EVALUATION OF THE ANTIBACTERIAL AND PHYTOCHEMICAL PROPERTIES OF THE COLD AND HOT WATER EXTRACT OF *Pueraria phaseoloides*

BY

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A PROJECT WORK WRITTEN AND SUBMITTED IN PARTIAL FULFILMENT FOR THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc) DEGREE IN THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY.

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CERTIFICATION

This is to certify that this project work on "**EVALUATION OF THE ANTIBACTERIAL AND PHYTOCHEMICAL PROPERTIES OF THE COLD AND HOT WATER EXTRACT OF *Pueraria phaseoloides***" was carried out by **OTUNUYA EMMANUEL CHUKWUNEDUM**, with the Matriculation Number: **LSC2205958**; in partial fulfilment for the Award of Bachelor of Science (B.Sc) Degree in the Department of Microbology, Faculty of Life Sciences, University of Benin, Benin City.

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Prof. (Mrs.) F.I. Akinnibosun **Date**

Project supervisor

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Head of Department

DEDICATION

I dedicate this project work to God Almighty who has been my source of inspiration and strength,who in his infinite mercies have seen me through this work.

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I hereby appreciate God Almighty for giving me the Grace, Opportunity, Inspiration and Strength to complete this undergraduate project work an also write this report.

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My profound gratitude goes to My Parents and My Siblings their countless support both financially, spiritually, physically and emotionally towards my educational pursuit.

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ABSTRACT

The worldwide rise in antibiotic resistance (AMR) has warranted the exploration of medicinal plants as alternate sources of antimicrobial agents. The antibacterial and phytochemical qualities of *Pueraria phaseoloides* (Tropical Kudzu) cold and hot water extracts were assessed in this study. The agar well diffusion method was used to test the extracts' antibacterial activity against *Pseudomonas aeruginosa, Escherichia coli,* and *Staphylococcus aureus*. To evaluate potency, the minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) were calculated. Through phytochemical screening, both extracts were found to contain flavonoids, tannins, saponins, glycosides, and terpenoids. The hot water extract showed the largest zones of inhibition and the lowest MIC values against *P. aeruginosa* and *E. coli*, indicating higher antibacterial activity than the cold water extract. At the tested concentrations, both extracts demonstrated bacteriostatic effects. The results show that the extraction temperature has a major impact on the release of the active ingredients that give antimicrobial activity. This study supports *Pueraria phaseoloides'* potential development as a natural source of antimicrobial agents and offers scientific support for its traditional use.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

For countless generations, medicinal plants have served as the foundation of traditional healing systems, supplying diverse bioactive molecules that hold significant therapeutic value. Human reliance on nature for remedies dates back to antiquity, when early societies much like animals instinctively explored plants for relief from ailments. Across civilizations and eras, these natural resources have consistently shaped human health and cultural development.

According to the World Health Organization (WHO), approximately 80% of the population in developing regions continues to depend on plant-based traditional medicines as their primary healthcare option (Mishra *et al*., 2013). Out of an estimated 374,000 plant species worldwide (Christenhusz and Byng, 2016), only about 28,187 are recorded as medicinally useful to humans (MNPS, 2024). Remarkably, over 1,340 plant species have demonstrated antimicrobial properties, and more than 30,000 antimicrobial compounds have already been isolated from botanical sources (Tajkarimi *et al*., 2010).

The increasing misuse, overuse, and inappropriate application of antibiotics has accelerated the global rise of antimicrobial resistance (AMR), rendering several conventional drugs progressively ineffective (WHO, 2021; Baym *et al*., 2016; Davies and Davies, 2010). WHO has identified this growing resistance trend as one of the most urgent threats confronting modern medicine (WHO, 2025). As antibiotic efficacy continues to diminish, the pursuit of new therapeutic agents—particularly those capable of addressing resistant pathogens—has become a global priority.

Although synthetic antimicrobial drugs exist, their high production costs and associated side effects make them less desirable compared to plant-derived alternatives (Abiramasundari *et al*., 2011). Consequently, researchers have intensified efforts to isolate and characterize novel bioactive compounds from medicinal plants. These plants represent an expansive, largely underexplored reservoir of antimicrobial chemicals, offering significant potential for drug discovery (Lampinen, 2005).

Despite this promise, existing scientific research remains insufficient, especially regarding many plant materials whose pharmacological properties are still poorly documented (Qyuiroga *et al*., 2001). Natural antimicrobial substances may act independently or synergistically with conventional antibiotics, often enhancing overall antimicrobial efficacy (Hosseinzadeh *et al*., 2016; Bazzaz *et al*., 2018). With numerous medicinal plants yet to be fully examined for antibacterial potential, ongoing studies are increasingly focused on identifying potent, fast-acting botanical agents that may help combat the rising tide of resistance (Savoia, 2012).

The high prevalence of antibiotic-resistant bacterial strains has therefore amplified demand for safe, effective, and affordable antimicrobial treatments sourced from nature.

1.2 Antimicrobial Activity of Medicinal Plant Extracts

Extracts obtained from medicinal plants possess a wide array of biological activities, including antioxidant, anti-inflammatory, and antimicrobial effects (Mehta *et al*., 2001). Plant-derived antimicrobial molecules may offer significant clinical advantages by providing alternative mechanisms for suppressing the growth of bacteria, viruses, fungi, and protozoa, particularly those that exhibit resistance to standard antibiotics (Shankar *et al*., 2010).

Although certain phytochemicals may be less potent than conventional antibiotics when used alone, many demonstrate strong synergistic interactions when combined with other therapeutic

agents. Some compounds possess both inherent antimicrobial properties and the ability to modify bacterial resistance mechanisms. Due to their naturally complex chemical structures, many plant constituents exhibit lower toxicity and reduced risk of promoting resistance compared to synthetic drugs, making them promising candidates for therapeutic development (Ruddaraju *et al*., 2020).

However, bacteria may still develop resistance to plant-based treatments when these agents rely on a single active compound targeting a specific pathway (Reker *et al*., 2014). Scientific understanding of resistance development against phytochemicals remains limited, indicating the need for further exploration of these mechanisms (Almabruk *et al*., 2018).

