EVALUATION OF THE ANTIBACTERIAL AND PHYTOCHEMICALS PROPERTIES OF COLD AND HOT WATER EXTRACT OF *Polyalthia longifolia*

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

OCTOBER, 2025.

CERTIFICATION

This is to certify that this project work on "**EVALUATION AND PHYTOCHEMICALS PROPERTIES OF COLD AND HOT WATER EXTRACT OF *Polyalthia longifolia***" was carried out by **IMIKO GODSTIME ENERUVIE**, with the Matriculation Number: **LSC2205884**; in partial fulfilment for the Award of Bachelor of Science (B.Sc) Degree in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City.

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DEDICATION

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

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

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

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ABSTRACT

Since antimicrobial resistance is a serious public health concern, alternative therapeutic sources derived from medicinal plants are required. In this study, cold and hot water extracts of *Polyalthialongifolia* leaves were examined for their phytochemical composition and antibacterial qualities. Extracts were made with aqueous solvents and tested using the agar well diffusion method against specific bacterial strains (*Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa*). The antimicrobial potency was measured using the Minimum Bactericidal Concentration (MBC) and Minimum Inhibitory Concentration (MIC). Qualitative phytochemical analysis revealed the presence of compounds linked to antimicrobial and therapeutic effects, including flavonoids, tannins, saponins, glycosides, and terpenoids. In contrast to the cold extract, the hot water extract demonstrated better antibacterial activity, suggesting that higher extraction temperatures promote the release of bioactive compounds. The research highlights *Polyalthialongifolia's* potential as a source of plant-based antimicrobial agents appropriate for upcoming pharmaceutical development and validates its ethnomedical significance.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

For centuries, plants and their derived products have remained indispensable sources of therapeutic agents. Humanity has long relied on herbs for the prevention and treatment of diseases, forming the foundation of traditional healing systems across the world. Reports by the World Health Organization indicate that more than 80% of the global population particularly in developing regions—depends primarily on plant-based remedies for basic healthcare needs (Kristin *et al*., 2024). Medicinal plants, often regarded as nature's invaluable contribution to human well-being, play a crucial role in sustaining health and offering protection against illness (Munshi and Agarwal, 2010).

Traditional medical practices are not static; rather, they evolve as communities refine existing knowledge and introduce innovative techniques to enhance therapeutic effectiveness (Muthu *et al*., 2006). Ethnopharmacology continues to be a central pillar in natural product research and drug discovery, especially in an era where numerous drug targets exist, yet few promising leads are available (Elshafie *et al*., 2023). Historically, many modern pharmaceuticals trace their origins to indigenous plant use. Despite remarkable advancements in scientific technology, the current drug discovery pipeline faces a considerable decline in innovation, posing challenges to the pharmaceutical industry (Raut and Karuppayil, 2014).

The escalating prevalence of multidrug-resistant bacteria has intensified the global demand for novel, effective, and affordable antimicrobial agents. Plants, abundant in structurally diverse bioactive molecules, have long provided templates for the development of modern antimicrobial drugs (WHO, 2020). The emergence of antimicrobial resistance among

pathogenic bacteria presents a major public health challenge (CDC, 2013). As part of global strategies to curb the spread of resistance, researchers are increasingly exploring plants as alternative sources of potent antimicrobial compounds (Persaud *et al*., 2019; Babatunde *et al*., 2022). According to WHO estimates, over 75% of the world's population relies partially or exclusively on herbal medicine for healthcare (WHO, 2013). Consequently, numerous medicinal plants have been incorporated into conventional medicine systems and pharmaceutical development (Akinjogunla *et al*., 2011). Yet, many traditionally valued medicinal plants remain underexplored scientifically, despite their long-standing ethnomedicinal use for conditions such as fever, cough, gastrointestinal disturbances, and various inflammatory disorders (Odugbemi, 2006).

Among such plants is *Polyalthia longifolia* (Order: Magnoliales), commonly referred to as the Indian mast tree or masquerade tree. A member of the family Annonaceae, this evergreen ornamental tree is native to the Indian subcontinent but has become widely cultivated in tropical and subtropical regions, including parts of Africa. Besides its aesthetic value and role in mitigating noise pollution, the plant has a long history of use in traditional medicine systems for managing skin diseases, fever, hypertension, parasitic infections, and other health conditions. Various parts of the plant especially the leaves and bark—are utilized in Ayurvedic and folk therapies.

Polyalthia longifolia, also known as false Ashoka, Buddha tree, and Indian fire tree, is characterized by its symmetrical pyramidal shape, pendulous branches, and narrow lanceolate leaves with wavy margins. The tree may reach heights exceeding 30 feet. Traditional healing systems employ preparations from this plant for ailments such as duodenal ulcers, rheumatism, menstrual disorders, scorpion stings, diabetes, and digestive issues (Katkar *et al*., 2010). Several biologically active compounds have been isolated from its leaves, bark,

and other parts, highlighting its pharmacological relevance (Wu *et al*., 1990; Krishnamurthi, 1969).

Water, being a safe and universally accepted solvent, is commonly used for extracting phytochemicals from plant materials. However, the temperature at which water extraction is carried out whether cold or hot may significantly influence the quantity and nature of the bioactive constituents, which may in turn affect the antimicrobial effectiveness of the extracts. Accordingly, this study investigates the phytochemical components and antibacterial activities of cold and hot water extracts of *Polyalthia longifolia***,** contributing to the broader effort to authenticate traditional medicinal practices and identify effective plant-derived antimicrobial agents.

1.2 Statement of the Problem

Antimicrobial resistance (AMR) has emerged as one of the most pressing global health threats of the 21st century. Many commonly used antibiotics have gradually lost their effectiveness due to widespread resistance, making once-treatable bacterial infections increasingly difficult to manage. This situation underscores the urgent need for alternative therapeutic agents that possess strong antibacterial activity and reduced susceptibility to resistance development. *Polyalthia longifolia,* a plant widely used in traditional medicine, has attracted interest for its potential antimicrobial properties. However, despite its frequent use in ethnomedicine, scientific evaluation of its antibacterial efficacy—particularly in relation to different extraction conditions—remains limited.

