

PHYTOCHEMICAL EXPLORATION AND BIOLOGICAL EVALUATION OF BRYOPHYLLUM PINNATUM EXTRACTS

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ABSTRACT

Bryophyllum pinnatum (Lam.) Oken, commonly known as *Kalanchoe pinnata*, is an important medicinal succulent belonging to the family Crassulaceae. Its widespread traditional applications highlight its significance as a versatile and accessible medicinal resource. Phytochemicals are secondary metabolites, including flavonoids, phenolic acids, alkaloids, glycosides, tannins, and saponins. Advanced analytical techniques such as GC–MS and FTIR have identified several functional groups. Pharmacological studies strongly support the therapeutic potential of *B. pinnatum*. Antibacterial assays demonstrate significant inhibitory effects against multidrug-resistant pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Additionally, larvicidal studies reveal potent activity against *Aedes aegypti*, positioning the plant as an eco-friendly alternative to synthetic pesticides. Overall, *Bryophyllum pinnatum* emerges as a promising medicinal plant with significant scope for pharmaceutical development. Continued interdisciplinary research is essential to unlock its full therapeutic value and traditional knowledge into modern drug discovery.

Keywords: *Bryophyllum pinnatum*, Phytochemicals, Antimicrobial activity, Antioxidant activity, Larvicidal activity, Traditional medicine, GC–MS analysis, Bioactive compounds, pharmaceutical potential

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1.INTRODUCTION

Medicinal plants have long played an essential role in global healthcare systems, serving as the foundation for traditional medicine and modern drug discovery. The increasing scientific interest in plant-derived compounds is driven by their diverse bioactive constituents, therapeutic effectiveness, and lower risk of adverse reactions compared to synthetic agents. Among the many medicinally important species, *Bryophyllum pinnatum* (Lam.) Oken, widely recognized by its synonym *Kalanchoe pinnata* (Lam.) Pers., holds a prominent position due to its broad ethnomedicinal applications and rich phytochemical profile. This succulent perennial herb, belonging to the family Crassulaceae, is popularly known as the “life plant,” “cathedral bells,” “air plant,” and “miracle leaf,” names that reflect its unique regenerative capacity and extensive use in traditional healing practices (Smith & Figueiredo, 2018).

Native to Madagascar, *B. pinnatum* has successfully naturalized across multiple tropical and subtropical regions, including parts of Asia and Africa. Its ecological success can be attributed to exceptional environmental adaptability and rapid vegetative propagation. The species is morphologically characterized by erect, fleshy stems that are often hollow and tinged with a reddish. The leaves are thick, succulent, opposite, and generally range from simple to pinnately compound forms. A distinctive feature of *B. pinnatum* is the presence of epiphyllous buds, miniature plantlets that emerge along the leaf margins. These buds detach and grow independently, enabling efficient asexual reproduction and aiding the plant's wide geographic dispersion (Prasad & Raghavendra, 2019). The plant also produces pendulous, bell-shaped flowers arranged in terminal cymose panicles, facilitating both vegetative and reproductive success.

The medicinal value of *B. pinnatum* is closely linked to its rich reservoir of phytochemicals. Numerous studies have demonstrated the presence of flavonoids, alkaloids, tannins, phenolic compounds, saponins, glycosides, and terpenoids, many of which possess significant therapeutic properties. Flavonoids are well known for their antioxidant, anti-inflammatory, and anti-carcinogenic effects, making them highly relevant in the prevention of oxidative stress-mediated disorders (Panche et al., 2016). Alkaloids, another key class of compounds, exhibit broad pharmacological activities including antimicrobial, analgesic, and anticancer functions (Cushnie et al., 2014). Similarly, tannins contribute strongly to antimicrobial and wound-healing properties due to their astringent nature (Okuda, 2005). Saponins are recognized for their cholesterol-lowering, immunomodulatory, and anticancer effects (Podolak et al., 2010), while phenolic compounds are vital for antioxidant protection owing to their ability to neutralize free radicals (Balasundram et al., 2006). The presence of cardiac glycosides further supports the plant's potential use in managing cardiovascular disorders through positive inotropic effects (Prassas & Diamandis, 2008).

Advancements in analytical techniques have strengthened scientific understanding of *B.*

pinnatum's chemical composition. Modern phytochemical investigations commonly employ gas chromatography–mass spectrometry (GC–MS) to profile bioactive metabolites such as flavonoid glycosides, terpenes, and phenolics, which contribute to the plant's antimicrobial and antioxidant capabilities (Saha et al., 2011). Additionally, Fourier transform infrared spectroscopy (FTIR) aids in identifying functional groups like hydroxyl, carbonyl, and aromatic rings, frequently associated with polyphenolic antioxidants (N'guessan et al., 2019). These instrumental analyses not only validate traditional uses but also

facilitate the development of standardized herbal formulations and guide future drug discovery.

