EVALUATION OF ANTIBACTERIAL AND PHYTOCHEMICAL PROPERTIES OF COLD AND HOT WATER EXTRACT OF *Mimosa pudica*

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UNIVERSITY OF BENIN

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF B.Sc. (HONS) IN MICROBIOLOGY, UNIVERSITY OF BENIN, BENIN CITY.

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CERTIFICATION

This is to certify that this project work was carried out by Victory Osama Osarobo with Matriculation number LSC2104010 in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, under the supervision of Mrs. I. Akhigbe

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(Head of department)

DEDICATION

This work is dedicated to God almighty, for his wisdom, guidance and provision in my academic journey and all through the duration of my study in the University of Benin.

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ABSTRACT

Medicinal plants have long served as a vital source of bioactive compounds with therapeutic potential against a wide range of microbial pathogens. The increasing incidence of antibiotic resistance among pathogenic bacteria has renewed scientific interest in exploring plant-derived compounds as alternative antimicrobial agents. *Mimosa pudica* (commonly known as the "Sensitive Plant" or "Touch-Me-Not") is a medicinal herb traditionally used in folk medicine for its reported antimicrobial, anti-inflammatory, and wound-healing properties. This study evaluated the phytochemical constituents and antibacterial activity of hot and cold water extracts of *Mimosa pudica* against three clinically significant bacterial isolates: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Qualitative phytochemical screening revealed the presence of saponins, flavonoids, and tannins, while terpenoids and glycosides were absent. Tannins were moderately present (++) in both extracts, suggesting their possible contribution to the plant's antimicrobial potential. The antibacterial assay demonstrated concentration-dependent inhibitory effects against all test organisms. The cold extract exhibited higher antibacterial activity than the hot extract, with *Staphylococcus aureus* showing a maximum zone of inhibition of 26.00 ± 5.29 mm at 2000 mg/ml, compared to 8.00 ± 5.29 mm for the hot extract. Similarly, the cold extract showed superior inhibition against *Escherichia coli* (27.33 ± 6.11 mm) and *Pseudomonas aeruginosa* (18.67 ± 7.57 mm) at the highest concentration. The Minimum Inhibitory Concentration (MIC) values revealed that *Staphylococcus aureus* was most susceptible to the cold extract (80 mg/ml), whereas *Pseudomonas aeruginosa* exhibited the least susceptibility (175 mg/ml for both extracts). The Minimum Bactericidal Concentration (MBC) results indicated that both extracts were bacteriostatic rather than bactericidal at the tested concentrations (2000 mg/ml). Comparative antibiotic sensitivity tests showed that the bacterial isolates displayed varying degrees of resistance and susceptibility to standard antibiotics, underscoring the need for effective plant-based alternatives. Overall, the findings demonstrate that *Mimosa pudica* possesses promising antibacterial activity, particularly in its cold water extract, likely due to the presence of tannins, saponins, and flavonoids. These results support the traditional use of *Mimosa pudica* in herbal medicine and suggest its potential as a natural source of antimicrobial compounds for combating bacterial infections, especially those caused by antibiotic-resistant pathogens.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

The global health landscape is grappling with the escalating threat of antimicrobial resistance (AMR), a crisis that claims approximately 700,000 lives annually and is projected to cause 10 million deaths by 2050 if unaddressed (World Health Organization, 2020). The overuse of synthetic antibiotics, coupled with a dwindling pipeline of new antimicrobial agents, has fueled the rise of multidrug-resistant pathogens, including *Staphylococcus aureus* (methicillin-resistant, MRSA), *Escherichia coli* (extended-spectrum beta-lactamase, ESBL), *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* (Fatima *et al*., 2023; Rana *et al*., 2024; Gajic *et al*., 2025). This has shifted scientific attention toward natural products, particularly medicinal plants, which offer a diverse array of bioactive compounds with potential to combat bacterial infections while minimizing resistance development (Cowan, 1999). Plants have been a cornerstone of traditional medicine for millennia, with an estimated 80% of populations in developing countries relying on herbal remedies for primary healthcare due to their accessibility, affordability, and cultural significance (World Health Organization, 2013).

Among these medicinal plants, *Mimosa pudica* L., a member of the Fabaceae family, stands out for its extensive ethnobotanical use and pharmacological potential. Commonly known as the sensitive plant, touch-me-not, or shame plant, this perennial herb is native to Central and South America but has become widely naturalized in tropical and subtropical regions, including Africa, South Asia, and Southeast Asia (Barboni *et al*., 2011). Its characteristic seismonastic movement—rapid folding of leaves upon mechanical stimulation—not only underscores its ecological adaptability but also suggests a robust chemical defense system, likel y linked to its

rich phytochemical profile. *M. pudica* is a repository of secondary metabolites, including alkaloids (e.g., mimosine), flavonoids (e.g., quercetin, luteolin), tannins, saponins, phenolic acids, terpenoids, and glycosides, which are implicated in its wide-ranging therapeutic applications (Mahajan *et al*., 2009).

The medicinal value of *Mimosa pudica* is deeply rooted in traditional medicine systems across diverse cultures, reflecting its versatility and efficacy. In Ayurveda and Siddha systems of India, the plant is revered for its multifaceted therapeutic properties. Decoctions of its leaves, stems, and roots are used to treat gastrointestinal disorders (e.g., diarrhea, dysentery), urogenital infections, wounds, and hemorrhoids, while its anti-inflammatory and analgesic properties make it a remedy for arthritis and muscle pain (Muthukumar *et al*., 2018). In African ethnomedicine, particularly in Nigeria, Ghana, and Kenya, *M. pudica* is employed as a poultice for wound healing, a febrifuge for malaria, and an antidote for snake venom (Burkill, 1995). In Latin American and Caribbean traditions, notably in Brazil and Jamaica, the plant is used as an antispasmodic, anticonvulsant, and emmenagogue, addressing conditions like menstrual irregularities and epilepsy (Ahmad *et al*., 2012). These traditional uses are supported by preliminary pharmacological studies that attribute the plant's efficacy to its bioactive phytochemicals.

Traditionally, whole *M. pudica* plants are used as medicines. The plant leaves, flowers, stems, roots, and fruits are used in the treatment of leprosy, dysentery, vaginal and uterine complaints, inflammations, burning sensation, asthma, leucoderma, fatigue and blood pressure. It is very useful in diarrhea, amoebic dysentery, bleeding piles and urinary infections (Chauhan *et al*., 2009). It has potent pharmacological activities like antidiabetic, antitoxin, antihepatotoxin,

antioxidant and wound healing activities (Meenatchisundaram *et al*., 2009). Apart from that, many research articles reported its antioxidant, antimicrobial, anti-venom, anticonvulsant, antiulcer, Antihyperglycemic activities (Gandhiraja *et al*., 2009; Ngo Bum *et al*., 2004; Chowdhury, 2008; Meenatchisun daram *et al*., 2009; Aarthi and Murugan, 2011; Elango *et al*., 2012). These activities are mainly due to the presence of various plant secondary metabolites such as the presence of bioactive constituents like terpenoids, flavonoids, glycosides, alkaloids, quinines, phenols, tannins, saponins and coumarin (Gandhiraja *et al*., 2009).

The alkaloids, particularly mimosine, are noted for their antimicrobial and antiproliferative effects, potentially disrupting bacterial cell membrane integrity and inhibiting protein synthesis (Robinson *et al*., 2005). Flavonoids, such as quercetin and luteolin, contribute to antioxidant and anti-inflammatory activities, neutralizing free radicals and modulating immune responses, which are critical in managing infections and associated tissue damage (Kumar *et al*., 2013). Tannins and phenolic compounds confer astringent and antimicrobial properties, aiding in wound contraction and pathogen inhibition, while saponins exhibit membrane-disrupting effects against microbes (Cowan, 1999). These compounds collectively underpin *M. pudica*'s therapeutic potential, making it a candidate for addressing infections in resource-limited settings where synthetic drugs are scarce.

Beyond infectious diseases, *M. pudica* has shown promise in other therapeutic domains. Studies have reported its hypoglycemic effects in diabetes management, hepatoprotective activity against liver damage, and anxiolytic properties for mental health disorders (Genest *et al*., 2008). Its antidepressant-like effects, mediated by flavonoids interacting with serotonin receptors, further highlight its neuropharmacological potential (Molina *et al*., 1999). The plant's broad-spectrum

medicinal value, combined with its widespread availability as a weed, positions it as a valuable resource for developing sustainable, plant-based therapeutics, particularly in combating the AMR crisis.

The antibacterial potential of *Mimosa pudica* has garnered significant interest due to its activity against both Gram-positive and Gram-negative bacteria, which are responsible for a wide range of community- and hospital-acquired infections. Common bacterial pathogens, such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*, are implicated in conditions ranging from skin infections and food poisoning to urinary tract infections and nosocomial pneumonias (Todar, 2008). The rise of resistance in these organisms necessitates alternative agents, and *M. pudica* extracts have shown promising in vitro activity against these strains.

Preliminary studies indicate that *M. pudica* extracts, particularly those prepared with polar solvents like water and ethanol, exhibit significant antibacterial activity. For instance, Nair *et al*. (2016) reported that hot water extracts of *M. pudica* leaves inhibited *S. aureus* with zones of inhibition ranging from 15–20 mm in agar well diffusion assays, comparable to standard antibiotics like streptomycin. This activity is attributed to tannins and phenolic compounds, which disrupt bacterial cell walls and inhibit enzymatic functions (Scalbert, 1991). Similarly, *B. subtilis*, a Gram-positive spore-forming bacillus, is susceptible to *M. pudica* extracts, with minimum inhibitory concentrations (MICs) as low as 0.5 mg/mL for aqueous extracts, suggesting potential for controlling foodborne infections (Tamilarasi *et al*., 2017).

Against Gram-negative bacteria, *M. pudica* demonstrates moderate to high efficacy, though typically less potent than against Gram-positive strains due to the outer membrane barrier in

Gram-negative bacteria. *E. coli*, a leading cause of urinary tract infections and gastroenteritis, is inhibited by *M. pudica* leaf extracts, with studies reporting MICs of 1–2 mg/mL for hot water extracts, likely due to flavonoids and saponins disrupting membrane permeability (Muthukumar *et al*., 2018). *P. aeruginosa*, notorious for its intrinsic resistance to many antibiotics, shows variable susceptibility, with zones of inhibition ranging from 10–15 mm for hot water extracts, suggesting that higher concentrations or synergistic combinations may be required for effective control (Nair *et al*., 2016). These findings highlight the plant's potential against common pathogens but also underscore the need for systematic evaluation of extraction methods to optimize antibacterial efficacy.

The choice of extraction method cold versus hot water significantly influences the yield and bioactivity of *M. pudica*'s phytochemicals. Hot water extraction (100°C) enhances the solubility of polar compounds like tannins and polysaccharides, which are effective against Gram-positive bacteria due to their ability to precipitate proteins and disrupt cell walls (Azwanida, 2015). Cold water extraction (25°C), however, preserves heat-labile compounds like certain flavonoids and volatile terpenoids, which may contribute to activity against Gram-negative bacteria by penetrating their outer membranes (Harborne, 1998). Despite these insights, comparative studies on the antibacterial effects of cold versus hot water extracts against common bacteria are limited, creating a knowledge gap that this study aims to address. By evaluating both extraction methods, this research seeks to elucidate how temperature affects the antibacterial potency of *M. pudica* against *S. aureus*, *B. subtilis*, *E. coli*, and *P. aeruginosa*, providing insights into optimal preparation techniques for traditional and pharmaceutical applications.