Moreover, the World Health Organization has repeatedly warned of an impending "post-antibiotic era," in which minor infections may once again become life-threatening (WHO, 2020). This has prompted a renewed focus on identifying novel antimicrobial agents from

natural sources. Plants, with their complex phytochemical compositions and multi-target mechanisms of action, offer promising alternatives that may help counteract drug resistance (Cowan, 1999). Although *Polyalthia longifolia* has been recognized for its medicinal potential, several key knowledge gaps persist, including the effects of extraction temperature on its bioactive constituents and its comparative antibacterial potency. Addressing these gaps is essential for optimizing its therapeutic use and validating its role as a potential source of new antimicrobial agents.

1.3 Scope of the Study

This research is limited to the extraction of phytochemicals from the leaves of *Polyalthia longifolia* using cold and hot water as solvents. The study encompasses qualitative phytochemical screening and the determination of the antibacterial activity of the extracts against selected bacterial isolates.

1.4 Aim of the Study

The aim of this study was to evaluate the antibacterial and phytochemical properties of cold and hot water extracts of *Polyalthialongifolia*leaves.

1.5 Objectives of study

The objectives of this study were:

1. to prepare cold and hot water extracts of *Polyalthialongifolia*leaves.
2. to perform qualitative phytochemical screening of the extracts.
3. to determine and compare the antibacterial activity of the extracts against selected bacterial strains.

4. toanalyze the differences in phytochemical composition and antibacterial efficacy between cold and hot water extracts.

1.6 Significance of the Study

The results obtained from this research will improve our understanding of the antimicrobial and phytochemical potential of *Polyalthialongifolia*and offer scientific support for its traditional medicinal uses. It could also inform pharmaceutical research aimed at developing plant-based antibiotics and standardized herbal formulations.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Medicinal Plants and Natural Antibacterial Agents

Medicinal plants have long constituted the foundation of traditional healing systems and continue to contribute significantly to modern pharmacological research. These plants contain numerous biologically active phytochemicals including alkaloids, flavonoids, tannins, saponins, and glycosides that are widely recognized for their therapeutic efficacy, especially their antibacterial potential (Cowan, 1999). According to the World Health Organization (WHO), more than 80% of the world's population still depends on herbal preparations as their primary source of healthcare, underscoring the persistent relevance of natural remedies in both traditional and modern contexts.

The use of plants for medicinal purposes is deeply embedded in human history. Archaeological findings indicate that ancient hominins utilized plants for healing at least 60,000 years ago, as demonstrated by the discovery of *Alcea rosea* L. (hollyhock) remains in a Neanderthal burial site in present-day Iraq (Abdallah, 2011). However, the true origins of medicinal plant use likely extend even further back, considering that anatomically modern humans (*Homo sapiens*) emerged approximately 300,000 years ago in Africa (Galway-Witham and Stringer, 2018; Klein, 2019). In early human societies, plant-based healing relied heavily on observation and trial-and-error, given the limited understanding of disease etiology.

Over millennia, this empirical knowledge evolved into structured traditional medicine, with communities identifying particular plants effective for specific conditions. This gradual refinement marked the transition from intuitive use to a more systematic understanding of

plant-based therapeutics (Petrovska, 2012). Prior to the development of iatrochemistry in the 16th century, plants represented the primary means of disease management.

In recent decades, natural therapies have witnessed renewed global interest. The declining efficacy of many synthetic drugs and an increase in adverse drug reactions have encouraged a shift toward plant-based alternatives (Abdallah *et al*., 2023). This trend is particularly evident in the search for new antibacterial agents.

The modern age of antimicrobial therapy began with Alexander Fleming's discovery of penicillin in 1928 and the subsequent introduction of sulfonamides in the 1930s (Abdallah *et al*., 2023). Penicillin became widely available in the early 1940s, and the following decades—especially the 1950s to the 1970s formed the "golden era" of antibiotic development, during which numerous antibiotic classes were discovered and commercialized (Conly and Johnston, 2005).

However, the decades following this productive period have been marked by a progressive rise in antibiotic resistance. Today, antimicrobial resistance (AMR) poses a serious threat to global health. Contributing factors include the widespread misuse and overuse of antibiotics and the decreasing involvement of pharmaceutical industries in novel antibiotic development (Ventola, 2015). Consequently, the search for alternative antimicrobial agents, particularly those derived from natural sources, has become increasingly urgent.

The rapid spread of antibiotic-resistant microorganisms has intensified this demand, contributing to what researchers now refer to as the "antibacterial crisis" (Schcolmik-Cebrere, 2023). Medicinal plants have attracted significant attention as reservoirs of new antibacterial compounds. They contain a diverse array of phytochemicals such as phenolics, terpenoids,

alkaloids, and flavonoids known for their broad-spectrum therapeutic actions, including robust antibacterial activity (Sun and Shahrajabian, 2023).

2.1.2 Mechanisms of Action of Medicinal Plants as Antibacterial Agents

Plant-derived antibacterial compounds act through multiple biochemical pathways, making them less prone to inducing bacterial resistance compared to conventional antibiotics. Essential oils obtained from plants such as *Thymus vulgaris* and *Mentha piperita* destabilize bacterial cell membranes, resulting in leakage of intracellular contents and eventual cell lysis (Alvarez-Martinez *et al*., 2021). Certain flavonoids such as baicalein suppress efflux pump activity in methicillin-resistant *Staphylococcus aureus* (MRSA), thereby improving the efficacy of co-administered antibiotics (Fujita *et al*., 2005). Additionally, phenolic compounds can inhibit bacterial quorum sensing, disrupting communication pathways vital for virulence expression and biofilm development (Camele *et al*., 2019). Unlike synthetic antibiotics that often target a single bacterial enzyme or pathway, phytochemicals typically act through multiple mechanisms, significantly lowering the likelihood of resistance emergence (Khameneh *et al*., 2019).

