EVALUATION OF ANTIOXIDANT CAPACITY, PHYTOCHEMICAL ACTIVITY OF THE AQUEOUS AND ETHANOLIC EXTRACT OF *Bryophyllum pinnatum*

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A PROJECT WORK SUBMITTED TO DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILMENT FOR THE REQUIREMENTS FOR THE AWARD OF BACHELORS OF SCIENCE DEGREE (B.Sc.) IN SCIENCE LABORATORY TECHNOLOGY (MICROBIOLOGY TECHNIQUES)

October, 2025.

CERTIFICATION

This is to certify that this project work, titled "EVALUATION OF ANTIOXIDANT CAPACITY, PHYTOCHEMICAL ACTIVITY OF THE AQUEOUS AND ETHANOLIC EXTRACT OF *Bryophyllum pinnatum*" was carried out by YAMAH Justus Onimisi with Matriculation Number LSC2009852 of the Department of Science Laboratory Technology (Microbiology Techniques), Faculty of Life Sciences, University of Benin, Benin City, Edo state. Under the supervision of Prof. E.O Oshomoh

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DEDICATION

This work is dedicated to God Almighty for His grace and mercies upon me throughout my undergraduate studies, and also to my parents, siblings and friends for their ever-loving support.

ACKNOWLEDGEMENTS

To God Almighty, the giver and sustainer of life, for His goodness, mercy, love, and tender care. To Him I owe the successful completion of this project work. To my supervisor, Prof. E O Oshomoh, thank you, sir, for your mentorship and unwavering support. I also wish to thank the entire staff of Microbiology option and department for their contributions to my academic journey. To my amazing and ever supportive parents and siblings, I owe this milestone to you all. And to my friends and coursemates, thank you for making this journey a memorable one, see you all at the top.

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ABSTRACT

Bryophyllum pinnatum, commonly known as "Miracle Leaf," has long been employed in traditional medicine for the treatment of infections and various oxidative stress-related ailments. This study aimed to evaluate and compare the phytochemical composition, antioxidant capacity, and antimicrobial activity of the aqueous and ethanol leaf extracts of *B. pinnatum*. Fresh leaves were collected, authenticated, air-dried, pulverized, and subjected to Soxhlet extraction using ethanol and distilled water. The extraction yields were determined, revealing a higher yield for the aqueous extract (18.3%) compared to the ethanol extract (14.3%). Preliminary qualitative phytochemical screening indicated the presence of diverse bioactive compounds, including alkaloids, flavonoids, phenols, tannins, saponins, glycosides, terpenoids, steroids, and anthraquinones in both extracts. Quantitative analysis showed that the ethanol extract contained higher concentrations of flavonoids (31.63 mg/g), phenolics (37.06 mg/g), and alkaloids (21.06 mg/g), whereas the aqueous extract exhibited elevated saponin content (31.57 mg/g). The antioxidant potential of the extracts was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) assays. The ethanol extract demonstrated superior free radical scavenging activity (IC_{50} = 63.11 µg/mL) and reducing power (345.5 µmol Fe^{2+}/g) compared to the aqueous extract, correlating with its higher phenolic and flavonoid contents. Antimicrobial activity was evaluated against clinically relevant pathogens, including *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Candida albicans,* and *Aspergillus niger* using agar well diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration/minimum fungicidal concentration (MBC/MFC) methods. Both extracts displayed dose-dependent, broad-spectrum antimicrobial effects, with Gram-positive bacteria being more susceptible than Gram-negative bacteria, and fungi showing the least sensitivity. Notably, the ethanol extract exhibited greater potency, requiring lower concentrations to inhibit and kill test organisms. These findings collectively validate the ethnomedicinal use of *B. pinnatum* and highlight the influence of extraction solvent on bioactivity. The study underscores the potential of the ethanol leaf extract as a promising source of natural antioxidants and antimicrobial agents, warranting further pharmacological and mechanistic investigations for therapeutic development

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Global rise in antimicrobial resistance and oxidative stress–related diseases has become a major public health concern, leading to an increased interest in natural products with therapeutic potential. Medicinal plants have long been a valuable source of bioactive compounds with antioxidant and antimicrobial properties, offering alternative approaches to combating infections and free radical damage (Atanasov *et al*., 2021). *Bryophyllum pinnatum* (Lam.) Oken, commonly known as the "miracle leaf" or "life plant," is a perennial herb belonging to the family Crassulaceae. It is widely distributed in tropical regions and traditionally used in the treatment of various ailments such as wounds, ulcers, infections, and inflammatory conditions (Nguyen *et al.*, 2019). The plant's leaves are rich in secondary metabolites like flavonoids, alkaloids, phenolic compounds, and glycosides, which are associated with antioxidant and antimicrobial activities (Chinelo *et al*., 2020). Antioxidants are molecules capable of neutralizing reactive oxygen species (ROS) that cause oxidative damage to cellular components. Excess ROS leads to oxidative stress, contributing to chronic diseases such as cancer, diabetes, and cardiovascular disorders (Masek *et al.*, 2022). Similarly, microbial resistance to existing antibiotics continues to limit treatment options, making it crucial to identify new sources of antimicrobial compounds (World Health Organization, 2023). Around the globe, it is consuming for the treatment and management of various pathologies such as conjunctivitis, edema, piles, cuts, eczema, constipation, epilepsy, cholera, asthma, chest colds, menstrual disorders, chicken pox and fever (Quazi *et al*., 2011). The plant parts are frequently applied for the cure of burns, rheumatoid arthritis, anti- septic, blisters, cough suppression, insect bites, psychiatric disorders and abdominal discomforts (Sadhana *et al.*, 2017). It is well-known for its anti-inflammatory, wound healing, analgesic and hemostatic qualities (Ferreira *et al*., 2014). Leaves extracts are useful for the remedy of jaundice, hypertension, renal stones and diabetes. Slightly heated leaves are applied on superficial skin infections and also used for the dropping of placenta in Southeast Nigeria, hence it act as a tocolytic agent to prevent the premature labor (Gupta *et al*., 2016; Mouhssin *et al.*, 2015). The

plant is also used for the cure of leg edema, fever, gout, abscesses, otitis and palpitations (Afzal *et al*., 2012). *Bryophyllum pinnatum* is widely utilized in ayurvedic medicines for the treatment of numerous conditions such as menorrhagia, hemorrhoids, vomiting, corns, ophthalmia and hematemesis. Root extract is being used for its hepatoprotective, laxative, diuretic and anti-psychotic effects (Afzal *et al.*, 2013). Paste of the crushed leaves is applied on skin for the treatment of boils and abscess (Saikia *et al*., 2006). In Germany, anthroposophic physicians prescribed *Bryophyllum pinnatum* Preparations for tocolysis and behavioral disorders (Simões *et al.*, 2012).

Medicinal plants have played a significant role in human health since ancient times and continue to serve as essential sources of therapeutic compounds for modern medicine. The increasing global demand for natural remedies has intensified research into plants with potent pharmacological activities, as they are often safer, more accessible, and cost-effective compared to synthetic drugs (Adeleye *et al*., 2020). Over the years, oxidative stress and microbial infections have emerged as major contributors to chronic diseases and public health challenges, highlighting the need for natural antioxidant and antimicrobial agents (Okechukwu *et al*., 2022). Herbal medicine has remained a vital aspect of primary healthcare, particularly in developing countries where up to 80% of the population depends on plant-based remedies for disease management (World Health Organization, 2019). Among these plants, *Bryophyllum pinnatum* has gained increasing scientific interest for its ethnomedicinal uses and pharmacological potential. Traditionally, the leaves of *B. pinnatum* are used in the treatment of wounds, ulcers, infections, inflammation, and hypertension (Eze *et al*., 2021). Recent studies have confirmed that its aqueous and ethanol extracts exhibit significant antioxidant and antimicrobial activities due to the presence of bioactive secondary metabolites (Oluwole *et al*., 2023). Consequently, the study of *Bryophyllum pinnatum* aligns with the global shift toward natural and sustainable sources of therapeutic agents for combating oxidative stress and microbial infections (Adekunle *et al.*, 2023).

