportunistic pathogen, especially in hospitals; *K. pneumoniae* can colonize and infect patients with weakened immune defenses or those with invasive devices like ventilators or catheters (Gorrie *et al.*, 2022).

2.7 Pathogenicity of *Klebsiella pneumoniae*

Hypervirulent strains,often carrying capsule types K1 or K2 can cause invasive infections (like liver abscesses and meningitis) even in healthy individuals, due to their enhanced defenses (Zhu *et al*., 2021). *K. pneumoniae* produces siderophores (aerobactin, yersiniabactin), which help it capture iron from the host, supporting its growth and boosting its virulence (Sohrabi *et al*., 2022). Fimbriae and LPS further enhance tissue attachment and trigger inflammation, aiding in infection establishment (Riwu *et al*., 2022). Surface structures called fimbriae (type 1 and type 3) also promotes colonization and biofilm formation (Guerra *et al.*, 2022). Some strains are hypervirulent (hvKp): they carry extra genes that make them more aggressive and able to cause severe infections even in healthy people (Choby *et al.*, 2019). Its lipopolysaccharide triggers strong inflammation and also helps the bacterium resist immune attacks (Abbas *et al*., 2024).

2.8 Resistance Mechanisms in *Klebsiella pneumoniae*

K. pneumoniae resists antibiotics in several ways, often at the same time, which makes treatment hard. It commonly produces extended-spectrum β-lactamases

(ESBLs) that break down many penicillins and cephalosporins (Zhang *et al.*, 2025). Powerful carbapenemases also occur and can destroy carbapenem antibiotics used as last-line drugs (Lazar *et al.*, 2024). Resistance genes are often carried on plasmids and mobile elements, so they spread quickly between bacteria and across hospitals (Ikhimiukor *et al.,* 2024). Efflux pumps actively pump antibiotics out of the bacterium and contribute to multidrug resistance (Almiyah, 2023). Biofilm formation protects bacterial communities from antibiotics and immune attack, causing persistent, hard-to-clear infections (Li *et al*., 2024). These combined resistance mechanisms (ESBLs, carbapenemases, efflux pumps, and biofilm formation) result in multi-drug-resistant (MDR) and extensively drug-resistant (XDR) strains of *K. pneumoniae*. MDR strains are resistant to at least three classes of antibiotics, while XDR strains are resistant to nearly all commonly used drugs. This makes routine antibiotics like penicillins, cephalosporins, fluoroquinolones, and even carbapenems often ineffective (J. Li *et al*., 2025). This poses a serious public health threat, especially in regions with limited infection control and antibiotic stewardship programs.

2.9 Botanical Description and Taxonomy of Garlic (*Allium sativum*)

Garlic is believed to have originated in Central Asia, with regions like Iran and Central Asia being the primary centers of diversity and early cultivation (Filyushin *et al.*, 2016). Garlic, scientifically named *Allium sativum L.*, is classified within the family Amaryllidaceae and the genus Allium, a large group that includes onions, leeks, and chives. It belongs to the subfamily Allioideae, and its taxonomy is rooted in the Linnaean system.

This bulbous perennial herb typically attains about 60 cm in height. Its underground bulb consists of multiple cloves encased in a papery sheath. Above the ground, it

bears long, sword-like leaves and often a flowering stalk (scape) topped with a spherical cluster (umbel) of small flowers or bulbils,its structure includes bulbs adapted for storage, leaves capable of photosynthesis, and a bulbil-rich flowering structure that supports its survival and spread in diverse climates (Atif *et al.,* 2020). Unlike many plants, cultivated garlic is generally sterile and is propagated vegetatively via cloves or bulbils,as they do not produce viable seeds.

Taxonomy

Rank	**Classification**
Kingdom	Plantae
Phylum	Tracheophyta
Class	Liliopsida
Order	Asparagales
Family	Amaryllidaceae
Subfamily	Allioideac
Genus	Allium
Species	*Allium sativum L.* (Schoch CL *et al.,2020).*

Figure 2.2: **Image of *Allium sativum L. plant* (Choudhary *et al.*, 2022*).***

2.10 Phytochemical Constituents of *Allium sativum*

Garlic (*Allium sativum* L.) contains a complex array of organosulfur compounds, especially alliin, allicin, diallyl sulfide, ajoene, and related derivatives, which provide its potent antimicrobial and antioxidant properties (Martins *et al*., 2016). It also includes phenolic acids and flavonoids, such as caffeic, p-coumaric, ferulic acids, and compounds like quercetin and kaempferol, which contribute significant antioxidant and anti-inflammatory effects (Shang *et al*., 2019). Furthermore, garlic contains saponins, notably compounds like proto-eruboside B and eruboside B, which supplement its antimicrobial and cardiovascular benefits through synergy with organosulfur constituents (Tavares *et al.*, 2021). Garlic is nutritionally enriched with amino acids, including non-protein sulfur amino acids that serve as precursors to allicin, alongside polysaccharides, vitamins, and minerals contributing to its health-supporting and immune-enhancing roles (Shang *et al*., 2019).

Flavonoids

Garlic contains measurable flavonoids such as quercetin and kaempferol alongside other phenolic compounds (Sasi *et al.*, 2021). These flavonoids act as antioxidants, scavenging free radicals and protecting cells from oxidative damage (Jan *et al*., 2022). They also modulate inflammatory signalling (for example NF-κB and Nrf2 pathways), helping reduce tissue inflammation (Mir *et al.*, 2023). Flavonoids can work together with garlic's sulfur compounds to enhance antimicrobial effects against bacteria and fungi (El-Saadony *et al.*, 2024). The amounts of individual flavonoids vary by cultivar, growing conditions and processing (heat or ageing change the profile) (Manoonphol *et al.,* 2023). Modern studies quantify garlic flavonoids using HPLC-MS and metabolomics, which supports reproducible analysis across samples (Bar *et al*., 2022).

Saponins in *Allium sativum*

Garlic bulbs contain steroidal saponins (furostanol and spirostanol glycosides) that are distinct from the more abundant sulfur compounds (Wang *et al.,* 2023). Saponin levels differ by garlic variety and bulb part, with some cultivars (e.g., Voghiera/purple types) showing much higher total saponins (Diretto *et al.*, 2017). Laboratory studies show garlic saponins have antifungal and antibacterial activity, acting by disrupting microbial membranes (El-Saadony *et al.,* 2024). Animal studies demonstrate that saponin fractions from garlic can lower cholesterol and reduce atherosclerosis risk (Miao *et al.,* 2020). Because saponins are relatively stable to cooking, they are being explored as natural food preservatives and adjuvants to boost other garlic antimicrobials (Jain *et al.*, 2025).