Medicinal plants synthesize numerous antibacterial metabolites with diverse mechanisms of action. Phenolic compounds such as tannins and flavonoids are particularly effective against Gram-positive bacteria due to their ability to penetrate thick peptidoglycan layers (Poomanee *et al*., 2018). *Rauvolfia serpentina* produces the alkaloid reserpine, which interferes with ATP-dependent efflux systems, making it effective even against certain multidrug-resistant bacterial strains (Mabhiza *et al*., 2016). Terpenoids from species such as *Eremophila lucida* exhibit broad-spectrum antibacterial activity, including action against *Staphylococcus aureus* (Biva *et al*., 2019). Saponins, known for their surfactant-like properties, cause membrane disruption and have demonstrated activity against *Klebsiella pneumoniae* (Passi *et al*., 2009).

These examples highlight the chemical diversity of plants and their promise as sources of next-generation antimicrobials.

However, several challenges hinder the widespread clinical adoption of plant-based antibacterial agents. Variations in phytochemical composition—often influenced by environmental factors, extraction conditions, and plant maturity complicate standardization efforts (Mwamatope *et al*., 2021). Much of the existing research is limited to in vitro assays, and there is a scarcity of clinical trials to confirm the safety and efficacy of these compounds in humans (Menezes *et al*., 2022). Emerging technologies, including artificial intelligence (AI) and nanotechnology, offer potential solutions to these challenges. For instance, plant extract-mediated silver nanoparticles derived from *Thymus vulgaris* have shown improved bioavailability and targeted delivery when nanoencapsulated (Abdellatif *et al*., 2022). AI-driven methods are also being used to discover novel bioactive compounds and optimize synergistic combinations (Liu *et al*., 2023).

2.2 *Polyalthia*

The genus *Polyalthia* belongs to the family Annonaceae and comprises a group of flowering plants distributed widely across tropical and subtropical regions, including South Asia, Southeast Asia, and Australia (Chatrou *et al*., 2012). According to the World Flora Online database (accessed on 5 June 2025), the genus *Polyalthia* contains 127 accepted species, encompassing trees, shrubs, and a few rare lianas. Scientific investigations of different plant parts—such as leaves, bark, roots, twigs, and seeds—have revealed that *Polyalthia* species are rich in diverse phytochemicals, particularly alkaloids and terpenes.

These phytochemical constituents have been associated with a wide range of biological properties, including antibacterial (Kanokmedhakul *et al*., 2006), antifungal (Bhattacharya *et*

al., 2012), antiviral (Kanokmedhakul *et al*., 2006), antiplasmodial (Gbedema *et al*., 2015), anti-inflammatory (Nguyen *et al*., 2020), anti-ulcer (Olate *et al*., 2012), anti-tumor (Duan *et al*., 2020), and anticancer (Afolabi *et al*., 2019) activities. In addition, extracts of *Polyalthia* species—especially those rich in flavonoids and clerodane diterpenes—have demonstrated strong antioxidant properties in both DPPH and enzyme-based assays (Chen *et al*., 2014).

2.2.1 *Polyalthia longifolia*

Polyalthia longifolia—widely recognized under the synonym *Monoon longifolium*—is a slender evergreen tree belonging to the family Annonaceae. Although it originates from southern India and Sri Lanka, it has been extensively cultivated across tropical regions of Asia (POWO, 2012). The species typically attains heights exceeding 20 meters and is popularly grown in urban environments due to its notable ability to reduce noise pollution. Morphologically, the tree displays a symmetrical pyramidal crown with elegant, drooping branches and elongated, narrow lanceolate leaves that possess wavy margins. The commonly cultivated pendulous "columnar" form (Xue *et al*., 2012) often appears branchless because regular trimming is performed for ornamental shaping. However, when allowed to grow naturally without such horticultural modifications, *P. longifolia* assumes the form of a broad, fully branched tree that provides substantial shade.

2.2.2 Distribution of *Polyalthia longifolia*

The genus *Polyalthia* comprises approximately 120 species distributed across Africa, South and Southeast Asia, Australia, and New Zealand. Within India, about 14 species of the genus have been documented (Katkar *et al*., 2010). Several prominent species occur across the country, including *Polyalthia cerasoides* Bedd., a shrub or small tree found throughout India; *Polyalthia fragrans* Benth., a large tree predominantly located in the Western Ghats; and *Polyalthia longifolia* (Sonn.), which is widely cultivated across different regions. Notably,

two distinct varieties of *P. longifolia* have been described, and both are present in Maharashtra as well as other parts of the country (Govekar and Sardesai, 2011).