1.2 Statement of the Problem

The relentless spread of antibiotic-resistant bacteria, coupled with limited access to effective treatments in developing countries, necessitates the exploration of natural alternatives like *Mimosa pudica*. Despite its widespread use in traditional medicine for treating infections, the antibacterial efficacy of *M. pudica* against common pathogens remains inconsistently documented, largely due to variations in extraction protocols. For instance, while hot water extracts have shown promise against *S. aureus* and *B. subtilis*, cold water extracts are understudied, potentially overlooking unique bioactive fractions (Tamilarasi *et al*., 2017). The lack of comparative data on cold versus hot water extracts hinders the optimization of *M. pudica*'s antibacterial potential, limiting its integration into evidence-based therapeutics. This study addresses these gaps by systematically evaluating the phytochemical profiles and antibacterial effects of both extracts against common bacterial pathogens, aiming to validate traditional claims and inform standardized preparation methods.

1.3 Aim and Objectives of the Study

The main aim of this study is to evaluate the phytochemical composition and antibacterial properties of cold and hot water extracts of *Mimosa pudica*.

The specific objectives of this study was to:

1. Qualitatively determine the phytochemical constituents present in cold and hot water extracts of *Mimosa pudica*.
2. Evaluate the antibacterial activity of cold and hot water extracts of *Mimosa pudica* against selected bacterial isolates.

3. Determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts against the test bacterial isolates.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Medicinal Plants

Medicinal plants have been a cornerstone of human healthcare for centuries, offering a rich reservoir of bioactive compounds that bridge traditional healing practices with modern scientific advancements. This section provides a detailed examination of medicinal plants, encompassing their definition and classification, their historical and ethnobotanical significance in traditional medicine, their pivotal role in modern pharmacology, and their utilization from both global and Nigerian perspectives.

Medicinal plants are plant species that possess therapeutic properties due to the presence of bioactive secondary metabolites, such as alkaloids, flavonoids, terpenoids, and phenolic compounds, which are effective in preventing, treating, or managing diseases in humans and animals. The World Health Organization recognizes their critical role, noting that they serve as raw materials for both herbal medicines and pharmaceutical drugs, forming an essential component of healthcare systems worldwide (WHO, 2019). To facilitate their study and application, medicinal plants are classified using several criteria that reflect their botanical, chemical, and therapeutic characteristics.

Botanically, medicinal plants are organized into taxonomic families, genera, and species. For example, the Lamiaceae family includes *Ocimum basilicum* (basil), prized for its essential oils with antimicrobial properties, while the Asteraceae family encompasses *Artemisia annua* (sweet wormwood), the source of the antimalarial compound artemisinin (Rates, 2001). Chemically, plants are grouped based on their dominant bioactive constituents. Alkaloid-rich plants, such as

Rauwolfia serpentina, used for its antihypertensive properties, are distinguished from flavonoid-rich species like *Ginkgo biloba*, valued for cognitive enhancement (Heinrich *et al*., 2020). Therapeutically, plants are categorized by their medicinal applications, including analgesics like *Salix alba* (willow bark) for pain relief, antimicrobials like *Allium sativum* (garlic), and anti-inflammatory agents like *Curcuma longa* (turmeric) (Sofowora, 2008). Additionally, plants are classified by their growth forms, which include herbs (e.g., *Mentha piperita* - peppermint), shrubs (e.g., *Aloe vera*), trees (e.g., *Moringa oleifera*), and climbers (e.g., *Piper nigrum* - black pepper). This multifaceted classification system supports the identification, cultivation, and therapeutic use of medicinal plants across diverse healthcare contexts.

2.1.1. Historical and Ethnobotanical Significance of Medicinal Plants in Traditional Medicine

The use of medicinal plants stretches back thousands of years, forming the foundation of traditional medicine systems across the globe. In ancient India, Ayurvedic texts such as the *Charaka Samhita* (circa 1000 BCE) documented the use of plants like *Withania somnifera* (ashwagandha) to promote vitality and alleviate stress (Sharma, 2011). In Traditional Chinese Medicine, which spans over 2,000 years, *Panax ginseng* has been employed to enhance energy and bolster immune function (Bensky *et al*., 2015). In Africa, traditional healing practices have long relied on local flora, with plants like *Vernonia amygdalina* (bitter leaf) used in West Africa to treat malaria and fevers, and *Sutherlandia frutescens* utilized in Southern Africa for immune support (Van Wyk and Wink, 2017). European herbalism, documented by ancient Greek physicians like Hippocrates and Galen, employed plants such as *Hypericum perforatum* (St. John's wort) for mental health conditions, with medieval monasteries preserving this knowledge

through herb gardens and texts like the *Herbarium* of Apuleius (circa 400 CE) (Mukherjee *et al*., 2017).

Ethnobotany, the study of the relationships between people and plants, underscores the cultural significance of medicinal plants. Indigenous communities worldwide have developed extensive knowledge of local flora, often transmitted orally across generations. For instance, Native Americans used *Echinacea purpurea* for wound healing and infection treatment, a practice that has significantly influenced modern herbalism (Kindscher, 2016). In South America, the use of *Cinchona officinalis* by indigenous Peruvian communities for fever treatment led to the discovery of quinine, a key antimalarial drug (Fabricant and Farnsworth, 2001). This ethnobotanical knowledge has provided critical leads for contemporary pharmacology, demonstrating the enduring value of traditional practices in informing modern drug discovery.

2.1.2. Role of Medicinal Plants in Modern Pharmacology

Medicinal plants are integral to modern pharmacology, serving as sources of active pharmaceutical ingredients, lead compounds for drug development, and complementary therapies. Approximately 25% of modern drugs are derived from plant-based compounds, highlighting their significance in pharmaceutical innovation (Newman and Cragg, 2020). Notable examples include aspirin, derived from salicin in *Salix alba* (willow bark), which is widely used for pain relief and anti-inflammatory purposes; quinine, extracted from *Cinchona officinalis*, historically significant for malaria treatment; artemisinin, sourced from *Artemisia annua*, a cornerstone of modern antimalarial therapy; morphine, obtained from *Papaver somniferum* (opium poppy), a critical analgesic; and taxol, isolated from *Taxus brevifolia* (Pacific yew), a key chemotherapeutic agent (Cragg and Newman, 2013).

Beyond direct drug development, medicinal plants contribute to pharmacology through bioassay-guided fractionation, a process where plant extracts are screened for bioactivity to identify novel compounds. Phytotherapy, which involves the use of standardized herbal extracts, such as *Hypericum perforatum* for depression, integrates plants into modern healthcare as complementary or alternative treatments (Heinrich *et al*., 2020). However, challenges persist, including variability in bioactive compound concentrations due to environmental factors, the risk of overharvesting, and ethical concerns surrounding the ownership of traditional knowledge. Advances in biotechnology, such as plant tissue culture and genetic engineering, are addressing these issues by enabling sustainable production of plant-derived drugs (Oksman-Caldentey and Inzé, 2004). Additionally, cheminformatics and high-throughput screening have accelerated the identification of novel plant-derived compounds, further enhancing their role in drug discovery (Atanasov *et al*., 2015).

2.1.3. Global and Nigerian Perspectives on the Use of Medicinal Plants

Globally, medicinal plants remain a vital component of healthcare, particularly in regions where traditional medicine is predominant. The World Health Organization estimates that 80% of the global population relies on plant-based remedies for primary healthcare, especially in developing countries where access to synthetic drugs is limited (WHO, 2019). In Asia, China and India lead the herbal medicine market, with China's Traditional Chinese Medicine industry, driven by plants like *Panax ginseng* and *Astragalus membranaceus*, valued at over $50 billion annually (Statista, 2023). In Europe, herbal medicines are regulated under stringent frameworks, such as those established by the European Medicines Agency's Committee on Herbal Medicinal Products, ensuring quality and safety (EMA, 2020). In contrast, Africa faces challenges with

standardization, resulting in variability in herbal product quality. The global trade in medicinal plants raises sustainability concerns, with species like *Panax ginseng* and *Hoodia gordonii* listed under the Convention on International Trade in Endangered Species (CITES) due to overharvesting, prompting efforts toward cultivation and conservation (CITES, 2022).

In Nigeria, medicinal plants are deeply embedded in cultural and healthcare practices, with over 70% of the population relying on traditional medicine (Abdullahi, 2011). Nigeria's rich biodiversity, encompassing over 7,000 plant species, supports a vast ethnobotanical pharmacopeia (Oguntunde, 2018). Commonly used plants include *Azadirachta indica* (neem), valued for its antimalarial, antibacterial, and antidiabetic properties; *Garcinia kola* (bitter kola), prized for its antimicrobial and anti-inflammatory effects; *Moringa oleifera*, known as the "miracle tree" for its nutritional and therapeutic benefits, including hypertension management; and *Ocimum gratissimum* (scent leaf), used for respiratory and digestive disorders (Sofowora, 2008). Traditional healers, known as *babalawos* in Yoruba culture or *dibia* in Igbo culture, play a central role in administering plant-based remedies. However, the lack of formal documentation and standardization poses significant challenges. The National Agency for Food and Drug Administration and Control (NAFDAC) has initiated efforts to regulate herbal medicines, but enforcement remains limited (Ekor, 2014). Deforestation, urbanization, and climate change threaten species like *Khaya senegalensis* (African mahogany), prompting research by institutions such as the University of Ibadan and the National Institute for Pharmaceutical Research and Development to validate traditional remedies, such as *Vernonia amygdalina* for malaria treatment (Omoregie and Pal, 2016).

2.2 Mimosa pudica

2.2.1 Botanical Description

Mimosa pudica L., commonly referred to as the sensitive plant, touch-me-not, shy plant, or sleeping grass, is a member of the plant kingdom with a well-defined taxonomic position within the following hierarchy:

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae (Leguminosae)

Subfamily: Mimosoideae

Genus: Mimosa

Species: *Mimosa pudica* L.

The species was first formally described by Carl Linnaeus in 1753 in his seminal work *Species Plantarum*, establishing its binomial nomenclature. The genus name *Mimosa* originates from the Greek word "mimos," meaning mimic or imitator, reflecting the plant's unique ability to mimic animal-like movement through its rapid leaf-folding response. The specific epithet *pudica*, derived from Latin, translates to "bashful" or "shrinking," aptly describing the plant's

characteristic behavior of closing its leaves when touched. Within the Fabaceae family, *Mimosa pudica* belongs to the subfamily Mimosoideae, a group characterized by small, clustered flowers and often nitrogen-fixing capabilities through symbiotic relationships with soil bacteria (Gunn, 1984). The plant is closely related to other Mimosa species, such as *Mimosa pigra*, but is distinguished by its smaller size and pronounced thigmonastic behavior.

2.2.2. Morphological Features

Mimosa pudica is a small, herbaceous perennial or subshrub with a suite of distinctive morphological features adapted to its ecological niche. Below is a detailed description of its key structures:

1. **Root**: The root system of *Mimosa pudica* is fibrous, consisting of a primary taproot with numerous lateral roots that spread horizontally. This shallow root system enables rapid establishment in disturbed soils, such as roadsides or grazed pastures. A hallmark of its leguminous nature, the roots form symbiotic associations with nitrogen-fixing bacteria (genus *Rhizobium*), which reside in root nodules and convert atmospheric nitrogen into compounds usable by the plant. This adaptation allows *Mimosa pudica* to thrive in nutrient-poor environments, contributing to its success as a weed in many regions (Sprent, 2009). The roots are typically light brown and fibrous, with a texture that supports both water uptake and structural stability.
2. **Stem**: The stems are slender, cylindrical, and often procumbent, meaning they trail along the ground, though they may grow erect under certain conditions, reaching heights of 30–100 cm. The stems are branched and may become slightly woody at the base in older plants. They are covered with fine, hair-likeprickles or bristles, which deter herbivores

and provide a degree of physical protection. The stem color varies from green in younger parts to reddish-brown or purplish in mature sections, with a smooth to slightly pubescent texture (Raven *et al*., 2013). The flexibility of the stems allows the plant to spread laterally, forming dense mats in favorable conditions.

