PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT AND ANTIMICROBIAL EVALUATION OF THE LEAF EXTRACT OF *Bryophyllum pinnatum*

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A DISSERTATION SUBMITTED TO THE DEPARTMENT OF PHARMACEUTICAL CHEMISTRY, FACULTY OF PHARMACY, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF DOCTOR OF PHARMACY DEGREE HONOURS IN PHARMACY.

NOVEMBER, 2025

CERTIFICATION

This is to certify that this work was done by Obayagbona Favour Eseosa in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City.

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(Head of Department)

DEDICATION

This work is dedicated to God almighty for his love and guidance throughout my stay in this institution and to my cherished family for their unwavering support and motivation.

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First and foremost, I give all glory and thanks to God Almighty for His grace, guidance, wisdom, and strength throughout the course of this project. Without Him, this would not have been possible.

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ABSTRACT

This study investigated the phytochemical composition, antioxidant capacity, and antimicrobial activity of the methanol leaf extract of *Bryophyllum pinnatum* (Crassulaceae), a plant widely used in traditional medicine for the treatment of infections, inflammation, and wounds. The crude extract was prepared by maceration in methanol and screened for secondary metabolites using standard phytochemical tests. The extract showed the presence of alkaloids, saponins, flavonoids, terpenoids, phenols, carbohydrates, and anthraquinones, while tannins were absent. Quantitative analyses revealed a total phenolic content of 0.040 ± 0.008 mg GAE/g and a total flavonoid content of 0.197 ± 0.005 mg QE/g, confirming the predominance of flavonoid constituents.The antioxidant potential of the extract was evaluated using DPPH and FRAP assays. The DPPH radical scavenging test showed concentration-dependent activity with an IC_{50} value of 189 µg/mL compared to 120 µg/mL for ascorbic acid, while the FRAP assay demonstrated moderate reducing power. Antimicrobial screening using the agar well diffusion method against selected bacterial and fungal isolates (*S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *B. subtilis*, *C. albicans*, and *A. niger*) revealed that the methanol extract exhibited no detectable inhibitory zones at 1000 mg/mL, whereas standard drugs ciprofloxacin and ketoconazole showed significant activity.

The findings indicate that *Bryophyllum pinnatum* contains bioactive secondary metabolites with measurable antioxidant capacity but limited antimicrobial effect under the tested conditions. Nonetheless, its strong phytochemical and antioxidant profile supports its ethnomedicinal use and suggests potential for further purification and evaluation of its individual constituents as sources of novel therapeutic agents.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Bryophyllum pinnatum also known as resurrection plant is an environmental weed, traditionally employed to treat headaches, coughs, fevers, gastrointestinal issues like diarrhea and dysentery, kidney and prostate ailments, and as a sedative or coagulant to address bleeding disorders. In Nigeria, for instance, it has been used to facilitate placental delivery, underscoring its role in postpartum *Bryophyllum pinnatum* (synonym *Kalanchoe pinnata*), belonging to the Crassulaceae family, is a succulent plant long revered in various traditional medicinal systems worldwide for its broad therapeutic applications. Indigenous communities across tropical regions, including parts of Africa, India, the Caribbean, and South America, have traditionally employed this plant to treat a spectrum of ailments from wounds and infections to gastrointestinal disorders and hypertension (Sharma *et al*., 2024).

Among its most widespread applications is its use in managing inflammatory and ulcerative conditions. In Brazil, for example, *B. pinnatum* is frequently used for gastritis, ulcers, and other inflammatory disorders, a use supported by its rich flavonoid profile particularly quercetin- and kaempferol-derived glycosides which contribute to its antioxidant and anti-inflammatory potency (Fernandes *et al.*, 2019)
Additionally, the plant shows potent antibacterial and wound-healing properties. Traditional applications such as applying heated leaves over boils or abscesses and using expressed leaf juice to treat infected wounds, insect bites, and eye or ear inflammations exemplify its antimicrobial usage (Oladejo et al., 2021). Moreover, studies have confirmed its in vitro antibacterial activity against common pathogens

implicated in infant respiratory infections demonstrating inhibitory effects against *Staphylococcus* and *Streptococcus* species thus reinforcing its traditional use in treating respiratory conditions (Etim *et al*., 2016) .

Ethnobotanical records further underscore its versatility: *B. pinnatum* has been care (Namadina et al., 2020). Notably, in vitro research supports its traditional use in hemostasis, with crude extracts demonstrating blood coagulant activity by significantly shortening clotting times in standard assays (Emeka, 2021).

Bryophyllum pinnatum serves as a multifaceted traditional remedy with scientifically acknowledged roles in anti-inflammatory, antimicrobial, wound healing, hemostatic, and gastroprotective contexts. Its diverse phytochemical composition including flavonoids, triterpenes, bufadienolides, phenolics, alkaloids, and saponins underpins its wide-ranging medicinal value, validating centuries of indigenous use and informing contemporary pharmacological interest.

1.2 LITERATURE REVIEW

1.2.1 Plant description

Bryophyllum pinnatum (also known as *Kalanchoe pinnata*) is a succulent, perennial herb in the Crassulaceae family, native to Madagascar and subsequently naturalized across many tropical and subtropical regions (Salauniyan et al., 2019) . The plant typically reaches a height of approximately 0.3 to 1.2 m, though stems may grow up to 2 m tall in favorable conditions (Res. J Pharm. Phytochem, 2020).

The stems are fleshy and succulent young stems often reddish and quadrangular, while older stems become pale and may become woody at the base (Res. J. Pharm. Phytochem., 2020) .

The leaves exhibit notable variation from youth to maturity. In young plants, leaves are simple, elliptic or ovate, fleshy, and oppositely arranged (Kenneth et al., 2020). In more mature plants, leaves may become pinnately compound, featuring 3–5 leaflets borne on long petioles (World of Succulents, 2024). Leaf blades are typically fleshy, oblong to elliptic, with distinctly scalloped (crenate) margins that may be tinged with shades of red or orange (Salauniyan et al., 2019). The leaves' margins are well known for producing characteristic tiny plantlets (propagules), which aid in vegetative reproduction.

Inflorescences are terminal panicles or cymes, bearing pendulous, bell-shaped flowers (Res. J. Pharm. Phytochem., 2020). The flowers have tubular corollas, typically yellowish- green with red or purple streaks, encased by a bell- shaped calyx that may display reddish mottling (World of Succulents, 2024). Flowering usually occurs in winter and spring (Res. J. Pharm. Phytochem., 2020)

The reproductive structures include a corolla with four lobes, eight stamens fused to the corolla tube's base, and a superior ovary composed of four carpels with slender styles; fruits are follicles containing numerous seeds.

1.2.2 Taxonomy

Kingdom: Plantae (Plants)

Subkingdom: Tracheobionta (vascular plants)

Division (Phyllum): Spermatophyta (seed plants)

Subdivision (Subphyllum):Magnoliophyta (flowering plants)

Class: Magnoliopsida (dicotyledons)

Subclass: Rosidae.

Order: Rosales

Family: Crassulaceae (Stone crop)

Genus: *Bryophyllum*

Species: *Bryophyllum pinnatum*

Source: Dhumane *et al.* (2024)

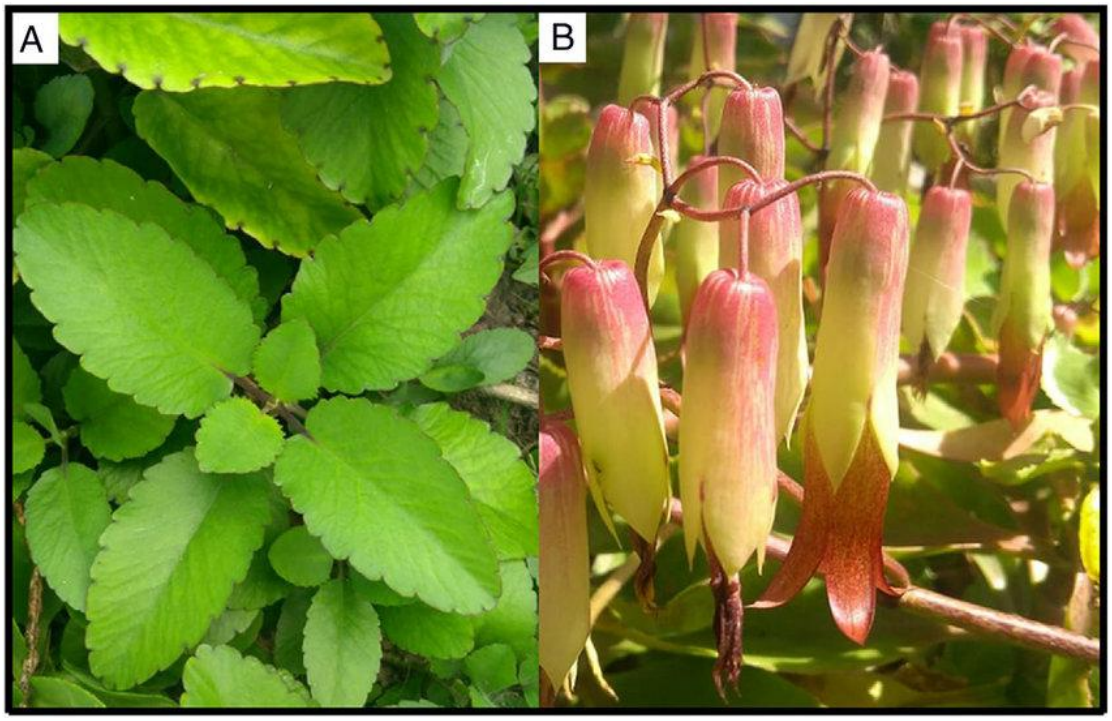

Figure 1 *Bryophyllum pinnatum* (A) Leaves (B) Inflorescences

1.2.3 Ethnomedicinal Uses of *Bryophyllum pinnatum*

Traditionally *B. pinnatum* is used as medicine across countries in Africa, Asia, South America and the Pacific. It is used in various forms including fresh leaf juice, poultices, decoctions and infusions. A central ethnomedicinal use of the plant is wound healing and treatment of burns, cuts, boils and skin infections, where the fresh juice or crushed leaves are applied topically to promote healing and reduce inflammation. This traditional practice is supported by recent scientific findings showing wound-healing, anti-inflammatory and antimicrobial properties of its leaf extract (Araújo et al., 2023; Sharma, 2024).

The plant is also traditionally used for pain and inflammatory disorders, including arthritis, muscle aches and general body pain. In vivo studies demonstrate antinociceptive and anti-inflammatory activity of the plant's leaf extract, supporting its analgesic uses (Selvakumar, 2022; da Silva et al., 2024).