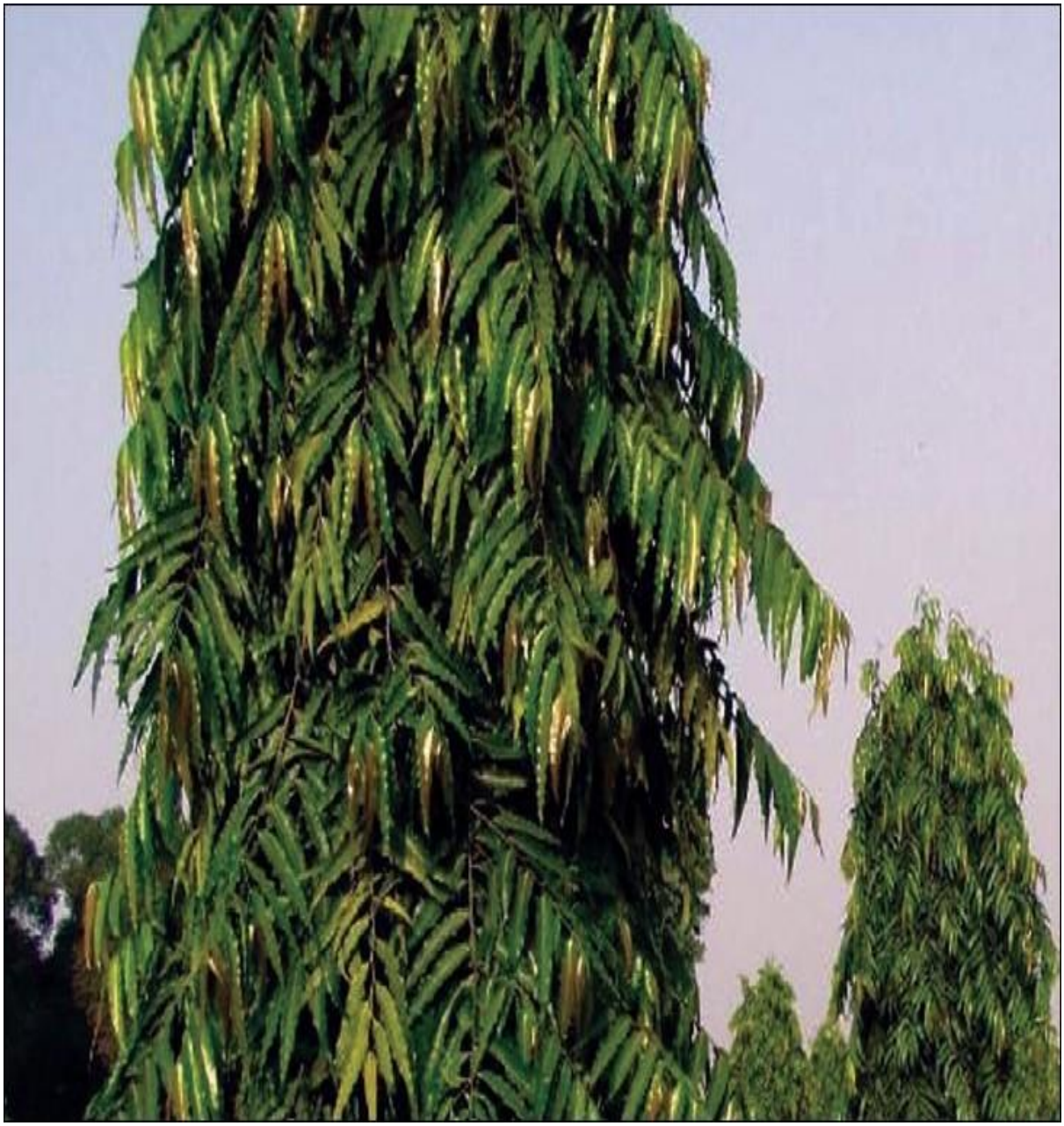

Plate 2.1: The whole tree of *Polyalthialongifolia*

(Source: Katkar, *et al*. 2010)

Plate 2.2: Leaves of *Polyalthialongifolia*

(Source: Katkar, *et al*. 2010)

Plate 2.3: Seeds of *Polyalthialongifolia*

(Source: Katkar, *et al*. 2010)

2.3 Taxonomical classification of *Polyalthialongifolia*(Sonn.)

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Sub class: Magnoliidae

Order: Mognoliids

Family: Annonaceae

Genus: *Polyalthia*

Species: *Longifolia*

(Source: Subramanion*et al*., 2013)

2.4 Phytochemical Constituents of *Polyalthia longifolia*

Polyalthia longifolia is rich in a wide spectrum of phytochemicals, particularly diterpenoids, alkaloids, tannins, and mucilaginous compounds, which collectively contribute to its broad medicinal relevance. Numerous structurally diverse alkaloids and diterpenoids have been isolated from the plant, including O-methylbulbocapnine-N-oxide, polyfothine, N-methylnandigerine-N-oxide, oliveroline-N-oxide, pendulamine A and B, 8-oxopolyalthiane, 16-oxo-5(10),13-halimadien-15-oic acid, 16-oxo-3,13-clerodadien-15-oic acid, and 16-hydroxycleroda-3,13-dien-16,15-olide. Advanced spectroscopic analyses have further identified two clerodane-type diterpenoids with strong antifeedant activity—16α-hydroxycleroda-3,13(14)Z-dien-15,16-olide and 16-oxo-cleroda-3,13(14)E-dien-15-oic acid.

The precise positioning of the olide functional group at carbon-16 was conclusively verified using X-ray crystallography (Katkar *et al*., 2010).

Further investigations led to the discovery of a novel γ-methoxybutenolide clerodane diterpene from the petroleum ether fraction of the bark. Its structure was elucidated through comparative spectroscopic methods, using a previously isolated γ-hydroxybutenolide diterpene as a reference (Mostafa *et al*., 2022).

Phytochemical evaluations across multiple plant parts—leaves, stems, bark, and seeds—have revealed a rich profile containing leukocyanidin, clerodane scaffolds, ent-helimanes, proanthocyanidins, β-sitosterol, and a broad array of apomorphine- and azafluorene-based alkaloids. Seed extracts additionally contain significant carbohydrate fractions. Three newly classified aporphine N-oxide alkaloids—(+)-O-methylbulbocapnine-β-N-oxide, (+)-O-methylbulbocapnine-α-N-oxide, and (+)-N-methylnandigerine-β-N-oxide—have been isolated from the leaves, along with a unique azafluorene alkaloid termed polylongine (5-hydroxy-6-methoxy-1-methyl-4-azafluoren-9-ol) (Katkar *et al*., 2010; Lúcio *et al*., 2015).