Amino acids & γ-glutamyl peptides in *Allium sativum*

Garlic is rich in γ-glutamyl peptides (for example γ-glutamyl-S-allylcysteine) which act as precursors to important stable sulfur compounds (Kodera *et al.*, 2019). These γ-glutamyl peptides are enzymatically converted (by γ-glutamyl transpeptidase) into S-allyl cysteine (SAC) during processing and ageing (Kosuge, 2019). SAC is water-soluble, bioavailable and a major antioxidant constituent of aged garlic preparations (Manoonphol *et al*., 2023). Processing (heat, ageing, fermentation) shifts the balance from volatile thiosulfates (allicin) to stable peptides like SAC (Liu *et al.*, 2025). Because of their stability and antioxidant actions, these amino-acid derivatives are favored in clinical studies that examine garlic's long-term protective effects.

2.11 Pharmacological and Antimicrobial Properties of Garlic

2.11.1 Antibacterial Activity

Garlic has broad-spectrum antibacterial activity demonstrated against both Gram-positive and Gram-negative species (Bhatwalkar *et al*., 2021). Much of this activity stems from organosulfur compounds produced when garlic tissue is damaged, especially allicin, which is formed from alliin by the enzyme alliinase (Borlinghaus *et al*., 2014). Allicin is a reactive thiosulfate that modifies accessible thiol groups in low-molecular-weight thiols (like glutathione) and protein cysteines, a process called S-thioallylation that disrupts essential microbial enzymes and redox balance (Gruhlke *et al.*, 2016). Beyond enzyme inactivation, allicin and other hydrophobic sulfur compounds disturb bacterial membranes, causing leakage of intracellular contents and rapid cell death (Nakamoto *et al.,* 2019). Garlic derivatives show activity against difficult and clinically important pathogens, including multidrug-resistant organisms and members of the Burkholderia cepacia complex (Wallock-Richards *et al*., 2014).

Ajoene and related compounds inhibit quorum-sensing pathways, reducing virulence gene expression and impairing biofilm establishment, which helps overcome tolerance mechanisms in chronic infections (Jakobsen *et al.*, 2012). Garlic extracts and isolated compounds often act synergistically with conventional antimicrobials and with nanoparticles (e.g., silver), lowering effective doses and improving kill rates in vitro and in animal wound models (Alfatemi *et al*., 2014). Small clinical and animal studies show topical garlic preparations can accelerate wound closure and improve scar appearance, although results vary by formulation and concentration (Alhashim and Lombardo, 2019).

2.11.2 Antifungal Activity

Garlic and its derivatives show consistent antifungal activity in modern laboratory and animal studies against yeasts (for example Candida spp.), cryptococci and a range of filamentous fungi (Khounganian *et al.,* 2023). The organosulfur compound allicin, produced when garlic is crushed, is a primary antifungal agent and acts by reacting with thiol groups in fungal enzymes and proteins, impairing metabolism (Yang *et al*., 2023)

Allicin also damages fungal cell membranes and organelles, causing leakage and loss of viability in susceptible species (Z. Li *et al*., 2022). Ajoene and other sulfur derivatives inhibit fungal virulence traits such as hyphal formation and biofilm development, which are important for persistent surface/skin infections (N. Li *et al.*, 2024) Recent translational work shows that delivery systems including garlic-loaded nanoparticles and chitosan-garlic hydrogels improve local retention and antifungal efficacy in wound and topical models (J. Wang *et al.*, 2024). Novel antifungal peptides and other non-volatile garlic components have been identified and show activity in experimental assays, expanding the pool of candidate antifungal molecules from garlic (S. Li *et al.*, 2023). Safety data indicate that properly formulated topical garlic derivatives are generally tolerated, but concentrated raw garlic or poor formulations can cause skin irritation or chemical burns, so careful formulation is essential (Madke and Das, 2021).

2.11.3 Antiviral activity

Garlic has demonstrated antiviral activity in recent studies, with several clinical and preclinical reports describing beneficial effects against respiratory and other viral infections (Vázquez-Blanquiño *et al.,* 2024). Allicin and other organosulfur

compounds can inhibit viral replication and interfere with viral proteomes, as shown in proteomic analyses of SARS-CoV-2 infected cells treated with allicin (Mösbauer *et al.*, 2021). Controlled trials and observational studies during the COVID-19 era have explored concentrated garlic derivatives as adjuncts, reporting preliminary improvements in some clinical parameters and immune markers (Hoh, 2025). In vitro and in vivo work published recently shows allicin reduces replication of animal and human RNA viruses (for example PRRSV in pigs), supporting a direct antiviral mechanism in multiple virus families (Hu *et al.,* 2023). Some garlic-derived molecules (for example ajoene-like fractions) have been reported to protect immune cells and reduce virus-induced cytopathicity in cell culture, suggesting both direct antiviral and host-protective effects (S. Liu *et al.,* 2024). Mechanistically, recent studies indicate antiviral actions include direct virucidal effects on envelopes, inhibition of viral enzymes/proteins, and modulation of host innate immunity (interferons, antiviral gene expression) (Rouf *et al.,* 2020).

2.11.4 Immunomodulatory and Wound-Healing Properties

Garlic stimulates innate immunity by enhancing macrophage function and increasing interferon production (Ansary *et al.*, 2020). Garlic compounds lower excessive inflammation by reducing pro-inflammatory cytokines such as TNF-α and IL-6 in cellular and animal studies (Avendaño-Ortiz *et al.*, 2023). Topical garlic preparations stimulate fibroblast proliferation and increase collagen deposition, which helps close wounds and strengthen repaired tissue (Alhashim and Lombardo, 2017).

2.12 Mechanisms of Antimicrobial Action of Garlic

When garlic tissue is crushed, the enzyme alliinase converts alliin to allicin, a reactive thiosulfinate that underlies most antimicrobial effects (Seki and Hosono, 2025). Allicin reacts with exposed cysteine (–SH) groups on bacterial proteins to form S-allylmercapto adducts ("S-thioallylation"), which inactivates key enzymes and depletes cellular thiol buffers like glutathione (Müller *et al.*, 2016). This thiol-stress triggers oxidative and heat-shock responses in bacteria and is a major reason allicin stops growth (Müller *et al.,* 2016). Because allicin is small and relatively hydrophobic, it diffuses across microbial membranes and can directly reach intracellular targets (Müller *et al.*, 2016). Garlic also interferes with biofilms and quorum sensing (QS), which are critical for chronic infection persistence (Seki and Hosono, 2025). The garlic-derived molecule ajoene down-regulates QS-controlled virulence programs by acting on the Hfq–sRNA pathway, thereby reducing biofilm formation and virulence factor expression in *Pseudomonas aeruginosa (*Jakobsen *et al.,* 2017). Computational/experimental analyses further support that ajoene analogs target Hfq–RNA interactions, consistent with QS inhibition and reduced virulence output (Fiori-Duarte *et al*., 2023). Across Gram-positive and Gram-negative bacteria, allicin shows broad-spectrum activity, including against resistant strains, due to this multi-target, thiol-directed mechanism (Müller *et al.*, 2016). Comprehensive recent reviews concur that organosulfur compound from garlic (allicin, ajoene, and related thiosulfinates) act through covalent thiol modification, membrane effects, and QS/biofilm interference, explaining activity in wounds and other infections (Seki and Hosono, 2025).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This experiment was conducted in the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Benin City, Edo state, Nigeria.