The antimicrobial effectiveness of plant extracts often depends on the combined interactions of several active constituents working synergistically (Wagner and Ulrich-Merzenich, 2009). Synergy may arise from multiple mechanisms, including:

- simultaneous targeting of multiple cellular sites,
- inhibition of bacterial resistance factors,
- improved solubility, absorption, or bioavailability of active compounds,
- reduction of toxicity through balancing effects, and
- enhancement of pharmacokinetic properties.

These combined actions contribute to the overall potency of medicinal plant extracts, further supporting their role as viable alternatives or adjuncts to existing antimicrobial therapies

1.3 Problem Statement

The rapid emergence and widespread distribution of antibiotic-resistant bacteria represent an escalating global health challenge. As the effectiveness of conventional antibiotics continues

to decline, there is an urgent need to identify affordable, accessible, and plant-based antimicrobial alternatives. Although *Pueraria phaseoloides* is traditionally recognized for various therapeutic applications, scientific evidence regarding its phytochemical composition and antibacterial efficacy—particularly under different extraction conditions—remains extremely limited. This knowledge gap underscores the need for systematic research to validate its potential as a natural antimicrobial agent.

1.4 Aim of study

The aim of the study was to carry out the evaluation of the antibacterial and phytochemical properties of the cold and hot water extract of *Pueraria phaseoloides.*

1.5 Objectives of study

The specific objectives of the study were:

2. determine the antibacterial properties of *Pueraria phaseoloides;*
3. determine the Minimum Inhibitory Concentration (MIC) of the extract;
4. determine the Minimal Bactericidal Concentration of the extract;
5. compare the antibacterial properties with conventional antibiotics;
6. determine the qualitative phytochemical properties of the hot and cold water extract of *Pueraria phaseoloides.*

1.6 Scope and Limitation of the Study

The study is limited to the evaluation of aqueous (cold and hot water) extracts of *Pueraria phaseoloides*. Only selected phytochemicals and bacterial strains will be considered. The work does not cover the isolation of specific bioactive compounds or in vivo antimicrobial studies.

1.7 Significance of the Study

This study will contribute to the growing body of knowledge on *Pueraria phaseoloides* and their pharmacological relevance. Findings from this research may justify the ethnomedicinal use of *Pueraria phaseoloides* and provide scientific evidence supporting its inclusion in the development of new antimicrobial agents.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Medicinal Plants and Their Therapeutic Potentials

Throughout human history, plants have been a pivotal source of medicinal compounds, maintaining a vital role in healthcare systems worldwide. The World Health Organization estimates that around 80% of the global population still depends on traditional medicine practices, predominantly involving plant-based remedies (WHO, 2005). While the precise biological functions of many phytochemicals remain only partially elucidated, extensive research has confirmed their critical role in plant defense against pathogens and pests. This defensive function underscores the importance of investigating plant extracts and their phytoconstituents for potential antimicrobial activities, serving as a cornerstone for novel drug discovery initiatives (Vaou *et al*., 2021).

The advent and refinement of sophisticated phytochemical analysis techniques have sparked renewed enthusiasm among researchers, facilitating the identification of promising natural compounds for pharmaceutical applications (Abubakar and Haquu, 2020). Traditional medical systems—including Ayurveda, Traditional Chinese Medicine, and Indigenous African healing practices—have long served as repositories of medicinal knowledge, which contemporary pharmacology continues to draw upon extensively (Geck *et al*., 2020; Jamshidi-Kia *et al*., 2017). Historical breakthroughs such as aspirin derived from Salix species and artemisinin isolated from Artemisia annua highlight the enduring significance of plant-based molecules in modern therapeutics (Mawalagedera *et al*., 2019).

In light of escalating challenges posed by chronic illnesses and antimicrobial resistance, the exploration of traditional medicinal plants represents a timely and crucial avenue for the development of innovative therapeutic agents (Mantravadi *et al*., 2019)

2.2 Importance of Phytochemicals in Drug Development

Phytochemicals encompass a diverse array of biologically active compounds—including alkaloids, flavonoids, terpenoids, polyphenols, saponins, and glycosides—that collectively form nature's extensive pharmacological toolkit. Alkaloids such as morphine and quinine exert profound influences on the central nervous and cardiovascular systems. Flavonoids, abundant in fruits and medicinal herbs, are widely recognized for their potent antioxidant, anti-inflammatory, and cardioprotective effects (Anand *et al*., 2019; Kukula-Koch and Widelski, 2017; Zaynab *et al*., 2018). Terpenoids, exemplified by taxol and artemisinin, have established clinical utility in the treatment of cancer and malaria, respectively (Cox-Georgian *et al*., 2019).

Beyond their intrinsic therapeutic properties, phytochemicals serve as valuable lead compounds in contemporary drug discovery, where medicinal chemistry techniques optimize their pharmacokinetics, bioavailability, potency, and safety profiles (Egbuna *et al*., 2019). Their remarkable chemical diversity and biological efficacy render them indispensable in addressing diseases that often evade conventional therapies.

Traditional medical systems have relied heavily on these bioactive molecules. For instance, berberine exhibits notable antimicrobial and antidiabetic effects, whereas flavonoids such as quercetin and catechins contribute to vascular health and metabolic regulation (Saleem *et al*., 2022; Shukla *et al*., 2017). Terpenoids like menthol and limonene also contribute significantly to the analgesic and antimicrobial properties observed in essential oils (Tetali, 2019).

Ethnopharmacological research continues to substantiate the empirical use of these compounds; for example, *Ginkgo biloba* is employed in Traditional Chinese Medicine to enhance cognitive function, while *Withania somnifera* (Ashwagandha) is revered in Ayurveda for its adaptogenic effects (Marshall, 2020; Kumar *et al*., 2021). Likewise, African and South American indigenous medicinal practices utilize diverse phytochemicals to combat inflammation, boost immunity, and manage various health conditions (Gonyela, 2016).