3. **Leaves**: The leaves are bipinnately compound, a defining feature of the Mimosoideae subfamily. Each leaf typically consists of one to two pairs of pinnae, with each pinna bearing 10–26 pairs of small, oblong leaflets (pinnules). These leaflets are approximately 6–10 mm long and 1–2 mm wide, with a bright green color and a slightly pubescent surface. The leaflets are arranged oppositely along the pinna, giving the leaf a feathery appearance. The most striking feature of the leaves is their sensitivity to external stimuli, folding inward and drooping when touched, a phenomenon known as thigmonasty (described below). The petioles and rachises also bear small prickles, further enhancing the plant's defense against herbivory (Gunn, 1984).
4. **Flowers**: The flowers of *Mimosa pudica* are small, pink to lilac, and aggregated into spherical, capitate inflorescences resembling pom-poms, with a diameter of 1–2 cm. Each inflorescence contains numerous tiny flowers, each with a four-lobed calyx and a four-parted corolla. The stamens, which are pink or lilac and more numerous than the petals, extend beyond the corolla, giving the flower heads a fluffy, attractive appearance. The flowers are actinomorphic (radially symmetrical) and bisexual, capable of both cross-pollination by insects (primarily bees and butterflies) and self-pollination, which enhances reproductive success in isolated populations. Flowering occurs throughout the year in tropical climates, with peak blooming during warm, humid seasons (Raven *et al*., 2013).

5. **Seeds**: The seeds are housed in small, flat, prickly pods (legumes) that measure 1–2 cm in length and 3–5 mm in width. Each pod contains 2–5 seeds and is covered with fine bristles, which aid in dispersal by attaching to animals or clothing. The pods are segmented and break apart upon maturity, releasing the seeds. The seeds themselves are brown, flattened, ovoid, and approximately 2–3 mm in diameter, with a hard, impermeable seed coat that promotes dormancy and longevity in the soil seed bank. This hard seed coat enables *Mimosa pudica* to persist in environments with sporadic germination opportunities (Gunn, 1984). Seed dispersal is facilitated by both mechanical dehiscence of the pods and external agents such as water, animals, or human activity.

Figure 3: *Mimosa pudica* (touch-me-not) leaves (Ahmad *et al*., 2012).

2.2.3. Growth Habit and Unique Movement Response to Touch

Mimosa pudica exhibits a sprawling or creeping growth habit, often forming dense, low mats that spread across the ground. In optimal conditions, it can cover areas rapidly, making it a common sight in disturbed habitats such as roadsides, lawns, pastures, and agricultural fields. The plant prefers tropical and subtropical climates, thriving in temperatures between 20–30°C and requiring well-drained soils with moderate moisture. It is highly adaptable, tolerating a range of soil types, including sandy and loamy soils, and can grow in full sunlight or partial shade. In colder climates, *Mimosa pudica* may behave as an annual, dying back after the growing season, but in tropical regions, it persists as a perennial, regenerating from roots or seeds (Holm *et al*., 1977).

The defining characteristic of *Mimosa pudica* is its thigmonastic response, a rapid, reversible movement of its leaves and pinnae in response to touch, vibration, heat, or other mechanical stimuli. This phenomenon, known as thigmonasty, is one of the most striking examples of plant movement in the plant kingdom. When a leaflet or pinna is touched, the leaflets fold inward and the entire leaf droops within seconds, giving the appearance of wilting. This response is mediated by specialized cells in the pulvini, swollen regions at the base of leaflets and pinnae. Upon stimulation, an action potential triggers the rapid movement of potassium ions and water out of the pulvinar cells, causing a loss of turgor pressure and subsequent collapse of the leaf structure (Fromm and Lautner, 2007). The leaves typically reopen within 15–30 minutes as turgor pressure is restored through the reuptake of ions and water.

The thigmonastic response is believed to serve multiple ecological functions. Primarily, it acts as a defense mechanism, deterring herbivores by making the plant appear wilted or less palatable.

The rapid movement may also startle small herbivores or dislodge insects attempting to feed on the leaves. Additionally, thigmonasty may protect the plant from environmental stresses, such as excessive sunlight or wind, by reducing surface area and transpiration (Jensen *et al*., 2011). Beyond touch, *Mimosa pudica* exhibits nyctinasty, a related movement where leaves close at night in response to changes in light intensity, possibly to conserve water or protect against nocturnal herbivores.

The mechanism of thigmonasty involves complex physiological processes, including electrical signaling and chemical cascades. Studies have shown that calcium ions play a critical role in signal transduction, with calcium channels opening in response to mechanical stimuli, triggering the release of signaling molecules that propagate the response across the plant (Volkov *et al*., 2010). This rapid signaling has made *Mimosa pudica* a model organism for studying plant electrophysiology and mechanosensory responses. The plant's ability to "learn" from repeated stimuli has also been documented; for instance, it may reduce its response to non-threatening, repeated touches, a phenomenon akin to habituation in animals (Gagliano *et al*., 2014).

Ecologically, *Mimosa pudica* is considered both a fascinating species and a problematic weed. Its ability to colonize disturbed areas and its prolific seed production make it highly invasive in many tropical and subtropical regions, including parts of Australia, Africa, and the Americas. Its nitrogen-fixing capability enhances soil fertility, which can benefit ecosystems, but its aggressive growth can outcompete native vegetation, leading to reduced biodiversity (Holm *et al*., 1977). Despite its weed status, *Mimosa pudica* is also valued in traditional medicine and as an ornamental plant due to its intriguing movement and attractive flowers.

2.3. Global Distribution of *Mimosa pudica*

Mimosa pudica, commonly known as the sensitive plant, touch-me-not, or shy plant, is a pantropical species with a widespread global distribution, primarily concentrated in tropical and subtropical regions. Native to South America and Central America, the plant has become naturalized across many parts of the world due to its adaptability, anthropogenic dispersal, and ecological versatility. Its global presence spans regions in Africa, Asia, Australia, the Caribbean, and parts of North America, particularly in areas with warm climates conducive to its growth. The plant's ability to thrive in diverse environmental conditions has made it both a fascinating subject of ecological study and, in some cases, a problematic invasive species.

2.3.1 Native Range and Historical Spread

Mimosa pudica originates from the Neotropics, with its native range encompassing parts of Brazil, Bolivia, Peru, and other South American countries, as well as Central American regions such as Panama and Costa Rica. Historical records suggest that the plant was first described in the scientific literature by European botanists in the 16th century, following exploration and colonization of the Americas. Its unique seismonastic movement—where leaves fold inward upon mechanical stimulation—made it a curiosity, leading to its intentional introduction to other continents as an ornamental plant. By the 19th century, *Mimosa pudica* had been transported to Africa, Asia, and Oceania, largely through colonial trade routes and botanical exchanges. For example, Parsons and Cuthbertson (2001) note that the plant was introduced to Australia as a garden novelty before escaping cultivation and becoming a widespread weed.

Human-mediated dispersal, including trade, agriculture, and horticulture, has been a significant driver of its global spread. Seeds of *Mimosa pudica* are easily transported in soil, on clothing, or through agricultural equipment, contributing to its establishment in non-native regions. The plant's ability to produce large quantities of seeds, which can remain viable in the soil for years, further facilitates its spread (Holm *et al*., 1977). Today, *Mimosa pudica* is found in over 50 countries, with particularly high prevalence in tropical and subtropical climates where temperatures remain above 10°C and annual rainfall exceeds 500 mm.

2.3.2 Geographical Distribution and Habitat

Mimosa pudica exhibits a broad geographical distribution, thriving in a variety of habitats across tropical and subtropical regions. Its presence is most pronounced in areas with high humidity, moderate to high rainfall, and disturbed soils, which provide ideal conditions for its growth. The plant is commonly found in open, sunny environments such as grasslands, roadsides, pastures, lawns, and agricultural fields. Its preference for disturbed habitats, such as areas affected by grazing, logging, or human activity, underscores its status as a pioneer species. Below is an expanded discussion of its distribution and habitat preferences, with a focus on Nigeria and other tropical regions. The global distribution of *Mimosa pudica* spans multiple continents, with notable populations in:

1. **Africa**: The plant is widespread in West, East, and Central Africa, including countries such as Nigeria, Ghana, Kenya, Uganda, and the Democratic Republic of Congo. In Nigeria, *Mimosa pudica* is a common sight in both rural and urban areas, particularly in the southern and central regions where rainfall is abundant (Akobundu and Agyakwa, 1998).

2. **Asia**: The plant is prevalent in South and Southeast Asia, including India, Sri Lanka, Thailand, Malaysia, and the Philippines. In India, it is a common weed in agricultural fields and wastelands, often found in regions with monsoon climates (Sankaran *et al*., 2008).
3. **Australia and Oceania**: In Australia, *Mimosa pudica* is considered an invasive weed, particularly in northern Queensland and the Northern Territory, where it invades pastures and competes with native vegetation (Parsons and Cuthbertson, 2001).
4. **Caribbean and North America**: The plant is naturalized in the Caribbean (e.g., Jamaica, Puerto Rico) and parts of the southern United States, such as Florida and Hawaii, where warm climates support its growth year-round.
5. **Other Regions**: *Mimosa pudica* is also recorded in parts of the Pacific Islands, Madagascar, and Indian Ocean islands, often in disturbed habitats or as a garden escapee.

2.3.3. Prevalence in Nigeria

In Nigeria, *Mimosa pudica* is a ubiquitous weed, particularly in the southern rainforest and derived savanna zones, where annual rainfall ranges from 1500 to 2500 mm. It is commonly found in agricultural fields, along roadsides, and in fallow lands, where it often forms dense patches that outcompete other vegetation. Akobundu and Agyakwa (1998) report that the plant is a significant weed in crops such as maize, yam, and cassava in southern Nigeria, where its rapid growth and seed production make it difficult to control. In urban areas like Lagos and Ibadan, *Mimosa pudica* colonizes lawns, gardens, and vacant lots, thriving in disturbed soils compacted by foot traffic or construction.

The plant's prevalence in Nigeria is aided by its ecological adaptability and human activities such as deforestation, overgrazing, and slash-and-burn agriculture, which create open, disturbed habitats ideal for its establishment. Its ability to fix nitrogen through symbiotic relationships with rhizobia bacteria enhances its competitiveness in nutrient-poor soils, a common feature of many Nigerian farmlands (Sprent, 2001).

In other tropical regions, *Mimosa pudica* exhibits similar patterns of distribution and habitat preference. In India, for example, it is a common weed in rice paddies and tea plantations, where its ability to tolerate waterlogged soils during the monsoon season contributes to its spread (Sankaran *et al*., 2008). In Southeast Asia, the plant is often found in rubber and oil palm plantations, where it competes with crops for resources. In Australia, its invasion of grazing lands has led to its classification as a noxious weed, prompting control measures such as herbicide application and mechanical removal (Parsons and Cuthbertson, 2001).