3.2 Materials for Antimicrobial Studies

3.2.1 Apparatus and Equipment

Portable autoclave, weighing balance, Hot air oven, mortar, pestle, micropipette, Incubator, Whatman filter paper, Cotton wool, Sterile pipette tip, Sterile cork borer (10mm diameter), transparent millimeter rule, grease pencil, Sterile swab sticks, Sterile Petri dishes, Tripod stand, Sterile inoculating wire loop, Bunsen burner, foil paper, Dried plant material.

3.2.2 Glassware

Conical flask, bottles (MacCartney, universal and Bijou) as well as test tubes, pipettes, glass stirrers, porcelain dish, maceration jars, glass funnels, beakers, measuring cylinders.

3.2.3 Chemicals and Reagents

Tween 80, distilled water. Disinfectant: Purit, soap, detergent.

Positive Control (Standard) Drugs:

Standard Antibiotic: Ciprofloxacin powder

3.2.4 Microbiological Media

Nutrient agar, Nutrient broth, and Mueller-Hinton Agar (MHA).

3.2.5 Clinical Isolates

Clinical isolates include *Klebsiella spp* and *Pseudomonas aeruginosa*

3.3 Methods

3.3.1 Collection and Identification

The bulbs of *Allium sativum* Linn. with Common name as Garlic,were bought from the Ring road market in Benin, Edo State, Nigeria, during the month of June, 2025. Care was taken to select healthy, bulb free from disease, insect damage, or physical blemishes.The identity of the plant was formally confirmed by Prof. Akinnibosun Henry Adewale (FLS, MRSB; London) of the Faculty of Life Sciences, Department of Plant Biology and Biotechnology, University of Benin, Edo State. A Voucher Specimen was prepared, assigned the voucher number, UBH-A388 and deposited for future reference and verification.

3.3.2 Preparation of Ethanolic and Aqueous Extracts

The bulbs were then spread out in thin layers, protected from direct sunlight, and allowed to air-dry at room temperature (25 ± 2°C) for a period of four days. The bulbs were then dried in a thermostat-controlled hot air oven at 40°C for 30 mins to render them brittle. It was then reduced to a uniform, coarse powder with the aid of a mechanical grinder. The resulting powder was stored in a cool, dark, and dry place until needed for extraction. The powdered material was extracted using maceration method for the extraction with Ethanol as solvents of choice. 800 g of the dried plant was soaked in ethanol for maceration.1 L of ethanol was used for the maceration.

Same maceration method was used for the aqueous extract and 700g of the dried plant was soaked in 1 L of sterile distilled water.

3.3.3 Preparation of Extraction Yield

The percentage yield of each extract was calculated to determine the efficiency of the extraction carried out using ethanol as solvent. The yield is calculated using the formula:

Percentage Yield (%) = (Weight of the dried extract / Weight of the initial powdered plant material) x 100

3.4 Antimicrobial Assay

3.4.1 Specimen Collection

Microorganisms used in this study were selected bacterial isolates obtained from the University of Benin Teaching Hospital (UBTH), Benin City, Edo state, Nigeria. They are: *Pseudomonas aeruginosa* and *Klebsiella pneumoniae.*

3.4.2 Preparation of Test Organisms

All test bacterial isolates were maintained in 20 % glycerol broth and frozen. Prior to use, test microorganisms were sub-cultured from stock into sterile nutrient agar plates and were incubated overnight at 37°C. After incubation, colonies from the overnight plates were suspended in sterile broth for 12 hours and adjusted to 0.5 McFarland standard to give an inoculum size of approximately 10^7 CFU/ml.

3.4.3 Preparation of McFarland Solution

A 0.5 McFarland standard solution is prepared by adding 0.5ml of 1.175 % (weight/volume). Barium Chloride dihydrate salt (BaCl2.2H2O) to 9.95 ml of 1 % Sulfuric acid (H2SO4) was used.

The two solutions are mixed completely to form a turbid suspension in a test tube which is then placed in a test tube rack and kept at room temperature before use.

3.4.4 Antimicrobial Susceptibility Tests

An antimicrobial susceptibility test was carried out using the ditch plate method. A total of 2 different bacterial isolates were used, sterile Mueller-Hinton agar was prepared and 30ml was poured into each petri dish and allowed to solidify. The surface of the media was dried in an hot air oven at 40°C for about 5 minutes to remove excess moisture. A ditch of 10mm wide and 50mm long was created in the centre of the agar plate using a sterile scalpel blade and the base of the ditch was sealed with drops of molten agar. A concentration of 800mg/mL of the extract was prepared and 1 mL dispensed into the ditch. The bacterial isolates were then streaked outward from the ditch toward the edge of the plate. The plates were incubated at 37℃ for 24 hrs. The growth of the organism was observed to assess its resistance or susceptibility to 800mg/mL of the extract for all the fraction. Same procedure was repeated for the aqueous extract.

3.4.5 Determination of Inhibitory Zone Diameter (IZD)

The inhibitory zone diameter was determined using the cup plate method with some modifications. Sterile Mueller-Hinton agar was prepared and 30 ml was poured into Petri dishes aseptically and allowed to solidify. The petri dishes were dried in a hot air oven at 40°C for about 5 minutes. The dried agar surface was then streaked with the test organism using a swab stick aseptically. A sterile cork borer (10mm) was used to bore 6wells in each agar plate. The base of the wells was sealed with 0.02 ml of molten agar. Five of the wells were filled with 0.25 ml of 25mg/0.25mL,50 mg/0.25mL, 100 mg/0.25mL, 200mg/0.25mL, 400mg/0.25mL respectively. A standard antibiotic Ciprofloxacin at 5ug was used in standardizing the work with the volume of the solution 0.25ml. The plates were incubated at 37°C for 24 hours. The inhibition zone diameters (IZD) were measured using a ruler in millimeters. Same method was carried out for all the different fractions of the plant (Ethanolic and Aqueous extract) and their inhibitory zone diameter were determined.