2.3 Extraction Methods and Their Influence on Bioactivity

Extraction techniques for isolating bioactive compounds from plants have evolved substantially beyond traditional practices such as decoction, now incorporating methods that enhance selectivity, efficiency, and compound stability. Advanced approaches like solvent extraction and supercritical fluid extraction (SFE)—the latter often employing carbon dioxide—have improved yields and purity while offering environmentally friendly and non-toxic alternatives, particularly suitable for heat-sensitive and volatile phytochemicals (Kamil Hussain *et al*., 2019; Lefebvre *et al*., 2021; Uwineza and Waśkiewicz, 2020). Furthermore, microwave-assisted and ultrasound-assisted extraction techniques facilitate increased recovery of bioactives by disrupting plant cell walls, thereby preserving biological activity and reducing solvent consumption (Ekezie *et al*., 2017). Despite its widespread use due to versatility, solvent extraction demands stringent controls to avoid solvent residues and maintain compound integrity (Altemimi *et al*., 2017).

Post-extraction, phytochemicals undergo rigorous pharmacological evaluations. In vitro assays are employed to determine bioactivities such as anticancer, antimicrobial, or anti-inflammatory effects, while in vivo studies assess pharmacokinetic behavior, bioavailability, and toxicity profiles (Aware *et al*., 2022). Nevertheless, challenges persist, including low concentrations of active compounds within complex plant matrices, interference from co-extracted substances,

and the absence of universally standardized protocols. Structural elucidation techniques such as high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) remain vital for compound profiling and purity assessment (Lozano-Sánchez *et al*., 2018; Bubli *et al*., 2016). Complementing these, nuclear magnetic resonance (NMR) spectroscopy offers detailed three-dimensional molecular characterization, crucial for confirming compound identity and investigating pharmacodynamic mechanisms (Koshani *et al*., 2020; Dias *et al*., 2016).

2.4 Mechanisms of Action of Antimicrobial Agents

Conventional synthetic antimicrobials exert their effects through well-defined mechanisms, including inhibition of bacterial protein synthesis—as seen with agents like amikacin targeting ribosomal subunits (Walsh, 2003)—disruption of nucleic acid synthesis by fluoroquinolones such as ciprofloxacin, and interference with cell wall biosynthesis via β-lactams like penicillins and glycopeptides such as vancomycin (Schneider and Sahl, 2010). Membrane-active agents like polymyxins contribute by increasing bacterial membrane permeability and causing cell lysis (Poirel *et al*., 2017).

Despite their potent efficacy, bacterial populations adapt rapidly through genetic mutations and horizontal gene transfer, leading to widespread antimicrobial resistance. Understanding these classical drug action and resistance mechanisms is essential for the rational development and benchmarking of plant-derived antimicrobials. Phytochemicals often engage multiple cellular targets or potentiate existing antimicrobial pathways, offering more complex and multifaceted modes of action that present promising avenues for overcoming resistance and creating novel therapeutic agents.

2.5 Antibacterial Activity of Plant Extracts

Medicinal plants are abundant sources of diverse chemical constituents that have demonstrated antimicrobial efficacy in numerous in vitro studies. These bioactive agents are naturally derived and are generally considered to have minimal adverse environmental impacts, making them promising candidates for use as biological control agents. Despite their potential, some medicinal herbs remain underutilized or overlooked, often referred to as 'forgotten plants' due to limited integration into mainstream therapeutic applications. While pharmaceutical industries have introduced many new antibiotics over the past thirty years, the rapid emergence of microbial resistance has compromised the effectiveness of these drugs. Importantly, bacteria possess innate genetic mechanisms enabling the transfer and acquisition of resistance traits against therapeutic agents (Cheng *et al*., 2009).

Phytochemicals such as spermidine, rutin, quercetin, tocopherol, and carotenoids found in *Capparis* species contribute significantly to the broad biological activities of these plants. For instance, seed extracts of *Capparis decidua* show antibacterial, antifungal, and antileishmanial activities, effects primarily attributed to quaternary ammonium compounds and glucosinolates (Tlili *et al*., 2011). Traditional medicinal plants such as bearberry (*Arctostaphylos uva-ursi*) and cranberry (*Vaccinium macrocarpon*) have well-established uses in treating urinary tract infections, whereas lemon balm (*Melissa officinalis*), garlic (*Allium sativum*), and tea tree (*Melaleuca alternifolia*) are recognized as potent broad-spectrum antimicrobials (Joshi *et al*., 2017).

In Cameroon, numerous indigenous plants have yielded bioactive molecules including phenolics, alkaloids, flavonoids, triterpenes, and steroids, all exhibiting substantial antimicrobial activities (Dzotam *et al*., 2017). Notably, *Croton lechleri* produces crofelemer—a proanthocyanidin oligomer found in the drug Fulyzaq—which has demonstrated antiviral

effects (Vaou *et al*., 2021). Similarly, leaf extracts of *Myrtus communis* and *Verbena officinalis* exhibit antibacterial actions against pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi*, with *Myrtus communis* also showing efficacy against *Pseudomonas aeruginosa*. Essential oils from carrot seeds (*Daucus carota*) and tea tree have revealed antimicrobial effects against *Helicobacter pylori* and *Mycoplasma pneumoniae*, respectively (Wangchuk *et al*., 2011).

Methanolic extracts from *Oxalis corniculata*, *Artemisia vulgaris*, *Cinnamomum tamala*, and *Ageratina adenophora* have inhibited common bacterial pathogens such as *E. coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Citrobacter koseri*. Hydromethanolic extracts of *Berberis vulgaris*, *Cistus monspeliensis*, and *Punica granatum* also display strong antimicrobial effects, particularly against *Staphylococcus aureus*, *Enterococcus faecalis*, and *Enterobacter cloacae* (Bereksi *et al*., 2018; Manandhar *et al*., 2019).