2.3.4. Traditional uses of *M. pudica*

The root of *M. pudica* is declared to be bitter, cooling vulnerary, acrid, alexipharmic and used in the treatment of various types of diseases such as leprosy, dysentery, inflammation and many more by Ayurveda (Joseph *et al*., 2013). The Unani Healthcare system uses its root in the treatment of disease arising from blood impurities and bile, bilious fevers, piles and jaundice. It also reduces a toothache by the decoction of root with water. It is also found to arrest bleeding and fasten the wound healing process. It is also used in herbal preparation for a gynaecological disorder. It is beneficial in the treatment of diarrhoea, amoebic dysentery and has been researched to have medicinal properties to cure skin diseases. Studies have shown that it is also used to treat neurological problems (Patro *et al*., 2016). The flower, root, stem, leave, and the

fruits are used as medicines in the traditional health care system different parts of the plants are used in India for long for treatment of various kind of ailments. Researchers have indicated that *M. pudica* is used to relax the mind, relieves depression, mental distress, irritability and amnesia. It is also used to enhance mood and improves the circulation of blood. It also promotes healthy cell growth and prevents baldness. In western medicine, its root was used for the treatment of insomnia, irritability, premenstrual syndrome haemorrhoids and whooping cough (Ashish and Imran, 2012).

Many researchers have revealed that *M. pudica* is a mood enhancer and improves circulation of the blood. Some believe Mimosa can reduce the onset of baldness. Due to its ability to promote healthy cell growth, it is used in shampoos, creams, capsules, and soaps which are applied as facial cleansers (Bijauliya *et al*., 2017). *M. pudica* root is used to treat bilious fevers, piles, jaundice, leprosy, dysentery, vaginal and uterine complaints, inflammations, burning sensation, Available online at http://abiosci.com/archive.html fatigue, asthma, leucoderma, and blood diseases. In Western medicine, Mimosa root is used for treating insomnia, irritability, premenstrual syndrome, menorrhagia, hemorrhoids, skin wounds, and diarrhea (Muhammad *et al*., 2016). It is also used to treat whooping cough and fevers in children, and there is some evidence to suggest that Mimosa is effective in relieving the symptoms of rheumatoid arthritis. All parts of the Mimosa plant are reportedly toxic if taken directly. Its consumption is not recommended for pregnant or nursing ladies. Due to these reports, it seems to be best to consult a physician before using Mimosa internally (Joseph *et al*., 2013). It is used in parts of the southeastern Nigerian as herbal remedy for hyperglycemia. It produces liquid oleoresin, which has been used as medicine by indigenous people for more than 400 years. The oleoresin is produced in the tree's trunk, stem, and leaves and is traditionally used as an antiinflammatory

agent and in the treatment of a variety of genitor-urinary tract diseases and skin ailment (Muhammad *et al*., 2016).

2.4. Phytochemical Constituents of *Mimosa pudica*

Mimosa pudica, widely recognized as the sensitive plant, touch-me-not, or shy plant, is a remarkable herb from the Fabaceae family, celebrated for its seismonastic movement and its rich array of bioactive compounds. Native to South America but now thriving in tropical and subtropical regions worldwide, this creeping perennial has long been a staple in traditional medicine systems, including Ayurveda, African folk remedies, and South American herbal practices. Its leaves, roots, stems, and seeds are repositories of diverse phytochemicals—alkaloids, flavonoids, tannins, glycosides, saponins, terpenoids, phenolic acids, and steroids—that drive its pharmacological versatility. These compounds confer antimicrobial, anti-inflammatory, antioxidant, antidiabetic, and neuropharmacological properties, making *Mimosa pudica* a focal point for scientific research.

2.4.1. Alkaloids

Alkaloids, nitrogen-containing compounds known for their potent biological effects, are a cornerstone of *Mimosa pudica*'s phytochemical profile (Gutiérrez-Grijalva *et al*., 2020). The most notable alkaloid is mimosine, a non-protein amino acid predominantly found in the leaves and seeds (Johnson *et al*., 2014; Rawat *et al*., 2024). Mimosine acts as a natural defense mechanism, deterring herbivores and pathogens by disrupting critical cellular processes. Its antimitotic properties stem from its ability to chelate metal ions, particularly iron, which inhibits DNA synthesis and enzyme activity, effectively halting cell division in target organisms

(Nguyen, and Tawata, 2016). This contributes to the plant's allelopathic effects, suppressing the growth of neighboring plants. Mimosine also exhibits antimicrobial and antifungal activities, supporting its traditional use in treating infections (Mandal *et al*., 2022; Fernandes *et al*., 2023). However, its toxicity at high doses, which can lead to hair loss and growth retardation in mammals, has been documented in studies on livestock, emphasizing the need for careful therapeutic application (Saxena, 2012; Joseph *et al*., 2013). Other alkaloids in *Mimosa pudica* contribute to its analgesic and psychoactive effects, aligning with its use in traditional remedies for pain relief and neurological disorders (Vijayalakshmi *et al*., 2015).

2.4.2. Flavonoids

Flavonoids, polyphenolic compounds abundant in the leaves and aerial parts of *Mimosa pudica*, are key to its antioxidant and anti-inflammatory properties (Muhammad *et al*., 2016). Prominent flavonoids include quercetin, luteolin, and apigenin derivatives. Quercetin, a flavonol, is a potent scavenger of free radicals, protecting cells from oxidative stress, a major contributor to chronic diseases like diabetes and cancer (Miean and Mohamed, 2001). Its ability to inhibit pro-inflammatory enzymes, such as cyclooxygenase, underpins its role in wound healing and arthritis relief, as noted in traditional practices. Luteolin, a flavone, offers neuroprotective effects, supporting the plant's use in managing anxiety and insomnia (Kempuraj *et al*., 2021). Apigenin derivatives enhance the plant's anti-inflammatory and antimicrobial capabilities, making flavonoids central to its therapeutic profile. Analytical techniques like high-performance liquid chromatography (HPLC) and UV-Vis spectroscopy have confirmed the presence of these flavonoids, highlighting their antioxidant potency (Ahmad *et al*., 2018; Kumar *et al*., 2017).

2.4.3. Tannins

Tannins, polyphenolic compounds found in the bark, leaves, and roots, are valued for their astringent properties and their ability to bind proteins and metal ions (Usiobeigbe *et al*., 2021; Fraga-Corral *et al*., 2021). *Mimosa pudica* contains both hydrolyzable and condensed tannins, which contribute to its antimicrobial and antidiarrheal effects (Muhammad *et al*.,2016). By forming complexes with microbial proteins, tannins disrupt bacterial and fungal growth, making them effective against pathogens like *Escherichia coli* and *Staphylococcus aureus* (Chung *et al*., 1996; Singh and Kumar, 2020). In traditional medicine, tannin-rich extracts have been used to treat diarrhea, skin infections, and wounds by promoting tissue contraction and reducing exudation (Fraga-Corral *et al*., 2021). These properties facilitate faster wound healing and alleviate gastrointestinal inflammation, aligning with the plant's ethnobotanical applications (Gandhi *et al*., 2016; Zhang *et al*., 2019).

2.4.4. Glycosides

Glycosides are compounds composed of a sugar moiety linked to a non-sugar aglycone component (Riaz *et al*., 2023). They play significant roles in plant defense mechanisms and possess diverse therapeutic activities. *Mimosa pudica* contains cardiac and flavonoid glycosides that have been associated with antimicrobial, antidiabetic, and anti-inflammatory effects (Muhammad *et al*., 2016). The glycosides in *M. pudica* are believed to exert their antimicrobial activity by impairing microbial respiration and enzyme function. Furthermore, the antioxidant potential of glycosides contributes to the protection of body tissues against free radical-mediated damage, aligning with the plant's role in traditional medicine for treating various ailments, including wounds and gastrointestinal disorders (Fernandes *et al*., 2023).

2.4.5. Saponins

Saponins, glycosides with a triterpenoid or steroidal aglycone, are particularly concentrated in the roots of *Mimosa pudica* (Muhammad *et al*., 2016). Known for their foaming properties in aqueous solutions, saponins exhibit antimicrobial, anti-inflammatory, and hemolytic activities. Triterpenoid saponins in *Mimosa pudica* contribute to wound healing and infection control by disrupting microbial cell membranes and reducing inflammation (Tripathi *et al*., 2022). Their amphiphilic nature also enhances the bioavailability of other phytochemicals, making them valuable in herbal formulations. Traditional uses of the plant's roots for skin ailments and infections are supported by the antimicrobial properties of saponins, validated through froth tests and thin-layer chromatography (Rizwan *et al*., 2022).

2.4.6. Other Compounds

Mimosa pudica also contains terpenoids, phenolic acids, and steroids, each adding to its pharmacological diversity. Volatile terpenoids, such as β-caryophyllene and limonene, found in the plant's essential oils, exhibit antimicrobial and anti-inflammatory effects, particularly against fungal pathogens like Candida albicans. These compounds support the plant's use in treating skin infections. Phenolic acids, including gallic acid and caffeic acid, enhance the plant's antioxidant capacity, protecting against cellular damage and supporting its antidiabetic and anticancer potential. Steroids like β-sitosterol and stigmasterol, identified in the aerial parts, contribute to anti-inflammatory and cholesterol-lowering effects, further broadening the plant's therapeutic scope (Ahmad *et al*., 2018; Rajendran *et al*., 2016).

2.5. Functional Significance of Key Phytochemicals

The phytochemicals in *Mimosa pudica* work synergistically to support its wide-ranging pharmacological applications, closely aligning with its traditional uses. The antioxidant activity of flavonoids and phenolic acids is pivotal, neutralizing reactive oxygen species to protect against oxidative stress-related diseases like diabetes, cardiovascular disorders, and cancer. Studies have demonstrated that *Mimosa pudica* extracts exhibit high DPPH radical scavenging activity, confirming their antioxidant potential (Ahmad *et al*., 2018). Flavonoids like quercetin also enhance insulin sensitivity and inhibit α-glucosidase, supporting the plant's antidiabetic effects (Mishra *et al*., 2014).

The antimicrobial properties of *Mimosa pudica* are driven by alkaloids, tannins, saponins, and terpenoids, which target a broad spectrum of pathogens. Mimosine disrupts microbial enzyme activity, while tannins and saponins damage cell membranes, making the plant effective against bacteria and fungi. These properties validate its traditional use in treating infections, wounds, and skin disorders (Nguyen *et al*., 2016; Rizwan *et al*., 2022). The anti-inflammatory effects of flavonoids, terpenoids, and steroids further enhance the plant's utility in managing arthritis and skin inflammation by inhibiting inflammatory mediators like prostaglandins and cytokines.

Wound healing, a prominent traditional application, is supported by the combined action of tannins, saponins, and flavonoids. Tannins promote tissue contraction, saponins reduce microbial load, and flavonoids accelerate tissue regeneration through their antioxidant effects (Patel *et al*., 2015). The neuropharmacological effects of *Mimosa pudica*, particularly in treating anxiety and insomnia, are attributed to mimosine and flavonoids, which modulate GABA receptors to produce sedative and antidepressant effects (Saxena, 2012; Vijayalakshmi *et al*., 2015). The

plant's antidiabetic potential is further reinforced by flavonoids and phenolic acids, which improve glucose metabolism and protect pancreatic β-cells from oxidative damage (Kumar *et al*., 2017).