3.4.6 Determination of Minimum Inhibitory Concentration (MIC)

Agar dilution method of Afoyan and Meyer (1997) was used in this study for the determination of Minimum Inhibitory Concentration (MIC) of the extract. A 2-fold serial dilution of the test ethanolic extract was prepared to give concentrations of 25mg/mL,50mg/mL, 100mg/mL, 200mg/mL and 400mg/mL. Double strength Mueller-Hinton agar was prepared according to the manufacturer's instruction. Calculated volumes of the extract and double strength agar (1gram of extract + 20ml molten agar) was poured into a petri-dish and rocked gently to mix properly and then allowed to set. This was repeated for 25mg,50mg,100mg and 200mg plates. Bacteria prepared to a standard concentration was streaked with the aid of a sterile wire loop

on the surface and different sections of each plate properly labeled. The dilution plates were incubated at 37°C for 18-24 hours for the bacteria populations. After incubation, the plates were visually examined for growths on the inoculated spots. The lowest concentration of the extract that inhibits growth was considered as the same procedure was repeated for the aqueous extract of the plant.

3.4.7 Determination of Minimum Bacteriocidal Concentration (MBC)

The MBC was determined using agar plate method. It was determined from the agar dilution of the MIC tests by sub-culturing from the agar plates showing no growth into fresh, sterile agar plates. The dilution plates was then incubated at 37°C for 18-24 hours for bacteria isolate. After incubation, the plates was visually examined for growths in the inoculated spots. The lowest concentration from the MIC plates that showed no growth on the sub-cultured plates was considered the MBC.

CHAPTER FOUR

4.0 RESULTS

The antibacterial effect of garlic extract on *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* isolated from wound swabs was assessed using both aqueous and ethanolic extracts. The extraction process yielded different concentrations of crude extract, with 700 g of garlic macerated in sterile distilled water producing 25 g of aqueous extract (3.57% yield), while 800 g of garlic macerated in ethanol yielded 18 g of ethanolic extract (2.25% yield).

Table 4.1 shows that both aqueous and ethanolic garlic extracts exhibited concentration-dependent antibacterial activity. For *Klebsiella pneumoniae*, the aqueous extract showed no inhibition at concentrations between 25–100 mg/mL, but inhibition was observed at 200 mg/mL (11 mm) and further increased at 400 mg/mL (14 mm). Similarly, the ethanolic extract showed no activity at 25–50 mg/mL, but inhibition zones appeared at 100 mg/mL (12 mm) and increased to 14 mm at 400 mg/mL. In both cases, the inhibition zones were lower than that of the standard drug ciprofloxacin (15 mm). For *Pseudomonas aeruginosa*, the aqueous extract produced no inhibition at lower concentrations but showed zones of 11–13 mm at 200–400 mg/mL, while the ethanolic extract demonstrated activity earlier, with inhibition beginning at 100 mg/mL (11 mm) and increasing to 14 mm at 400 mg/mL. However, these values were still markedly lower compare to the standard ciprofloxacin(24mm).

Table 4.1: Inhibition Zone (mm) of Aqueous and Ethanolic Garlic Extract Against *Klebsiella spp. and Pseudomonas aeruginosa*

Test Organism	**Extract Type**	**25 mg/mL**	**50 mg/mL**	**100 mg/mL**	**200 mg/mL**	**400 mg/mL**	**STD**
Klebsiella spp.	Aqueous	0	0	0	0	11	15
	Ethanolic	0	0	0	12	14	15
Pseudomonas aeruginosa	Aqueous	0	0	0	11	13	24
	Ethanolic	0	0	11	13	14	24

Standard = Ciproflaxin (0.25ml)

As presented in Table 4.2, the mean inhibition zones were 5.58 ± 6.98 mm for *K. pneumoniae* and 9.17 ± 9.14 mm for *P. aeruginosa*. The statistical analysis revealed that concentration had a significant effect on inhibition ($p = 0.000$), while differences between bacterial species were not statistically significant ($p = 0.292$).

Table 4.2 Mean Zone of Inhibition of Garlic Extract Against *Klebsiella pneumoniae and Pseudomonas aeruginosa at Different Concentrations*

Concentration (mg)	***K. pneumoniae* (mm)**	***P. aeruginosa* (mm)**	**p-value**
25	0	0	Conc: p = 0.000
50	0	0	Bacteria: p = 0.292
100	0	11	
200	12	13	
400	14	13	
std	15	24	
Overall	5.58 ± 6.98	9.17 ± 9.14	

Significant $p < 0.05$

Table 4.3 further compares aqueous and ethanolic extracts. For *K. pneumoniae*, ethanolic extract produced a higher mean inhibition (5.2 ± 7.15 mm) compared to aqueous (2.2 ± 4.91 mm), though the difference was not statistically significant ($p = 0.5$). Similarly, for *P. aeruginosa*, ethanolic extract showed greater inhibition (7.6 ± 7.02 mm) than aqueous extract (4.8 ± 6.61 mm), but again without statistical significance ($p = 0.607$). Thus, although ethanolic extract showed greater potency, the variation was not strong enough to reach statistical significance at $p < 0.05$.

Table 4.3. Zone of Inhibition of Garlic Extract by Extract Type Against *Klebsiella pneumoniae and Pseudomonas aeruginosa*

organism	**Extract**	**N**	**Mean ± SEM**	**p value**
Klebsiella spp	Aqueous	5	2.2 ± 4.91	0.5
Pseudomonas aeruginosa		5	4.8 ± 6.61	
Klebsiella spp	Ethanolic	5	5.2 ± 7.15	0.607
Pseudomonas aeruginosa		5	7.6 ± 7.02	

Significant $p < 0.05$, Values are presented as mean ± Standard error of mean.

The MIC values in Table 4.4 reveal that ethanolic extract inhibited both *K. pneumoniae* and *P. aeruginosa* at 100 mg/mL, while aqueous extract required a higher concentration (200 mg/mL) for inhibition.

Table 4.4: Minimum Inhibitory Concentration (MIC) of Ethanolic and Aqueous of Garlic Extracts Against *Klebsiella spp. and Pseudomonas aeruginosa*

Test Organism	Extract Type	12.50 mg/mL	25 mg/mL	50 mg/mL	100 mg/mL	200 mg/mL	MIC (mg/mL)
Klebsiella spp.	Ethanolic	G	G	G	NG	NG	100
Pseudomonas aeruginosa	Ethanolic	G	G	G	NG	NG	100
Klebsiella spp.	Aqueous	G	G	G	G	NG	200
Pseudomonas aeruginosa	Aqueous	G	G	G	G	NG	200

G: Growth

NG:No Growth

For bactericidal activity (Table 4.5), ethanolic extract achieved MBC at 200 mg/mL for both organisms, whereas aqueous extract showed no bactericidal effect even at the maximum tested concentration (>200 mg/mL).