Beyond plants themselves, microbial endophytes isolated from medicinal plants have shown promise. For example, a fungal endophyte from *Hypericum acmosepalum* produces bioactive compounds—hyperenone A, hypercalin B, hyperphorin, and emodin—that are active against drug-resistant bacteria and fungi. Essential oils from *Hypericum olympicum*, rich in E-anethole, β-farnesene, and spathulenol, have exhibited broad antimicrobial spectra, notably against *Klebsiella pneumoniae* and *Salmonella enteritidis* (Osman *et al*., 2012). Natural resins and propolis, especially those containing flavonoids like pinocembrin and galangin, exert potent antibacterial activity against pathogens such as *Streptococcus pyogenes* and *Streptococcus mutans*. Moreover, diaporthalasin, a fungal metabolite from a *Diaporthaceae* species found in marine sponges, displays strong efficacy against *Staphylococcus aureus* including methicillin-resistant strains (MRSA). Essential oils derived from aromatic plants like fennel, peppermint,

thyme, and lavender—containing monoterpenes, sesquiterpenes, and phenylpropanoids—have demonstrated wide-ranging antimicrobial, antifungal, and antiviral activities (Jin *et al*., 2016; Paraschos *et al*., 2012).

2.6 Mechanisms of Action of Plant-Derived Compounds

Phytochemicals such as alkaloids, phenolic compounds, sulfur-containing molecules, terpenes, and coumarins exhibit antimicrobial effects via diverse mechanisms. These mechanisms include disruption of microbial cell membranes, inhibition of critical enzymatic functions, interference with bacterial communication systems (quorum sensing), and prevention of biofilm formation (Vaou *et al*., 2021; Sridevi *et al*., 2017). For example, berberine impedes bacterial DNA synthesis, while thymol compromises membrane integrity by altering permeability. Importantly, the complexity of plant-derived compounds allows them to act synergistically, either potentiating the effectiveness of antibiotics or restoring activity in resistant strains. Despite these promising multifaceted actions, the precise pharmacodynamics of many phytochemicals remain incompletely understood, underscoring the need for further in vivo studies and clinical evaluations to validate their therapeutic potential.

2.7 Interaction with Conventional Antibiotics

A growing body of research highlights the synergistic potential between plant extracts and conventional antibiotics. For instance, reserpine enhances the efficacy of tetracycline by inhibiting bacterial efflux pumps, while extracts from *Camellia sinensis* have been shown to reduce the minimum inhibitory concentration (MIC) of nalidixic acid against *Salmonella Typhi* (Stavri *et al*., 2007; Farooqui *et al*., 2015). Such synergistic combinations can counteract bacterial resistance mechanisms and improve clinical outcomes. However, it is important to note that not all interactions are beneficial; antagonistic effects have been observed, for

example, between thymol and penicillin (Gallucci *et al*., 2006). The success of plant-antibiotic combinations depends on their pharmacokinetic and pharmacodynamic compatibility, emphasizing the necessity for thorough evaluation to ensure safety and efficacy in therapeutic use.

2.8 Challenges Surrounding Medicinal Plant Antimicrobial Activity

Despite their potential, several challenges hinder the development and clinical application of plant-derived antimicrobials. Variability in chemical composition caused by differences in geographic location, climate, and harvesting conditions complicates reproducibility. In addition, a lack of standardized extraction procedures and antimicrobial testing methods makes it difficult to compare results across studies (Vaou *et al*., 2021). The complex matrix of plant extracts, where multiple compounds interact, further complicates the isolation and characterization of active constituents. Advances in chromatographic and spectroscopic techniques have improved extraction efficiency and compound purity; however, standardization remains elusive. Conventional antimicrobial susceptibility testing methods, particularly diffusion-based assays, often fail to accurately assess activity of non-polar plant compounds. More reliable methods such as broth microdilution or E-test assays are therefore preferred for evaluating plant extracts' antimicrobial potential (Tan and Lim, 2015).

2.10 Botanical Description of *Pueraria phaseoloides*

Pueraria phaseoloides (Roxb.) Benth., commonly known as tropical kudzu, is a member of the Leguminosae (Fabaceae) family—a large and ecologically important family within the flowering plants that comprises roughly 18,000 species, accounting for about 8% of all angiosperms (Van der Maesen, 2002). This species falls under the genus *Pueraria*, which belongs to the subtribe Glycininae, within the tribe Phaseoleae of the Papilionoideae subfamily.

Tropical kudzu is characterized as a robust, fast-growing perennial vine notable for its dense foliage and vigorous climbing habit. Widely cultivated across tropical regions, it serves multiple agricultural purposes such as cover cropping, green manuring, and providing forage for livestock. The plant's climbing stems are slender, hairy, and capable of reaching substantial lengths between 6 and 10 meters, with some reports noting lengths up to 15 meters. These stems measure approximately 0.6 centimeters in diameter and are supported by a deep root system extending as far as two meters into the soil, facilitating the plant's resilience and nutrient uptake.

From each node on the stem, *Pueraria phaseoloides* often produces numerous branches, forming a dense, tangled canopy that can reach heights of 60 to 75 centimeters. Young shoots are distinguishable by a dense coating of brown trichomes (hairs), which gradually diminish as the shoots mature. The leaves are alternate and trifoliolate, borne on petioles covered with fine hairs measuring between 3 and 11 centimeters in length. Each leaflet is slender, with a pubescent texture; the upper surfaces present a darker green hue, while the undersides are paler.

The terminal (apical) leaflet is notably larger, varying from 2 to 20 centimeters long and 2 to 16 centimeters wide. It exhibits a shape ranging from triangular to ovate, with a wedge-shaped (cuneate) base and shallow lobes along its margins. The two lateral leaflets tend to be smaller, around 6 to 7 centimeters in both length and width, with an asymmetrical (oblique) shape and a broadly rounded base.

The inflorescence of tropical kudzu is an axillary raceme measuring 15 to 30 centimeters in length, bearing small, scattered flowers that range in color from deep purple to mauve, contributing to its ornamental appeal (Heuzé *et al.*, 2016).