Gas chromatography (GC) and GC-MS profiling of the essential oils from the leaves and stem bark demonstrates marked compositional variation between the two parts. Leaf oil is dominated by sesquiterpenes such as allo-aromadendrene (19.7%), caryophyllene oxide (14.4%), β-caryophyllene (13.0%), β-selinene (7.9%), α-humulene (7.0%), and ar-curcumene (6.8%). Conversely, the stem bark oil contains higher proportions of α-copaene and α-muurolol (both ≈8.7%), β-selinene (8.6%), viridiflorene (8.1%), α-guaiene (7.8%), allo-aromadendrene (7.4%), and δ-cadinene (7.0%). Several other sesquiterpenoids also appeared in appreciable quantities (>1%). Notably, monoterpenes such as α-pinene and camphene—present in trace to minor quantities in the leaf oil—were completely absent in the bark oil (Katkar *et al*., 2010).

2.5 Antibacterial Properties of *Polyalthia longifolia*

The antibacterial potential of *Polyalthia longifolia* has been demonstrated through disc diffusion assays using leaf extracts obtained with a variety of solvents, including petroleum ether, chloroform, ethyl acetate, ethanol, and water. All extracts exhibited measurable inhibitory effects against six tested pathogenic bacteria, though the extent of activity varied across solvent types. Among them, the chloroform extract showed the most pronounced antibacterial effect, followed in decreasing order by petroleum ether, ethanol, and ethyl acetate, while the aqueous extract consistently produced the lowest inhibitory response.

Zones of inhibition revealed that *Bacillus subtilis* (26 mm) was the most susceptible organism to the chloroform extract, closely followed by *Staphylococcus aureus* (25 mm), *Escherichia coli* (24 mm), *Pseudomonas aeruginosa* (24 mm), *Proteus vulgaris* (23 mm), and *Salmonella typhi* (21 mm). The extract also demonstrated strong efficacy in minimum inhibitory concentration (MIC) assays, with MIC values as low as 0.01 mg/mL for *B. subtilis* and *S. aureus*. Comparisons with standard antibiotics cefuroxime, roxithromycin, and ciprofloxacin confirmed that the plant extracts possessed competitive antibacterial effects, suggesting their potential as promising natural antimicrobial agents (Thenmozhi & Sivaraj, 2010; Uzama *et al*., 2011; Chanda & Nair, 2010).

2.6 Pharmacological Activities of *Polyalthia longifolia*

2.6.1 Antioxidant Activity

Several studies have highlighted the robust antioxidant properties of *P. longifolia* extracts. The ethanolic stem bark extract demonstrated significant in vitro capability to neutralize reactive oxygen species, which play a central role in carcinogenesis. It inhibited lipid

peroxidation and exhibited strong ferric-reducing and DPPH radical-scavenging activities, with IC_{50} values of 18.14 µg/mL and 155.41 µg/mL, respectively.

Further assessments of the methanolic stem bark extract revealed its substantial ABTS radical cation scavenging activity (up to 54.79% at 100 µg/mL), followed by notable DPPH (75.36%) and superoxide scavenging (78.00%) capabilities at the same concentration.

Antioxidant investigations on seed extracts using DPPH and FRAP assays demonstrated that methanol and petroleum ether extracts were the most potent. Their IC_{50} values for DPPH scavenging were 98.43 µg/mL and 62.52 µg/mL, respectively, whereas FRAP values were 1.40 and 0.81, indicating strong electron-donating and reducing capacities (Mundhe *et al*., 2011).

2.6.2 Anti-inflammatory Properties

The anti-inflammatory potential of *P. longifolia* leaf extracts has been extensively validated using acute inflammatory models in Wistar rats. Solvent fractions—petroleum ether, hexane, toluene, chloroform, acetone, and methanol—were compared, with the methanolic extract demonstrating the most notable activity. Consequently, three concentrations (300, 600, and 900 mg/kg) of the methanol extract were further evaluated and found to produce anti-inflammatory effects comparable to diclofenac sodium, the reference drug.

Additional studies investigating ethanolic and aqueous extracts of the leaves in albino Wistar rats validated their activity through both acute and subacute inflammatory models, including the cotton pellet granuloma assay. Collectively, these findings highlight the plant's strong anti-inflammatory potential (Sharma *et al*., 2011; Tanna *et al*., 2009).

2.6.3 Hepatoprotective Activity

The hepatoprotective efficacy of *P. longifolia* leaf extracts has been demonstrated using Wistar rats exposed to hepatotoxic agents such as diclofenac sodium and carbon tetrachloride (CCl_4). Among multiple solvent fractions tested (petroleum ether, hexane, toluene, chloroform, acetone, and methanol), the methanolic extract exhibited the greatest protective effect. Doses of 300, 600, and 900 mg/kg were evaluated based on preliminary results.

Biochemical analyses indicated that the methanolic extract significantly ameliorated alterations in liver glycogen and serum markers associated with hepatic injury. In the CCl_4-induced damage model, ethanolic extracts effectively reduced elevated levels of SGOT, SGPT, ALP, and bilirubin restoring them toward normal physiological ranges.

Further corroborative evidence came from studies in mice challenged with paracetamol, where methanolic extracts of *P. longifolia* markedly preserved hepatic architecture and restored antioxidant enzyme levels. Histopathological examinations supported these biochemical findings, demonstrating reduced necrosis and improved liver morphology. These cumulative results suggest that *P. longifolia* enhances endogenous antioxidant defenses and provides substantial protection against oxidative liver damage (Verma *et al*., 2008).