Table 4.5: Minimum Bactericidal Concentration (MBC) of Ethanolic and Aqueous Garlic Extracts Against *Klebsiella spp. and Pseudomonas aeruginosa*

Test Organism	Extract Type	12.50 mg/mL	25 mg/mL	50 mg/mL	100 mg/mL	200 mg/mL	MBC (mg/mL)
Klebsiella spp.	Ethanolic	G	G	G	G	NG	200
Pseudomonas aeruginosa	Ethanolic	G	G	G	G	NG	200
Klebsiella spp.	Aqueous	G	G	G	G	G	>200
Pseudomonas aeruginosa	Aqueous	G	G	G	G	G	>200

G: Growth

NG:No Growth

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CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 Discussion

This study investigated the antimicrobial properties of aqueous and ethanolic extracts of *Allium sativum* (garlic) against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Results demonstrated that both extracts exhibited concentration-dependent inhibition of bacterial growth, with ethanolic extracts consistently more potent than aqueous extracts. The ethanolic extract inhibited both test organisms at a minimum inhibitory concentration (MIC) of 100 mg/mL and displayed bactericidal activity at 200 mg/mL, whereas the aqueous extract required higher concentrations (200 mg/mL) to inhibit growth and showed no bactericidal effect within tested concentrations. The antimicrobial effects observed can be attributed to sulfur-containing bioactive compounds, particularly allicin, diallyl sulfides, and ajoene, known to exert broad-spectrum antibacterial activities.

The inhibitory effect of garlic against *K. pneumoniae* in this study agrees with previous research. Abubakar (2009) reported that garlic extracts exerted significant activity against *K. pneumoniae* and other nosocomial pathogens, with ethanol extracts producing greater inhibition zones than aqueous extracts (Abubakar, 2009). Similarly, Okunye *et al*. (2020) evaluated garlic against extended-spectrum β-lactamase (ESBL)-producing *K. pneumoniae* and observed MIC values that mirrored the present study, with ethanolic extracts consistently outperforming aqueous extracts (Okunye *et al*., 2020). The current findings are also comparable to those of Oyawoye *et al*. (2022), who observed antibacterial effects of garlic extracts on multidrug-resistant strains of *K. pneumoniae*, highlighting ethanol as a superior solvent for extraction (Oyawoye *et*

al., 2022). Garlic's ability to inhibit *P. aeruginosa* has been consistently reported. Eyengho and Ayanlere (2025) showed that aqueous and ethanolic garlic extracts demonstrated inhibitory activity against *P. aeruginosa*, with ethanolic extracts exhibiting stronger effects (Eyengho & Ayanlere, 2025). Likewise, Al-Yas (2011) demonstrated that aqueous garlic extract inhibited *P. aeruginosa* isolated from otitis media, though at higher concentrations than ethanolic extracts (Al-Yas, 2011).

These results are further supported by Jabeen *et al*. (2014), who observed that ethanolic garlic extracts were more effective against *P. aeruginosa* compared to aqueous forms, suggesting that ethanol extracts bioactive compounds responsible for disrupting bacterial cell walls and interfering with quorum sensing (Jabeen *et al*., 2014). Shakib *et al*. (2021) provided additional evidence, demonstrating that garlic exhibited antimicrobial activity even against carbapenemase-producing *P. aeruginosa* strains, underlining its potential in managing drug-resistant pathogens (Shakib *et al*., 2021).

A consistent theme in the literature also confirmed by this study is that ethanolic extracts are more effective than aqueous extracts. Ethanol dissolves a wider spectrum of garlic phytochemicals, including non-polar sulfur-containing compounds like diallyl disulfide and diallyl trisulfide, which possess higher antimicrobial potency than water-soluble components. Liu *et al*. (2021) observed that ethanolic garlic extracts produced larger inhibition zones and lower MIC values against Gram-negative bacteria compared to aqueous extracts (Liu *et al*., 2021).

In the present study, ethanolic extracts achieved MIC values at 100 mg/mL for both test organisms, while aqueous extracts required 200 mg/mL. The lack of bactericidal

activity with aqueous extracts also underscores their weaker potency relative to ethanol extracts.

The antimicrobial activity of garlic is largely attributed to allicin, a reactive sulfur compound produced when alliin is converted by the enzyme alliinase upon crushing garlic bulbs. Allicin disrupts bacterial cell walls, inhibits thiol-dependent enzymes, and interferes with quorum sensing, thereby suppressing virulence in *P. aeruginosa*. Other organosulfur compounds such as ajoene and diallyl sulfides contribute synergistically by targeting bacterial DNA replication and membrane integrity.

Garlic's broad-spectrum activity against Gram-negative bacteria, including *K. pneumoniae* and *P. aeruginosa*, demonstrates its potential as a natural antimicrobial agent. However, the observed weaker inhibition compared to ciprofloxacin highlights limitations in crude extracts, which may require concentration, purification, or synergistic formulations with antibiotics to achieve clinical relevance. The study highlights the importance of garlic extracts as potential complementary agents in the management of bacterial infections, particularly those involving multidrug-resistant strains. The greater activity of ethanolic extracts emphasizes the need for careful selection of extraction solvents to optimize the therapeutic potential of plant-based antimicrobials.

Additionally, the ability of garlic to inhibit *P. aeruginosa*, a pathogen known for high intrinsic resistance, suggests its possible role in managing infections where conventional antibiotics fail. This aligns with the growing global emphasis on exploring natural products as reservoirs for novel antimicrobial agents.

5.2 Conclusion

This research provides compelling evidence that *Allium sativum* extracts, particularly the ethanolic extract, possesses significant antibacterial and bactericidal properties against two resistant pathogens, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The key finding is the superior efficacy of the ethanolic extract, which demonstrated a minimum inhibitory concentration (MIC) of 100 mg/mL, half the concentration required by the aqueous extract (200 mg/mL) to achieve the same effect. More importantly, only the ethanolic extract exhibited a bactericidal effect at the tested concentrations, which is a highly desirable trait for a therapeutic agent.

These results are particularly significant in light of the growing global threat of antimicrobial resistance. As conventional antibiotics like Ciprofloxacin face increasing ineffectiveness against these multidrug-resistant strains, our findings highlight the potential of plant-based compounds as a viable alternative. The unique mechanism of action of garlic's active compounds, which can disrupt bacterial defenses and biofilms, positions it as a promising candidate for further development. This study provides a strong scientific foundation for the continued exploration of garlic's therapeutic potential, paving the way for future research into in-vivo applications and the development of standardized, effective phytomedicines to combat infectious diseases.