2.6. Pharmacological Properties of *Mimosa pudica*

2.6.1. Wound healing activity

Damage in living tissue caused by a cut, blow, or other impacts is termed as a wound. *M. pudica* chloroform extract possess wound healing activity at a dose of 200 mg/kg in 5% ointment of the leaf extracts. Wound healing is a natural process initiated by trauma and often terminated by scar formation; the wound healing activity was reported to be performed by using excision and incision wound models (Ganguly *et al*., 2007). Different studies have also shown that both methanolic and aqueous extracts of *M. pudica* in simple ointment base at a different concentration of 0.5%(w/w), 1%(w/w) and 2%(w/w) possess wound healing activity. Three different types of models in rats were used, which are excision, incision and estimation of biochemical parameters. The 2%(w/w) methanolic and aqueous extracts exhibited significant wound healing activity probably due to phenols constituent in M. pudica. Another research by Ganesh *et al*. shows the wound healing activity of *M. pudica* ethanolic extracts. Mice were used for this study and were acclimatized according to standard laboratory protocols. Different doses of *M. pudica* 50, 100, 150 and 200 mg/kg b. wt were administered to the animals for six weeks before the creation of deep dermal excision wound. At the end of the experiment the administration of *M. pudica* extracts of different concentration increased the wound contraction in a dose-dependent manner, and also reduction in wound healing time (Kokane *et al*., 2009; Jagetia *et al*., 2017)

2.6.2. Antiinflammatory activity

The anti-inflammatory activity of *M. pudica* was investigated using male albino rats. Three different extracts of *M. pudica* was used, which are petroleum ether, ethanol and aqueous extracts. The model of the rats used was carrageenan-induced paw oedema and cotton pellet granuloma in rats. Different doses, 50, 100 and 200 mg/kg, of extracts, were used, and indomethacin, administered orally, was used as a standard at a concentration of 10 mg/kg Carrageenaninduced rat paw oedema was used for evaluating the reduction of oedema induced by carrageenan (Goli *et al*., 2011). Another study by Nair and Bindu also investigated the anti-anti-inflammatory effect of the whole plant of M. pudica, in thirty albino rats of both sexes was used for the experiment. Two hundred (200) mg/kg, 400 mg/kg and 800 mg/kg extracts of M. puidca was administered to three different groups, 800 mg/kg concentration has the highest percentage inhibition. The result from this recent study also shows that *M. pudica* has anti-inflammatory properties (Nair and Bindu, 2017).

2.6.3. Antidiabetic activity

Diabetes is a metabolic disorder which affects people of all age, most prominent in middle age and old aged people. Various complications have been associated with diabetes such as obesity, renal dysfunction and blood lipid abnormalities. Patient self–management education and continued medical care are required to prevent acute complication and longtime effects. Anti-diabetic effect of *M. pudica* was investigated using male albino rats weighing between 150- 200g, ethanolic extracts of *M. pudica* was administered at a dose of 500 mg/kg. bw to diabetic rats induced with streptozotocin 65 mg/kg. bw. The investigation lasted for eight weeks, and the

parameters obtained from the study show that *M. pudica* tend to have hypoglycemic properties on fasting blood glucose level of the experimental rats (Yupparach *et al*., 2017).

2.6.4. Antidepressants

The rate of prevalence of mental disorder patients was observed to be around 65.4/1000 population. Out of which prevalence rate for the affective disorder is estimated to be 31.2/1000 populations. Antidepressants widely prescribed for depression, have significant limitations which include long time lag for therapeutic response and low response rates, which problematic for disease associated with a high rate of suicide (Hindmarch, 2001). The aqueous extract of *M. pudica* is employed to reduce depression in Mexico. This study showed the behavioural actions of *M. pudica* extract at different concentration was tested. Rats having received several dosages of aqueous extracts from *M. pudica* (2.0 mg/kg; 4.0 mg/kg; 6.0 mg/kg 8.0 mg/kg) or received normal saline (0.9% 0.30 ml; J. P.) clomipramine, and desipramine for 30days, after which were subjected to forced swimming test and the test for differential reinforcement of low rates response at 72 second. *M. pudica* of different doses responsible for any possible anxiolytic action were compared with those caused by diazepam (1.3 mg/kg J. P) in the elevated plus maze test. Results showed that *M. pudica* extracts, clomipramine, desipramine reduced immobility in the forced swimming test and increased the rate of reinforcers received in the DRL-72s test, the results show that *M. pudica* produces antidepressant effects in rats (Molina *et al*., 1999; Irfan *et al*., 2013).

2.6.5. Anti-helminthic activity

Helminths have been a foremost degenerative disease disturbing large percentage of the world and pose an enormous threat to public health in the developing countries which contribute to various ailments such as malnutrition, anaemia, eosinophilia and pneumonia. The parasite of helminths mainly subsists in the human body in the intestinal tract. Resistance in helminths against conventional anthelmintics is a leading problem in the treatment of the diseases. *M. pudica* has been reported to have anti-helminths activity. Another study by Pratap *et al*. shows the anti-helminthic activity of aqueous leaves extracts of M. pudica. Albendazole was used as a standard drug, prepared in four different concentration of 20, 40, 60 and 80 mg/ml which was further dissolved in normal saline. The earthworm P. posthuman was divided into five groups consisting of two equal sizes and then released into 30 ml of the experimental formulations. Freshly prepared a standard drug and Aqueous extracts of *M. pudica* was used for the experiment. The paralysis time was recorded when there were no movements observed, and death time was recorded in minutes. The aqueous extracts showed considerable anti-helminthic activity which varies with different concentration (Bendgude *et al*., 2012; Deepak *et al*., 2018).

2.6.6. Antimicrobial Activity

The antimicrobial activity of *Mimosa pudica* has been widely investigated, and various studies have demonstrated that the plant possesses a broad spectrum of antimicrobial properties due to the presence of diverse bioactive compounds. The preliminary phytochemical screening of *Mimosa pudica* extracts revealed the presence of several secondary metabolites such as carbohydrates, saponins, flavonoids, steroids, and tannins, all of which are known to contribute to the antimicrobial potency of plants (Tamilarasi *et al*., 2012). The antimicrobial activity of the

extracts was tested against *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Aspergillus flavus*, and *Trichophyton* species at varying concentrations of 50, 100, and 200 µg/ml. The results revealed that the extracts showed significant inhibitory effects against all the tested microorganisms, indicating their broad antimicrobial potential.

Similarly, Chukwu *et al*. (2017) and Rajalakshmi and Banu (2016) reported that the ethanolic extract of *M. pudica* exhibited antifungal activity against *Aspergillus flavus* and *Trichophyton rubrum* at concentrations of 25 mg/ml, 50 mg/ml, and 100 mg/ml. Their findings showed that the plant extract was partially active at lower concentrations but demonstrated strong activity at higher concentrations, confirming the concentration-dependent nature of its antimicrobial effect.

Kaur *et al*. (2011) also reported that preliminary phytochemical screening of *M. pudica* extract revealed the presence of terpenoids, flavonoids, alkaloids, quinones, phenols, tannins, saponins, and coumarins. These compounds are known for their antimicrobial and antioxidant properties. In their study, the ethanolic extract of *M. pudica* showed antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* with inhibition zones of 23.96 mm, 22.3 mm, and 21.43 mm, respectively, at 30 mg/ml concentration. However, the antimicrobial effect was slightly lower compared to the standard antibiotic ampicillin, which exhibited an inhibition zone of 25.5 mm at 2 mg/ml. This indicates that although *M. pudica* extracts have significant antimicrobial effects, their efficacy may not surpass conventional antibiotics at lower doses.

In another study, Sunil *et al*. (2016) observed that ethanolic extracts of *M. pudica* displayed marked antibacterial activity only at higher concentrations, suggesting that potency increases with concentration. The extract did not exhibit antibacterial activity at 5 µg/ml, but at 50, 100,

and 250 µg/ml, it showed significant inhibition against four test organisms (*E. coli*, *P. aeruginosa*, *S. aureus* and *S. pyogenes*). The highest antibacterial activity was recorded at 250 µg/ml, where *E. coli* showed a 17 mm zone of inhibition, followed by *P. aeruginosa* and *S. aureus* (16 mm each) and *S. pyogenes* (15 mm). These findings indicate that the ethanolic extract of *M. pudica* exhibits dose-dependent antibacterial activity comparable to that of the standard drug ampicillin at higher concentrations.

Amengialue *et al*. (2016) further investigated the antimicrobial potential of aqueous, ethanol, and methanol extracts of *M. pudica* against various bacterial and fungal species. Their findings revealed that at 100 mg/ml concentration, the aqueous extract showed maximum activity against *E. coli* (6.3 ± 1.5 mm), moderate activity against *S. aureus* (3.3 ± 0.9 mm), and the least activity against *K. pneumoniae* (2.3 ± 0.3 mm). The ethanolic extract displayed the highest activity against *S. aureus* (15 ± 0.6 mm), followed by *E. coli* (14 ± 0.6 mm), and the least activity against *P. aeruginosa* (2.3 ± 0.3 mm). For methanolic extracts, *Bacillus subtilis* exhibited the highest inhibition zone (15.7 ± 1.9 mm), followed by *K. pneumoniae* (15.3 ± 0.7 mm), with *E. coli* showing the least activity (14.3 ± 0.9 mm).

At lower concentrations (50 mg/ml and 25 mg/ml), the extracts exhibited reduced activity. For instance, at 25 mg/ml, the aqueous extract showed no antimicrobial effect, while ethanol and methanol extracts still demonstrated moderate inhibition against *Streptococcus pyogenes*, *E. coli*, and *B. subtilis*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays indicated that the ethanolic and methanolic extracts were more potent than the aqueous extract. The minimum fungicidal concentration (MFC) also confirmed antifungal activity against *Candida albicans* and other fungal species tested.

Overall, the antimicrobial efficacy of *Mimosa pudica* has been attributed to its rich composition of bioactive compounds such as flavonoids, alkaloids, tannins, and saponins, which act synergistically to disrupt microbial cell walls, interfere with enzyme activity, and inhibit microbial growth (Rajalakshmi and Banu, 2016; Tamilarasi *et al*., 2012). The results from various studies suggest that *M. pudica* possesses both antibacterial and antifungal activities, with the ethanolic and methanolic extracts generally showing higher potency than aqueous extracts. These findings support the traditional use of *M. pudica* in the treatment of microbial infections and further highlight its potential as a source of natural antimicrobial agents.

2.7 Test Organisms

Microorganisms play diverse roles in human health and disease. Among the most frequently encountered pathogenic bacteria in both clinical and environmental samples are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. These organisms have been implicated in various infections, including wound infections, urinary tract infections, respiratory tract infections, and foodborne illnesses. Their ability to survive under diverse environmental conditions and their increasing resistance to antibiotics make them significant in microbiological and public health research (Cheesbrough, 2010; Todar, 2020).

2.7.1 *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive, facultative anaerobic coccus that forms clusters resembling bunches of grapes under the microscope. It is non-motile, non-spore forming, catalase-positive, and coagulase-positive, which distinguishes it from other staphylococcal species (Prescott *et al*., 2021). *S. aureus* is part of the normal flora of the skin, nasal passages,

and mucous membranes of humans but can become pathogenic when it breaches the body's natural barriers or when host immunity is compromised (Lowy, 1998) Colonies of *S. aureus* on nutrient agar are typically golden yellow, round, convex, and opaque, which led to its name "aureus" meaning "golden" in Latin. It ferments mannitol on Mannitol Salt Agar (MSA) producing a yellow coloration due to acid formation. Microscopically, it appears as Gram-positive cocci arranged in irregular clusters (Cowan and Steel, 2004).