Ethnomedicinal records report the use of *B. pinnatum* in treating gastrointestinal disorders such as dyspepsia, ulcers and gastritis. Experimental studies showing gastroprotective and antioxidant effects of the leaf extract align with these traditional uses (Selvakumar, 2022; Sharma, 2024).

Another important traditional application is in urinary and renal ailments, including urinary tract discomfort and kidney stones. Traditional healers commonly administer leaf infusions as a natural diuretic, and scientific studies indicate diuretic properties that support these uses (Sharma, 2024; JDDT, 2024).

In women's health, *B. pinnatum* is used culturally for menstrual pain relief and reproductive health support. Recent scientific interest has explored its potential role in dysmenorrhea management, driven by observed uterine-relaxant and analgesic actions (Zurfluh et al., 2023; Folami et al., 2024).

The plant is also reported in folk medicine for respiratory issues such as cough, asthma, bronchitis and ear ache. Ethnobotanical surveys present *B. pinnatum* as a frequently used remedy among traditional healers for respiratory complaints, and supporting pharmacological data show bronchodilatory and anti-inflammatory effects (Folami et al., 2024; Selvakumar, 2022).

In addition, *B. pinnatum* is traditionally applied to infected wounds, bites and abscesses, based on its perceived antiseptic effects. Contemporary antimicrobial

screenings report variable activity, yet support topical ethnomedical use (Araújo et al., 2023; Global Research Online, 2024).

Beyond these uses, the plant is traditionally employed for fever, hypertension, diabetes and detoxification, reflecting its wide ethnopharmacological significance. Preclinical studies support potential antihyperglycaemic, antioxidant and cardioprotective effects, although clinical validation remains limited (Selvakumar, 2022; Nnaebue et al., 2024).

Traditional preparations are mainly fresh-leaf based, but modern research has translated these into standardised gels and ointments for topical applications, confirming traditional relevance in wound care (Araújo et al., 2023; Gadge and Gadge, 2025). However, authors emphasise the importance of dose standardisation and phytochemical profiling due to variation in bioactive content across regions, preparation methods and harvest conditions (Sharma, 2024; JDDT, 2024).

1.2.4 Phytochemistry

Bryophyllum pinnatum leaves are rich in secondary metabolites chiefly flavonoids and phenolic acids, bufadienolide (steroidal) glycosides, triterpenes/steroids, saponins, tannins and alkaloids which together likely explain many of the plant's antioxidant, anti-inflammatory, antimicrobial and bioactive properties (Selvakumar, 2022; Sharma, 2024). Quantitative phytochemical surveys report high total phenolic and flavonoid contents in methanolic and aqueous extracts, while targeted analyses have identified flavonols such as quercetin derivatives (e.g. rutin) and characteristic bufadienolides (e.g. bryophyllin-type glycosides) that are pharmacologically important and may contribute both therapeutic and toxic effects (Nnaebue et al., 2024; JDDT, 2024).

Variation in compound profile and concentration is common and depends on geography, harvest time and extraction method, so standardisation is recommended for any therapeutic development (Sharma, 2024).

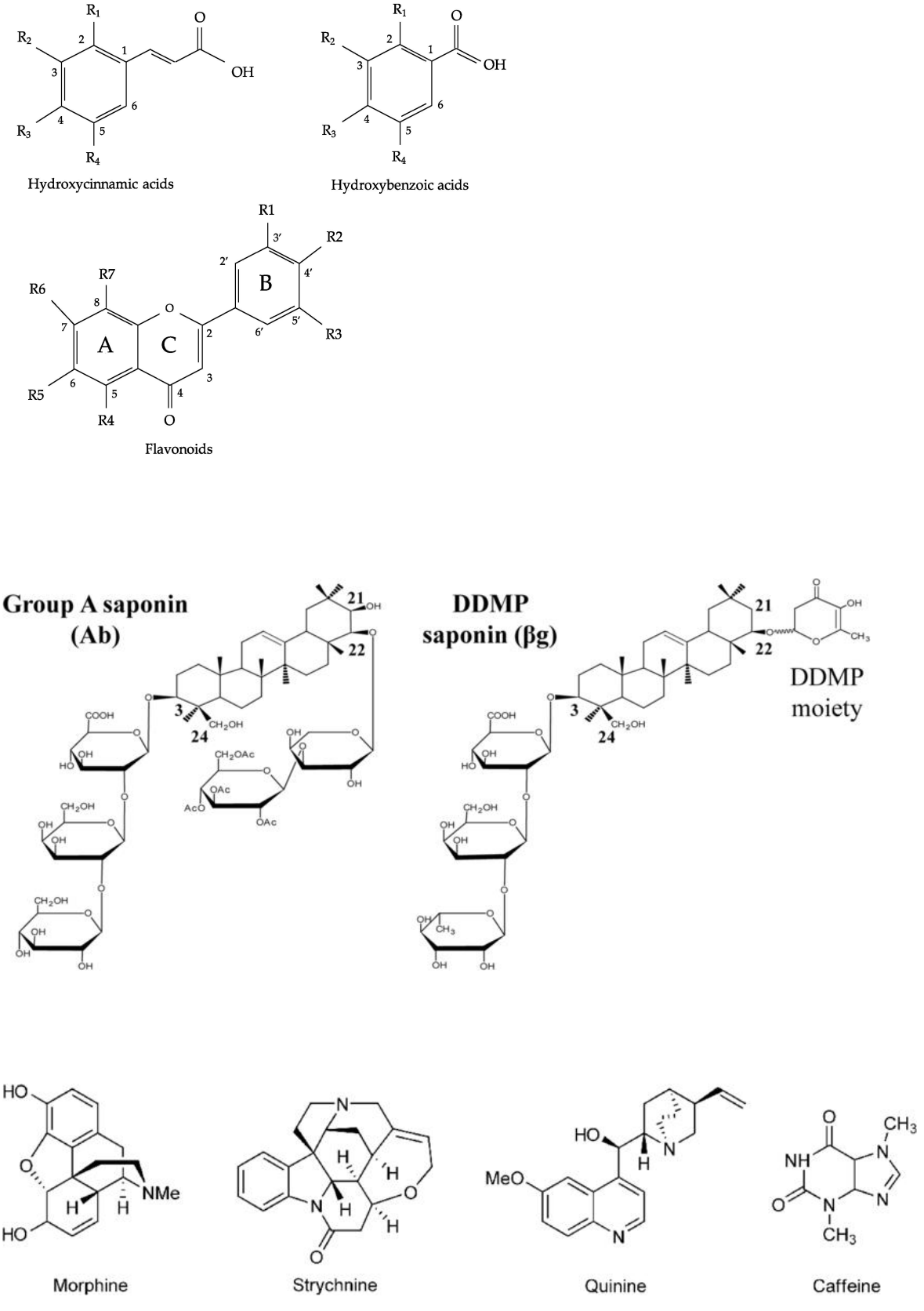

Figure 1.2.4.1 Chemical structures isolated from *Bryophyllum pinnatum*

1.2.5 Pharmacology

Generally, *B. pinnatum* extracts, oils and other products from the plant is used for a variety of illnesses traditionally. This has prompt researchers to further investigate the phytochemical constituents of this plant for possible remedies to ailments.

1.2.5.1 Anti-inflammatory activity

Multiple experimental studies and reviews report that *B. pinnatum* leaf extracts reduce inflammation in acute and chronic models likely via inhibition of pro-inflammatory mediators and antioxidant effects. Animal models (paw-edema, cotton-pellet granuloma) showed significant reduction in inflammation after administration of hydro-ethanolic or methanolic extracts. (Elufioye, 2022; Johnson et al., 2021).

1.2.5.2 Analgesic / antinociceptive effects

Extracts of *B. pinnatum* have shown antinociceptive effects in rodent pain models (hot-plate, acetic-acid writhing), suggesting central and peripheral analgesic activity. These effects are commonly attributed to flavonoids, terpenoids and phenolic compounds present in the leaves. (Selvakumar, 2022; Elufioye, 2022).

1.2.5.3 Antimicrobial activity

Several in-vitro studies report antibacterial and antifungal activity of *B. pinnatum* extracts against organisms such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida* spp. but results are inconsistent across studies (depend on solvent, concentration and microbial strain). Some recent work reports little or no activity against certain clinical isolates, while others show measurable zones of inhibition;

therefore antimicrobial activity appears extract- and strain-dependent. (Sharma, 2024; multiple experimental studies 2021–2025).

1.2.5.4 Antioxidant activity

Leaves of *B. pinnatum* are rich in phenolics and flavonoids and demonstrate free-radical scavenging (e.g., DPPH, FRAP) in vitro. These antioxidant properties are cited as important contributors to other observed pharmacological effects (anti-inflammatory, wound healing, cytoprotection). Environmental factors (e.g., drought stress) may alter phenolic content and antioxidant potency. (da Silva et al., 2024; Selvakumar, 2022).

1.2.5.5 Antidiabetic / hypoglycaemic effects

Several in-vitro and animal studies report glucose-lowering effects of *B. pinnatum* extracts and improved biochemical markers in diabetic models; mechanisms proposed include enhanced insulin sensitivity, inhibition of carbohydrate-digesting enzymes and antioxidant protection of pancreatic tissue. These findings are promising but require clinical validation. (da Silva et al, 2024;).

1.2.5.6 Wound-healing and tissue-repair activity

Topical preparations and gel formulations containing *B. pinnatum* extracts have been shown to accelerate wound closure, enhance collagen deposition and reduce microbial contamination in some experimental studies effects attributed to combined anti-inflammatory, antimicrobial and antioxidant actions. (Selvakumar, 2022; formulation studies 2025).

1.2.5.7 Immunomodulatory / haematological effects and cytoprotection

Some studies report that *B. pinnatum* extracts modulate immune-related parameters and improve certain haematological indices in models of chronic inflammation; additional reports also indicate hepatoprotective and antiulcer potentials in animal studies. These are plausible given the plant's phytochemical profile but again need more targeted mechanistic and clinical work. (Elufioye, 2022; recent experimental reports 2022–2025).

1.2.6 Antioxidants

Antioxidants are substances that prevent or slow oxidative damage by neutralising reactive oxygen species (ROS) and other free radicals, thereby protecting lipids, proteins and nucleic acids from oxidative modification (Chaudhary, 2023).

They arise from two complementary sources: endogenous defence systems (enzymatic antioxidants such as superoxide dismutases, catalase and glutathione peroxidases, plus small molecules like glutathione and coenzyme Q) and exogenous (dietary) antioxidants, notably vitamins (C, E), carotenoids and a broad range of plant polyphenols found in fruits, vegetables, tea and other whole foods (Gülçin, 2025; Rudrapal et al., 2024).