5.3 Recommendations

The findings of this research provide a strong foundation for continued investigation into the antimicrobial potential of *Allium sativum.* The study recommends the following to translate these promising in-vitro results into a practical solution for combating antimicrobial resistance:

Based on the results obtained from this study, it is recommended that future researchers continue to investigate the antimicrobial potential of *Allium sativum* using broader and more diverse wound isolates. This study focused mainly on determining the MIC and MBC of the ethanolic and aqueous garlic extracts, so it would be useful for future work to look at other extraction approaches and purification methods. Doing so may help identify whether higher yield or more refined extracts could produce greater inhibitory effects on wound pathogens. Researchers may also consider testing different concentrations, preparation techniques, and storage conditions to understand how these factors influence antimicrobial activity.

It is also recommended that further studies compare garlic extracts with a wider range of antibiotics, especially those commonly used in wound management within local healthcare settings. Although this study provided a basic comparison with selected standard antibiotics, more detailed comparisons could help determine where garlic extracts may offer an advantage or where they could serve as an alternative when antibiotic resistance becomes a problem. In addition, since laboratory conditions cannot fully represent what happens in actual wounds, future investigations should try to simulate real wound environments to understand how garlic extracts behave in the presence of bodily fluids, tissue debris, and mixed microbial populations.

Another important recommendation is for future researchers to assess the safety and possible side effects of garlic extracts when applied directly to wounds. While garlic is generally seen as safe, its concentrated forms may cause irritation or delayed healing in some individuals. Therefore, toxicity tests, stability tests, and assessments of how the extracts interact with the skin should be included in further studies. This

would provide clearer information for their potential use as complementary wound-care agents.

Finally, it is recommended that more attention be given to evaluating the cost-effectiveness and practical usability of garlic extracts in clinical and community settings, especially in regions where antibiotics are expensive or resistance is widespread. By addressing these areas in future studies, the potential role of *Allium sativum* in managing wound infections can be understood more clearly and applied more confidently.

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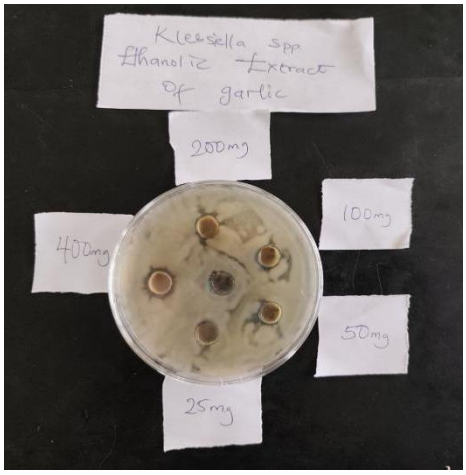
APPENDIX



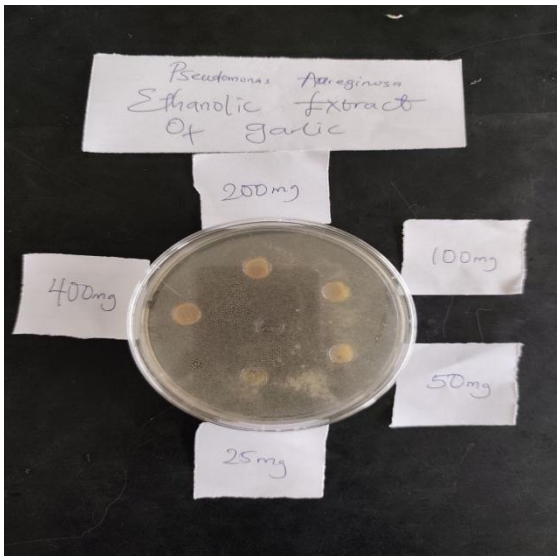


PLATE: a). Antibiogram of *Klebsiella spp* using the ethanolic extracts of *Allium sativum* in various concentrations (200mg/mL,100mg/mL,50mg/mL,25mg/mL,12.5mg/mL).

b). Antibiogram of *Pseudomonas aureginosa* using the ethanolic extracts of *Allium sativum* in various concentrations (200mg/mL,100mg/mL,50mg/mL,25mg/mL,12.5mg/mL).