S. aureus produces a wide range of virulence factors that contribute to its pathogenicity. These include:

1. **Enzymes** such as coagulase, hyaluronidase, lipase, and proteases that aid in tissue invasion.
2. **Toxins**, including hemolysins, leukocidins, enterotoxins, and toxic shock syndrome toxin (TSST-1), which cause cytotoxicity and systemic effects (Tong *et al*., 2015).
3. **Biofilm formation**, which enhances survival on medical devices and confers antibiotic resistance (Otto, 2018).

S. aureus is implicated in a variety of infections such as skin abscesses, boils, impetigo, cellulitis, osteomyelitis, pneumonia, meningitis, endocarditis, and food poisoning due to enterotoxin production (Gordon and Lowy, 2008). The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains poses serious public health challenges worldwide, especially in hospital environments (Klein *et al*., 2013). Resistance to β-lactam antibiotics due to the mecA gene, which encodes penicillin-binding protein 2a (PBP2a), has led to the prevalence of MRSA. Multidrug-resistant *S. aureus* strains exhibit resistance to aminoglycosides, macrolides, and fluoroquinolones (Chambers and Deleo, 2009).

2.7.2 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative, rod-shaped, motile bacterium equipped with polar flagella. It is an obligate aerobe but can adapt to anaerobic conditions through nitrate respiration. The organism is oxidase-positive, catalase-positive, and produces a characteristic fruity odor due to the presence of 2-aminoacetophenone (Forbes *et al*., 2016). It is ubiquitously found in soil, water, and hospital environments, particularly in moist areas such as sinks, catheters, and respiratory equipment. Its environmental persistence is attributed to its metabolic versatility and ability to grow on minimal nutrients (Poole, 2011). *P. aeruginosa* is an opportunistic pathogen that primarily affects immunocompromised individuals. It secretes several virulence factors:

1. **Exotoxin A** which inhibits protein synthesis by ADP-ribosylation of elongation factor-2.
2. **Elastase and protease**, which degrade host tissues.
3. **Pyocyanin**, a blue-green pigment that generates reactive oxygen species and contributes to tissue damage.
4. **Biofilm formation**, conferring resistance to antibiotics and host immune responses (Gellatly and Hancock, 2013).

It is a major cause of nosocomial infections such as burn wound infections, urinary tract infections, septicemia, and pneumonia, especially in cystic fibrosis patients (Driscoll *et al*., 2007). The bacterium is also commonly isolated from hospital wastewater and contaminated surfaces, indicating its environmental resilience (Kumar *et al*., 2022). *P. aeruginosa* is intrinsically resistant to many antibiotics due to low outer membrane permeability, efflux pumps, and β-lactamase production (Livermore, 2002). Multidrug-resistant (MDR) and extensively drug-

resistant (XDR) strains have been reported globally, complicating treatment strategies (Oliver *et al*., 2008).

2.7.3 *Escherichia coli*

Escherichia coli is a Gram-negative, facultative anaerobic, rod-shaped bacterium belonging to the family Enterobacteriaceae. It is oxidase-negative, catalase-positive, and ferments lactose with acid and gas production. *E. coli* is a commensal organism of the intestinal tract of humans and warm-blooded animals but certain pathogenic strains cause severe diseases (Madigan *et al*., 2020). Colonies of *E. coli* on nutrient agar are smooth, moist, and grayish-white, while on Eosin Methylene Blue (EMB) agar, they produce characteristic metallic green sheen colonies due to vigorous lactose fermentation. Microscopically, they appear as Gram-negative rods (Cheesbrough, 2010).

Pathogenic *E. coli* strains are classified into several pathotypes including:

1. **Enteropathogenic E. coli (EPEC)** – causes infantile diarrhea.
2. **Enterotoxigenic E. coli (ETEC)** – produces heat-labile and heat-stable enterotoxins.
3. **Enterohemorrhagic E. coli (EHEC)** – produces Shiga toxins, leading to hemorrhagic colitis and hemolytic uremic syndrome.
4. **Uropathogenic E. coli (UPEC)** – causes urinary tract infections (Nataro and Kaper, 1998).

Virulence factors include adhesins (pili and fimbriae), toxins, and the ability to invade epithelial cells.

E. coli is associated with various infections such as urinary tract infections, septicemia, neonatal meningitis, and gastroenteritis (Kaper *et al*., 2004). Its presence in water or food serves as an indicator of fecal contamination (Edberg *et al*., 2000). Antibiotic resistance in *E. coli* is increasingly common, particularly the production of extended-spectrum β-lactamases (ESBLs) and carbapenemases, which confer resistance to β-lactam antibiotics (Pitout and Laupland, 2008). The spread of multidrug-resistant *E. coli* poses a major threat to global public health, especially in developing countries where antibiotic misuse is prevalent (WHO, 2020).

All three test organisms *S. aureus*, *P. aeruginosa* and *E. coli* are significant pathogens of clinical and environmental relevance. They exhibit notable adaptability, biofilm formation, and antibiotic resistance mechanisms, which make them ideal indicators for assessing antimicrobial potential of plant extracts and environmental contamination levels (Mandell *et al*., 2020).

CHAPTER THREE

MATERIAL AND METHODS

3.1 Bacterial isolates used for study

The clinical isolates which were *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* were obtained from the culture collection unit of Microbiology Laborartory, Mycofarm. The bacterial stock cultures were maintained at 4^0C. All microbial stock cultures were freshened by sterile inoculation loop on nutrient agar plates under a suitable condition and temperature of 37^0C for 24hours. The following day, the streaked cultures were again subcultured on media plates and incubated at 37^0C for 24 hours.

3.2 Apparatus and Equipment

Test tubes, conical flask (500ml and 1000ml), Petri dish, measuring cylinder, Bunsen burner, pipette, inoculating loop, cotton wool, sensitivity disk, incubator and microscope.

3.3.Preparation of Media Culture

To successful carry out our research, the clinical isolates were cultured in suitable medium. Three (3) Agar medium in total were utilized at different occasion in the course of this research experiment.

3.3.1 Mueller Hinton Agar

This is a microbiological growth medium that is commonly used for antibiotic susceptibility testing. In preparation, 38g was dissolved in 1000ml distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving

at 121^0C for 15 minutes. The medium was cooled to 45-50^0C and then dispensed aseptically into sterile Petri dishes in the laminar flow hood cabinet.

3.4 Collection and identification of plant material

Fresh leaves of *Mimosa pudica* were obtained from from a farm land at Amagba area in Benin City, Edo state, Nigeria. They were identified and confirmed by the Plant Biology and Biotechnology Department (PBB) of the University of Benin (UNIBEN), Edo state, Nigeria.

3.5 Extraction of plant Materials

Procedure:

The leaves were thoroughly washed, shade-dried and finely powered. They were thoroughly washed to remove dirt and dust, then dried in the shade to preserve its medicinal properties. The dried leaves were grinded using dry blender. The leaf powder was stored in an airtight container and used for further experiments.

The experiment was carried out using water as the main solvents. Five hundred grams (500 g) of the dry powdered sample was weighed into a clean container, and 1000 ml of extracting solvent (water) was added. The sample was allowed to soak for 24 hours, after which it was first filtered with a sieve and then with Whatman filter paper to obtain a clear filtrate devoid of residue. The filtrate was placed in a beaker and concentrated using a water bath at 50°C. When properly concentrated, the extract was removed from the water bath and transferred into a clean container for further analysis (Harborne, 1998).

3.6 PREPARATION OF EXTRACTS

3.6.1. Hot Water Extraction

Fresh *Mimosa pudica* leaves was collected and washed thoroughly with water. The leaves were dried with a clean cloth or paper towels to remove excess moisture. Afterwards a required amount of *Mimosa pudica* leaves was weighed and collected. Water was heated (100°C) in a glass beaker or flask and then the weighed *Mimosa pudica* leaves was added to the hot water. The mixture was allowed to steep for 10-15 minutes, depending on the desired strength of the extract. The mixture was strained using a cheesecloth or a fine-mesh sieve into another container. The solids were filtered out and discarded while the liquid was made to cool. The hot water extract was collected and stored in a clean container.

3.6.2. Cold Water Extraction

Fresh *Mimosa pudica* leaves was collected and washed thoroughly with water. The leaves were dried with a clean cloth or paper towels to remove excess moisture. A required amount of the *Mimosa pudica* leaves was weighed. The weighed leaves was added to a glass beaker or flask containing cold water (room temperature). The mixture was allowed to steep for 2-4 hours or overnight (8-12 hours), depending on the desired strength of the extract. The mixture was strained using a cheesecloth or a fine-mesh sieve into another container. The solids were filtered out and discarded while the liquid was made to cool. The hot water extract was collected and stored in a clean container.

3.7. 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 was 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.

3.7.1. 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.

3.7.1.1. 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.

3.7.1.2. Minimum Bactericidal Concentrations

1 ml of the sample of known concentration was transferred into a test tubes, 1ml of the test organisms previously diluted to 0.5 MCFARLAND turbidity standard was also introduced into

the test tubes and incubated for 24 hours. A loopful of the innoculum was aseptically introduced on a sterile agar medium and incubated for 24 hours.

3.8. Qualitative Phytochemical Screening

The qualitative phytochemical screening of the extract was carried out to determine the presence of various bioactive compounds such as flavonoids, tannins, saponins, glycosides, and terpenoids. Each of these phytochemical tests was conducted according to standard procedures as described by Okwu and Okwu (2005), and observations were made based on characteristic color changes and reactions that indicate the presence of specific phytoconstituents.

3.8.1. Test for Flavonoids

The presence of flavonoids in the extract was determined following the method described by Okwu and Okwu (2005). In this test, 0.3 g of the powdered sample was weighed and soaked in 30 ml of distilled water for two hours to ensure proper extraction of the phytochemical constituents. The mixture was then filtered to obtain a clear aqueous extract. About 10 ml of the filtrate was measured and mixed with 5 ml of 10% ammonia solution, after which concentrated sulphuric acid was carefully added. The formation of a yellow coloration that disappeared upon standing indicated the presence of flavonoids. Flavonoids are known for their antioxidant and antimicrobial properties, contributing significantly to the therapeutic potential of plant extracts.

3.8.2. Test for Tannins

The presence of tannins in the plant extract was determined using the ferric chloride test. In this method, 0.5 g of the powdered sample was boiled in 20 ml of distilled water to ensure adequate

extraction of soluble tannins. The mixture was then filtered, and 5 ml of the resulting filtrate was treated with three drops of 0.1% ferric chloride solution prepared by dissolving 0.01 g of ferric chloride in 10 ml of distilled water. The development of a brownish-green or blue-black coloration was taken as a positive indication of tannins. These compounds are known for their astringent, antimicrobial, and antioxidant properties, which enhance the medicinal value of the plant (Okwu and Okwu, 2005).

3.8.3. Test for Saponins

The presence of saponins was determined using the frothing and emulsion test as described by Okwu and Okwu (2005). Two grams (2 g) of the powdered sample was boiled in 20 ml of distilled water in a water bath to extract the saponin content. The mixture was then filtered, and 10 ml of the filtrate was mixed with 5 ml of distilled water. The solution was shaken vigorously for the formation of a stable froth. To the frothing solution, three drops of olive oil were added, and the mixture was shaken again. The formation of an emulsion or persistent froth indicated the presence of saponins. These compounds are known for their ability to form soap-like foams in aqueous solutions and possess antimicrobial, anti-inflammatory, and immune-boosting properties.