The therapeutic potential of *Bryophyllum pinnatum* can be attributed to its rich phytochemical composition, including flavonoids, glycosides, tannins, alkaloids, and terpenoids, which are known to exhibit diverse biological properties (Akinyemi *et al.*,

2022). These bioactive constituents have been linked to the plant's antioxidant and antimicrobial capabilities, making it a promising candidate for drug discovery and development. The comparative evaluation of aqueous and ethanol extracts of *Bryophyllum pinnatum* is particularly important because solvent polarity significantly affects the extraction yield and concentration of active compounds (Adesina *et al*., 2021).

Therefore, this research focuses on the evaluation of antioxidant capacity, phytochemical constituents, and antimicrobial activity of the aqueous and ethanol extracts of *Bryophyllum pinnatum.* This study aims to scientifically validate its traditional medicinal uses and to contribute to the growing field of natural product research by identifying potential leads for pharmacological applications.

1.2 Aim and Objectives of the Study

The aim is to evaluate the antioxidant capacity, phytochemical constituents, and antimicrobial activity of the aqueous and ethanol leaf extracts of *Bryophyllum pinnatum.*

Objectives:

The following are the objectives of the study which include, to;

1. to perform qualitative phytochemical screening of the aqueous and ethanol leaf extracts of *Bryophyllum pinnatum.*

2. to determine the antioxidant activity of both extracts using standard assays.

3. to assess the antimicrobial activity of the extracts against selected pathogenic microorganisms.

CHAPTER TWO

LITERATURE REVIEW

2.1 Bryophyllum pinnatum (Lam.) Oken.

The increasing interest in medicinal plants is largely driven by their rich phytochemical composition and therapeutic properties. Among such plants is *Bryophyllum pinnatum*, a succulent perennial herb belonging to the family Crassulaceae. It is widely known for its use in traditional medicine across tropical and subtropical regions. The plant is commonly referred to as the "miracle leaf" or "life plant" because of its numerous health benefits, including wound healing, anti-inflammatory, antimicrobial, antidiabetic, and antioxidant effects. Medicinal plants are valuable sources of bioactive compounds that have shown significant pharmacological properties and potential for drug development. Due to the growing concern over the side effects and antimicrobial resistance associated with synthetic drugs, research into natural alternatives has intensified. The evaluation of antioxidant capacity, phytochemical constituents, and antimicrobial activity of plants such as *Bryophyllum pinnatum* has therefore become increasingly important (Adeleye *et al*., 2021).

2.2 Botanical Description and Distribution of *Bryophyllum pinnatum*

Bryophyllum pinnatum is a fleshy herb that can grow up to 1.5 meters in height. It has thick, succulent leaves that are simple, opposite, and serrated along the edges. Each leaf margin produces plantlets capable of developing into new plants through vegetative propagation. The flowers are bell-shaped, pendulous, and usually greenish-purple or reddish in color (Nnamani *et al.*, 2019). The plant is native to Madagascar but is now widely distributed across tropical Africa, Asia, and the Americas. It thrives in warm climates and is commonly found in gardens, roadsides, and rocky areas. In Nigeria, it is known by various local names such as “Odundun” in Yoruba, “Abamoda” in Igbo, and “Ewe Abamoda” in southwestern regions (Okechukwu *et al*., 2022). Its adaptability and ease of propagation make it readily available for medicinal and research purposes.

2.3 Taxonomy of Bryophyllum pinnatum

Kingdom: Plantae

Phylum: Spermatophyta (also referred to as Tracheobionta or Anthophyta)

Class: Magnoliopsida (also referred to as Dicotyledoneae)

Order: Rosales

Family: Crassulaceae

Genus: Bryophyllum (formerly a separate genus, now sometimes considered a subgenus of Kalanchoe)

Species: pinnatum (sometimes listed as Kalanchoe pinnata)

2.4 Ethnomedicinal Properties of *Bryophyllum pinnatum*

Bryophyllum pinnatum (Lam.) Oken, also known as the "miracle leaf," "life plant," or "resurrection plant," is widely used in traditional medicine across tropical and subtropical regions. It holds a prominent place in ethnomedicine because of its remarkable healing abilities and its wide range of therapeutic applications. Various parts of the plant particularly the leaves are used in herbal formulations to treat numerous diseases such as wounds, ulcers, kidney stones, hypertension, diabetes, and microbial infections (Aye *et al*., 2020). In Africa, *Bryophyllum pinnatum* is commonly used as a household remedy. The leaves are chewed or boiled in water to relieve cough, chest pain, and asthma symptoms, while the fresh leaf juice is often applied directly to wounds, burns, boils, and skin infections to promote healing (Akinpelu *et al*., 2019). Traditional healers in Nigeria and Ghana also use the aqueous extract to treat malaria, fever, ear-ache, and dysentery (Saha *et al*., 2021). In some West African communities, women use decoctions of the leaves as a uterotonic agent to aid childbirth and to prevent postpartum infections (Agbor *et al*., 2020). Similarly, in Indian Ayurvedic medicine, *B. pinnatum* is known as "Parnabija" and is prescribed for the treatment of urinary disorders, ulcers, dysentery, and cough (Mohan *et al.*, 2018). The plant is also valued for its anti-inflammatory, analgesic, and diuretic properties. In traditional Chinese medicine, the leaves are used to clear toxins, stop bleeding, and treat conditions like high blood

pressure and gastric ulcers (Sultana *et al.*, 2018). Among Caribbean populations, *B. pinnatum* is popularly called “leaf of life” and is used as a tea for treating colds, bronchial infections, and hypertension (Saha *et al*., 2021). The ethnomedicinal relevance of *Bryophyllum pinnatum* is strongly linked to its phytochemical composition. Studies have shown that its leaves contain a wide array of bioactive compounds including flavonoids, alkaloids, glycosides, phenols, tannins, terpenoids, and saponins (Aye *et al.*, 2020; Alhassan *et al.*, 2021). These constituents are known to contribute significantly to the plant’s pharmacological effects. For instance, the flavonoids and phenolic compounds exhibit strong antioxidant properties, which help in scavenging free radicals and reducing oxidative stress (Sultana *et al.*, 2018). The saponins and tannins are responsible for antimicrobial and anti-inflammatory activities, while alkaloids contribute to its analgesic and hypotensive actions (Mohan *et al*., 2018).

Modern pharmacological investigations have validated many of these traditional claims. Extracts of *B. pinnatum* have demonstrated wound healing potential by enhancing fibroblast proliferation and collagen synthesis (Ojewole, 2005). Its antimicrobial activity has been confirmed against several pathogenic bacteria and fungi, including *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*, and *Candida albicans* (Akinpelu *et al*., 2019). Additionally, the plant exhibits hepatoprotective, nephroprotective, and antidiabetic properties in experimental models (Saha *et al*., 2021; Alhassan *et al*.,2021). These findings support the ethnobotanical knowledge that *Bryophyllum pinnatum* is an important natural remedy with broad therapeutic applications. Beyond its medicinal uses, *B. pinnatum* also holds cultural and spiritual importance in several traditional societies. In parts of Nigeria and India, the plant is regarded as sacred and is believed to possess protective powers against evil spirits. It is sometimes planted around homes or used in religious rituals for its symbolic association with healing and renewal (Agbor *et al*., 2020). This deep-rooted cultural significance underscores the long-standing relationship between humans and medicinal plants, and the reliance on nature for holistic health management. Overall, the ethnomedicinal properties of *Bryophyllum pinnatum* demonstrate the plant’s immense value in traditional healthcare systems. Its widespread use across continents, coupled with increasing scientific validation, makes it a promising candidate for drug development and pharmacological

research. Continued studies on its bioactive compounds, safety profile, and therapeutic mechanisms could lead to the development of effective natural remedies for various diseases, aligning traditional knowledge with modern scientific evidence (Saha *et al.*, 2021).