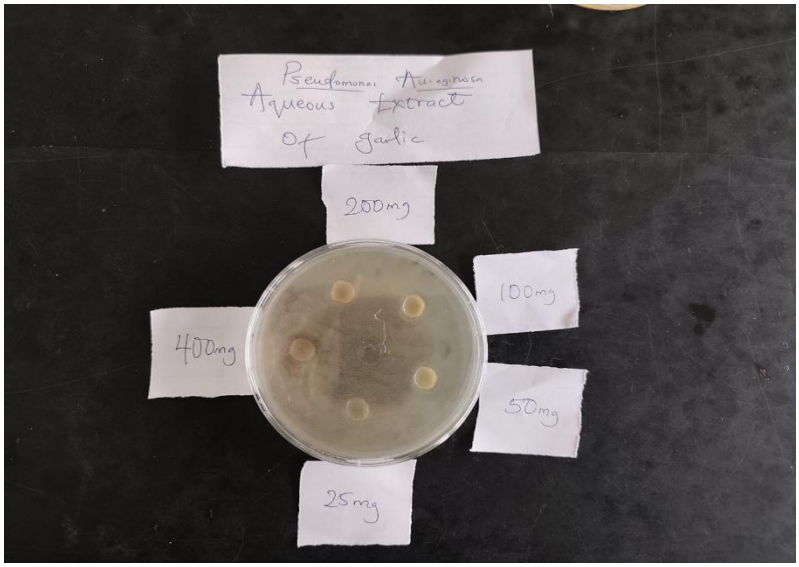
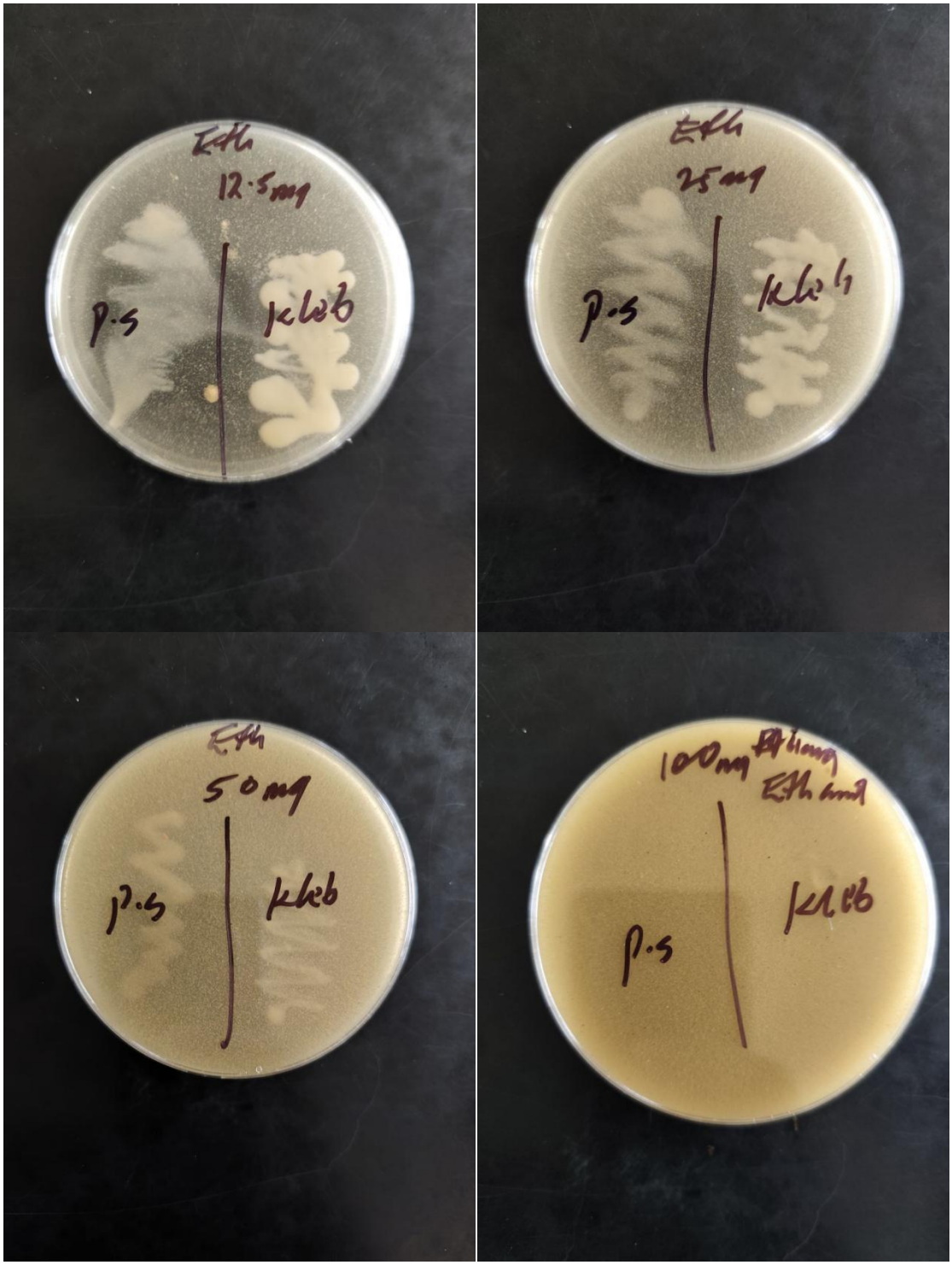


PLATE: a). Antibiogram of Pseudomonas aureginosa using the aqueous extracts of *Allium sativum* in various concentrations (200mg/mL,100mg/mL,50mg/mL,25mg/mL,12.5mg/mL). b). Antibiogram of

Klebsiella spp. using the aqueous extracts of *Allium sativum* in various concentrations (200mg/mL,100mg/mL,50mg/mL,25mg/mL,12.5mg/mL).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P.s
Klebb
Eth
25mg
P.s
Eth
50mg
P.s
Klebb
100mg
Eth
P.s

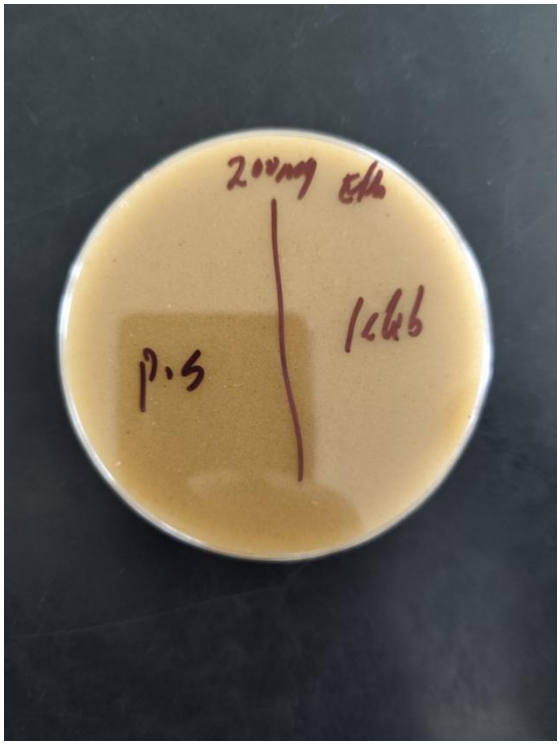

PLATE: Determination of Minimum Inhibitory Concentration (MIC) of *Pseudomonas aureginosa* and *Klebsiella spp* using the ethanolic extracts of *Allium sativum* in various concentrations a) 12.5mg/mL b). 25mg/mL c). 50mg/mL d). 100mg/mL e). 200mg/mL.