Antioxidants act by direct radical scavenging, metal-ion chelation, breaking lipid-peroxidation chains and modulating redox-sensitive signalling pathways that control endogenous defence (for example via Nrf2). These actions link antioxidant biology to ageing and to many chronic conditions in which oxidative stress is implicated (Tumilaar et al., 2024; Chaudhary, 2023).

Contemporary research highlights two important caveats: first, assays for "total antioxidant capacity" and in-vitro antioxidant activity are methodologically diverse

and do not reliably predict in-vivo effects; second, blunt suppression of ROS can disturb physiological redox signalling (the “antioxidant paradox”), so beneficial interventions increasingly emphasise dietary patterns and targeted modulation rather than high-dose single compounds (Gülçin, 2025; Wan et al., 2024).

1.2.6.1 Antioxidant Classification

Antioxidants can be classified in several complementary ways: by origin (endogenous versus exogenous), by biochemical mechanism (primary/chain-breaking versus secondary/preventive), by molecular/functional type (enzymes, small molecules, minerals, peptides, polyphenols), and by physicochemical behaviour (water-soluble versus lipid-soluble) (Tumilaar et al., 2024; Gülçin, 2025).

- **Origin: endogenous vs exogenous**

  Endogenous antioxidants are produced within the organism and include enzymatic systems (superoxide dismutases, catalase, glutathione peroxidases) and non-enzymatic intracellular molecules (reduced glutathione, uric acid, coenzyme Q). Exogenous (dietary or supplemental) antioxidants are obtained from foods or industrial formulations and include vitamins (C, E), carotenoids (β-carotene, lycopene), polyphenols (flavonoids, phenolic acids), antioxidant peptides and certain minerals (selenium, zinc). Both sources work together to maintain redox balance. (Korczowska-Łącka et al., 2023; Tumilaar et al., 2024).

- **Mechanistic classification:** primary (chain-breaking) and secondary (preventive) antioxidants.

  Primary antioxidants (chain-breaking) directly scavenge free radicals or peroxyl radicals to interrupt radical-propagation reactions (for example, vitamin E acting in lipid membranes). Secondary or preventive antioxidants act upstream to reduce

radical formation by chelating transition metals that catalyse Fenton chemistry, decomposing hydroperoxides, or regenerating other antioxidants (examples: metal chelators, some phenolic antioxidants, and enzymes that remove ROS precursors). This mechanistic split is widely used in food chemistry and biological contexts. (Gülçin, 2025; Balkan Med J editorial, 2025).

- **Molecular/functional classes**

• Enzymatic antioxidants: superoxide dismutases (SOD), catalase, glutathione peroxidase: catalyse conversion of reactive species to less reactive molecules and form the first line of defence in cells. (Tumilaar et al., 2024).

• Small-molecule, non-enzymatic antioxidants: vitamins (C, E), glutathione, ubiquinone, uric acid: they act as direct radical scavengers, co-factors or redox buffers. (Korczowska-Łącka et al., 2023).

• Phytochemicals and polyphenols: flavonoids, phenolic acids, stilbenes (e.g., resveratrol): often act both as direct scavengers and as modulators of redox-sensitive signalling (e.g., Nrf2 pathway). Their bioactivity depends on structure, metabolism and microbiome conversion. (Duan et al., 2025).

• Carotenoids and pigments: lipophilic antioxidants that protect membranes and lipoproteins from peroxidation. (Gülçin, 2025).

• Minerals and cofactors: selenium (as part of GPx), zinc (stabilises antioxidant enzymes), iron chelators — act indirectly by supporting enzymatic systems or preventing metal-catalysed radical formation. (Korczowska-Łącka et al., 2023).

• Antioxidant peptides and engineered/biotechnological antioxidants: small peptides derived from food proteins and recombinant enzymes are an expanding class with potential therapeutic and food-preservative applications. (Antioxidants special issue, 2024; Paraschiv et al., 2025).

- **Physicochemical classification: water-soluble vs lipid-soluble**

  Antioxidants are often grouped by solubility because location determines function: water-soluble antioxidants (vitamin C, glutathione, many phenolics) neutralise radicals in cytosol and plasma, whereas lipid-soluble antioxidants (vitamin E, carotenoids, ubiquinone) protect membranes and lipoprotein particles from lipid peroxidation. (Duan et al., 2025).

- **Natural vs synthetic and applied classifications**

  In industry and research, a practical distinction is made between natural antioxidants (from plant, animal or microbial sources) and synthetic antioxidants (BHT, BHA, propyl gallate) used in food and pharmaceutical preservation. Ongoing research also treats novel nanomaterials and engineered enzymes as a separate applied category because of unique delivery and stability properties. (Baschieri et al., 2021; Paraschiv et al., 2025).

A single compound may belong to several classes (e.g., vitamin E is lipophilic, chain-breaking, dietary and non-enzymatic). Contemporary literature emphasises context bioavailability, metabolism, tissue localisation and redox signalling when assigning functional significance to any antioxidant in vivo (Gülçin, 2025; Duan et al., 2025).

1.2.6.2 Antioxidant Mechanisms

Antioxidants protect biological systems through several complementary mechanisms that together prevent or limit oxidative damage. One primary mechanism is direct radical scavenging (chain-breaking), where antioxidants donate an electron or hydrogen atom to neutralise reactive oxygen species (ROS) or lipid peroxyl radicals,

forming a more stable, less reactive species and terminating propagation of radical chain reactions (Gülçin, 2025). Closely related is the role of phenolic and chromanol-containing antioxidants (for example flavonoids and vitamin E) which stabilise the resulting radical by resonance or by localisation within membranes, thereby interrupting lipid peroxidation cascades (Baschieri, 2021; Gülçin, 2025).

A second mechanism is preventive: antioxidants reduce the rate of radical formation rather than scavenging radicals after they form. This includes metal-ion chelation (binding Fe^{2+} or Cu^{2+} to block Fenton chemistry) and enzymatic removal of ROS precursors; both approaches limit the generation of the most damaging radical species. Dietary polyphenols, certain peptides and small molecules can act as metal chelators in biological and food systems. (Chaudhary, 2023).

Enzymatic antioxidant systems constitute another major defence: superoxide dismutases (SOD) convert superoxide into hydrogen peroxide, which is subsequently converted to water by catalase and glutathione peroxidases; these coordinated enzymatic steps detoxify ROS and maintain cellular redox balance (Tumilaar et al., 2024). Beyond these enzymes, cells regulate expression of many antioxidant and phase-II detoxification proteins through redox-sensitive transcriptional programmes most notably the Keap1–Nrf2 pathway so that exposure to oxidants or electrophiles induces a protective, gene-expression response (Bonay et al., 2024; Signorini et al., 2024).

Antioxidants also act indirectly by regenerating each other (for example vitamin C can regenerate oxidised vitamin E), repairing oxidatively damaged biomolecules via dedicated repair enzymes, and modulating redox-dependent signalling networks and inflammatory responses rather than simply acting as stoichiometric radical sinks (Tumilaar et al., 2024; Gülçin, 2025). Finally, contemporary literature emphasises that

ROS perform physiological signalling roles and that antioxidant interventions can have hormetic effects low-level oxidative signals can be adaptive so mechanistic understanding must account for localisation, dose, metabolism and timing to predict biological outcomes (Signorini et al., 2024; Baschieri, 2021).

1.2.6.3 Health Benefits of Antioxidants

Increasing evidence suggests that adequate antioxidant intake especially from antioxidant-rich diets such as fruits, vegetables, whole grains, nuts and olive oil supports the maintenance of redox balance, promotes immune health and improves physiological resilience against disease development and progression (Hu et al., 2025; Kwaśniewska, 2023). While food-based antioxidants consistently demonstrate health benefits, high-dose isolated antioxidant supplementation may produce mixed or adverse outcomes in specific populations, emphasising the importance of dietary sources and personalised nutrition strategies (Yang et al., 2023; Middha et al., 2018). Therefore, antioxidants are recognised as key contributors to long-term wellness and chronic disease prevention within balanced dietary patterns. Some of the health benefits of antioxidants include:

- **Cardiovascular health**

  Antioxidant-rich diets (high in fruits, vegetables, nuts, whole grains and tea) are associated with lower incidence of cardiovascular disease and reduced cardiovascular mortality in observational studies; mechanisms include reduced LDL oxidation, improved endothelial function and lower systemic inflammation (Kalogerakou et al., 2024; Kwaśniewska, 2023). However, benefits are most consistent for whole-food dietary patterns rather than routine high-dose single-nutrient supplements (Kalogerakou et al., 2024).

- **Metabolic health (diabetes, insulin resistance, metabolic syndrome)**

  Dietary antioxidants and polyphenol-rich foods are linked with improved markers of insulin sensitivity, lower fasting glucose and reduced oxidative markers in several cohort and intervention studies; proposed mechanisms include attenuation of oxidative stress–driven insulin signalling disruption and reduced inflammation (Kalogerakou et al., 2024; Tumilaar et al., 2024). Clinical outcomes vary by compound, dose and population.

- **Neuroprotection and cognitive ageing**

  Higher dietary intakes of certain antioxidants (vitamins C and E, carotenoids, flavonoids/polyphenols) are associated in observational studies with lower risk of cognitive decline and dementia or with slower decline on cognitive tests; preclinical models show neuroprotection against oxidative injury, but large randomized trials showing clear prevention of dementia are lacking (Nazzi et al., 2024; Houldsworth, 2024).

- **Cancer: potential benefits and important cautions**

  Some antioxidant-rich dietary patterns correlate with lower cancer risk in observational studies, plausibly via reduced oxidative DNA damage and modulation of inflammation (Gülçin, 2025). Critically, randomized trials of high-dose single antioxidants have sometimes shown harm in specific groups (notably increased lung cancer risk with β-carotene supplements in smokers), emphasising that isolated high-dose supplementation can have pro-oxidant or adverse effects in certain contexts (Middha et al., 2018; Yang et al., 2023).

- **Immune function and inflammation**

  Antioxidants modulate redox-sensitive signalling (for example Nrf2) and can reduce pro-inflammatory cytokines and oxidative biomarkers, thereby supporting

immune homeostasis in chronic inflammatory states; clinical results are heterogenous and likely depend on baseline nutritional and inflammatory status, the specific antioxidant, and the dosing regimen (Frontiers Immunology editorial, 2024; Kalogerakou et al., 2024).