2.5 Pharmacological Properties of *Bryophyllum pinnatum*

Beyond its antioxidant and antimicrobial properties, *Bryophyllum pinnatum* exhibits a wide range of pharmacological activities including anti-inflammatory, antidiabetic, antihypertensive, analgesic, and anticancer effects (Eze *et al*., 2021). Its leaf extracts have been shown to reduce pain and inflammation by modulating prostaglandin synthesis and suppressing cytokine production (Akinyemi *et al.*, 2020). Studies have also reported hepatoprotective effects, suggesting that the plant may support liver function by mitigating toxin-induced damage (Oluwole *et al*., 2022). The antidiabetic potential of *Bryophyllum pinnatum* has been linked to its ability to stimulate insulin secretion and improve glucose tolerance (Adekunle *et al*., 2023). Moreover, its anticancer activity is thought to involve the induction of apoptosis and inhibition of angiogenesis, particularly in breast and colon cancer cell lines (Adewale *et al*., 2019). These findings underline the pharmacological versatility of *Bryophyllum pinnatum* and its relevance in drug development.

2.5.1 Phytochemical Constituents of *Bryophyllum pinnatum* ;

Phytochemicals are naturally occurring compounds in plants that contribute to their color, flavor, and resistance to diseases. The major phytochemicals found in *Bryophyllum pinnatum* include flavonoids, alkaloids, tannins, saponins, glycosides, terpenoids, and phenolic compounds (Oluwole *et al*., 2021). These bioactive constituents play crucial roles in the plant's pharmacological effects and are responsible for its antioxidant and antimicrobial properties. Flavonoids and phenolic compounds are well-known antioxidants that help in neutralizing free radicals, thereby reducing oxidative stress and cellular damage (Akinyemi *et al.*, 2022). Alkaloids possess antimicrobial and analgesic activities, while saponins exhibit surface-active properties that contribute to antibacterial and antifungal actions. The presence of these compounds in both aqueous and ethanol extracts makes *Bryophyllum pinnatum* a potential candidate for natural drug formulation.

The qualitative and quantitative presence of these phytochemicals may vary depending on environmental conditions, extraction methods, and solvent polarity. Ethanol, being an organic solvent, tends to extract more nonpolar compounds, while aqueous extraction favors polar compounds. Therefore, comparative studies of both extracts provide insight into their chemical diversity and biological potential (Adesina *et al*., 2021).

2.5.2 Antioxidant Activity of *Bryophyllum pinnatum ;*

Antioxidants are substances that inhibit or delay oxidative processes caused by reactive oxygen species (ROS) and free radicals. Excessive ROS generation can lead to oxidative stress, which contributes to the development of chronic diseases such as cancer, diabetes, and cardiovascular disorders (Olowokudejo *et al*., 2020). The antioxidant capacity of *Bryophyllum pinnatum* has been attributed to its rich composition of phenolic and flavonoid compounds that scavenge free radicals and chelate metal ions.

Studies have demonstrated that both aqueous and ethanol extracts of *Bryophyllum pinnatum* exhibit significant antioxidant activities through assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing antioxidant power (FRAP), and hydrogen peroxide scavenging (Eze *et al.*, 2020). The ethanol extract generally shows higher antioxidant potential, likely due to the enhanced solubility of phenolic compounds in organic solvents. These findings suggest that *Bryophyllum pinnatum* may serve as a natural source of antioxidant agents.

2.5.3 Antimicrobial Activity of *Bryophyllum pinnatum* ;

Antimicrobial resistance has become a major global health threat, driving the search for new therapeutic agents from natural sources (World Health Organization, 2020). Plants like *Bryophyllum pinnatum* have gained research interest due to their rich phytochemical composition that confers broad-spectrum antimicrobial properties. The antimicrobial effect of *Bryophyllum pinnatum* has been reported against various bacterial and fungal pathogens, including *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, and Aspergillus niger* (Nwankwo *et al.*, 2020).
The antimicrobial mechanisms of *Bryophyllum pinnatum* extracts are often linked to the disruption of microbial cell membranes, inhibition of enzyme activity, and interference

with nucleic acid synthesis (Okafor *et al*., 2021). Phytochemicals such as alkaloids, saponins, and tannins are believed to act synergistically to enhance these effects (Nwankwo *et al*., 2020). For instance, saponins may increase cell membrane permeability, allowing other active constituents to penetrate and exert bactericidal or fungicidal actions. Comparative studies have revealed differences in antimicrobial efficacy between solvent extracts. Ethanol extracts of *B. pinnatum* generally show stronger inhibitory effects than aqueous extracts, possibly due to better solubility of phenolic compounds in organic solvents (Okechukwu *et al*., 2023). These findings suggest that extraction method significantly influences antimicrobial potency, and optimizing solvent systems can enhance the therapeutic applications of the plant.

2.6 Mechanisms of Action of Plant-Derived Extracts

The pharmacological activities of plant extracts are the result of complex interactions among different phytochemicals that target multiple cellular pathways. The antioxidant and antimicrobial properties of *Bryophyllum pinnatum* are primarily attributed to its flavonoids, phenols, and alkaloids, which act through mechanisms such as free radical scavenging, enzyme inhibition, and metal ion chelation (Nnamani *et al*., 2019). Flavonoids inhibit lipid peroxidation and neutralize reactive species, thereby protecting cell membranes and proteins from oxidative damage (Ogunbiyi *et al*., 2021). Phenolic compounds also contribute to the redox balance of cells by donating hydrogen atoms or electrons, stabilizing free radicals (Awodoyin *et al*., 2020). The synergistic effects of these compounds enhance the bioactivity of plant extracts, often yielding results comparable to or exceeding those of synthetic drugs.

In the context of antimicrobial activity, secondary metabolites such as alkaloids and tannins interfere with bacterial quorum sensing, inhibit protein synthesis, and alter cell wall permeability (Olowokudejo *et al*., 2021). These biochemical mechanisms provide a scientific basis for the traditional use of *B. pinnatum* in treating infections and wounds.

2.7 Phytochemicals

Phytochemicals are naturally occurring compounds in plants that are responsible for their distinctive color, flavor, and aroma. These compounds are not classified as essential nutrients, but they play important roles in maintaining plant and human health. In plants,

they act as defense agents against pathogens, herbivores, and ultraviolet radiation, while in humans, they exert numerous biological effects that can help prevent or manage diseases (Balasundram *et al*., 2022).

Phytochemicals are secondary metabolites, meaning they are not directly involved in the plant's primary metabolic processes such as growth and reproduction, but are essential for its adaptation and survival in the environment. They are broadly grouped into major classes such as flavonoids, alkaloids, tannins, phenolics, terpenoids, saponins, and glycosides, each with its unique structure and biological function (Tungmunnithum *et al*., 2018). Flavonoids and phenolic compounds, for example, are well-known for their antioxidant activity and their ability to scavenge free radicals that cause oxidative damage to cells. Alkaloids often display antimicrobial, analgesic, and anti-inflammatory effects, while terpenoids and saponins possess properties that enhance immune function and reduce inflammation (Abarikwu, 2021). The biological importance of phytochemicals arises from their ability to interact with cellular signaling pathways, modulate enzyme activity, and influence gene expression. Through these mechanisms, phytochemicals can help in the prevention of chronic illnesses such as cancer, cardiovascular diseases, diabetes, and neurodegenerative disorders (Rahman *et al*., 2021). Some phytochemicals act as natural antibiotics, inhibiting the growth of bacteria and fungi by disrupting their membrane structures or interfering with their metabolic enzymes (Bello *et al.*, 2019). Others play chemopreventive roles by inducing detoxifying enzymes that help eliminate carcinogenic compounds from the body (Rani *et al*., 2022).