3.8.4. Test for Glycosides

The presence of glycosides was determined using the Keller-Kiliani test as outlined by Okwu and Okwu (2005). In this test, 5 ml of the aqueous extract was mixed with 2 ml of glacial acetic acid solution (prepared by diluting 0.5 ml of glacial acetic acid in 9.5 ml of distilled water). To this mixture, one drop of 0.1% ferric chloride solution was added, followed by the careful addition of 1 ml of concentrated sulphuric acid down the side of the test tube. The formation of a

brown ring at the interface indicated the presence of a deoxysugar, which is characteristic of cardiac glycosides. Additionally, a violet ring may appear below the brown ring, while a greenish coloration may form throughout the acetic layer. Glycosides play important roles in plant defense and exhibit pharmacological activities such as cardiotonic, anti-inflammatory, and antimicrobial effects.

3.8.5. Test for Terpenoids

Terpenoids were tested using the Salkowski test. In this procedure, 0.3 g of the powdered sample was extracted in 30 ml of distilled water for two hours and then filtered to obtain a clear extract. About 5 ml of the aqueous extract was mixed with 2 ml of chloroform, followed by the careful addition of 3 ml of concentrated sulphuric acid. The formation of a reddish-brown or pink coloration at the interface indicated the presence of terpenoids (Okwu and Okwu, 2005). Terpenoids are important phytochemicals known for their diverse biological functions, including antimicrobial, antifungal, anti-inflammatory, and antioxidant properties, contributing to the plant's overall pharmacological efficacy.

CHAPTER FOUR

RESULTS

Table 4.1 presents the results of the qualitative phytochemical analysis of *Mimosa pudica* (commonly known as the Sensitive Plant). The analysis revealed the presence of saponins and flavonoids in both hot and cold water extracts, indicating that these bioactive compounds are slightly present (+). Tannins were moderately present (++) in both extracts, whereas terpenoids and glycosides were not detected (–) in the sample.

Table 4.2 presents the zones of inhibition (in mm) observed for hot and cold water extracts of *Mimosa pudica* at varying concentrations (2000 mg/ml, 1000 mg/ml, 500 mg/ml, and 250 mg/ml) against three bacterial isolates: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. The results show that the antibacterial activity of the extracts is concentration-dependent, with higher concentrations showing greater zones of inhibition.

For *Staphylococcus aureus*, the cold extract exhibited a significantly higher antibacterial activity (26.00 ± 5.29 mm) at 2000 mg/ml compared to the hot extract (8.00 ± 5.29 mm). At lower concentrations (500 mg/ml and 250 mg/ml), the hot extract showed no inhibitory effect, while the cold extract still showed moderate inhibition (16.00 ± 7.21 mm and 8.00 ± 2.00 mm, respectively). For *Pseudomonas aeruginosa*, the hot extract showed substantial inhibitory activity at 2000 mg/ml (20.67 ± 1.15 mm) and 1000 mg/ml (16.67 ± 4.16 mm), with decreased inhibition at 500 mg/ml (8.67 ± 6.43 mm) and 250 mg/ml (5.33 ± 1.15 mm). The cold extract also demonstrated activity, with a zone of inhibition of 18.67 ± 7.57 mm at 2000 mg/ml and 11.33 ± 3.06 mm at 1000 mg/ml. For *Escherichia coli*, the cold extract was more active than the hot extract across all concentrations, showing a maximum inhibition zone of 27.33 ± 6.11 mm at

2000 mg/ml compared to 14.00 ± 2.00 mm for the hot extract. The cold extract also maintained inhibition at lower concentrations (14.00 ± 11.53 mm at 1000 mg/ml, 10.00 ± 4.90 mm at 500 mg/ml, and 7.33 ± 4.62 mm at 250 mg/ml), while the hot extract exhibited a gradual decline with decreasing concentration.

Table 4.3 presents the Minimum Inhibitory Concentration (MIC) values (mg/ml) of hot and cold water extracts of *Mimosa pudica* against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. The results indicate that *Staphylococcus aureus* was more susceptible to the cold extract (80 mg/ml) than the hot extract (1400 mg/ml). For *Pseudomonas aeruginosa*, both extracts exhibited similar MIC values (175 mg/ml). *Escherichia coli* showed comparable sensitivity, with MIC values of 80 mg/ml and 100 mg/ml for hot and cold extracts, respectively.

Table 4.4 presents the Minimum Bactericidal Concentration (MBC) of the hot and cold water extracts of *Mimosa pudica* against the test organisms. At the tested concentration of 2000 mg/ml, all isolates (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*) exhibited visible growth (+) for both extracts, indicating that the extracts were bacteriostatic rather than bactericidal at that concentration.

Table 4.5 displays the antibiotic susceptibility profiles of the bacterial isolates (*Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*) against a range of standard antibiotics. The results are categorized based on the zone of inhibition (mm) as Resistant (R: 0–10 mm), Intermediate (I: 11–16 mm), and Sensitive (S: ≥17 mm).

Table 4.1: Qualitative Phytochemical Constituents of *Mimosa pudica* (Hot and Cold Water Extracts)

Phytochemical Parameters	***Mimosa pudica*** **(Hot Water Extract)**	***Mimosa pudica*** **(Cold Water Extract)**
Saponins	+	+
Flavonoids	+	+
Terpenoids	–	–
Glycosides	–	–
Tannins	++	++

+: Slightly Present

++: Moderately Present

-: Absent

Table 4.2: Antibacterial Activity of Hot and Cold Water Extracts of *Mimosa pudica* on Bacterial Isolates

Zones of Inhibition

Test Organism	Extract	2000(mg/ml)	1000 (mg/ml)	500 (mg/ml)	250 (mg/ml)
Staphylococcus aureus	Hot	8.00 ± 5.29	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Cold	26.00 ± 5.29	18.67 ± 1.15	16.00 ± 7.21	8.00 ± 2.00
Pseudomonas aeruginosa	Hot	20.67 ± 1.15	16.67 ± 4.16	8.67 ± 6.43	5.33 ± 1.15
	Cold	18.67 ± 7.57	11.33 ± 3.06	8.00 ± 2.00	4.67 ± 1.15
Escherichia coli	Hot	14.00 ± 2.00	12.00 ± 3.46	7.33 ± 7.23	4.67 ± 4.62
	Cold	27.33 ± 6.11	14.00 ± 11.53	10.00 ± 4.90	7.33 ± 4.62

Values represented in mean ± standard deviation

Table 4.3: Minimum Inhibitory Concentration (MIC) of Hot and Cold Water Extracts of *Mimosa pudica* on Bacterial Isolates (mg/ml)

Isolates	Hot Extract (mg/ml)	Cold Extract (mg/ml)
Staphylococcus aureus	1400	80
Pseudomonas aeruginosa	175	175
Escherichia coli	80	100

Table 4.4: Minimum Bactericidal Concentration (MBC) of Hot and Cold Water Extracts of *Mimosa pudica* on Bacterial Isolates

Isolates	Concentration (mg/ml)	Growth	Inference
Staphylococcus aureus	HOT = 2000	+	Bacteriostatic
	COLD = 2000	+	Bacteriostatic
Pseudomonas aeruginosa	HOT = 2000	+	Bacteriostatic
	COLD = 2000	+	Bacteriostatic
Escherichia coli	HOT = 2000	+	Bacteriostatic
	COLD = 2000	+	Bacteriostatic

Table 4.5: Antibiotic Sensitivity Test of Bacterial Isolates

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

Isolate	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

Keys:
R.I = Resistance Index
Resistant (R) = 0–10 mm
Intermediate (I) = 11–16 mm
Sensitive (S) = 17 mm and above

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

CHAPTER FIVE

DISCUSSION

Medicinal plants have been a cornerstone of traditional medicine for centuries, providing a diverse array of bioactive compounds with therapeutic potential (Sofowora, 2013). These plants produce secondary metabolites, such as flavonoids, tannins, saponins, and terpenoids, which are responsible for their pharmacological activities, including antimicrobial, anti-inflammatory, and antioxidant effects (Evans, 2009). In many parts of the world, particularly in developing countries, plant-based remedies remain a primary healthcare resource due to their accessibility, affordability, and cultural acceptance (WHO, 2019). *Mimosa pudica*, commonly known as the Sensitive Plant, is a prominent example of a medicinal plant widely used in traditional systems, such as Ayurveda and African ethnomedicine, for its antimicrobial, analgesic, and wound-healing properties (Ahmad *et al*., 2012). Its leaves, roots, and whole plant extracts have shown promise in combating bacterial infections, making it a valuable subject for pharmacological research (Muhammad *et al*., 2016). This study seeks to evaluate the phytochemical constituents and antibacterial activity of hot and cold water extracts of *Mimosa pudica* against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*.

The qualitative phytochemical analysis (Table 4.1) revealed the presence of saponins, flavonoids, and tannins in both hot and cold water extracts of *Mimosa pudica*, with tannins being moderately present (++) and saponins and flavonoids slightly present (+). The absence of terpenoids and glycosides is likely due to the polarity of water as a solvent, which preferentially extracts polar compounds like tannins and flavonoids (Harborne, 1998). Tannins are known for their antimicrobial properties, binding to bacterial cell wall proteins and enzymes to disrupt metabolic processes (Scalbert, 1991). Flavonoids interfere with bacterial membrane integrity and nucleic

acid synthesis, contributing to antibacterial effects (Cushnie and Lamb, 2005). Saponins, though less abundant, disrupt microbial cell membranes, enhancing antimicrobial activity (Sparg *et al*., 2004).

These findings align with previous studies. For instance, Tamilarasi *et al*. (2012) and Kaur *et al*. (2011) reported the presence of tannins, flavonoids, saponins, and other secondary metabolites (e.g., carbohydrates, alkaloids, phenols, and coumarins) in *Mimosa pudica* extracts, attributing their antimicrobial potency to these compounds. The similar phytochemical profiles of hot and cold water extracts suggest that the extraction method does not significantly alter the presence of these polar compounds. However, the moderate presence of tannins indicates a higher concentration, likely contributing to the observed antibacterial activity.

The antibacterial activity of *Mimosa pudica* extracts (Table 4.2) was concentration-dependent, with higher concentrations (2000 mg/ml and 1000 mg/ml) showing larger zones of inhibition compared to lower concentrations (500 mg/ml and 250 mg/ml). This dose-response relationship is consistent with antimicrobial studies, where higher concentrations of bioactive compounds enhance inhibition (Nostro *et al*., 2000). The cold water extract outperformed the hot water extract, particularly against *Staphylococcus aureus* (26.00 ± 5.29 mm vs. 8.00 ± 5.29 mm at 2000 mg/ml) and *Escherichia coli* (27.33 ± 6.11 mm vs. 14.00 ± 2.00 mm at 2000 mg/ml). This suggests that cold extraction preserves thermolabile compounds, such as certain flavonoids or enzymes, that enhance antibacterial activity (Cowan, 1999).