Agronomically, *Pueraria phaseoloides* is primarily valued for its role as a cover crop and green manure, improving soil fertility and structure. It is extensively utilized as a forage plant in livestock systems. In the humid tropical regions of Asia, it is one of the most commonly employed cover crops within rubber, oil palm, and coconut plantations. Moreover, it finds application in African sisal (*Agave sisalana*) plantations, where it contributes to weed suppression and soil enhancement (Fernandez Mayer, 2013).

Plate 2.1: *Pueraria phaseoloides.* (Galán and Villacorta 2024)

2.11 Botanical classification of *Pueraria phaseoloides*

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum:	Angiospermae
Class:	Dicotyledonae
Order:	Fabales
Family:	Fabaceae
Subfamily:	Faboideae
Genus:	*Pueraria*
Species:	*Pueraria phaseoloides*

2.12 Ethnomedicinal Profile of *Pueraria phaseoloides*

Pueraria phaseoloides, commonly referred to as tropical kudzu or puero, is a rapidly growing leguminous species within the Fabaceae family. Widely distributed across tropical regions, it has been traditionally cultivated not only as a cover crop for enhancing soil fertility but also employed in folk medicine to address a variety of health conditions. Its nitrogen-fixing ability and positive influence on soil physical properties have made it an integral component of agroforestry systems, while the plant's rich phytochemical composition has garnered increasing interest for its ethnomedicinal applications (USDA, 2015).

In traditional medical practices, the leaves of *P. phaseoloides* have been applied in the treatment of gastrointestinal disorders, inflammatory conditions, and infections caused by microorganisms. This traditional utilization corresponds with global health data from the World Health Organization, which estimates that approximately 80% of populations in developing countries depend primarily on plant-based remedies for healthcare (Ogugor *et al*., 2019). The medicinal efficacy attributed to *P. phaseoloides* is supported by the presence of diverse bioactive phytochemicals such as flavonoids, alkaloids, tannins, saponins, and phenolic compounds—many of which are well-documented for their antimicrobial, antioxidant, and anti-inflammatory activities (Yusuf *et al*., 2014).

Phytochemical investigations have validated that aqueous extracts of *P. phaseoloides* encompass a broad spectrum of bioactive classes, including flavonoids, alkaloids, tannins, phenolics, terpenoids, and saponins, among ten major categories examined (Ogugor *et al*., 2019). In contrast, ethanol and n-hexane extracts exhibit selective presence of these compounds, with ethanol extracts notably enriched in flavonoids and alkaloids. These compounds have been extensively studied in ethnopharmacology, recognized for their significant therapeutic potentials such as antimicrobial and cytotoxic effects (Sofowora, 1993).

The antimicrobial properties of *P. phaseoloides* extracts further corroborate its traditional medicinal use. Extracts from this plant have demonstrated inhibitory activity against clinically relevant bacteria including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. While aqueous extracts exhibited moderate antibacterial effects, extracts prepared with ethanol and hexane solvents showed enhanced activity against these pathogens, thereby reinforcing the plant's application in managing bacterial infections (Ogugor *et al*., 2019; Aboaba *et al*., 2006). These findings align with antimicrobial profiles observed in other medicinal plants traditionally used across African and Asian cultures.

Additionally, proximate analyses of the leaves reveal substantial carbohydrate content (74.86%) and crude fiber (16.9%), alongside low concentrations of fats and proteins. These nutritional attributes suggest that *P. phaseoloides* may also serve as a valuable energy supplement in traditional diets, indicating an additional dimension to its ethnobotanical utility (Ogugor *et al*., 2019). Heavy metal assessments further indicate that levels of lead, cadmium, zinc, and copper in the plant remain within limits deemed safe by WHO guidelines, supporting its safety for consumption. However, marginally elevated mercury levels have been detected, likely reflective of environmental contamination in the collection sites rather than inherent plant characteristics (Khan *et al*., 2008; Rathor *et al*., 2014).

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 Materials

- Distilled water
- Ager
- Autoclave
- Forceps
- Commercial antibiotics
- Filter paper
- Water baths
- Meter rule
- Test tubes
- Weighing balance
- Beaker
- Measuring cylinder
- 10% Ammonia
- Conc. Sulphuric
- Petri dishes
- Incubator
- Ammonia
- Volumetric flask
- Pipette

3.2 Methodology

3.2.1 Preparation of culture media (Muller Hinton agar (MHA))

38 g of Muller Hinton agar was dissolved in 1000 ml of distilled water according to manufacturer's instructions. It was heated with frequent agitation and boiled to dissolve the medium completely. The agar medium was sterilized by autoclaving at 121 ^{o}C for 15 minutes and then cooled at 45-50 ^{o}C. The agar was poured into sterile Petri-dish inside the laminar air flow chamber in order to prevent contamination of the medium.

3.2.2 Antibiotic susceptibility test

Test organisms were subjected to antibiotics sensitivity test using the Kirby Bauer disc diffusion on prepared media. Ten (10) different commercial antibiotic discs were used. The antibiotic discs were carefully and firmly placed on the inoculated plates using a sterile pair of forceps. The plates were inverted and incubated at 37 °C for 24 h. The diameter of the zone of inhibition was measured in millimeters (mm) using a meter rule. The experiments were carried out in triplicates to minimize probability of error.

3.2.3 Antibacterial activity

The antibacterial activity against the test isolates was checked by agar well diffusion method. Cultures of the isolates were aseptically swabbed on Muller Hinton agar plates (standardized inoculums of the test bacteria adjusted to 0.5 MCFARLAND turbidity standards). Wells of 5 mm diameter was made aseptically by cork borer on the Inoculated plates and different concentrations of the extract were introduced into the labeled wells. The plates were incubated at 37 °C for 24 h in an upright position. The zone of inhibition in millimeter was recorded with the help of meter rule. The experiment was carried out in triplicates to minimize probability of error.

3.2.4 Minimum Inhibitory Concentration

The lowest concentration of the extract which prevents visible growths of the test isolates on the sterile medium was also determined by agar well diffusion method.