The increasing attention given to plant-based diets and herbal remedies in modern medicine is largely due to the health benefits derived from phytochemicals. A diet rich in fruits, vegetables, and medicinal plants has been consistently associated with reduced risk of chronic diseases and improved overall well-being (Balasundram *et al*., 2022). In particular, medicinal plants such as *Bryophyllum pinnatum* have been shown to contain significant amounts of phytochemicals including flavonoids, alkaloids, and phenolic compounds that are linked to its anti-inflammatory, antimicrobial, and wound-healing properties (Adesegun *et al.*, 2020). The synergy among these bioactive compounds enhances the overall therapeutic potential of *Bryophyllum pinnatum*, making it a valuable

component in traditional medicine. These phytochemicals not only support the plant's survival but also contribute to its pharmacological efficacy in humans. As research continues to advance, phytochemicals are increasingly recognized as vital contributors to preventive healthcare and the development of plant-based pharmaceuticals (Ogunyemi *et al*., 2020).

Antioxidants

Antioxidants are substances that help protect the body's cells and tissues from damage caused by free radical's unstable molecules produced during normal metabolic reactions or through exposure to environmental stressors such as pollution, radiation, and infections (Pizzino *et al.,* 2017). When free radicals accumulate beyond the body's natural defense capacity, oxidative stress occurs, leading to cellular injury and the onset of various diseases such as diabetes, cancer, cardiovascular disorders, and premature aging (Rani *et al*., 2022). Antioxidants can be classified as either endogenous or exogenous, depending on their source. Endogenous antioxidants are synthesized naturally in the body and include enzymatic molecules such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which function as the body's first line of defense against oxidative damage. Exogenous antioxidants, on the other hand, are obtained from the diet, primarily through the consumption of plant-based foods rich in vitamins C and E, carotenoids, phenolic compounds, and flavonoids (Uddin *et al*., 2020). These compounds protect cellular components by scavenging reactive oxygen and nitrogen species, chelating metal ions that catalyze oxidation, and upregulating the body's antioxidant enzymes (Rahman *et al.,* 2021). Plant-derived antioxidants are particularly important because they not only neutralize harmful radicals but also boost the body's own defense systems. Their mechanisms involve donating hydrogen atoms or electrons to stabilize free radicals, interrupting oxidative chain reactions, and enhancing the repair of oxidized biomolecules (Gülçin, 2023). Furthermore, antioxidants can influence cellular signaling pathways such as Nrf2, which regulates the expression of protective enzymes and stress-response genes (Pizzino *et al.*, 2017).

In herbal medicine, antioxidants are considered the cornerstone of many therapeutic actions. They play crucial roles in tissue protection, wound healing, and the prevention of

inflammation-related disorders. For instance, studies have shown that *Bryophyllum pinnatum* possesses strong antioxidant potential due to the presence of flavonoids and phenolic compounds capable of scavenging DPPH and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radicals (Olabinri *et al.*, 2019). These antioxidant activities help reduce oxidative stress markers and maintain cellular redox balance, supporting its traditional use in the management of various ailments such as ulcers, burns, and inflammatory conditions. Antioxidants also contribute to the preservation of cellular integrity by preventing lipid peroxidation a process that damages cell membranes and accelerates aging. By maintaining membrane stability and protecting biomolecules, antioxidants enhance metabolic efficiency and overall vitality. The regular consumption of antioxidant-rich plants and foods, therefore plays a major role in disease prevention and the promotion of long-term health. Medicinal plants like *Bryophyllum pinnatum* stand out as potent sources of natural antioxidants, bridging the connection between nutrition and therapy in both traditional and modern medicine (Uddin *et al.*, 2020).

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

The following materials were used in this study:

3.1.1 Apparatus/ Equipment

Bench autoclave (Gallenkamp, U.K.), Binocular Microscope (Olympus), Incubator (size 2, Gallenkamp, U.K.), Hot air oven (size 2, Gallenkamp, U.K.), Weighing balance (H80, Mettler, Switzerland), Centrifuge (MSE High speed 18), Water bath (Gallenkamp, U.K.), Spectrophotometer (SP8-400 uv/ visible, PYE UNICAM England), Soxhlet apparatus, Glass wares (pyrex burettes, pipettes, beakers, microscopic slides, glass petri diches, measuring cylinders, flasks, separating funnels, bijou, universal and Macartney bottles).

3.1.2 Microbiological Media

Nutrient Agar (BIOTECH, TM 341, India), Mueller Hinton Agar (BIOTECH, TM 339, India), Nutrient Broth (BIOTECH, TM 350, India), Sabouraud Dextrose Broth (BIOTECH, TM 361, India), Potato Dextrose Agar (BIOTECH, TM 387, India),

3.1.3 Chemicals/Reagents

All chemicals used were of analytical grade and they include, Ethanol (99.89%), Distilled water, n-Hexane, Hydrogen peroxide, 1% Tetramethyl-p-phenylenediamine hydrochloride (Oxidase reagent), Phenolphthalene, 0.1N NaOH, Tween-80 (10%), Picric acid, wagner reagent, Dragendroff's reagent, Methylated spirit, Crystal violet(0.5%$^{w}/_{v}$, BEMA), Safranine (BEMA), Grams iodine, Plasma, Kovac's reagent (Merk 6029259559), 1% Barium chloride, 1% sulfuric acid (H_2SO_4), Dettol, Glycerol, Starch, Glycerin, Sodium chloride, Fehling's solution A and B, Ferric chloride, Sodium picrate, dilute ammonia solution.

All media and reagents used were prepared according to the manufacturer's direction.

3.1.4 Antimicrobial agents

Ciprofloxacin (Sigma-Aldrich Biochemika, USA), Nystatin (Sigma-Aldrich Biochemika, USA) and *Bryophylum pinnatum* – Miracle leave (ethanol and distilled water extracts).

3.1.5 Source of Test Microorganisms

The microbial isolates used were obtained from stock cultures of clinical isolates from cases of Nosocomial infections from University of Benin teaching Hospital (UBTH) and stored as stock cultures in Pharmaceutical Microbiology and Biotechnology Department of Faculty of Pharmacy, University of Benin (UNIBEN). The selected isolates include

Staphylococcus aureus, *Bacillus subtilis, Klebsiella pneumonia, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger*.

3.1.6 Sterilization of materials

The oven and autoclave were used to sterilize various materials. Glass-wares such as test tubes, glass rod, pipette, measuring cylinder, beakers and conical flasks used for the research work were soaked and washed using detergent and rinsed several times with distilled water. They were then wrapped with aluminium foil paper and dried in the oven in an inverted position at 160-170^{o}C for 45-60 minutes.

3.2 METHODS

3.2.1 Source, collection and Identification of Plant Samples

The *Bryophylum pinnatum* were sourced from home Gardens within University of Benin Residential communities. After acquisition of sufficient quantity, the samples were transported to the Plant Biology and Biotechnology laboratory, where it was Identified and Authenticated by Prof. H. Akinnibosun as well as my supervisor (Prof. Oshomoh) as *Bryophylum pinnatum* (Mirale Leave).

3.2.2 Preparation and Extraction of Plant Sample

The plant samples without the roots were obtained for the experiment and rinsed with distilled water and air dried for two week in the laboratory before pulverization (milling into fine powder) and Sohxlet extraction using ethanol and distilled water. In the process, specific mass of the powdered sample was weighed and poured into an extraction thimble of a sohxlet apparatus, the solvents (2500 mL) were separately introduced into the round bottom flask, placed in the heating mantle and the apparatus was coupled to the Julabo recirculating cooling system and the apparatus was turned on and the extraction process continued until the solvent leaving the thimble became clear. After which the liquid extracts collected was evaporated to dryness using rotary evaporator and thermostatically regulated water-bath. The yields upon concentration to dryness were weighed, the percentage yield calculated and the extracts were stored in sterilized sample bottles and kept in the refrigerator at 4°C for subsequent assay (Alara *et al*., 2012; Dowe *et al*., 2020).