2.6.4 Anti-leishmanial Activity

Investigations into the bioactivity of *Polyalthia longifolia* have shown that the clerodane diterpene 16α-hydroxycleroda-3,13(14)Z-dien-15,16-olide exhibits promising anti-leishmanial potential without inducing cytotoxic effects, as evidenced by the survival of treated laboratory animals for more than six months. The compound produced rapid, dose-responsive parasite mortality at concentrations between 2 and 50 mg/mL, achieving an IC_{50} of 8.04 mg/mL, a potency comparable to the standard drug miltefosine. Similarly, methanolic

leaf extracts of *P. longifolia* displayed significant inhibitory activity against *Leishmania donovani* promastigotes when evaluated using the MTT-based promastigote viability assay. The extract suppressed parasite proliferation in a concentration-dependent manner, yielding an IC_{50} value of 4.18 µg/mL, thereby confirming its strong in vitro antileishmanial efficacy (Pal *et al*., 2011).

2.6.5 Antifungal Activity

The antifungal potential of *P. longifolia* has been demonstrated using extracts derived from multiple solvents, including petroleum ether, benzene, chloroform, methanol, and ethanol. Among these, the petroleum ether fraction consistently showed superior inhibitory effects. Further assessments of aqueous extracts (10–50%) against ten fungal contaminants associated with paddy (*Oryza sativa L.*) revealed substantial pathogen suppression, with *Alternaria alternata* exhibiting the highest level of inhibition at 50% concentration. Other species—*Fusarium solani*, *F. moniliforme*, *Drechslera halodes*, *F. oxysporum*, *Curvularia lunata*, and *D. tetramera*—also showed inhibition values exceeding 80%, comparable to commonly applied synthetic fungicides such as Dithane M-45, Captan, Benlate, Thiram, and Bavistin at 2% treatment levels. Additional experiments evaluating aqueous extracts from the leaves and pericarp against *Fusarium oxysporum* and *Pythium aphanidermatum* (isolated from ginger rhizome rot) confirmed a concentration-dependent reduction in fungal growth (Dileep *et al*., 2013).

2.6.6 Antihyperglycemic Activity

The glucose-lowering potential of *P. longifolia* leaf extracts has been explored using alloxan-induced diabetic rat models. Diabetes was triggered by administering alloxan (180 mg/kg body weight) intraperitoneally at two 24-hour intervals, after which the extracts or powdered plant material were administered daily for one week. On the eighth day, biochemical

markers—including fasting blood glucose, serum lipids, creatinine, urea, total protein, alkaline phosphatase, and hepatic glycogen—were quantified. The results indicated that the plant extracts markedly reduced hyperglycemia, although they did not significantly alter most other biochemical indices associated with diabetes. The study concluded that, while the extracts lack strong holistic anti-diabetic activity, they do possess meaningful antihyperglycemic effects. Moreover, the extracts significantly countered sucrose-induced hyperglycemia, underscoring their potential therapeutic relevance (Nair *et al*., 2007).

2.6.7 Anti-ulcer Activity

The anti-ulcerogenic properties of *P. longifolia* were investigated using several animal ulcer models, including water-immersion stress-induced gastric lesions (300 mg/kg), aspirin-plus-pylorus ligation–induced ulcers in rats, and ethanol-induced ulcers in mice. Across all models, the ethanolic extract produced notable decreases in gastric volume, free acidity, and ulcer index relative to untreated controls. Specifically, inhibition values of 89.71% for HCl/ethanol-induced ulcers and 95.3% ulcer protection under stress-induced conditions were reported. Further evaluation using ethanol- and ethanol/HCl-induced ulcer models demonstrated that doses of 270 mg/kg and 540 mg/kg significantly mitigated mucosal injury, with the reductions in ulcer severity proving statistically substantial when compared with control animals. These findings collectively highlight the extract's strong gastroprotective efficacy.

2.7 Influence of Extraction Techniques on Phytochemical Composition and Antibacterial Activity (Cold Versus Hot Water Extraction)

The efficiency with which bioactive metabolites are recovered from plant materials is greatly affected by the extraction technique employed. Variations in temperature during extraction play a decisive role in determining both the quantity and type of phytochemicals obtained.

Hot-water extraction generally enhances the release of highly polar constituents—particularly phenolic acids, flavonoids, and related hydrophilic compounds—owing to improved solubility and diffusion at elevated temperatures (Zhang *et al*., 2018). Conversely, cold-water extraction is advantageous for conserving thermosensitive molecules that may degrade under heat, thereby producing a phytochemical spectrum that differs markedly from that of heat-assisted methods (Tiwari *et al*., 2011).

Empirical comparisons further support these distinctions. Rahman *et al*. (2020), in their investigation of *Azadirachta indica*, reported that hot-water extracts exhibited superior antimicrobial potency relative to those obtained through cold maceration. Their findings highlight the importance of extraction temperature in modulating the release and availability of antibacterial constituents, reinforcing the notion that extraction methodology can significantly influence the biological performance of plant-derived preparations.

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 Materials

Distilled water

Ager

Autoclave

Forceps

Commercial antibiotics

Filter paper

Water baths

Meter rule

Test tubes

Weighing balance

Beaker

Measuring cylinder

10% Ammonia

Conc. Sulphuric

3.2 Methodology

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

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

3.2.2Antibiotic susceptibility test

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

3.2.3 Antibacterial activity

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

3.2.4 Minimum Inhibitory Concentration

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

3.2.5 Minimum Bactericidal Concentrations

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

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

3.3 Extraction

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

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

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

3.4 Qualitative Phtyochemicals

3.4.1 Test for Flavonoids

Equipment: Weighing balance

Apparatus: Beaker, filter paper, measuring cylinder

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

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

Procedure:

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

Observation:

Appearance of yellow coloration which disappears on standing shows the presence of flavonoids (OkwuandOkwu2005).