- **Eye health and age-related macular degeneration (AMD)**

  Epidemiological and trial evidence supports protective roles for certain antioxidant combinations (notably lutein, zeaxanthin, vitamins C and E and zinc in formulated supplements) in slowing progression of intermediate to advanced AMD for at-risk individuals, though routine supplementation for everyone is not universally recommended (Kalogerakou et al., 2024; Gülçin, 2025).

- **Skin health and ageing**

  Topical and dietary antioxidants protect skin from ultraviolet (UV)-induced oxidative damage, reduce markers of photoageing and can improve some cosmetic outcomes; bioavailability and formulation influence efficacy, and combined antioxidant approaches (diet + topical) are often most effective (Tumilaar et al., 2024; Gülçin, 2025).

- **Mechanistic synthesis**

  Health benefits attributed to antioxidants arise from multiple mechanisms: direct radical scavenging, metal-ion chelation, inhibition of lipid peroxidation, regeneration of other antioxidants (e.g., vitamin C regenerating vitamin E), up-regulation of endogenous defences via transcriptional programmes (e.g., Nrf2), and modulation of inflammatory signalling (Tumilaar et al., 2024; Gülçin, 2025).

1.2.7 Bacterial infections

Bacterial infections represent a major cause of morbidity and mortality worldwide, affecting multiple organ systems and populations. Although antibiotics revolutionised the treatment of bacterial diseases in the 20th century, the challenge of antimicrobial resistance (AMR), shifts in pathogen epidemiology, and emerging co- infections have renewed urgency in understanding bacterial disease. This essay addresses the etiology, pathogenesis, epidemiology, diagnosis, treatment, complications, and control of bacterial infections, with a focus on developments since 2019.

1.2.7.1 Pathogenesis and Mechanisms of Disease

Bacteria cause disease by several successive steps:

- **Adhesion / colonization**: Bacteria first adhere to mucosal surfaces or injured tissue, using adhesins, fimbriae, pili, or biofilm formation.
- **Invasion or local proliferation**: Following adhesion, bacteria may invade deeper tissues or proliferate locally.
- **Evasion of host defences**: Many bacteria produce virulence factors such as capsules, exotoxins, endotoxins (lipopolysaccharide in Gram-negatives), and enzymes (e.g. proteases, hyaluronidases) that help them evade phagocytosis, complement, or antibodies.
- **Damage / dissemination**: Tissue damage may be direct (toxins) or indirect (inflammation, immune response). Bacteria may disseminate via lymphatics or bloodstream.

In bloodstream infections, bacteremia triggers systemic inflammatory response syndrome (SIRS), which may progress to sepsis and septic shock if not controlled.

1.2.7.2 Clinical Manifestations

Symptoms of bacterial infections vary widely, depending on organ involved. Common features include fever, chills, malaise, local signs (e.g. cough, dyspnea, dysuria, wound erythema). In bloodstream infections or sepsis, signs may include hypotension, tachycardia, altered mental status, oliguria, and organ dysfunction.

In pediatric patients, serious bacterial infections may present subtly with nonspecific signs such as lethargy, poor feeding, irritability, or high fever; hence clinical prediction tools are used (Keitel et al., 2019).

1.2.7.3 Challenges of Antimicrobial Resistance

AMR severely constrains therapeutic options. In many settings, empirical therapy fails due to resistance, leading to higher mortality. Also, development of new antibiotics has lagged. Further, antibiotic misuse (e.g. prescribing for viral illness) accelerates resistance (MacKinnon et al., 2020). Stewardship programmes, surveillance, and regulation of antibiotic use are essential.

One experimental direction is the development of nano- antibiotics or nanocarriers (e.g. silica nanoparticles carrying levofloxacin) to penetrate bacterial membranes more effectively (Gonzalez et al., 2021).

1.2.7.4 Prevalence of Multidrug Resistant Organisms

The prevalence of multidrug-resistant organisms (MDROs) has reached alarmingly high and increasingly documented levels worldwide. A global narrative review noted that the incidence and prevalence of antimicrobial-resistant bacterial infections have “attained incongruous levels” in the 21st century, posing a silent pandemic. A systematic analysis estimated that in 2021 there were roughly 1.27 million deaths

directly attributable to bacterial antimicrobial resistance globally, emphasising the heavy burden of resistance (Ahmad et al., 2023). In hospital-acquired infections in one large cohort, the prevalence of bacterial infections increased from 8.09% to 10.79% during the COVID-19 period, though the proportion of those infections that were multidrug-resistant dropped from 10.14% to 8.07% still indicating a significant baseline of MDROs among infected inpatients (Murray et al., 2024). In West Africa, a meta-analysis covering 50 studies found that the overall prevalence of multidrug-resistant bacteria in healthcare and community settings was 59% (95% CI: 48-69), with a higher rate in nosocomial infections (65%) compared with community-acquired ones (53%) (Schmidt et al., 2023). In a recent hospital-based study of Gram-negative Enterobacteriaceae bloodstream isolates, the overall MDR rate reached 83.2% and in one tertiary centre 93.2% (Adusei et al., 2025). A study focusing on multidrug-resistant Acinetobacter baumannii causing hospital and ventilator-associated pneumonia across 29 countries found a pooled prevalence of 79.9% (95% CI: 73.9-85.4% (Azerefegne et al., 2025)). Another study of sputum samples in Tanzania reported a high prevalence of multidrug-resistant bacteria among bacterial pneumonia patients, further underscoring the growth of MDR prevalence in resource-limited settings (Peleg et al., 2019). Together these findings demonstrate that MDR bacterial organisms are not only prevalent in hospitals but increasingly frequent in community and healthcare settings, with rates frequently exceeding 50% in many low and middle-income settings, and high single-pathogen rates approaching 80–90% (Kamori et al., 2024).

1.2.7.5 Challenges Associated With Antibiotic Research and Development

The development of new antibiotics faces a cluster of interlocking scientific, clinical, regulatory and economic challenges that together have produced a near-collapse of a sustainable commercial pipeline. Discovering chemically novel scaffolds that overcome intrinsic defence mechanisms of problematic Gram-negative pathogens (porin exclusion, efflux pumps, enzymatic degradation) has proven technically difficult and yields very high failure rates in lead optimisation and preclinical stages (Muteeb, 2023; Blaskovich, 2025). Clinical development is complicated because antibiotic trials must demonstrate effectiveness across heterogeneous infections, often rely on non-inferiority designs, and require rapid enrolment of patients with specific resistant pathogens problems that drive up cost, extend timelines and increase the chance of late failure (Piddock et al., 2024; Gargate, 2025). Economically, new antibiotics are poor commercial investments: appropriate stewardship (restricting use to preserve efficacy), low unit prices and short treatment courses mean that revenues rarely recoup R&D costs, which has led large pharmaceutical companies to largely abandon antibiotic R&D and left small biotech firms financially precarious (Anderson, 2023; WHO, 2024). Regulatory and market incentives remain fragmented and inadequate; while “push” funding (grants, public–private partnerships) helps early-stage science, the absence of robust “pull” mechanisms (market entry rewards, subscription/guaranteed payment models) limits later-stage investment and commercial viability (Anderson, 2023; Global AMR Hub, 2024). The lack of fast, reliable diagnostics that can identify causative pathogens and resistance mechanisms at the point of care undermines appropriate trial enrolment, stewardship and market adoption of narrow-spectrum agents creating both a scientific and commercial barrier to development (Alatawi, 2025). Manufacturing challenges (complex chemistry for

novel molecules, scalability, quality control) and supply-chain fragility further raise costs and can produce intermittent global shortages even for older agents, weakening investor confidence (Piddock et al., 2024). Finally, the human capital pipeline is fraying: experienced antimicrobial drug-discovery scientists are leaving the field because of scarce funding and limited career prospects, reducing capacity to run long, technically demanding discovery programs (Wasan, 2023; Gargate, 2025). Together, these factors explain why antibiotic R&D is scientifically hard, clinically cumbersome, regulatorily uncertain and economically unattractive and why coordinated policy actions (diagnostics scale-up, rebalanced incentives, targeted push/pull funding, and support for manufacturing and workforce) are repeatedly recommended to revive the pipeline.

1.2.8 Fungal Infections

Fungal infections, also known as mycoses, represent a significant and increasing global health burden. While superficial infections like athlete's foot or candidiasis are common and relatively benign, invasive fungal infections can cause severe disease, especially in immunocompromised individuals. The global rise in fungal infections has been exacerbated by increased use of immunosuppressive therapies, the COVID-19 pandemic, and emerging multidrug-resistant fungal species.

1.2.8.1 Epidemiology and Risk Factors

Fungal infections affect over a billion people worldwide annually, with over 1.5 million deaths due to invasive fungal diseases (Bongomin et al., 2021). Factors contributing to this include increasing numbers of immunocompromised patients, such as those undergoing chemotherapy, organ transplantation, or suffering from

HIV/AIDS. Environmental factors like climate change have also influenced the distribution and severity of fungal diseases (Rodrigues and Nosanchuk, 2020).

The COVID-19 pandemic has significantly increased fungal infection rates, particularly invasive pulmonary aspergillosis and mucormycosis. These are often seen in critically ill patients receiving corticosteroids or prolonged oxygen therapy (Hoenigl et al., 2022). The emergence of *Candida auris*, a multidrug-resistant yeast, has further complicated the clinical landscape, causing outbreaks in healthcare settings globally (Jeffery-Smith et al., 2019).

1.2.8.2 Common Fungal Pathogens and Types of Infections

Fungal infections can be classified into superficial, subcutaneous, and systemic (or invasive) categories. Superficial infections affect the skin, nails, and mucous membranes, typically caused by dermatophytes (*Trichophyton*, *Microsporum*) or *Candida* species. Although rarely life-threatening, these infections are widespread and can be chronic.

Systemic fungal infections, on the other hand, are more severe and often fatal if untreated. These include infections caused by *Aspergillus*, *Candida*, *Cryptococcus*, *Histoplasma*, and *Mucorales* species (Gonçalves et al., 2020). *Candida* species are the most common cause of fungal bloodstream infections (candidemia), especially in hospitalized patients (Clancy and Nguyen, 2020). Invasive aspergillosis, primarily caused by *Aspergillus fumigatus*, is a leading cause of morbidity and mortality in immunosuppressed individuals (Latgé and Chamilos, 2020).

In tropical regions, fungal diseases such as chromoblastomycosis, sporotrichosis, and mycetoma are more prevalent and are considered neglected tropical diseases

(Queiroz-Telles et al., 2019). Despite their impact, these infections often receive little public health attention.

1.2.8.3 Diagnosis and Treatment Challenges

Diagnosing fungal infections remains a significant. Symptoms are often nonspecific, and diagnostic tools are limited in many regions. Traditional methods like culture and histopathology are slow and may lack sensitivity. Molecular techniques, such as PCR and antigen detection (e.g., galactomannan for aspergillosis), offer better sensitivity but are costly and not widely available (Verweij et al., 2020).