3.2.3 Determination of Percentage yield

The percentage yield of the plant extracts was determined using the formula:

$$Percentage\ (\%)yield = \frac{Weight\ of\ plant\ extract}{Wt.\ of\ pulverised\ powder} x\ \frac{100}{1}$$

1.) Percentage yield of the ethanol extract:

Mass/weight of extract = 28.6g

Mass of pulverized powder = 200.0g

$$Percentage\ (\%)yield = \frac{28.6}{200} x\ \frac{100}{1}$$

Percentage yield = 14.3%

2.) Percentage yield of the aqueous extract

Mass/weight of extract = 36.6g

Mass of pulverized powder = 200g

Percentage (%) yield = 36.6/200 x 100

Percentage yield = 18.3%

3.2.4 Antimicrobial assay of the extracts

The modified agar well diffusion method described by Cheesbrough, (2006) and CLSI, (2010) was used to determine the antimicrobial sensitivity/potency of the ethanol and aqueous extracts of *Bryophylum pinnatum* against test organisms. In the process, wells of 6mm in diameter were made into seeded Mueller Hinton agar (antibacterial sensitivity) and Sabouraud dextrose agar (antifungal sensitivity) plates using a flamed cork borer. Prior to seeding, isolated colonies/spores stored in slants were sub-cultured into nutrient broth/sabouraud dextrose broth, vigorously shaken and adjusted to achieve 1:100 dilution of 0.5 Macfarland tubidity standard (containing approximately 10^6 cfu/spores per mL when counted using a cytometer) previously determined using a spectrophotometer. Sterile swab sticks was then dipped into the standardized microbial suspension and gently spread over (seeding) the surface of the agar plates in even strokes to obtain a uniform growth pattern across the entire surface of the plate. The 6mm wells were filled with equal volumes (100µL) of the stock concentration and lower dilutions of the sample corresponding to 100, 50 and 25 mg/mL concentrations. The same quantity of sterilized normal saline and 1 µg/mL Ciprofloxacin (bacterial plates)/10µg/mL Nystatin served as negative and positive controls respectively. All plates were appropriately incubated i.e

24hrs, 38°C for bacterial plates and ambient temperature (27±2°C) for 48-72hrs for fungal plates in an upright position to allow proper diffusion of extracts. All experiments were in triplicates. After incubation, the absence or presence of microbial growth around the wells were observed on the plates and the diameter of clear zones were measured using a millimetre (mm) calibrated ruler and the mean Inhibition zone diameters (IZDs) calculated and recorded.

3.2.5 Determination of Minimum Inhibitory Concentration (MICs) of the selected antimicrobial agent

The modified broth dilution method described by Firas *et al*. (2008), was used to determine the MICs of the extracts against the test isolates. Varying concentrations of the selected antimicrobial agent ranging from 0.1 -10 mg/mL were constituted in 10 ml of Mueller-Hinton broth in sterile capped tubes from the stock. 100µL of the overnight broth culture of the test standardized microbial suspension. In each round of experiment, a tube without the extract but with same volume of broth and inoculum served as controls. The same experiment was repeated for the fungal isolate but Sabouraud dextrose broth was used in place of Mueller-Hinton. All tubes were appropriately incubated. After incubation, tubes were observed for growth/turbidity. In all cases, the lowest concentration of the extract at which there was no observable bacterial or fungal growth was recorded as the MICs.

3.2.6 Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) of the Extracts

The broth tubes with no visible growth following MIC determination were inoculated into fresh Nutrient agar/SDA plates using a flamed inoculating loop. Three MIC experimental tubes with concentrations beginning from MIC and progressively higher than the MIC concentrations were considered after which all plates were appropriately incubated (bacterial plates at 38°C for 24 hours and fungal plates at 27±2°C/room temperature for 48 hours). After incubation, all plates were observed for growth and the MBC/MFC was recorded as the lowest concentration of extracts that completely destroyed the microbial cells indicated as no observable growth of test organisms inoculated from tubes into the fresh agar plates (Lalitha, 2004; CLSI, 2010; Dowe *et al*., 2016).

3.2.8 Qualitative Phytochemical Analyses

Qualitative screening of the phytochemical components of the plant extracts was carried out using the modified method described by sexena *et al*., (2013). Essentially, specific weight of the extracts was made up to 10 ml in a test tube and different reagents were added to specifications. Positive results were indicated by colour change and precipitate formation which were compared against standards. The extracts were tested for the presence of glycosides, alkaloids, tannins, saponins, anthraquinones, phenolics, steroids, resins, terpenoid and flavonoids.

1. **Test for saponins**

To 1g of the plant extract was added 20 ml of distilled water and heated for 5minutes. 4ml of the solution was measured into a test tube and 2ml of distilled water was added with vigorous shaking, after which it was allowed to stand for 6 minutes. A stable frothing or foaming indicates the presence of saponins.

2.Test for anthraquinone

One gram (1 g) of the extract was shaken vigorously with 10 ml of chloroform. To 4 ml chloroform extract was added 10 % ammonium hydroxide solution (2 ml). Observation of color change to orange indicates the presence of anthraquinones

3. **Test for steroids**

One gram (1 g) of the extract was extracted with 20 ml methanol, by heating on a water bath. It was filtered and the filtrate evaporated to dryness. A little quantity of the residue obtained from the filtrate was dissolved in 2 ml of chloroform. Sulphuric acid was carefully added by the side of the test tube to form a lower layer.

4.Test for tannins

One gram (1 g) of the extract dissolved in a tube up to 2ml plus two drops of 5 % ferric chloride. The presence of reddish brown precipitate confirmed the presence of tannins.

5. **Test for flavonoids**

To 2 ml of the filtrate obtained above, 1 ml of sodium hydroxide was added, and then 1 ml conc. HCl was added. Formation of cloudy precipitate confirms the presence of flavonoids.

6. Phenolics

One milliliter of the extract was added to 1 mL of 10% $FeCl_2$ and mixed together. The presence of blue precipitate confirmed the presence of phenols.

7. Tests for alkaloids

Two grams (2 g) of the extract was dissolved in 5 ml 1 % sulphuric acid and filtered. The filtrate was tested with alkaloidal reagents (Dragendorff, Wagner, Mayer and Hager). In the process, the filtrates are collected in various test tubes. To a tube containing the filterate, a few drops of Wagner's Reagent (Potassium-iodine solution) were added to one part of the filtrate in a test tube. A reddish brown precipitate formation gives a positive result. Generally, the formation of specific precipitate and coloration upon adding drops of Dragendrorff, Wagner, Mayer and Hager's reagent indicates positive results or presence of alkaloids.

8. Test for Resins

To 0.2g of the extract in the test tube was treated with 15 ml of ethanol (98%), vortexes for two minutes and 2ml of the alcoholic extract was then poured into 10 ml of distilled water in test tube, vortexed again for two minutes and allowed to stand for 5minutes undisturbed. The tube was then observed for precipitate formation. A precipitate occurring indicates the presence of resins.

9. Test for terpenoids

A quantity (9ml) of ethanol was added to 1g each of the extracts and refluxed for a few minute and filtered. Each of the filtrates was concentrated to 2.5ml in a boiling water bath. Distilled water, 5ml was added to each of the concentrated solution, each of the mixtures was allowed to stand for 1 hour and the waxy matter was filtered off. Each of the filtrates was extracted with 2.5ml of chloroform using a separating funnel. To 0.5ml each of the chloroform extract was evaporated to dryness on a water bath and heated with 3ml of concentrated sulphuric acid for 10 minutes on a water bath. A grey colour indicates the presence of terpenoids.

10. Test for glycosides

To 5ml of the extract in tubes treated with glacial acetic acid containing 1drop of ferric chloride (0.1%) was added to 1ml of concentrated H_2SO_4. A brownish to brick red ring or violet colour at the interphase indicates the presence of glycosides.