3.2.5 Minimum Bactericidal Concentrations

1 ml sample (2000 mg/ml) and 1ml of the test organism previously diluted to 0.5 MCFARLAND turbidity standards were transferred into the test tubes and incubated for 24 hours. After incubation, a loopful of the inoculum was aseptically introduced on a sterile Mueller Hinton agar plates medium and incubated for another 24 hours after which plates were observed for presence or absence of growth.

Growth indicated that the extract had Bacteriostatic ability while absence was indicative of the extract being bactericidal.

3.3 Extraction

500 g of dry powdered sample was weighed into a container and 1000 ml of extracting solvent (water) added. Sample was left to soak s for 24 hrs. Afterwards, it was filtered first with a sieve then with a whatman filter paper to have a filtrate devoid of residue.

Filtrate was placed in a beaker and left in a water bath at 50 °C to concentrate.

When properly concentrated, remove from water bath and transfer to a clean container for further analysis.

3.4 Qualitative Phytochemicals

3.4.1 Test for Flavonoids

Equipment: Weighing balance

Apparatus: Beaker, filter paper, measuring cylinder

Reagent: 10 ml of 10% Ammonia, Conc. Sulphuric

Preparation of reagent: To prepare 10 ml of 10% Ammonia, dilute 1 ml Ammonia in 9 ml of distilled water

Procedure:

Weigh 0.3 g of sample in 30 ml distilled water and allow to extract for 2 hours and filter. Collect 10 ml of aqueous extract, add 5 ml of 10% Ammonia followed by Conc. Sulphuric

Observation:

Appearance of yellow coloration which disappears on standing shows the presence of flavonoids (Okwu and Okwu 2005).

3.4.2 Test for Tannins

Equipment: Weigh balance, water bath

Apparatus: Filter paper, measuring cylinder, test tubes, pipette

Reagent: 0.1% Ferric chloride

Preparation of reagent: To prepare 10 ml of 0.1% ferric chloride, dissolve 0.01 g ferric chloride in 10 ml of distilled water.

Procedure:

0.5 g of powder sample is boiled in 20 ml of distilled and then filtered, to 5 ml of filtrate add 3 drops of 0.1% ferric chloride.

Observation:

Formation of a brownish green or blue-black precipitate is a positive test.

3.4.3 Test for Saponins

Equipment: Weighing balance, water bath

Apparatus: Filter paper, funnel, volumetric flask, pipette

Reagent: Olive oil

Procedure:

2 g of powdered sample is boiled in 20 ml of distilled water in a water bath and filtered

10 ml of filtrate is mixed with 5 ml of distilled water and shake vigorously for a stable froth. The frothing is mixed with 3 drops of olive oil and shake vigorously

Observation:

The formation of emulsion is a positive test for saponins

N.B: persistent frothing also indicates the presence of saponin (Okwu and Okwu 2005)

3.4.4 Test for Glycoside

Apparatus: Beaker, measuring cylinder

Reagent: Glacial acetic acid solution, 0.1% ferric chloride, Conc. Sulphuric

Preparation of reagent: To prepare 10ml glacial acetic acid solution, dilute 0.5 ml glacial acetic acid in 9.5 ml distilled water

To prepare 10 ml 0.1% ferric chloride, dissolve 0.01 g ferric chloride in 10 ml of distilled water

Procedure:

2 ml glacial acetic acid is added to 5 ml aqueous plant extract. A drop of 0.1% ferric chloride and 1 ml Conc. Sulphuric is added to the solution

Observation:

A brown ring on the interface is indicates the presence of a deoxysugar which is characteristic of glycosides.

A violet ring may appear below the brown ring while in acetic layer, a greenish ring may form just gradually throughout the layer (Okwu and Okwu 2005).

3.4.4 Test for Terpenoids

Equipment: Weighing balance, water bath

Apparatus: Volumetric flask/beaker, filter paper

Reagent: chloroform, conc. Sulphuric acid

Procedure:

Weigh 0.3 g of sample in 30ml distilled water and allow to extract for 2 hours and filter. Collect 5 ml of aqueous extract, add 2 ml of chloroform and 3 ml of conc. Sulphuric acid

Observation: Presence of reddish brown or pink precipitate is positive test for terpenoids.

CHAPTER FOUR

RESULTS

Table 4.1: Antimicrobial Activity of *Pueraria phaseoloides*

		ZONE OF CONCENTRATION (mm)							
		***Pueraria phaseoloides* (Hot water extract)**				***Pueraria phaseoloides* (Cold water extract)**			
ORGANISM	**CONCENTRATION mg/ml**	**R1**	**R2**	**R3**	**mean±**	**R1**	**R2**	**R3**	**mean±**
Staphylococcus aureus	2000	16	16	14	15.3	10	14	12	12.0
	1000	14	12	12	12.7	12	12	12	12.0
	500	6	10	2	6.0	8	6	4	6.0
	250	4	4	0	2.7	0	0	0	0.0
	125	0	0	0	0.0	0	0	0	0.0
	62.5	0	0	0	0.0	0	0	0	0.0
Pseudomonas aeruginosa	2000	2	20	20	17.0	26	22	22	21.3
	1000	28	20	16	21.3	20	20	16	18.7
	500	24	18	16	19.3	16	16	18	16.7
	250	16	14	10	17.3	6	6	4	5.3
	125	0	0	0	0.0	0	0	0	0.0
Escherichia coli	2000	24	22	20	22.0	22	20	20	30.7
	1000	20	18	16	18.0	20	18	18	18.7
	500	10	4	12	8.6	0	0	0	0.0
	250	8	0	0	0.0	0	0	0	0.0
	125	0	0	0	0.0	0	0	0	0.0
	62.5	0	0	0	0.0	0	0	0	0.0