Treatment of fungal infections is also complicated by limited antifungal drug classes: polyenes (e.g., amphotericin B), azoles (e.g., fluconazole, voriconazole), and echinocandins (e.g., caspofungin). Resistance to these drugs is increasing, particularly in *Candida auris* and *Aspergillus* species (Arastehfar et al., 2020). Drug toxicity and drug–drug interactions, especially in patients on multiple therapies, further complicate treatment.

Moreover, many low- and middle-income countries lack access to essential antifungal medications and diagnostic infrastructure, contributing to higher morbidity and mortality (Denning and Bromley, 2020).

1.2.8.4 Recent Advances and Public Health Implications

Recent years have seen progress in understanding fungal pathogenesis and in the development of new diagnostic tools and antifungal agents. For instance, ibrexafungerp, a novel antifungal agent approved in 2021, shows promise against resistant *Candida* species (Wiederhold, 2021). Research into fungal vaccines,

although still in early stages, is ongoing and may offer future preventive strategies (Nami et al., 2019).

On the public health front, the World Health Organization (WHO) published its first list of priority fungal pathogens in 2022, highlighting 19 fungal species that pose the greatest threat to human health due to drug resistance, lack of treatment options, and global burden (WHO, 2022). This is a crucial step toward guiding global efforts in surveillance, research, and response.

1.3 Justification of the Study

The increasing resistance of pathogens to conventional antibiotics and antifungals has created an urgent need for alternative antimicrobial agents. *Bryophyllum pinnatum* is widely used in traditional medicine for treating infections, but its antioxidant and antimicrobial properties remain underexplored scientifically. Investigating the methanol extract of this plant against clinically important organisms such as *E. coli*, *S. aureus*, *P. aeruginosa*, *C. albicans*, and *A. niger* may validate its traditional use and identify potential sources of novel antimicrobial compounds. This study could contribute to the development of accessible, plant-based treatments in the fight against antimicrobial resistance.

1.4 Aim and Objectives

1.4.1 Aim

The aim of the study was to evaluate the phytochemical antioxidant and antimicrobial properties of methanol extract of *Bryophyllum pinnatum* leaves.

1.4.2 Objectives

The objectives were to:

1. Prepare a methanol extract of *Bryophyllum pinnatum* leaves using standard solvent extraction methods.
2. Carry out preliminary phytochemical screening of the methanol extract to detect the presence of antimicrobial-related bioactive compounds such as flavonoids, alkaloids, tannins, saponins, and phenolics.
3. Determine the total phenolic and flavonoid content in the leaf of *B. pinnatum*
4. Determine antioxidant capacity of the methanol leaf extract via DPPH and FRAP assays
5. Assess the antimicrobial activity of the methanol leaf extract against the following microorganisms: *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Bacillus subtilis, Candida albicans, Aspergillus niger.*
6. Compare the antimicrobial activity of the methanol leaf extract with standard drugs: Ciprofloxacin as the positive control for bacterial strains and Ketoconazole as the positive control for fungal strains.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials

2.1.1 Glasswares

Measuring cylinder, conical flask, round bottom flask, test tube, test tube rack, tongs, spatula, porcelain dish, glass jars, Glass stirrer, funnels, bottles, pipettes, reagent bottles. (All glasswares are products of pyrex).

2.1.2 Microbiological Media

Mueller Hinton agar (Titan Biotech Ltd, India), Nutrient agar (Lifesave Biotech, USA).

2.1.3 Solvents and Reagents

1% alcoholic napthol, Acetic anhydride(sigma-aldrichgermany), Aluminum chloride, Ammonia, Ammonium hydroxide, Chloroform, Distilled water, Dragendorff's reagent,Fehling's solution A and B, Ferric chloride, Folin Ciocalteu's reagent, Gelatin solution,

Hager's reagent, Hydrochloric acid, Lead acetate, Mayer's reagent, Methanol, Petroleum ether, Sodium carbonate, Sodium chloride, Sodium hydroxide, Sulphuric acid, Wagner's reagent, Quercetin, Gallic acid, DPPH, Ferrous sulphate.

2.1.4 Equipment

Analytical Balance (Ohaus Corporation, Shanghai,) Water bath, Mechanical grinder (Viking Exclusive, Joncod), Refrigerator, Rotary evaporator (Staurt, England) incubator ,Autoclave, Weighing balance, UV - Visible spectrophotometer.

2.1.5 Other Materials

Whatman Filter paper, Masking Tape, Spatula, Cotton wool, Petri dishes, Cork borer, Bunsen flame, Swab, Wire loop, disinfectant.

2.1.6 Drug

Ciprofloxacin 1.5mg, ketoconazole 60mg.

2.1.7 Plant materials

Bryophyllum pinnatum leaf extract.

2.1.8 Study centers

The study was carried out in the following centres: Department of pharmaceutical microbiology, University of Benin, Nigeria. Department of Pharmaceutical Chemistry, Natural Product Research laboratory, University of Benin, Benin City.

2.2 Methods

2.2.1 Collection of plant materials

Fresh Leaves of *Bryophyllum pinnatum* were obtained by a herbal practitoner from Ugbowo in Ovia North East Local Government Area of Edo State in February, 2021. The botanical identification and authentication was carried out in Plant biology and biotechnology (PBB) department, university of Benin and the voucher specimen (voucher number UBH - B593).

2.2.2 Preparation of plant material

The leaves of *Bryophyllum pinnatum* obtained was allowed to dry for 3 weeks before being ground into powder and stored in air tight container until ready for use.

2.2.3 Extraction

The powdered plant materials (1000g) were extracted separately by methanol (3.5L) at room temperature for 1 week. The extracts were concentrated in rotary evaporator at 45°C. The concentrated extract was stored in refrigerator until ready for use.

2.3 Determination phytochemical constituent of the leaves of *Bryophyllum pinnatum* extract

Simple chemical tests to detect the presence of secondary metabolites were done according to standard methods (Stalh, 1973; Sofowora, 1982; Evans, 2002).

Approximately 5 g of the crude powdered sample was boiled with 75 mL of distill ed water for 30 minutes. The solution was filtered hot and allowed to cool. The filtrate obtained was used to carry out the following tests.

2.3.1 General Tests for Alkaloids

- To 2 mL of filtrate was added 2 drops of Dragendorff's reagent.

  Expected observation for a positive result; formation of a reddish brown precipitate.

- To 2 mL of filtrate was added 2 drops of Wagner's reagent.

  Expected observation for a positive result; formation of a brown precipitate.

- To 2 mL of filtrate was added 2 drops of Hager's reagent.

  Expected observation for a positive result; formation of a yellow precipitate.

- To 2 mL of filtrate was added 2 drops of Mayer's reagent.

Expected observation for a positive result; formation of a milky precipitate.

2.3.2 Tests for Carbohydrates

2.3.2.1 Molisch's Test

To 2 mL of filtrate was added 2 drops of 1% alcoholic naphthol followed by 2 mL of concentrated sulphuric acid at a slanty position.

Expected observation for a positive result; formation of a violet ring at the interface of two liquid layers.

2.3.3 Tests for Reducing Sugars

2.3.3.1 Fehling's Test

To 2 mL of filtrate was added 2 drops of Benedict's reagent (a mixture of equal volumes of Fehling's solution A and B). The resulting solution was heated over a boiling water bath for 3 minutes.

Expected observation for a positive result; formation of orange or brick red precipitate.

2.3.3.2 Keller Kiliani's Test for Deoxysugars

To 2 mL of filtrate was added few drops of dilute acetic acid containing a trace of 5% ferric chloride. The resulting mixture was transferred to the surface of concentrated sulphuric acid.

Expected observation for a positive result; formation of a violet ring at the interface of two liquid layers.

2.3.4 Test for Saponins

2.3.4.1 Frothing Test

1 mL of filtrate was diluted with 10 mL distilled water and shaken vigorously for one minute.

Expected observation for a positive result; formation of a persistent frothing.

2.3.4.2 Lieberman Burchard's Test for Steroidal saponins or Phytosterols

A mixture of 1 mL chloroform and few drops of acetic anhydride was added to 2 mL of the filtrate. To the final mixture was added 2 drops of concentrated sulphuric acid.

Expected observation for a positive result; Gradual change of colour from violet to blue and to green.

2.3.5 Test for Tannins

2.3.5.1 Gelatin Test

To 2ml of the aqueous filtrate was added 2ml of 1% gelatin solution in 10% NaCl.

Expected observation for a positive result: formation of precipitate.

2.3.6 Test for Phenolic Compounds

2.3.6.1 Ferric Chloride Test

To 2ml of filtrate was added 5ml of distilled water followed by 2 drops of 5% ferric chloride solution. A blank test was done by adding 2 drops of 5% ferric chloride to 5ml of distilled water.

Expected observation for a positive result; formation of intense coloration in the test sample.

2.3.7 Test for Flavonoids

2.3.7.1 Alkaline Reagent Test

To 2ml of filtrate was added few drops of 20% sodium hydroxide solution followed by few drops of dilute hydrochloric acid solution.

Expected observation for a positive result: formation of intense yellow precipitate which dissolves on addition of dilute acid.

2.3.8 Test for Terpenoids

2.3.8.1 Salkowski Test

The filtrate (5 mL) was mixed with 2 mL of chloroform and concentrate H_2SO_4 was carefully added (drop wise) to form a layer.

Expected observation for a positive result: formation of a reddish brown coloration at the interface.

2.3.9 Test for Anthraquinone Derivatives

2.3.9.1 Bontreger's Test

2 mL of filtrate was shaken with 2 mL of petroleum ether. The ether layer was washed with 2 mL distilled water and then shaken with dilute ammonia solution.

Expected observation for a positive result: formation of pink colour on addition of ammonia solution.

2.4 Determination of Polyphenolic Content

2.4.1 Total Phenol

Total phenol content in the extracts were determined by the method described by Kim *et al.*, 2003. The extract solution (0.5 mL) with a concentration of 1000 µg/mL was added to 4.5 mL of deionized distilled water and 0.5 mL of Folin Cioacalteu's reagent (previously diluted with water 1:10, v/v) which was then added to the solution. After mixing the tubes, they were maintained at room temperature for 5 minutes followed by the addition of 5mL of 7% sodium carbonate and 2 mL of deionized distilled water.

After mixing the samples, the samples were incubated for 90 minutes at room temperature. The absorbance was measured by spectrophotometer at 750 nm. The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of extract (mg GAE/g extract). The standard curve was prepared by gallic acid in six different concentration (12.5, 25, 50, 75, 100 and 150 mg/L).