3.4.2 Test for Tannins

Equipment: Weigh balance, water bath

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

Reagent: 0.1% Ferric chloride

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

Procedure:

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

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

3.4.3 Test for Saponins

Equipment: Weighing balance, water bath

Apparatus: Filter paper, funnel, volumetric flask, pipette

Reagent: Olive oil

Procedure:

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

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

Observation:

The formation of emulsion is a positive test for saponins

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

3.4.4 Test for Glycoside

Apparatus: Beaker, measuring cylinder

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

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

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

Procedure:

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

Observation:

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

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

3.4.5 Test for Terpenoids

Equipment: Weighing balance, water bath

Apparatus: Volumetric flask/beaker, filter paper

Reagent: chloroform, conc. Sulphuric acid

Procedure:

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

Observation:

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

CHAPTER FOUR

RESULTS

Table 4.1: Antimicrobial Activity of *Polyalthialongfolia*

		ZONE OF CONCENTRATION (mm)							
		***Polyalthialongfolia* (Hot water extract)**				***Polyalthialongfolia* (Cold water extract)**			
ORGANISM	**CONCENTRATION mg/ml**	**R1**	**R2**	**R3**	**mean ±**	**R1**	**R2**	**R3**	**mean ±**
Staphylococcus aureus	2000	80	60	60	66.7	50	50	40	46.7
	1000	70	70	40	60.0	20	20	20	20.0
	500	24	22	18	21.3	12	14	14	13.3
	250	16	20	14	13.3	12	4	6	7.3
	125	20	16	4	13.3	2	2	2	3.0
	62.5	0	0	0	0.0	0	0	0	0.0
Pseudomonas aeruginosa	2000	66	66	44	58.7	80	75	60	71.7
	1000	62	60	58	60.0	75	70	50	65.0
	500	50	50	48	49.3	60	60	24	48.0
	250	30	30	18	26.0	18	16	18	17.3
	125	0	0	0	0.0	12	10	2	8.0
Escherichia coli	2000	60	80	60	66.7	80	80	60	73.3
	1000	60	40	30	43.3	60	40	35	95.0
	500	22	22	18	20.7	20	14	12	15.3
	250	20	18	14	17.3	18	16	8	14.0
	125	0	0	0	0.0	12	10	2	8.0
	62.5	0	0	0	0.0	0	0	0	0.0

Table 4.2: Minimum Inhibitory Concentration

Isolates	Concentration (mg/ml)		ZONE OF CONCENTRATION (mm) *Polyalthialongfolia* (Hot water extract)			*Polyalthialongfolia* (Cold water extract)		
	HOT	COLD	R1	R2	R3	R1	R2	R3
Staphylococcus	120	120	12	10	8	10	8	6
aureus	100	100	10	8	6	4	4	2
	80	80	6	6	2	2	2	2
	60	60	0	0	0	0	0	0
Pseudomonas	225	225	15	18	8	22	10	16
aeruginosa	200	200	8	8	0	8	10	8
	175	175	0	0	0	10	2	4
	150	150	0	0	0	0	0	0
Escherichia	225	120	18	10	10	8	8	6
coli	200	100	14	12	0	6	6	2
	175	80	4	4	4	0	0	0
	150	60	0	0	0	0	0	0

Table 4.3: Minimum Inhibitory Concentration of *Polyalthialongfolia* (MIC).

	(mg/ml)	
Isolates	**Hot**	**Cold**
Staphylococcus aureus	80	80
Pseudomonas aeruginosa	200	175
Escherichia coli	175	100

Table 4.4: Minimum Bactericidal Concentration of *Polyalthialongfolia*

Isolates	Hot	Cold
Staphylococcus aureus	60	60
Pseudomonas aeruginosa	175	150
Escherichia coli	150	80

Table 4.5: Antibiotics Sensitivity of *Polyalthialongfolia*

Gram Positive Disc

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

Gram Negative Disc

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

Key:

R.I = Resistance Index

Resistant (R) = 0-10mm

Intermediate (I) = 11-16mm

Sensitive (S) = 17mm and above

Table 4.6: Qualitative Phytochemical Constituent of *Polyalthialongfolia*

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

Key:

+ = slightly present

++ = moderately present

- = Absent

CHAPTER FIVE

DISCUSSION AND CONCLUSION

This study investigated the antibacterial potential and phytochemical constituents of *Polyalthia longifolia* (Masquerade tree) leaves extracted using hot and cold aqueous methods. The activities of these extracts were evaluated against *Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus.* Both extraction methods produced extracts with notable antibacterial effects, although the degree of inhibition varied depending on the test organism and the extraction temperature. Phytochemical screening confirmed the presence of several bioactive secondary metabolites—particularly tannins, saponins, and flavonoids—which are widely recognized for their antimicrobial roles.

5.1 Antibacterial Activity

The antibacterial assay (Table 4.1) demonstrated that temperature influenced extract potency. Hot-water extracts generally produced larger inhibition zones against *S. aureus* and *E. coli,* reflecting the enhanced solubility and release of temperature-stable phytochemicals at elevated temperatures (Rahman *et al*., 2020). For example, at 2000 mg/mL, the hot extract inhibited *S. aureus* by 80 mm compared with 50 mm from the cold extract. Similar inhibition (80 mm) was recorded for *E. coli* in both extracts but occurred in a concentration-dependent pattern.

Interestingly, *P. aeruginosa* responded better to the cold-water extract, which produced an inhibition zone of 80 mm compared with 66 mm from the hot extract. This observation suggests that cold extraction may preserve heat-sensitive compounds crucial for inhibiting this notoriously resistant Gram-negative bacterium.

These findings align with previous studies by Uzama *et al*. (2011) and Chanda and Nair (2010), who reported that *P. longifolia* leaf extracts exhibit broad antimicrobial effects on diverse bacterial groups, although the magnitude varies with cell wall structure and extraction solvent. The reduced susceptibility of *P. aeruginosa* may be linked to its robust outer membrane and effective efflux pumps, which provide intrinsic resistance to many antimicrobial agents (Hancock & Speert, 2000).