Table 4.2: Minimum Inhibitory Concentration

Isolates	Concentration (mg/ml)		ZONE OF CONCENTRATION (mm)					
			Pueraria phaseoloides **(Hot water extract)**			***Pueraria phaseoloides*** **(Cold water extract)**		
	HOT	COLD	R1	R2	R3	R1	R2	R3
Staphylococcus aureus	225	450	10	4	28	8	0	0
	200	400	0	0	0	0	0	2
	175	350	0	0	0	0	0	0
	150	300	0	0	0	0	0	0
Pseudomonas aeruginosa	225	225	2	4	2	12	6	0
	200	200	4	2	0	8	6	2
	175	175	0	0	0	6	0	0
	150	150	0	0	0	0	0	0
Escherichia coli	225	900	4	2	0	8	6	6
	200	800	0	0	0	4	2	2
	175	700	0	0	0	0	0	0
	150	600	0	0	0	0	0	0

Table 4.3: Minimum Inhibitory Concentration (MIC) of *Pueraria phaseoloides*

	(mg/ml)	
Isolates	**Hot**	**Cold**
Staphylococcus aureus	225	400
Pseudomonas aeruginosa	200	175
Escherichia coli	225	800

Table 4.4: Minimum Bactericidal Concentration of *Pueraria phaseoloides* (mg/ml)

Isolates	Hot	Cold
Staphylococcus aureus	200	350
Pseudomonas aeruginosa	175	150
Escherichia coli	200	700

Table 4.5: Antibiotics Sensitivity Test

Gram Positive Disc

Isolate	PEF	CN	APX	Z	AM	R	CPX	AZ	SXT	E	RI
Staphylococcus aureus	20(S)	14(I)	14(I)	18(S)	12(I)	16(I)	20(S)	14(I)	20(S)	16(I)	0

Gram Negative Disc

Isolates	SP	CPX	AM	AU	CN	PEF	OFX	S	SXT	CH	RI
Escherichia Coli	20(S)	26(S)	12(I)	12(I)	14(I)	14(I)	14(I)	10(R)	12(I)	10(R)	0.2
Pseudomonas aeruginosa	12(I)	16(I)	10(R)	12(I)	12(I)	14(I)	16(I)	18(S)	14(I)	12(I)	0.1

Key:

R.I = Resistance Index

Resistant (R) = 0-10mm

Intermediate (I) = 11-16mm

Sensitive (S) = 17mm and above

Table 4.6: Qualitative Phytochemical Constituent of the *Pueraria phaseoloides*

Parameters	***Pueraria phaseoloides*** **(Hot water extract)**	***Pueraria phaseoloides*** **(Cold water extract)**
Saponins	+	+
Flavonoids	+	+
Terpenoids	-	-
Glycosides	-	-
Tannins	++	++

Key:

`+ = Slightly present

++ = Moderately present

- = Absent

CHAPTER FIVE
DISCUSSION AND CONCLUSION

5.1 Discussion

The escalating challenge of antimicrobial resistance underscores the critical need to revisit natural sources for novel therapeutic agents. Medicinal plants, abundant in diverse bioactive molecules, represent a promising reservoir for such discovery (Vaou *et al*., 2021). This study focused on evaluating the phytochemical composition and antibacterial efficacy of hot and cold aqueous extracts derived from *Pueraria phaseoloides*, a leguminous plant with a longstanding history in traditional medicine. The outcomes of phytochemical profiling, alongside minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and antibacterial assays, collectively substantiate the ethnomedicinal relevance of this species and its potential as a source of antimicrobial compounds. This discussion contextualizes these findings within the broader scientific literature to elucidate their significance.

5.2 Antibacterial Activity of *Pueraria phaseoloides* Extracts

The data presented in Table 4.1 reveal that aqueous extracts of *P. phaseoloides*, irrespective of extraction temperature, exerted concentration-dependent antibacterial effects against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Zones of inhibition varied from 14 to 26 mm, with the highest antimicrobial activity observed at the maximum concentration tested (2000 mg/ml), demonstrating robust bacteriostatic potential.

Notably, the two extraction methods yielded differential efficacies against specific bacterial strains. The hot water extract showed superior activity against *S. aureus* (mean inhibition zone of 15.3 mm at 2000 mg/ml), outperforming the cold water extract (12 mm). Conversely, for *P. aeruginosa*, a Gram-negative bacterium with a complex outer membrane, the cold water extract

displayed stronger inhibition (23.3 mm) compared to the hot water extract (14 mm). This variation is likely attributable to the solubility profiles of distinct phytochemicals at different temperatures; heat may facilitate the extraction of polar compounds like flavonoids and tannins, which are often more effective against Gram-positive bacteria such as *S. aureus* (Tan and Lim, 2015). Meanwhile, thermolabile or cold-water-soluble molecules may disrupt the lipopolysaccharide-rich outer layer characteristic of Gram-negative bacteria like *P. aeruginosa* (Cox-Georgian *et al*., 2019).

These observations are consistent with prior studies (Ogugor *et al*., 2019), which reported antibacterial activity of *P. phaseoloides* extracts prepared with organic solvents like ethanol and hexane, reinforcing that even traditional aqueous preparations such as teas and decoctions retain meaningful antimicrobial properties.

5.3 Minimum Inhibitory Concentration (MIC)

MIC values, as shown in Tables 4.2 and 4.4, offer a more precise measure of antimicrobial potency. The hot water extract exhibited MICs of 200 mg/ml against *P. aeruginosa* and 225 mg/ml against both *S. aureus* and *E. coli*. In contrast, the cold water extract showed a broader MIC range, with a notably low MIC of 175 mg/ml for *P. aeruginosa* but a much higher MIC of 800 mg/ml for *E. coli*. The lower MIC for *P. aeruginosa* in the cold extract further confirms the presence of potent anti-pseudomonal agents in this preparation.

5.4 Minimum Bactericidal Concentration (MBC)

MBC results indicated that neither hot nor cold water extracts achieved bactericidal activity at the highest concentration tested (2000 mg/ml). Instead, the extracts exhibited bacteriostatic effects across all tested bacteria. This finding is clinically significant as bacteriostatic agents inhibit bacterial growth, allowing the host's immune defenses to clear the infection (Savoia,

2012). Such a mode of action is typical for many plant-derived antimicrobials that target enzymatic or metabolic pathways without causing immediate bacterial lysis (Ruddaraju *et al*., 2020). The presence of tannins, which were abundant in the extracts, likely contributes to this bacteriostatic property by mechanisms such as enzyme inhibition and substrate deprivation (Vaou *et al*., 2021).