2.4.2 Total Flavonoid

Total flavonoid contents were estimated using the method described by *Ebrahimzadeh et al.,* 2008. Briefly, 0.5 mL 0f extract sample (1 mg/mL) was mixed with 1.5 mL of methanol and then , 0.1 mL of 10% aluminium chloride was added, followed by 0.1 mL of 1 M potassium acetate and 3.8 mL of distilled water. The mixture was incubated at room temperature for 30 minutes. The absorbanve was measured ny a spectropjotometer at 415 nm. The results were expressed as milligrams quercetin equivalents (QE) per gram of extract (mg QE/g extract). The standard curve was prepared by quercetin in six different concentrations (12.5, 25, 50, 75, 100 and 150 mg/L).

2.5 Determination of Antioxidant Activity

2.5.1 DPPH Radical Scavenging Assay

The scavenging effect of crude methanol extract on DPPH radical was estimated with method described by *Jain et al*., 2008. A solution of 0.1mM DPPH in methanol was prepared, and 1.0 mL of this solution was mixed with 3.0 mL of extract in methanol containing 0..01 - 0.2mg/mL of the extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 minutes. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used

as reference standard. The ability to scavenge DPPH radical was calculated by the following equation:

DPPH radical scavenging activity (%)={A_0-A_t/(A_0)} x 100

Where; A_0 was the absorbance of DPPH radical + methanol,

A_t was the absorbance of DPPH radical + sample extract/standard.

The 50% inhibitory concentration value (IC50) is indicated as the effective concentration of the sample that is required to scavenge 50% of the DPPH free radical

2.5.2 Ferric Reducing Antioxidant Power (FRAP) Assay

Reagents for FRAP assay

a) Acetate buffer 300 mM pH 3.6: Weigh 3.1 g sodium acetate trihydrate and add 16 mL of glacial acetic acid and make the volume to 1 L with distilled water

b) TPTZ (2, 4, 6-tripyridyl-s-triazine); (M.W, 312.34), 10 mMin 40 mM HCl (M.W, 36.46).

c) $FeCl_3$ 6 H_2O; 9M.W, 270.30), 20 mM.

The working FRAP reagent was prepared by mixing a, b and c in the ratio of 10:1:1 just before testing. Standard was $FeSO_4$. 7 H_2O: 0.1 - 1.5 mM in methanol.

2.5.3 Procedure

FRAP solution (3.6 mL) add to distilled water (0.4 mL) and incubated at 37^0C for 5 min. Then this solution mixed with certain concentration of the plant extract (80 mL) and incubated at 37^0C for 10 min. The absorbance of the reaction mixture was measured at 593 nm. For construction of the calibration curve, five concentration of FeSO4. 7H2O (0.1, 0.4, 0.8, 1, 1.2, 1.5 mM) were used and the absorbance values were measured as for sample solutions.

The FRAP assay was done according to Benzie and Strain (1996) with some modifications. The stock solutions included 300 mM acetate buffer (3.1g $C_2H_2NaO_2$.$3H_2O$ and 16 mL $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ solution. The fresh working was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL FeCl3.6H2O solution and then warmed at 370C before using. Fruit extracts (150 mL) were allowed to react with 2850 mL of the FRAP solution for 30 min in the dark condition. Readings of colored product [ferrous tripyridyltriazine complex] were then taken at 593 nm. The standard curve was linear between 25 and 800 mM Trolox. Results are expressed in mM TE/g fresh mass. Additional dilution was needed if the FRAP value measured was over the linear range of the standard curve.

2.6 Antimicrobial Activity Screening

2.6.1 Microbial Sample Collection

The organisms used in the course of the investigation were pathogenic clinical isolates (*S.aureus, E.coli, B.subtilis, P.aeruginosa, C. albican, A.niger, K. pneumonia*) from the University Of Benin Teaching Hospital (UBTH), they were collected and transferred to the Department Of Pharmaceutical Microbiology University Of Benin, Benin City.

2.6.2 Preparation of Stock Solution

Stock solution of 5ml Ciprofloxacin was prepared containing 20µg/ml. 100µg of Ciprofloxacin was dissolved in 0.5ml 10% DMSO and 4.5ml of sterile water. Fluconazole 2µg/ml was used as stock solution.

2.6.3 Preparation of Inoculum

The Inoculums were standardized to obtain 1.5 x 10^8 CFU/ml approximately. The bacteria were diluted to about 10^7 CFU/ml and a loopful of the inoculum was used for spotting on the agar plates.

2.6.4 Susceptibility Testing of Methanol Extract of *Bryophyllum pinnatum* Leaves

Agar well diffusion method was used to determine the antimicrobial activity of the extract using Muller Hinton agar.(Das, et al., 2013) The agar plate was inoculated with the microbial organisms over the entire agar surface, and seven wells were made with a sterile cork borer. The concentration of the extract was made by dissolving 2g of the extract in 2ml of 20% tweene 80 . 1000mg/ml was the concentration made from the extract, and were introduced into the wells made on the petri dish. They were incubated at 37°c for 24hours and the zone of inhibition was measured.

CHAPTER THREE

RESULTS

3.1 Phytochemical Constituents of *Bryophyllum pinnatum* Leaves Extract

The Crude extract of *Bryophyllum pinnatum* was shown to contain alkaloids, carbohydrates, reducing sugar, saponins, terpenoids, phenols, flavoinoids, and anthraquinones.

Table 3.1 Phytochemical Constituents of *Bryophyllum pinnatum* Leaves Extract

Phytochemical	**Inference**
Alkaloids	+
Carbohydrates	+
Reducing sugars	+
Deoxy sugars	+
Saponins	+
Tannins	-
Terpenoids	+
Phenols	+
Flavonoids	+
Anthraquinones	+

Key

+ Indicates presence of the componen

- Indicates absence of the component

3.2 Total Phenolic and Flavonoid Content in the Leaves Extract of *B. pinnatum.*

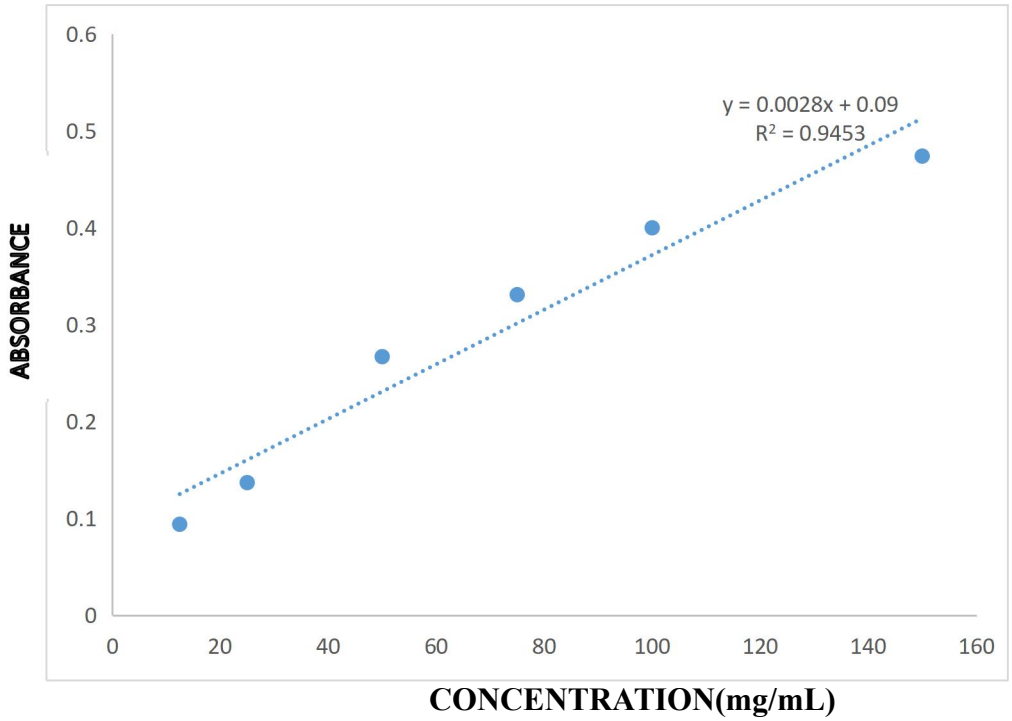

Figure 3.1 Gallic acid calibration curve for the determination of total phenol content.

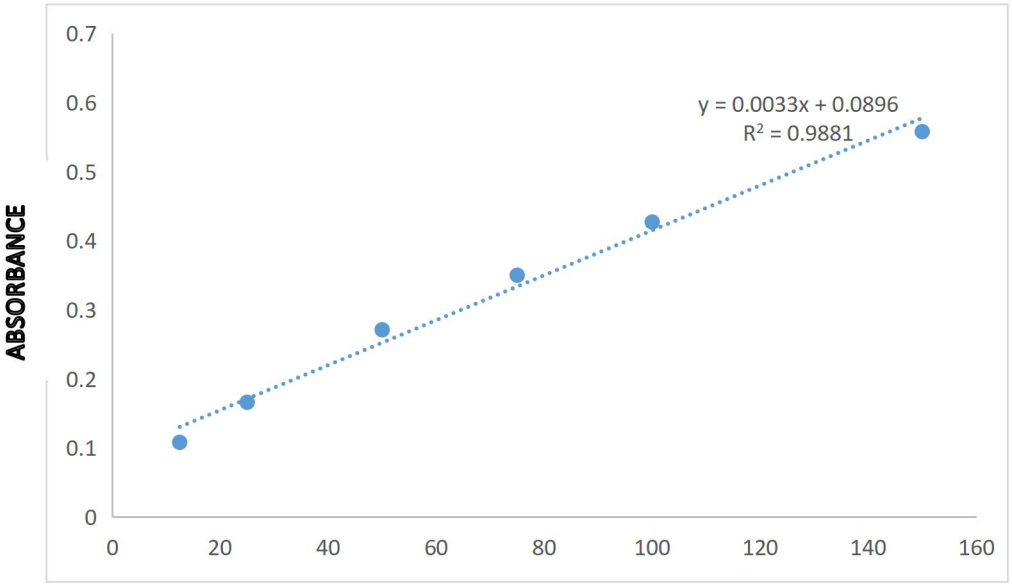

Figure 3.2 Quercetin calibration curve for the determination of total flavonoid content.