5.2 Minimum Inhibitory Concentration (MIC)

The MIC values for the extracts ranged between 80–225 mg/mL for hot-water extracts and 80–175 mg/mL for cold-water extracts. The lowest MIC (80 mg/mL) was observed against *S. aureus* for both extraction methods, indicating strong antibacterial potential. This finding corresponds with Thenmozhi and Sivaraj (2010), who also recorded very low MIC values for *P. longifolia* extracts against *S. aureus* and *Bacillus subtilis*, reinforcing the plant's high inhibitory capacity.

5.3 Minimum Bactericidal Concentration (MBC)

MBC results revealed that while most isolates experienced bacteriostatic inhibition, the hot-water extract exerted a bactericidal effect on *P. aeruginosa.* This pattern indicates that the mode of action of *P. longifolia* extracts varies between bacterial species, especially considering the structural complexity of Gram-negative cell envelopes (Mabhiza *et al*., 2016). The bactericidal effect of the hot extract suggests the presence of thermostable compounds—likely tannins or flavonoids—that can penetrate or destabilize bacterial membranes (Biva *et al*., 2019).

5.4 Antibiotic Sensitivity Test

Antibiotic susceptibility profiles revealed differing levels of sensitivity among the test organisms, with notable resistance in *P. aeruginosa.* This aligns with the global trend of increasing resistance to conventional antibiotics such as ampicillin and ofloxacin (Ventola, 2015). Remarkably, the inhibition zones produced by *P. longifolia* extracts were comparable to, or exceeded, those produced by some standard antibiotics, particularly against *S. aureus* and *E. coli.* This supports the plant's potential for therapeutic application, a conclusion echoed by Afolabi *et al*. (2019) and Elshafie *et al*. (2023), who attributed the antibacterial and cytotoxic activities of *P. longifolia* to its diterpenoids, alkaloids, and other phytochemicals.

5.5 Phytochemical Constituents

Phytochemical analysis revealed the presence of saponins and tannins in both extracts, while flavonoids were only detected in the hot-water extract. Neither extract contained glycosides or terpenoids. The exclusive presence of flavonoids in the hot extract supports earlier findings that higher temperatures enhance the extraction of polar, thermostable compounds (Zhang *et al*., 2018). Heat-induced disruption of plant cell matrices facilitates the release of bioactive molecules such as flavonoids and phenolic acids (Tiwari *et al*., 2011).

Flavonoids are known to exhibit strong antibacterial effects through mechanisms such as nucleic acid inhibition and membrane disruption (Álvarez-Martínez *et al*., 2021), which may explain the superior activity of the hot extract. Additionally, tannins and saponins detected in both extracts corroborate previous reports by Thenmozhi and Rajeshwari (2010), highlighting their contributions to broad-spectrum antimicrobial effects. Tannins inhibit microbial enzymes and precipitate cell wall proteins (Cowan, 1999), while saponins induce membrane leakage due to their surfactant nature (Passi *et al*., 2009).

5.6 Mechanisms Underlying Observed Antibacterial Activity

The antibacterial effect of *P. longifolia* extracts appears to result from synergistic interactions between multiple phytochemicals. Flavonoids disrupt metabolic processes and compromise membrane integrity, tannins form complexes with cell wall macromolecules, and saponins increase membrane permeability (Cowan, 1999; Passi *et al*., 2009; Khameneh *et al*., 2019). These multi-target mechanisms reflect the common antimicrobial action of plant-derived compounds and help reduce the likelihood of bacterial resistance (Álvarez-Martínez *et al*., 2021).

5.7 Implications for Traditional Medicine and Future Research

The findings scientifically validate the traditional use of *P. longifolia* in the management of infectious diseases. The pronounced antibacterial activity—especially from hot-water extracts—underscores the importance of extraction temperature in herbal medicine preparation. The variations in activity between extraction methods highlight the need for standardized extraction protocols in future therapeutic applications.

CONCLUSION

This study demonstrates that both hot and cold aqueous extracts of *Polyalthia longifolia* possess significant antibacterial activity, although the hot-water extract generally exhibited greater potency due to its higher flavonoid and tannin content. The results support the plant's long-standing use in traditional medicine and indicate its potential as a source of novel, plant-based antimicrobial agents capable of combating resistant pathogens. Overall, *P. longifolia* presents strong prospects for development into phytotherapeutic formulations aimed at addressing the growing challenge of antibiotic resistance.

RECOMMENDATIONS

Further studies should incorporate quantitative assays to determine exact concentrations of flavonoids, tannins, and saponins to better correlate compound levels with antibacterial strength. Solvents like ethanol, methanol, or acetone should be included to determine whether stronger antibacterial activity can be achieved beyond water-based methods. More sensitive antimicrobial assays – such as brother microdilution, time-kill kinetics, and biofilm inhibition tests – would give deeper insight into the extract's mechanism of action. Combining *Polyalthiakongifolia* extracts with standard antibiotics could reveal synergistic effects, especially against multi-drug resistant strains like *Pseudomonas.*

Before pharmaceutical application, toxicity testing using animals models or cell i=lines is recommended to confirm safe dosage limits.

Future research should aim to establish standardized extraction temperatures, durations, and concentrations for consistent herbal formulations.

CONTRIBUTIONS TO KNOWLEDGE

The research clearly demonstrates that hot water extractin releases more potent antibacterial compounds, especially flavonoids, compared to cold extraction. This adds new insight on how temperature influences phytochemical yield in *Polyalthialongifolia.*

It confirms that both extracts possess broad-spectrum antibacterial activity against *Staphylococcus aureus, E. coli* and *Pseudomonas aeruginosa.*This validates the plant's ethnomedicinal use and expands the scientific justification for its therapeutic relevance.

This study contributes new data explaining how extraction temperature influences bacteriostatic vs. bactericidal activity, especially the stronger effect of hot extracts on gram-positive organisms.

Although widely used traditionally, the plant is under-documented in African scientific literature. This project expands local research benchmarks for West African microbiology and Phytomedicine.

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