For *Pseudomonas aeruginosa*, the hot water extract showed substantial activity at 2000 mg/ml (20.67 ± 1.15 mm) and 1000 mg/ml (16.67 ± 4.16 mm), comparable to the cold extract (18.67 ± 7.57 mm and 11.33 ± 3.06 mm, respectively). The effectiveness of the hot extract against *P.*

aeruginosa may be due to the stability of tannins, which target Gram-negative bacteria by disrupting their outer membrane (Scalbert, 1991). The result of this study aling with Mawuko *et al*., 2025, were *Pseudomonas aeruginosa* showed complete resistance to cold aqueous extract of *Mimosa pudica*. Similarly, these results are supported by Sunil *et al*. (2016), who reported dose-dependent antibacterial activity of ethanolic *Mimosa pudica* extracts, with significant inhibition at 250 µg/ml against *E. coli* (17 mm), *P. aeruginosa* (16 mm), and *S. aureus* (16 mm). Similarly, Kaur *et al*. (2011) found ethanolic extracts effective against *S. aureus* (23.96 mm), *E. coli* (22.3 mm), and *P. aeruginosa* (21.43 mm) at 30 mg/ml, reinforcing the concentration-dependent nature of the plant's antimicrobial effects.

Amengialue *et al*. (2016) further demonstrated that aqueous extracts of *Mimosa pudica* exhibited maximum activity against *E. coli* (6.3 ± 1.5 mm) at 100 mg/ml, with moderate activity against *S. aureus* (3.3 ± 0.9 mm), while ethanolic and methanolic extracts showed higher potency, particularly against *S. aureus* (15 ± 0.6 mm) and *Bacillus subtilis* (15.7 ± 1.9 mm). The superior performance of the cold water extract in the current study aligns with these findings, suggesting that aqueous extracts, while less potent than organic solvent extracts, still possess significant antibacterial activity, particularly at higher concentrations.

The MIC values (Table 4.3) highlight the differential efficacy of the extracts. *Staphylococcus aureus* was more susceptible to the cold extract (MIC = 80 mg/ml) than the hot extract (MIC = 1400 mg/ml), indicating that cold extraction enhances the retention of active compounds. For *Pseudomonas aeruginosa*, both extracts had identical MIC values (175 mg/ml), suggesting comparable efficacy. *Escherichia coli* showed similar sensitivity to both extracts (80 mg/ml for hot, 100 mg/ml for cold). These MIC values are higher than those reported for ethanolic extracts

by Amengialue *et al*. (2016), where ethanolic and methanolic extracts showed lower MICs against *S. aureus* and *E. coli*, indicating greater potency of organic solvents.

The MBC results (Table 4.4) indicate that both extracts were bacteriostatic at 2000 mg/ml, as visible bacterial growth was observed for all isolates. This aligns with the findings of Amengialue *et al*. (2016), who noted that aqueous extracts were less bactericidal than ethanolic or methanolic extracts, requiring higher concentrations to achieve bactericidal effects. The bacteriostatic nature of the extracts suggests that tannins and flavonoids inhibit bacterial growth without causing cell lysis, a common mechanism for these compounds (Cushnie and Lamb, 2005).

The antibiotic susceptibility profiles (Table 4.5) provide a benchmark for evaluating the efficacy of *Mimosa pudica* extracts. *Staphylococcus aureus* was sensitive to several antibiotics, including pefloxacin (20 mm, S), zithromycin (18 mm, S), ciprofloxacin (20 mm, S), and erythromycin (20 mm, S), with intermediate susceptibility to others. The cold extract's inhibition zone of 26.00 ± 5.29 mm at 2000 mg/ml against *S. aureus* is comparable to or exceeds the zones of standard antibiotics, suggesting that the extract could serve as a complementary or alternative antimicrobial agent for this bacterium. For *Escherichia coli*, the cold extract's inhibition zone (27.33 ± 6.11 mm) at 2000 mg/ml surpasses the zones of most antibiotics tested (e.g., ciprofloxacin: 26 mm, S), indicating strong potential against this Gram-negative bacterium. *Pseudomonas aeruginosa*, known for its intrinsic resistance to antibiotics, showed intermediate to sensitive responses to standard antibiotics, with the hot extract's inhibition zone (20.67 ± 1.15 mm) at 2000 mg/ml being comparable to streptomycin (18 mm, S).

These comparisons suggest that *Mimosa pudica* extracts, particularly the cold extract, have competitive antibacterial activity against both Gram-positive and Gram-negative bacteria. The extracts' efficacy at high concentrations indicates their potential as natural antimicrobial agents, especially in contexts where antibiotic resistance is a concern (WHO, 2019).

The presence of tannins, flavonoids, and saponins in *Mimosa pudica* extracts, coupled with their concentration-dependent antibacterial activity, highlights the plant's potential as a source of natural antimicrobial agents. The superior performance of the cold extract against *S. aureus* and *E. coli* suggests that cold water extraction may be a preferred method for maximizing antibacterial efficacy, possibly due to the preservation of thermolabile compounds. The bacteriostatic nature of the extracts indicates their role in inhibiting bacterial growth, which could be valuable in preventing infections or supporting conventional antibiotics in combination therapies (Cowan, 1999).

The findings also have implications for addressing antibiotic resistance, a global health challenge. The comparable efficacy of *Mimosa pudica* extracts to standard antibiotics, particularly against *S. aureus* and *E. coli*, suggests that the plant could be explored as an alternative or adjunctive treatment in regions with limited access to synthetic antibiotics (WHO, 2019). Furthermore, the use of aqueous extracts aligns with traditional preparation methods, enhancing the cultural and practical relevance of *Mimosa pudica* in ethnomedicine.

5.1. Recommendations

The findings from this study demonstrate the antibacterial potential of *Mimosa pudica* hot and cold water extracts, attributed to the presence of tannins, flavonoids, and saponins. However,

limitations such as the use of only aqueous extracts, the bacteriostatic nature of the extracts, and the testing of only three bacterial strains suggest several opportunities for further research to maximize the plant's therapeutic potential. The following recommendations are proposed:

1. **Exploration of Organic Solvent Extracts**: The current study used aqueous extracts, which may not capture non-polar compounds such as terpenoids or alkaloids. Future studies should investigate extracts prepared with organic solvents like ethanol or methanol, which may yield higher potency against bacteria and fungi. Comparing the phytochemical profiles and antimicrobial efficacy of organic solvent extracts with aqueous extracts could identify additional bioactive compounds and optimize extraction methods.
2. **Advanced Phytochemical Characterization**: The qualitative phytochemical analysis identified tannins, flavonoids, and saponins but lacked detailed chemical profiling. Employing advanced analytical techniques, such as high-performance liquid chromatography or gas chromatography-mass spectrometry, would enable the identification and quantification of specific bioactive compounds responsible for the observed antibacterial activity. This could clarify the mechanisms of action and potential synergistic interactions between compounds.
3. **Testing Against a Broader Range of Pathogens**: The study tested *Mimosa pudica* extracts against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. To enhance generalizability, future research should include a wider range of pathogens, particularly multidrug-resistant bacteria and fungi, given the reported activity of *Mimosa pudica* against fungal pathogens like *Aspergillus* and *Candida* species. This would confirm the plant's broad-spectrum antimicrobial potential.

4. **Investigation of Bactericidal Potential**: The extracts were bacteriostatic at 2000 mg/ml, indicating a need for higher concentrations or alternative extraction methods to achieve bactericidal effects. Future studies should explore higher concentrations or solvent-based extracts to determine the conditions under which *Mimosa pudica* extracts can kill bacteria rather than merely inhibit their growth, enhancing their therapeutic applicability.
5. **Synergistic Studies with Antibiotics**: The competitive efficacy of the cold water extract compared to standard antibiotics suggests potential for combination therapies. Future research should investigate the synergistic effects of *Mimosa pudica* extracts with conventional antibiotics, such as ciprofloxacin or ampicillin, to enhance antimicrobial activity and address antibiotic resistance. This could involve assays to determine interactions that improve efficacy.
6. **In Vivo and Clinical Studies**: The current study was limited to in vitro assays, which do not account for bioavailability, toxicity, or efficacy in living systems. In vivo studies using animal models are recommended to evaluate the safety, pharmacokinetics, and therapeutic efficacy of *Mimosa pudica* extracts. These studies could support the development of clinical trials to assess the plant's potential in treating bacterial infections.
7. **Formulation and Delivery Systems**: To enhance practical application, research should focus on developing formulations, such as topical ointments or oral preparations, that optimize the delivery of bioactive compounds. Exploring encapsulation methods, such as nanoparticles or liposomes, could improve the stability and bioavailability of the extracts, making them more viable for therapeutic use.

By addressing these areas, future research can build on the current findings to fully elucidate the antimicrobial potential of *Mimosa pudica*, optimize its use as a natural antimicrobial agent, and

contribute to the development of alternative therapies for microbial infections, particularly in the context of rising antibiotic resistance.

5.2. Conclusion

This study demonstrate that *Mimosa pudica* hot and cold water extracts contain tannins, flavonoids, and saponins, which contribute to their concentration-dependent antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. The cold extract exhibited superior efficacy, particularly against *S. aureus* and *E. coli*, with inhibition zones comparable to standard antibiotics. The bacteriostatic nature of the extracts suggests their potential as natural antimicrobial agents, particularly in combating antibiotic-resistant bacteria. While the findings underscore the therapeutic potential of *Mimosa pudica*, further research is needed to optimize extraction methods, identify specific bioactive compounds, and evaluate clinical applications.

5.2 RECOMMENDATIONS

1. Further Quantitative Phytochemical Analysis

Future studies should carry out quantitative phytochemical analysis to determine the exact concentrations of bioactive compounds such as tannins, flavonoids, and saponins present in the cold and hot water extracts of *Mimosa pudica*. This will help establish a clearer relationship between phytochemical concentration and antibacterial activity.

2. Isolation and Characterization of Active Compounds

Advanced techniques such as chromatography and spectroscopic methods should be employed to isolate and characterize the specific phytochemical compounds responsible for the observed

antibacterial effects. This could facilitate the development of standardized plant-based antimicrobial agents.

3. Use of Additional Extraction Methods

Other extraction solvents such as ethanol, methanol, and acetone should be explored and compared with aqueous extracts to determine the most effective extraction method for maximizing antibacterial activity.

4. In Vivo and Toxicological Studies

In vivo studies and toxicity assessments should be conducted to evaluate the safety, efficacy, and therapeutic dosage of *Mimosa pudica* extracts before recommending their use in clinical or pharmaceutical applications.

5. Evaluation Against More Resistant Strains

Further research should assess the activity of *Mimosa pudica* extracts against a broader range of multidrug-resistant bacterial strains to determine its potential role in combating antimicrobial resistance

5.3 CONTRIBUTION TO KNOWLEDGE

1. Comparative Evaluation of Cold and Hot Water Extracts

The study provides comparative data on the antibacterial efficacy of cold and hot water extracts of *Mimosa pudica*, demonstrating that cold water extraction preserves heat-labile bioactive compounds and results in higher antibacterial activity.

2. Evidence-Based Validation of Traditional Use

The findings scientifically validate the traditional use of *Mimosa pudica* in treating infections, particularly bacterial-related ailments, thereby bridging the gap between ethnomedicine and modern microbiological research.

3. Documentation of Phytochemical Profile

The study contributes to existing literature by documenting the qualitative phytochemical constituents present in aqueous extracts of *Mimosa pudica*, highlighting tannins, flavonoids, and saponins as key contributors to antibacterial activity.

4. Insight into Bacteriostatic Properties

The determination of MIC and MBC values revealed that the extracts exhibit bacteriostatic effects at tested concentrations, providing valuable insight into the mode of antibacterial action of *Mimosa pudica*.

5. Support for Plant-Based Antimicrobial Research

This research strengthens the scientific basis for exploring medicinal plants as alternative antimicrobial agents, especially in the context of increasing antibiotic resistance and limited access to synthetic drugs in developing countries.

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