Table 3.2 Total Phenolic Content in the leaves of *B. pinnatum*

Sample	Mean ± SEM
Leaves	0.040 ± 0.0080

Table 3.3 Total Flavonoid Content in the leaves of B. pinnatum

sample	Mean ± SEM
Leaves	0.197 ± 0.0051

3.3 Antioxidant Activity:

3.3.1 DPPH Assay

Table 3.4 DPPH scavenging activity (% inhibition) of *B. pinnatum*

CONC. mg/mL	Ascorbic acid	Leaf Extract
0.01	28.34 ± 0.0047	12.03 ± 0.0056
0.03	35.56 ± 0.0022	24.60 ± 0.0015
0'07	42.78 ± 0.0021	27.01 ± 0.0036
0.1	48.93 ± 0.0038	32.62 ± 0.0027
0.12	50.00 ± 0.001	40.91 ± 0.0018
0.15	56.68 ± 0.0025	48.13 ± 0.0029
0.2	59.09 ± 0.0021	50.53 ± 0.0018

The Data represent Mean ± Standard Error of Mean(SEM) of triplicate analysis

Table 3.5 50% Inhibitory concentration of DPPH

Samples	1C50 (µg/mL)
Ascorbic acid	120
Leaf extract	189

Table 3.6 Ferric Reducing Antioxidant Power (FRAP)

Conc. mM	Ferrous Suiphate	Leaf Extract
0.1	0.358 ± 0.0049	0.052 ± 0.0006
0.4	0.434 ± 0.0311	0.189 ± 0.0015
0.8	0.495 ± 0.0198	0.391 ± 0.0024
1.0	0.568 ± 0.0088	0.499 ± 0.0024
1.2	0.642 ± 0.0025	0.612 ± 0.0020
1.5	0.713 ± 0.0070	0.781 ± 0.0026

The Data represent Mean ± Standard Error of Mean(SEM) of triplicate analysis

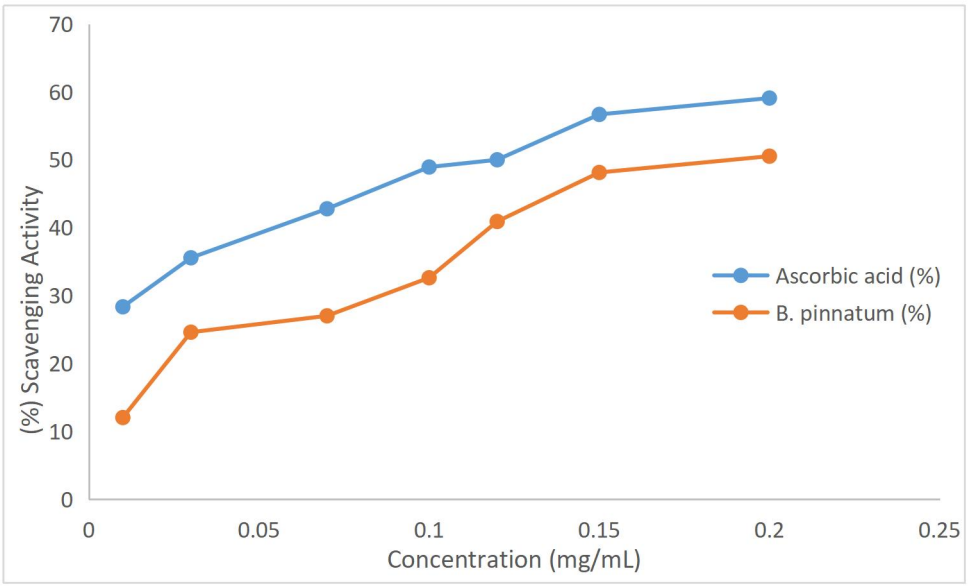

Figure 3.3 Percentage Scavenging Activity of DPPH and *B. pinnatum*

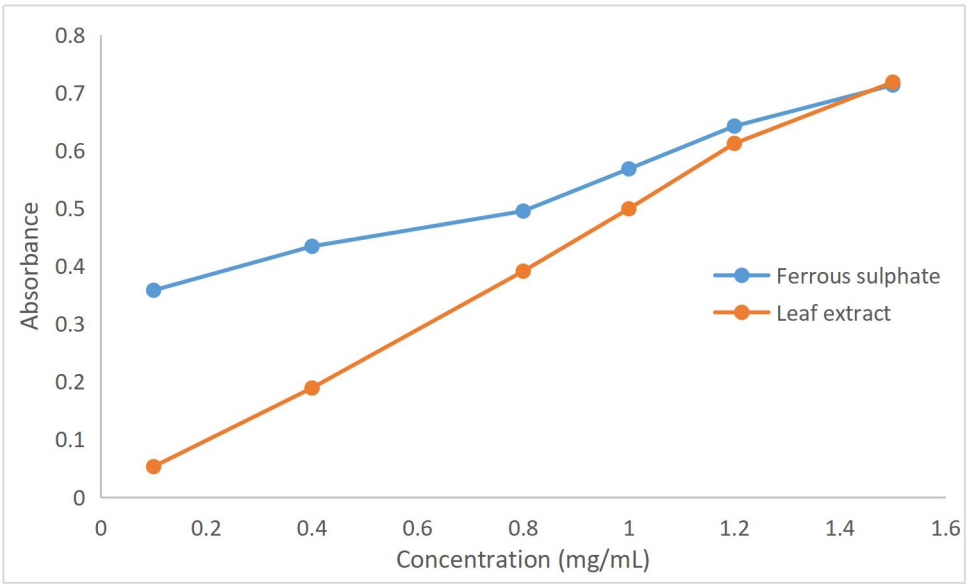

Figure 3.4 Plot of Ferrous sulphate concentration (mM) vs absorbance for determination of antioxidant activity.

3.4 Antimicrobial Susceptibility Testing of *Bryophyllum pinnatum*

The susceptibility of a microorganism to an antimicrobial agent is evident by the presence of growth inhibitory zone on seeded agar plate. This zone is measured in millimetre as an index of the killing or inhibitory action of the test agent against a given organism. The isolates investigated include *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Klebsilla pneumonia, Candida albican, and Aspergillus niger.*

Table 3.7 Zone of Inhibition (mm) Produced by Plant Extract and Standard Drugs Against Test Organisms

Organisms	Extract (1000mg/ml)	Standard drug	Standard used(Strenght)
P. Aeruginosa	ND	22	Ciprofloxacin (1.5mg/ml)
E. Coli	ND	20	Ciprofloxacin (1.5mg/ml)
K. Pneumonia	ND	19	Ciprofloxacin (1.5mg/ml)
S. Aureus	ND	40	Ciprofloxacin (1.5mg/ml)
B. Subtilis	ND	20	Ciprofloxacin (1.5mg/ml)
C. Albicans	ND	35	Ketoconazole (60mg/ml)
A. niger	ND	ND	Ketoconazole (60mg/ml)

Key

ND = Not Detected

CHAPTER FOUR

DISCUSSION

4.1 Phytochemical Constituents of *Bryophyllum pinnatum* Leaves Extract

The phytochemical screening of the leaf extract of *Bryophyllum pinnatum* revealed the presence of alkaloids, carbohydrates (including reducing and deoxy-sugars), saponins, terpenoids, phenols, flavonoids and anthraquinones; notably, tannins were not detected under the conditions used. This profile aligns in large part with previous studies of *B. pinnatum* and supports the plant's traditional use in treating infections. (Chaithra et al., 2020).

The detection of alkaloids is significant. Alkaloids are nitrogenous secondary metabolites that often display potent antimicrobial activities through mechanisms such as interfering with microbial nucleic acid synthesis or disrupting membrane integrity. Their presence in the extract therefore provides a plausible basis for antimicrobial effects observed in various *B. pinnatum* leaf extracts. (A Review On Pharmacological Activities of *B. pinnatum*, 2020).

The positive result for saponins likewise supports an antimicrobial mechanism. Saponins are amphiphilic compounds capable of interacting with microbial cell membranes, increasing permeability or causing lysis. Their presence suggests that part of the extract's antimicrobial effect could stem from membrane-perturbing actions. Numerous studies of *B. pinnatum* have linked saponin content to bioactivity. (Okafor et al., 2023).

The positive results for phenols and flavonoids are particularly important. Phenolic compounds and flavonoids are among the most widely studied plant antimicrobial agents: they can inhibit microbial enzymes, chelate metal ions necessary for microbial growth, generate oxidative stress on microbes, and disrupt cell walls. Given that your

extract contains both, it is highly plausible that these classes contribute substantially to any antimicrobial activity observed. Recent phytochemical reviews of *B. pinnatum* emphasise flavonoids as major active constituents.(*Phytochemical & Pharmacological Profile Review, 2022*).

The positive result for terpenoids corroborates further mechanistic possibilities: terpenoids (monoterpenes, diterpenes, triterpenes) often have membrane-disrupting properties and broad antimicrobial/anti-inflammatory effects; several *B. pinnatum* studies report terpenoids that correlate with activity. (*Chaithra et al., 2020*).

The presence of anthraquinones is noteworthy. Though less commonly emphasised in some *B. pinnatum* screenings, anthraquinones do have documented antimicrobial and laxative effects, and their detection strengthens the view that the leaf extract is chemically rich and potentially pharmacologically active. (*Okele et al., 2019*).

The detection of carbohydrates (reducing and deoxy-sugars) is expected: leaves generally contain substantial primary metabolites. Although these are not directly antimicrobial, they may affect the extract's solubility, viscosity, and perhaps synergise with secondary metabolites (for example by improving uptake or stabilising active compounds). It is also consistent with nutritional-/metabolite-profiling studies of the plant. (*Chaithra et al., 2020*).

An interesting point is the absence of tannins. Tannins are polyphenolic compounds known for their antimicrobial, astringent and protein-precipitating properties. Several studies of *B. pinnatum* report tannin presence; for example, quantitative screening found tannin levels in polar extracts. (*Okafor et al., 2023*). The negative result may reflect several factors: the extraction solvent used may not have efficiently extracted tannins; the detection threshold of the qualitative test may have been too high; or the

particular plant sample (e.g., geographic origin, harvest time, maturity) may have lower tannin content compared to other studies.

The phytochemical profile obtained strongly supports the antimicrobial screening of the extract. Multiple classes known to exert antimicrobial activity are present, which provides a mechanistic rationale for any positive antimicrobial results obtained. Literature review on *B. pinnatum* reports that methanolic leaf extracts show good antibacterial activity against both Gram-positive and Gram-negative bacteria. (Akinpelu, 2000).