3.2.9 Quantitative Phytochemical Composition

After preliminary analysis to determine presence of these phytochemicals, the samples were further subjected to quantitative analysis to determine the percentage of each of these secondary metabolites present the plant extracts. The following procedures were adopted:

1. Determination of total phenolics compounds

The total phenol content was determined using a standard caliberation curve as described by sexena *et al*., (2013). To 1ml of samples/extracts in test tube was mixed with methanol (5 g/L) and further mixed with ethanol solution of gallic acid (1 mL; 0.025-0.400 mg/mL) with 5 mL of Folin-Ciocalteu reagent (diluted tenfold) and sodium carbonate (4 mL, 0.7 M) solution and ultimately the volume was made up to 8 ml with distilled water followed by vigorous shaking and was allowed to stand for 30mins, after which absorbance values were measured at 765 nm using a spectrophotometer and the standard curve was plotted to determine the total phenolic contents. All experiments were carried out in triplicate. The total phenolics components in the extracts in gallic acid equivalents (GAE) were calculated by the formular:

$T = CxV/M$

Where:

T = total phenolic contents, milligram per gram of sample extract, in GAE

C = the concentration of gallic acid established from the calibration curve, mg/mL

V = the volume of extract, milliliter

M = the weight of sample/extract (g)

Or

percentage phenol extracted from powdered sample thus:

$$\text{Phenols (\%)} = \frac{100}{W} \times \frac{C}{1000} \times \frac{VF}{VA} \times \frac{D}{1}$$

Where:

W = Weight of sample analysed

C = Concentration of standard in mg/ml

VF = Total filtrate volume

VA = Volume of filtrate analysed

D = Dilution factor where applicable

2. Determination of tannin content

The tannin content was determined by Folin Denis colorimetric method described by Sexena *et al*. (2013). Briefly, Five grams of the powdered sample was measured into a volumetric flask and 50 mL of distilled water was added to the content of the volumetric flask. The mixture was shaken for 30 min at room temperature and filtered to obtain the filtrate. A standard tannic acid solution was prepared, 2 mL of the standard solution and equal volume of distilled water were dispersed into a separate 50 mL volumetric flasks to serve as a standard and reagent blank respectively. Then 2 mL of each of the respective experimental samples were measured into their respective labeled flasks. The content of each flask was mixed with 35 mL distilled water and 1 mL of the Folin reagent . This was followed by 2.5 mL of saturated Na_2CO_3 solution. Therefore, each flask was diluted to the 50 mL mark with distilled water and incubated for 90 min at room temperature. After which their absorbance was measured at 760 nm in a spectrophotometer with the reagent blank at zero. The tannin content was calculated as shown below:

$$\text{Tannin (\%)} = \frac{100}{W} \times \frac{au}{as} \times C \times \frac{Vt}{Va}$$

Where: W = Weight of sample

au = Absorbance of test sample

as = Absorbance of standard tanning solution

C = Concentration of standard tannin Solution

Vt = Total volume of extract

Va = Volume of extract analyzed

3. Determination of total flavonoids

The method is based on the formation of the flavonoids-aluminium complex which has an absorptivity maximum at 415nm. 100µl of the sample/extracts in methanol (10 mg/ml) was mixed with 100 µl of 20 % aluminum trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5ml. The absorbance at 415 nm was read after 40 minutes. Blank samples were prepared from 100 ml of plant extracts and a drop of acetic acid, and then diluted to 5ml with methanol. The absorbance of standard rutin solution (0.5 mg/ml) in methanol was measured under the same conditions. All experiments were carried out in triplicates.

4. Determination of total alkaloids

To 5g of the sample weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed

Percentage alkaloids were computed as follows:

$$\text{Alkaloids (\%)} = \frac{(W2-W1)}{\text{Weight of sample}} \times 100$$

Where:

(W_2- W_1) = Weight of residue

5. Determination of total saponins

The total saponin was done by the double solvent extraction gravimetric method decribed by Sexena *et al*. (2013). Briefly, 5g of sample was mixed with 50 mL of 20% aqueous ethanol solution and incubated for 12 h at a temperature of 55^{o}C with constant agitation. After that, the mixture was filtered through Whatman No. 42 grades of filter paper. The residue was re-extracted with 50 mL of the ethanol solution for 30 min and the extracts weighed together. The combined extract was reduced to about 40 mL by evaporation and then transferred to a separating funnel and equal volume (40 mL) of diethyl ether was added to it. After mixing well, there was a partition and the other layer was discarded while the aqueous layer was reserved. This aqueous layer was re-extracted with the ether after which its pH was adjusted with drop-wise addition of dilute NaOH solution. Saponin in the extract was taken up in successive extraction with 60 and 30 mL portion of normal butanol. The combine extract was washed with 5% NaCl solution and evaporated to dryness in a previously weighted evaporating dish. The saponin was then dried in the oven at 60^{o}C (to remove any residual solvent) cooled in a desiccators and re-weighed. The saponin was determined and calculated as a percentage of the original samples.

Saponin (%) = (W2-W1/W) x 100

Where: W = Weight of sample used

W1 = Weight of empty evaporation dish

W2 = Weight of dish + saponin extract

6. Determination of total glycosides

The digested glycoside content of the sample was determined using the method described by Gilliani *et al.*, 2007 and Sexena *et al*., 2013. In the process, 5g of the sample was dissolved in 250 ml of distilled water and treated with glacial acetic acid containing 1drop of ferric chloride (0.1%) and introduced into a beaker containing 1ml of concentrated H_2SO_4 with continuous agitated for 3hours using a shaker, followed by filtration. After which 10 ml of freshly prepared 0.10% Anthrone reagent was added, stoppered and mixed thoroughly by gently shaking. The experiment was repeated to obtain a blank using distilled water in place of sample. After which samples obtained were transferred to spectrophotometer and absorbance read at 630 nm against the blank. The total available glycosides was then calculated accordingly:

$$\text{Glycoside (\%)} = \frac{25\ A_1 \times 100}{W \times A_2}$$

Where : W = weight of sample

25 = Constant

A_1 = Absorbance of diluted sample

A_2 = Absorbance of diluted standard

3.3 Invitro Antioxidant assay

1.) DPPH radical scavenging assay

Free radical scavenging ability of the sample/extracts was tested by DPPH radical scavenging assay as described by Jha*et al*., (2018). Summarily, a solution of 0.1 mM DPPH in methanol was prepared, and 2.4 mL of this solution was mixed with 1.6 mL of extract in methanol making a whole volume of 3mL in per test-tubes of different concentrations (15–960 µg/mL). The reaction mixture was vortexed thoroughly and left in the dark or incubated with complete foil masking in the dark at ambient trmperature (27±2ºC) for 30 min. The hydrogen atom donating ability of the sample was determined by the decolorization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH produces violet/purple color in methanol solution and fades to shades of yellow color in the presence of antioxidants which indicates a positive result and characterized by decrease in absorbance readings. The absorbance of the mixture was measured

spectrophotometrically at 517 nm. Ascorbic acid was used as reference or positive control while tubes with reagents without sample served as (negative control). The blank correction was a preparation of the extract concentration in the reference solvent (without DPPH reagents). Percentage DPPH radical scavenging activity was calculated by the following equation:

% DPPH radical scavenging activity (% RSA) = {(A0− A1)/A0}×100

where A_0 is the absorbance of the control, and A_1 is the absorbance of the extractives/standard.

Or % Inhibition = $\frac{A_{control}-(A_{sample}-A_{sample\ blank})}{A_{control}} \times 100$

Where $A_{control}$ = Absorbance of DPPH in methanol (negative control)

Then % of inhibition was plotted against concentration, and from the graph IC_{50} was calculated. **IC_{50} estimation is given by** IC_{50} = concentration giving 50% inhibition. Determine by plotting % inhibition vs log(concentration) and interpolate, or rather from a linear interpolation between the two points that straddle 50%. All experiment was done in triplicates for each concentration.