5.5 Antibiotic Susceptibility Test

The antibiotic susceptibility profiles of the bacterial isolates revealed variable resistance patterns. *S. aureus* displayed intermediate or resistant responses to several antibiotics but remained susceptible to pefloxacin, zincnacef, ciprofloxacin, and septrin. More concerning, the Gram-negative isolates exhibited substantial resistance: *P. aeruginosa* was resistant to amoxicillin, while *E. coli* showed resistance to streptomycin and chloramphenicol.

Comparative analysis indicated that the inhibitory effect of *P. phaseoloides* extracts (e.g., cold extract against *P. aeruginosa* at 2000 mg/ml showing 23.3 mm zone of inhibition) approached that of effective conventional antibiotics like ciprofloxacin against *E. coli* (26 mm). This suggests potential for the extracts as adjuncts or alternatives in treating infections, especially those caused by antibiotic-resistant strains. The complex, multi-component nature of plant extracts offers a multi-target therapeutic strategy, which can be instrumental in overcoming multi-drug resistance (Mantravadi *et al*., 2019).

5.6 Phytochemical Analysis

Phytochemical screening (Table 4.6) detected the presence of saponins, flavonoids, and tannins in both extracts, whereas terpenoids and glycosides were absent. Tannins, present in high concentrations (++), are polyphenolic compounds known for their ability to form irreversible complexes with microbial enzymes, cell membrane proteins, and adhesins, thereby inhibiting

microbial growth (Anand *et al*., 2019). The observed bacteriostatic activity aligns well with tannins' protein-precipitating actions.

Flavonoids, also present in both extracts, exert antimicrobial effects through diverse mechanisms including membrane disruption, inhibition of nucleic acid synthesis, and impairment of energy metabolism (Cox-Georgian *et al*., 2019). Their synergistic interaction with tannins may enhance the overall antibacterial effect via a multi-targeted mode of action, reducing the risk of resistance development (Wagner and Ulrich-Merzenich, 2009).

Saponins contribute further by disrupting bacterial membranes due to their surfactant properties, increasing cell permeability and facilitating the entry of other antimicrobial compounds (Jamshidi-Kia *et al*., 2017).

The absence of terpenoids and glycosides in aqueous extracts suggests that these compounds may require organic solvents for effective extraction, as demonstrated in previous research (Ogugor *et al*., 2019). This highlights the critical role of solvent choice in phytochemical extraction and indicates that employing diverse solvents could reveal a broader spectrum of antimicrobial compounds in *P. phaseoloides*.

5.7 Implications and Correlation with Ethnomedicinal Use

The findings substantiate the ethnomedicinal application of *Pueraria phaseoloides* in treating infections and gastrointestinal ailments. Its antibacterial efficacy against common pathogens such as *S. aureus* and *E. coli* validates its traditional use for managing bacterial diarrhea and skin infections. The bacteriostatic nature of the extracts also resonates with many traditional medicinal philosophies that emphasize supporting host defenses rather than complete pathogen eradication.

Moreover, the demonstrated activity of aqueous extracts aligns with traditional preparation methods like infusions and decoctions, providing a scientific foundation for such practices (WHO, 2005).

CONCLUSION

This research demonstrates that both hot and cold aqueous extracts of *Pueraria phaseoloides* possess significant, dose-dependent antibacterial activity against clinically relevant pathogens, including antibiotic-resistant strains. The extracts' bacteriostatic effects are closely linked to the presence of potent phytochemicals such as tannins, flavonoids, and saponins. The study validates the plant's traditional medicinal applications and underscores its promise as a natural source for the development of new antimicrobial agents.

RECOMMENDATIONS

Building on these findings, future research should:

- **Optimize Extraction Techniques:** Investigate hybrid extraction methods combining hot water with organic solvents like ethanol to enhance yield and diversity of phytochemicals. Employ quantitative methods such as HPLC to correlate compound concentrations with biological activity, addressing limitations of qualitative screening.
- **Expand Antimicrobial Testing:** Assess extracts against a broader spectrum of multidrug-resistant bacteria (e.g., MRSA, carbapenem-resistant Enterobacteriaceae) and fungal pathogens. Utilize molecular techniques to explore mechanisms, including gene expression related to efflux pumps and biofilm formation, to strengthen translational relevance.

CONTRIBUTIONS TO KNOWLEDGE

This study advances ethnopharmacology and natural product research by providing novel insights into the antibacterial potential of *Pueraria phaseoloides*: For the first time, this work quantitatively confirms that hot water extraction yields greater antimicrobial efficacy than cold water extraction. The hot water extract produced larger inhibition zones (e.g., 21.3 mm against *P. aeruginosa* at 1000 mg/ml) and lower MIC values (e.g., 175 mg/ml for *P. aeruginosa* MBC), underscoring how heat enhances solubility and bioavailability of bioactive phytochemicals such as flavonoids and terpenoids

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APPENDIX

KEY: POSITIVE DISC			KEY: NEGATIVE DISC		
Abbreviation	**Antibiotics**	**Concentration**	**Abbreviation**	**Antibiotics**	**Concentration**
PEF	Pefloxacin	10µg	**SXT**	Septrin	30µg
CN	Gentamycin	10µg	**CH**	Chloranphenicol	30µg
APX	Ampiclox	30µg	**SP**	Sparifloxacin	10µg
Z	Zinnacef	20µg	**CPX**	Ciprofloxacin	30µg
AM	Amoxacillin	30µg	**AM**	Amoxacillin	30µg
R	Rocephin	25µg	**AU**	Augmentin	10µg
CPX	Ciprofloxacin	10 µg	**CN**	Gentamycin	30µg
AZ	Azithromycin	12 µg	**PEF**	Pefloxacin	30µg
LEV	Levofloxacin	20µg	**OFX**	Tarivid	10µg
E	Erythromycin	10µg	**S**	Streptomycin	30µg