4.2 Total Phenol Content

Total phenolic content was assayed using the Folin-Ciocalteu method. In this study, the absorbance of a series of concentrations of gallic acid were plotted to their concentration to yield a linear calibration curve of gallic acid ($y = 0.0028x + 0.09$) with coefficient of correlation (r) value of 0.9453 (Figure 3.1). The total phenolic content (TPC) of *Bryophyllum pinnatum* leaf extract was found to be 0.040 ± 0.008 mg GAE/g, indicating a moderate concentration of phenolic compounds when compared to other medicinal plants. Phenolic compounds are well known for their hydrogen-donating ability, which contributes significantly to antioxidant activity (Gülçin, 2025). The presence of phenols such as gallic acid derivatives and catechins in *B. pinnatum* supports its reported bioactive potential and therapeutic use in oxidative stress-related disorders (Araújo et al., 2023).

The relatively moderate phenolic content observed may be attributed to extraction solvent efficiency, plant maturity, and post-harvest storage conditions, all of which influence the recovery of phenolic compounds (da Silva et al., 2024). Similar phenolic ranges have been reported for methanolic extracts of *B. pinnatum*, suggesting that

methanol is an effective solvent for extracting moderately polar phenolics (Selvakumar, 2022). Phenolic compounds act as reducing agents and singlet oxygen quenchers, which explains their role in the antioxidant capacity demonstrated by the extract (Baschieri, 2021).

4.3 Total Flavonoid Content

Total flavonoid content (TFC) was determined by the aluminium chloride ($AlCl_3$) colorimetric method with quercetin standards (12.5–150). In this study, the absorbance of a series of concentrations of were plotted to their concentration to yield a linear calibration curve of quercetin acid ($y = 0.00334x + 0.0896$) with coefficient of correlation (r) value of 0.9881 (Figure 3.1). The TFC of *B. pinnatum* was 0.197 ± 0.005 mg QE/g, which is higher than its total phenol content, confirming that flavonoids form a major component of its antioxidant system. Flavonoids such as quercetin and kaempferol derivatives have been identified as key bioactive constituents of *B. pinnatum*, exhibiting strong free radical scavenging and metal-chelating abilities (Fernandes et al., 2019; Sharma, 2024).

The high flavonoid level correlates with previous studies linking flavonoid-rich *B. pinnatum* extracts to anti-inflammatory, hepatoprotective, and antimicrobial activities (Selvakumar, 2022; Nnaebue et al., 2024). Flavonoids' structural characteristics especially hydroxyl group number and position enhance their redox potential and stability as radical scavengers (Duan et al., 2025). Thus, the elevated flavonoid content in this extract supports its biological efficacy and justifies the traditional use of *B. pinnatum* in wound healing and infection management (Araújo et al., 2023).

4.4 Antioxidant Activity

The antioxidant potential of the extract was evaluated using DPPH radical scavenging and Ferric Reducing Antioxidant Power (FRAP) assays. The DPPH assay revealed a dose-dependent increase in scavenging activity, with an IC_{50} of 189 µg/mL, compared to 120 µg/mL for ascorbic acid, indicating moderate antioxidant efficiency. The FRAP assay showed a steady increase in absorbance with concentration, confirming the extract's reducing capability, albeit lower than the standard Fe^{2+} curve.

These results align with previous findings that *B. pinnatum* possesses appreciable antioxidant capacity primarily due to its phenolic and flavonoid constituents (*Selvakumar, 2022; da Silva et al., 2024*). Antioxidants from plant sources are crucial in neutralising reactive oxygen species (ROS), reducing lipid peroxidation, and protecting cellular macromolecules from oxidative stress (*Chaudhary, 2023*). The moderate antioxidant activity observed suggests a synergistic contribution of both phenolics and flavonoids, rather than a single compound effect (*Tumilaar et al., 2024*).

Environmental stress factors, extraction temperature, and solvent polarity have also been shown to influence antioxidant potential in *B. pinnatum* (*Gadge and Gadge, 2025*). Despite being less potent than the standard ascorbic acid, the extract's activity supports its ethnopharmacological relevance as a natural antioxidant remedy.

4.5 Antimicrobial Activity

The methanolic extract of *Bryophyllum pinnatum* leaves at a concentration of 1000 mg/ml showed no detectable antimicrobial activity against all the test organisms used in this study, including *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans*, and *Aspergillus niger*. This was evidenced by the absence of inhibition zones (ND) across

all microbial strains, indicating that under the experimental conditions, the extract exhibited no bactericidal or fungicidal effect.

The lack of activity at such a high extract concentration suggests that either the methanolic extract did not extract sufficient bioactive antimicrobial compounds, or the phytochemicals present were not potent enough to inhibit the growth of the tested organisms at this dose. This finding contrasts with previous studies where *B. pinnatum* methanol extracts demonstrated broad-spectrum antimicrobial activity. For instance, Adedayo et al. (2022) reported significant antibacterial effects of *B. pinnatum* methanol extract against *S. aureus* and *E. coli*, while Akinpelu et al. (2021) showed inhibition against *P. aeruginosa* and *B. subtilis*. Differences in plant age, environmental factors, extraction efficiency, and solvent polarity could account for this variation (Nwankwo et al., 2023).

In contrast, the standard antibiotics produced clear zones of inhibition against the respective organisms. Ciprofloxacin (1.5 mg/ml) showed zones ranging from 19–40 mm, with the highest activity against *S. aureus* (40 mm), demonstrating its well-established broad-spectrum potency (Okoro et al., 2021). Ketoconazole (60 mg/ml) inhibited *C. albicans* with 35 mm, showing strong antifungal action. However, *A. niger* showed no response to the extract or standard drug, consistent with the intrinsic resistance of filamentous fungi to many antimicrobial agents (Eze & Nwachukwu, 2022).

The absence of antimicrobial activity observed in this extract, despite documented bioactivity in literature, highlights the importance of phytochemical variation, solvent choice, and extraction time, as extraction was performed in February 2021, and

phytochemical potency may degrade over time if drying, storage, and extraction protocols are suboptimal (Chukwu et al., 2023).

Overall, while *B. pinnatum* is known to possess antimicrobial phytoconstituents such as flavonoids, alkaloids, and tannins (Ibrahim et al., 2022), this result suggest that the methanol extract prepared in February 2021 was not effective under the present laboratory conditions.

CHAPTER FIVE

CONCLUSION

The present study demonstrated that the methanolic leaf extract of *Bryophyllum pinnatum* possesses appreciable levels of phenolic and flavonoid compounds, both of which significantly contribute to its antioxidant potential. The total phenolic content (TPC) and total flavonoid content (TFC) correlated positively with the antioxidant activities determined through DPPH radical scavenging and ferric-reducing antioxidant power (FRAP) assays. These findings indicate that the extract is capable of donating hydrogen atoms or electrons to neutralize free radicals and reduce oxidative intermediates.

The observed antioxidant capacity validates the ethnomedicinal use of *B. pinnatum* in the treatment of oxidative stress-related disorders such as inflammation, wound infections, and hepatic dysfunction. The synergy between phenolic and flavonoid constituents may be responsible for the overall antioxidant efficiency observed in the assays.

Therefore, *Bryophyllum pinnatum* can be regarded as a promising natural source of antioxidant agents with potential pharmacological applications. However, further investigations are recommended to isolate, purify, and characterize the specific bioactive compounds responsible for these effects. Additionally, in vivo studies and toxicity evaluations should be conducted to establish its safety profile and therapeutic efficacy in biological systems.

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APPENDIX

The following presents the full calculations used to obtain the quantitative results in this study.

APPENDIX A: Total Phenol Content (TPC) Calculations (mg GAE/g)

Calibration curve used:

$y = 0.0028x + 0.09$

Where:

(y) = absorbance of sample

(x) = concentration of gallic acid (mg/L)

Given that sample absorbance from the data is 0.202

$0.202 = 0.0028x + 0.09$

$0.202 - 0.09 = 0.0028x$

$0.112 = 0.0028x$

$x = 0.112/0.0028 = 40$ mg/L

Since 1 mg/mL extract was used, concentration translates to:

$40/1000 = 0.040$

Thus; TPC = 0.040 mg GAE/g

APPENDIX B: Total Flavonoid Content (TFC) Calculations (mg QE/g)

Calibration curve used:

$y = 0.00334x + 0.0896$

Given that sample absorbance is 0.748

$0.748 = 0.00334x + 0.0896$

$0.748 - 0.0896 = 0.00334x$

$0.6584 = 0.00334x$

x = 0.6584/0.00334 = 197 mg/L

Since 1 mg/mL extract was used, concentration translates to:

197/1000 = 0.197

Thus; TFC = 0.197 mg QE/g

APPENDIX C: Calculation of % DPPH Radical Scavenging Activity

DPPH radical scavenging activity (%)={A_0-A_t/(A_0)} x 100

Where;

A_0 was the absorbance of DPPH radical + methanol,

A_t was the absorbance of DPPH radical + sample extract/standard.

Example; % inhibition of Leaf Extract at concentration of 0.10 mg/mL = 32.62% From the raw table below.

Table 3.3.1.1 DPPH scavenging activity (% inhibition) of *B. pinnatum*

CONC. mg/mL	Ascorbic acid	Leaf Extract
0.01	28.34 ± 0.0047	12.03 ± 0.0056
0.03	35.56 ± 0.0022	24.60 ± 0.0015
0'07	42.78 ± 0.0021	27.01 ± 0.0036
0.1	48.93 ± 0.0038	32.62 ± 0.0027
0.12	50.00 ± 0.001	40.91 ± 0.0018
0.15	56.68 ± 0.0025	48.13 ± 0.0029
0.2	59.09 ± 0.0021	50.53 ± 0.0018

The Data represent Mean ± Standard Error of Mean(SEM) of triplicate analysis

Where;

A_t = 0.252

$A_0 = 0.374$

$\frac{0.374 - 0.252}{0.374} \times 100 = 32.62\%$

APPENDIX D: Determination of IC_{50} for DPPH

IC_{50} = concentration of extract/standard required to achieve 50% inhibition.

For the leaf extract:

Concentration (mg/mL): 0.15 → 48.13%

Concentration (mg/mL): 0.20 → 50.53%

Steps:

$\Delta\% = 50.53 - 48.13 = 2.40$

Difference from 50% = $50 - 48.13 = 1.87$

Fraction = $1.87 / 2.40 = 0.77917$

$IC_{50} = 0.15 + 0.77917 \times 0.05 = 0.18896$ mg/mL

Convert to µg/mL: (muitiply by 1000)

$IC_{50} = 188.96$ µg/mL ≈ 189 µg/mL

For Ascorbic acid:

Concentration (mg/mL): 0.12 → 50.00%

At this concentration, 50% inhibition is been achieved

Thus; $IC_{50} = 0.120$ mg/mL

Convert to µg/mL: (muitiply by 1000)

$IC_{50} = 120$ µg/mL