2.) Ferrous reducing antioxidant Potential (FRAP) assay

The ferrous reducing antioxidant Potential (FRAP) of samples was evaluated by the method described by Baydar and Baydar (2013). Accordingly, the freshly prepared stock solution contain 300 mM acetate buffer (3.1g C2H3NaO2.3H2O and 16 M C2H4O2), pH 3.6, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 nMHCl, and 20 mM FeCl3.H2O solution. The extracts (1.5 ml) were allowed to react separately with 2.85 ml of the FRAP solution incubated for 5-30 min in the dark in a water bath at 37^{o}C and readings (absorbance) of the coloured product (ferrous tripyridyltriazine complex) were then taken at 593 nm.

The standard curve of FeSO4 (absorbance vs [Fe^{2+}] µM) was made after conversion of sample absorbance to µmol Fe^{2+} equivalent per gram of extract) according to the following:

FRAP ($\mu molFe^{2+}/g$) = $\frac{X\ \mu molFe^{2+}/mL}{mg\ sample/mL} \times 1000$

3.3.1 Data Analyses

Data analysis was carried out using Microsoft excel, Spss and Graphpad prism applications. All data were summarised by descriptive (mean, mean ± standard error of mean, etc.) into table charts and graphs and statistical significance at 0.05.

CHAPTER FOUR

RESULTS

4.1 Yields of Lemongrass extracts

Results obtained for the yield upon ethanol and aqueous extraction of the *Bryophyllum pinnatum* is presented in table 3.1

Table 4.1: Yield of the ethanol and aqueous extracts of *B. pinnatum*

Extraction Solvent	Weight of plant material (g)	Weight of extract (g)	Percentage yield (%)
Ethanol	200.0	28.6	14.3
Aqueous	200.0	36.6	18.3

Table 4.2: Antimicrobial activities of the Aqueous extract of *B. pinnatum* at different concentrations

Organisms	Zones of Inhibition (mean ± S.D mm)					
	Concentrations (mg/mL)			CIP	Nystatin	Sterilized
	25	50	100	1µg/mL	10µg/mL	D.H_2O
S. aureus	14.5±2.5	21.6±1.5	24.3±1.6	33.1±1.2	0.0±0.0	0.0±0.0
B. subtilis	18.6±1.3	24.1±2.9	28.5±2.5	30.5±1.6	0.0±0.0	0.0±0.0
K. pneumoniae	13.6±1.3	15.3±1.7	18.1±1.2	34.3±2.7	0.0±0.0	0.0±0.0
P. aeruginosa	7.2±1.8	13.3±2.1	14.3±2.6	31.6±1.3	0.0±0.0	0.0±0.0
C. albicans	12.5±1.6	16.3±1.5	18.1±1.9	0.0±0.0	29.5±1.6	0.0±0.0
A. niger	7.1±2.3	10.5±1.6	13.3±1.6	0.0±0.0	32.4±2.3	0.0±0.0

Key: S.D = Standard Deviation, 0.0 = No activity, CIP = ciprofloxacin, D.H_2O = Distilled water

Table 4.3: Antimicrobial activities of the Ethanol extract of *B. pinnatum* at different concentrations

Organisms	Zones of Inhibition (mean ± S.D mm)					
	Concentrations (mg/mL)			CIP	Nystatin	Sterilized
	25	50	100	1µg/mL	10µg/mL	D.H_2O
S. aureus	16.9±1.1	23.6±2.4	28.5±1.6	31.3±1.7	0.0±0.0	0.0±0.0
B. subtilis	21.5±1.6	24.1±1.1	31.1±1.3	35.6±3.1	0.0±0.0	0.0±0.0
K. pneumoniae	13.3±2.7	18.5±2.6	29.1±1.4	32.5±1.6	0.0±0.0	0.0±0.0
P. aeruginosa	9.1±1.4	14.1±1.9	15.5±1.5	29.1±1.9	0.0±0.0	0.0±0.0
C. albicans	12.5±2.5	18.3±2.1	20.3±1.7	0.0±0.0	28.5±1.6	0.0±0.0
A. niger	7.1±2.1	11.5±1.7	13.2±1.6	0.0±0.0	33.3±1.7	0.0±0.0

Key: S.D = Standard Deviation, 0.0 = No activity, CIP = ciprofloxacin, D.H_2O = Distilled water

Table 4.4: Minimum Inhibitory Concentration (MIC), Minimum Bactericidal/Fungicidal concentrations (MBCs/MFCs) of the ethanol and aqueous extract of *B. pinnatum* against the Test Organisms

Organisms	Aqueous		Ethanol	
	MIC	MBC	MIC	MBC
	(mg/mL)			
S. aureus	0.9	2	0.6	1
B. subtilis	0.5	0.6	0.2	0.4
K. pneumoniae	6	6	2	2
P. aeruginosa	6	8	4	7
C. albicans	7	7	5	6
A. niger	8	12	8	10

Table 4.5 : DPPH radical scavenging activity of *B. pinnatum* Determined spectrophotometrically at 517nm

Concentration (µg/mL)	Aqueous Extract	Ethanol Extract	Ascorbic acid (Standard Antiox.)
10	0.592±0.05	0.566±0.03	0.511±0.13
25	0.470±0.15	0.463±0.01	0.383±0.01
50	0.457±0.06	0.345±0.06	0.283±0.05
100	0.325±0.15	0.240±0.12	0.151±0.07
200	0.240±0.01	0.173±0.13	0.063±0.01

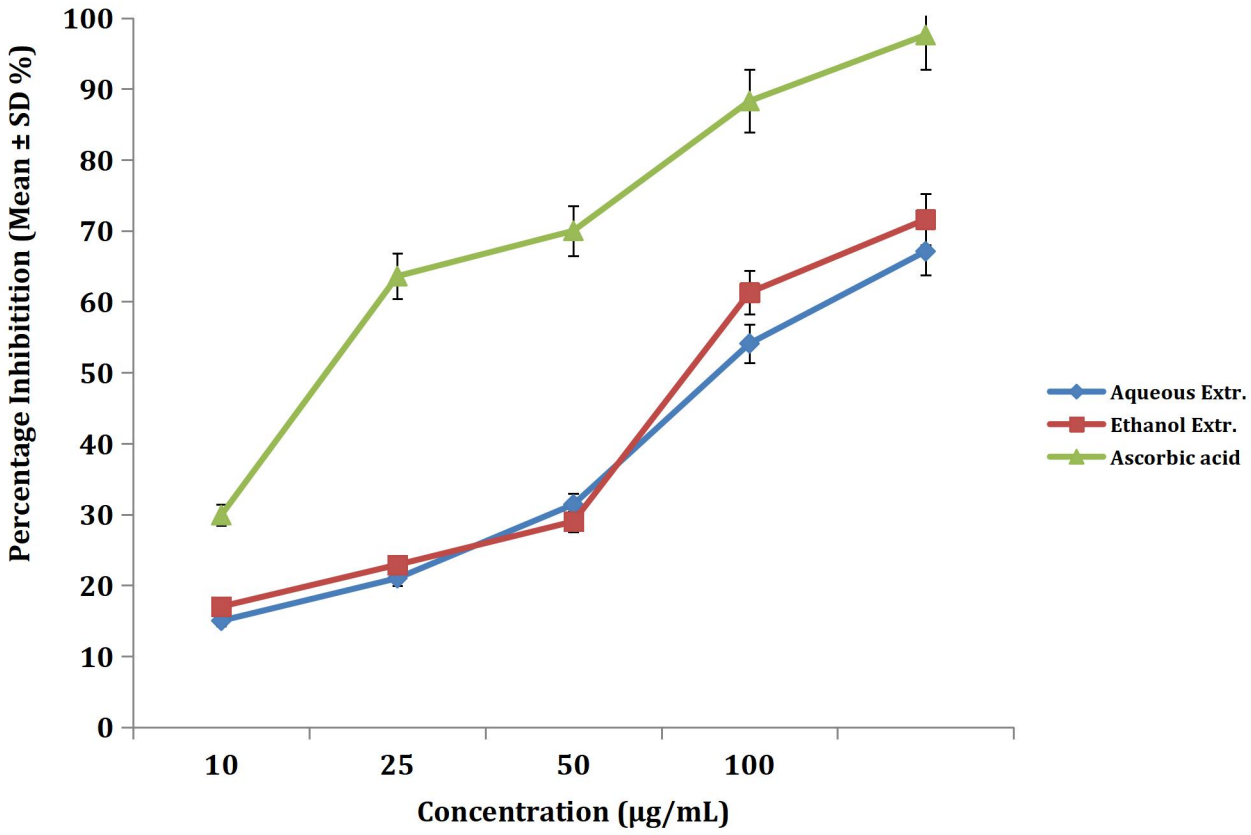

Figure : Percentage inhibition of DPPH radical/Radical Scavenging activity (RSA) of the Aqueous and Ethanol extracts of *B. pinnatum* at different concentrations