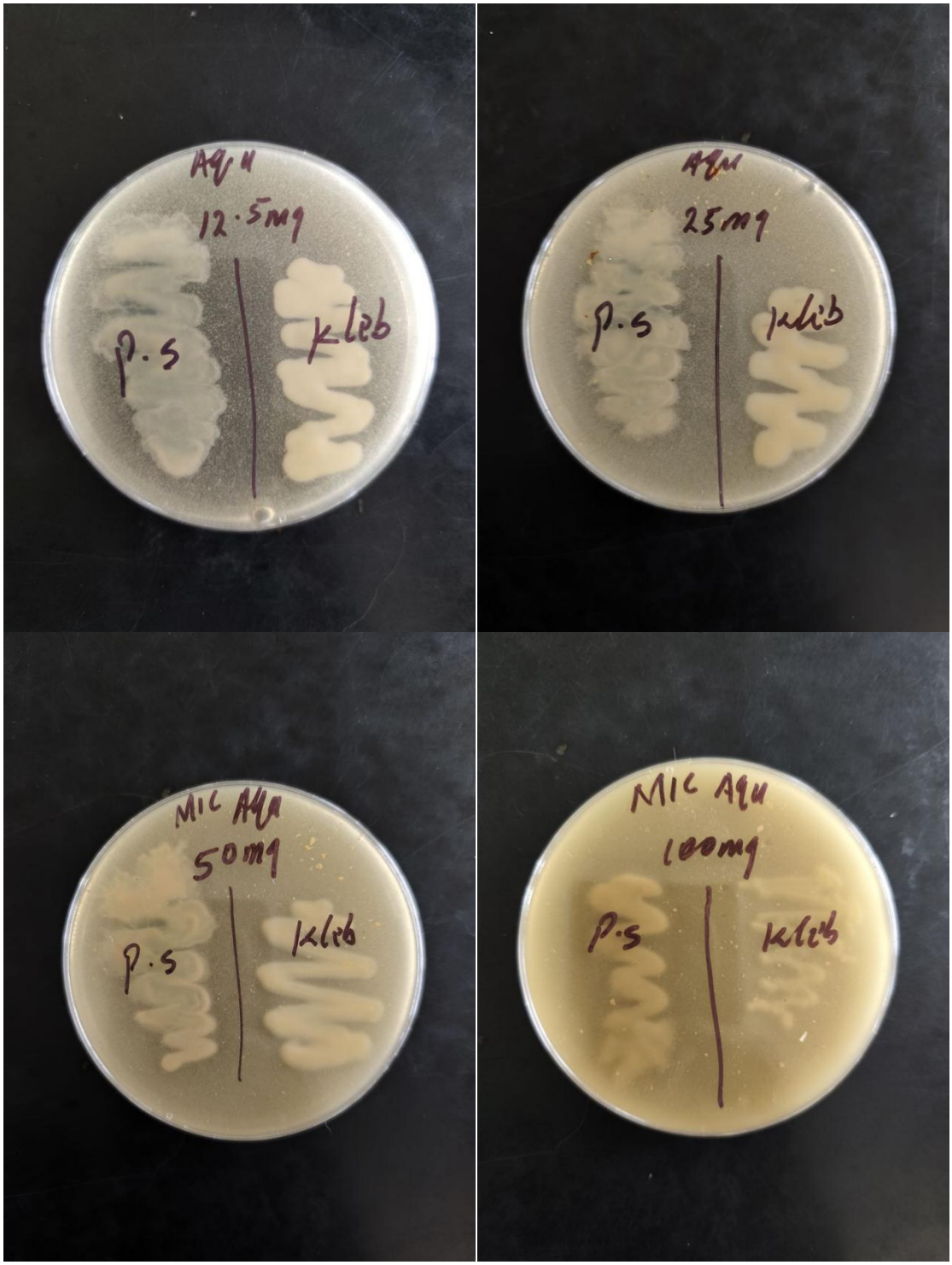
AQU
12.5mg
P.S
Klab
AQU
25mg
P.S
Klab
MIC AQU
50mg
P.S
Klab
MIC AQU
100mg
P.S
Klab

PLATE: Determination of Minimum Inhibitory Concentration (MIC) of Pseudomonas aureginosa and Klebsiella spp using the aqueous extracts of *Allium sativum* in various concentrations a) 12.5mg/mL b). 25mg/mL c). 50mg/mL d). 100mg/mL e). 200mg/mL.

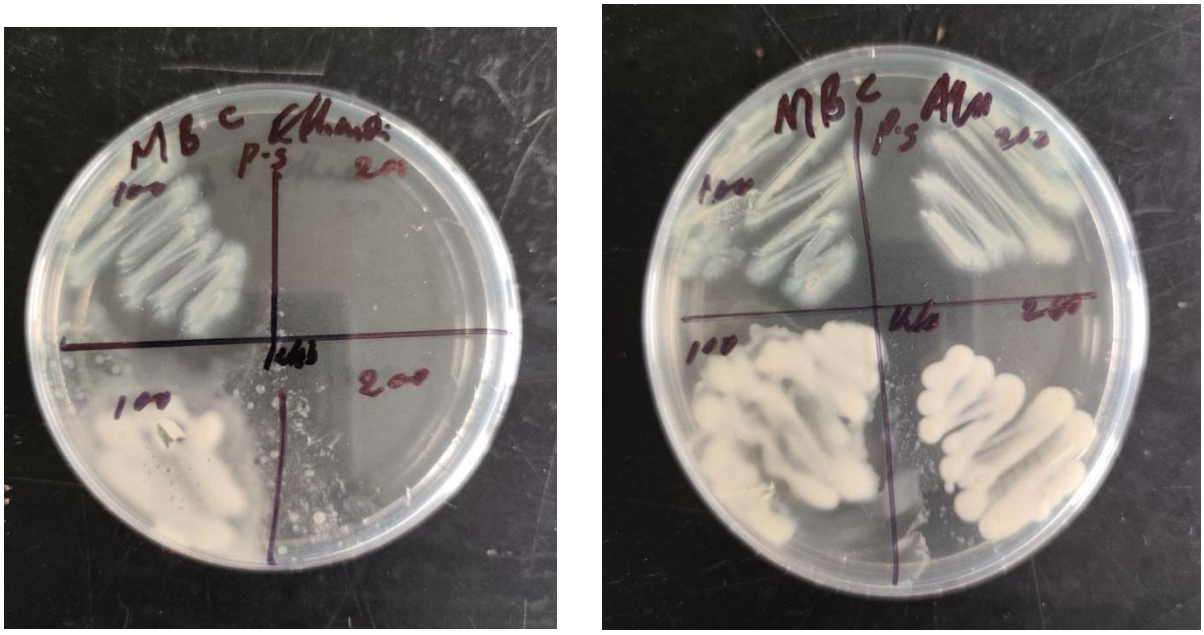

PLATE: a). Determination of Minimum Bacteriocidal Concentration(MBC) of *Pseudomonas aureginosa* and *Klebsiella spp* using the ethanolic extracts of *Allium sativum* in various concentrations of 100mg/mL and 200mg/mL b). Determination of Minimum Bacteriocidal Concentration (MBC) of *Pseudomonas aureginosa* and *Klebsiella spp* using the aqueous extracts of *Allium sativum* in various concentrations of 100mg/mL and 200mg/mL.

PLANT VERIFICATION CERTIFICATE

University of Benin

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Edo State, Nigeria.

Department of Plant Biology and Biotechnology

Herbarium Unit

Faculty of Life Sciences

University of Benin, Benin City, Edo State

Plant Name: *Allium sativum* Linn.

Family: Amaryllidaceae

Common Name: Garlic

Voucher Number: UBH-A388

Student Name: Ehiozua Nathan Osaebhue

Plant Identification and Voucher Number Issued by:

18/08/2025

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London, MSWS; USA, MBOSON, MAEIAN, MFBAN; Nigeria)