Table 4.6 : FRAP showing the reducing power of the *B. pinnatum* extracts by measuring their ability to reduce Fe^{3+} to Fe^{2+}

Sample	FRAP (µmol Fe^{2+}/g extract ± SD)
Ethanol extract	345.5 ± 10.3
Aqueous extract	258.1 ± 11.6
Ascorbic acid (standard)	663.6 ± 13.1

Overall, the ethanol extract exhibited stronger antioxidant potential than the aqueous extract, though both were less active than Ascorbic acid (Standard antioxidant).

Table 4.7: Phytochemical compounds in the extracts of *B. pinatum*

Plant constituents	Aqueous	Ethanol
Alkaloids	+	+
Flavonoids	+	+
Phenols	+	+
Saponins	+	+
Tannins	+	+
Anthraquinones	+	+
Terpenoids	+	+
Glycosides	+	+
Steroids	+	+

Key: + = positive (present), - = negative (absent)

Table 4.8 : Quantitative analysis of secondary metabolites in the extracts of *B. pinnatum* [mean ± SD (mg/g DW)]

Plant constituents	Aqueous	Ethanol
Alkaloids	15.61 ±1.35	21.06± 1.21
Flavonoids	20.51±2.16	31.63±2.18
Phenolics	24.61±2.06	37.06±5.11
Saponins	31.57± 3.54	21.45±4.51
Tannins	9.13±2.61	11.70±3.70
Anthraquinones	8.05±1.66	10.91±2.11
Terpenoids	16.51±3.61	18.21±3.16
Glycosides	16.14±1.03	18.17±3.15
Steroids	5.81±1.66	7.53±1.91

CHAPTER FIVE

SUMMARY, CONCLUSION, AND RECOMMENDATIONS

5.1 Summary of Findings

This study was undertaken to evaluate and compare the phytochemical constituents, antioxidant capacity, and antimicrobial activity of the aqueous and ethanol leaf extracts of *Bryophyllum pinnatum*. The objectives of the study were successfully met, yielding the following key findings:

1. **Extraction Yield**:

The aqueous extraction yielded a higher quantity of crude extract (18.3%) compared to the ethanol extraction (14.3%), suggesting a better extraction efficiency for water-soluble compounds.

2. **Phytochemical Screening**:

Qualitative Analysis: Both the aqueous and ethanol extracts tested positive for the presence of major bioactive compounds, including alkaloids, flavonoids, phenols, tannins, saponins, glycosides, terpenoids, steroids, and anthraquinones.

Quantitative Analysis: The ethanol extract consistently contained significantly higher concentrations of most phytochemicals, particularly flavonoids (31.63 mg/g), phenolics (37.06 mg/g), and alkaloids (21.06 mg/g). The aqueous extract, however, showed a higher saponin content (31.57 mg/g).

3. **Antioxidant Activity:**

DPPH Assay: Both extracts demonstrated dose-dependent free radical scavenging activity. The ethanol extract exhibited a significantly stronger antioxidant potency (IC_{50} = 63.11 µg/mL) compared to the aqueous extract (IC_{50} = 88.67 µg/mL), though both were less potent than the ascorbic acid standard (IC_{50} = 26.67 µg/mL).

FRAP Assay: The results confirmed the superior reducing power of the ethanol extract (345.5 µmol Fe^{2+}/g) over the aqueous extract (258.1 µmol Fe^{2+}/g), aligning with the DPPH findings and the higher phenolic and flavonoid content in the ethanol extract.

4. Antimicrobial Activity:

Zone of Inhibition: Both extracts exhibited broad-spectrum, dose-dependent antimicrobial activity against all test organisms (*Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger*). The ethanol extract consistently produced larger zones of inhibition across all concentrations.

Potency and Spectrum: The Gram-positive bacteria (S. aureus and B. subtilis) were more susceptible than the Gram-negative bacteria (*K. pneumoniae* and *P. aeruginosa*). The fungi (*C. albicans* and *A. niger*) were the least susceptible.

MIC and MBC/MFC: The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) values revealed that the ethanol extract was more potent, requiring lower concentrations to inhibit and kill the microorganisms. For instance, against B. subtilis, the ethanol extract had an MIC of 0.2 mg/mL and an MBC of 0.4 mg/mL, whereas the aqueous extract had an MIC of 0.5 mg/mL and an MBC of 0.6 mg/mL.

5.2 Conclusion

In conclusion, this study provides scientific validation for the traditional use of *Bryophyllum pinnatum* in treating infections and oxidative stress-related conditions. The research leads to the following major conclusions:

1. The leaves of *Bryophyllum pinnatum* are a rich repository of diverse phytochemicals with recognized therapeutic value.

2. The solvent used for extraction significantly influences the bioactivity of the extracts. Ethanol was a more effective solvent than water for extracting the antioxidant and antimicrobial principles from *Bryophyllum pinnatum*.

3. The strong antioxidant activity of the extracts, particularly the ethanol extract, is directly correlated with its high concentration of phenolic compounds and flavonoids.

4. The significant antimicrobial activity against a panel of clinically relevant pathogens, including multi-drug resistant nosocomial isolates, underscores the potential of *B. pinnatum* as a source of novel antimicrobial agents.

5. The findings collectively justify the ethnomedicinal applications of *Bryophyllum pinnatum* and position it as a promising candidate for further pharmacological investigation.

5.3 Recommendations

Based on the findings and conclusions of this study, the following recommendations are made:

1. **For Practice and Application:**

The ethanol extract of *Bryophyllum pinnatum* should be considered for development into topical formulations for the treatment of bacterial and fungal skin infections, given its potent activity against skin pathogens like *S. aureus* and *C. albicans.*

Herbal practitioners should prioritize ethanol-based tinctures over water-based infusions to maximize the therapeutic benefits of *B. pinnatum*.

2. **For Policy:**

Public health authorities and agricultural bodies should encourage the cultivation and conservation of *Bryophyllum pinnatum* as a valuable medicinal plant resource.

Further toxicological studies should be mandated to establish safe dosage levels, paving the way for its potential integration into formal healthcare systems as a standardized herbal remedy.

3. **For Future Research:**

Compound Isolation: Future work should focus on the bioassay-guided fractionation and isolation of the specific active compounds responsible for the antioxidant and antimicrobial activities.

Synergistic Studies: Research should investigate the synergistic effects of the *B. pinnatum* extracts with conventional antibiotics to combat antimicrobial resistance.

In-vivo Studies: The efficacy and safety of the most potent extract (ethanol) should be validated using in-vivo models of infection and oxidative stress.

Mechanistic Studies: Further studies are needed to elucidate the precise mechanism of antimicrobial action (e.g., cell wall disruption, protein synthesis inhibition) and the specific pathways of antioxidant activity.

Investigation of Aqueous Extract: The relatively high saponin content in the aqueous extract warrants specific investigation into its unique biological properties, such as its anti-inflammatory or immunomodulatory potential.

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