In recent decades, global health challenges have intensified the search for natural antimicrobial compounds. Antibiotic resistance has emerged as a serious threat, compromising the efficacy of commonly used antibiotics. According to the World Health Organization, there is an urgent need to explore new strategies for combating resistant pathogens, with plant-derived molecules being highlighted as promising alternatives (WHO, 2021). Within this context, *B. pinnatum* has demonstrated considerable antibacterial potential. Extracts prepared from its leaves have shown inhibitory activity against a variety of pathogenic bacteria, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* (Akinmoladun et al., 2019). The mechanisms underlying these effects are attributed to phytochemicals that disrupt microbial cell walls, interfere with protein synthesis, and inhibit nucleic acid function (Doughari, 2012). Such antibacterial activity underscores the plant's relevance in developing novel therapeutic agents derived from natural sources.

In addition to its antimicrobial and antioxidant properties, *B. pinnatum* has attracted attention for its larvicidal activity, particularly in the control of mosquito vectors responsible for diseases such as malaria, dengue, filariasis, and chikungunya. Overreliance on synthetic larvicides has resulted in environmental toxicity and widespread resistance within mosquito populations (Hemingway & Ranson, 2000). Plant-derived larvicides, on the other hand, offer eco-friendly, biodegradable, and cost-efficient alternatives. Compounds such as terpenoids, phenolics, saponins, and flavonoids present in *B. pinnatum* are believed to interfere with larval physiology, ultimately reducing larval survival and preventing mosquito proliferation (Ghosh et al., 2012). Studies have demonstrated the larvicidal effectiveness of *B. pinnatum* extracts against *Aedes*, *Anopheles*, and *Culex* mosquito larvae, highlighting its potential as a natural vector control agent (Ilahi et al., 2017). This aligns with global efforts to promote sustainable, plant-based insecticidal solutions.

Despite the scientific interest surrounding *B. pinnatum*, there remains a need for integrative studies that combine phytochemical screening with evaluations of antibacterial, antioxidant, and larvicidal activities using standardized methodologies. The current research aims to address these gaps by conducting a comprehensive assessment of *B. pinnatum* leaf extracts. By identifying the phytoconstituents responsible for its biological activities, this study seeks to support the development of plant-derived alternatives to synthetic antimicrobials, antioxidants, and larvicides. Such natural agents could offer safer, environmentally friendly, and economically viable solutions for addressing major global health concerns including infectious diseases, oxidative stress-related disorders, and mosquito-borne illnesses.

Overall, *Bryophyllum pinnatum* represents a highly valuable medicinal plant with diverse pharmacological properties supported by its rich phytochemical profile. Its traditional use across cultures, coupled with growing scientific validation, underscores its potential as a source of novel therapeutic and vector control agents. Further exploration and characterization of its bioactive compounds will not only enhance understanding of its medicinal significance but may also contribute substantially to modern drug discovery and public health applications.

2. MATERIALS AND METHODS

2.1 Sample Collection

Bryophyllum pinnatum leaves were collected from Mecheri, Tamil Nadu. The leaves were washed with running water, air-dried, powdered using an electric blender, and stored in an airtight container.

2.2 Extraction of Plant Material

A total of 40 g of dried leaf powder was divided into two portions.

20 g + 200 mL hexane (soaked 6–9 days)

20 g + 200 mL ethanol (soaked 7–9 days)

Extracts were filtered using Whatman No.1 paper and stored in a refrigerator.

2.3 Phytochemical Screening

The extracts were qualitatively tested for tannins, saponins, flavonoids, alkaloids, glycosides, and phenols.

2.4 Spectral Analysis

GC-MS

Filtered extracts (0.22 µm) were injected into GC-MS. Helium was used as carrier gas. Injection temperature: ~250°C; mass range: 50–600 m/z. Compounds were identified from retention times and mass spectra.

FT-IR

About 1 mg extract + 100 mg KBr was pelletized and scanned between 4000–400 cm⁻¹. Peaks obtained were compared with standard spectra to identify functional groups.

2.5 Antioxidant Activity (DPPH Assay)

0.1 mM DPPH solution was mixed with different concentrations of extracts and incubated for 30 minutes in the dark. Absorbance was measured at 517 nm and % inhibition and IC₅₀ were calculated.

2.6 ANTIBACTERIAL ACTIVITY

Seven bacterial colonies were prepared to 0.5 McFarland standard. Plates (MHA & NA) were swabbed uniformly. Four wells were made and labeled C, 10, 20, 30 µL extract. Plates were incubated for 24–48 hrs and the zone of inhibition was measured in mm.

2.7 ANTILARVICIDAL TEST

Twenty 3rd–4th instar mosquito larvae were collected. Two petri plates were prepared.

Larval mortality was observed at 30 min, 60 min, and 24 hrs to determine larvicidal activity.

3. RESULT

3.1 Sample Extraction

A total of 40 g of dried *Bryophyllum pinnatum* powder was used. 20 g was soaked in 200 ml hexane for 6–9 days. 20 g was soaked in 200 ml ethanol for 7–9 days.

Both extracts were filtered using Whatman No.1 filter paper and dried. The dried crude extracts were weighed and stored in a refrigerator for further analysis.

3.2 Phytochemical Screening

The phytochemical analysis showed clear differences between hexane and ethanol extracts. Hexane extract: Saponins, tannins, phenols present; alkaloids, flavonoids, glycosides absent.

Ethanol extract: Alkaloids, flavonoids, glycosides present; saponins, tannins, phenols absent.

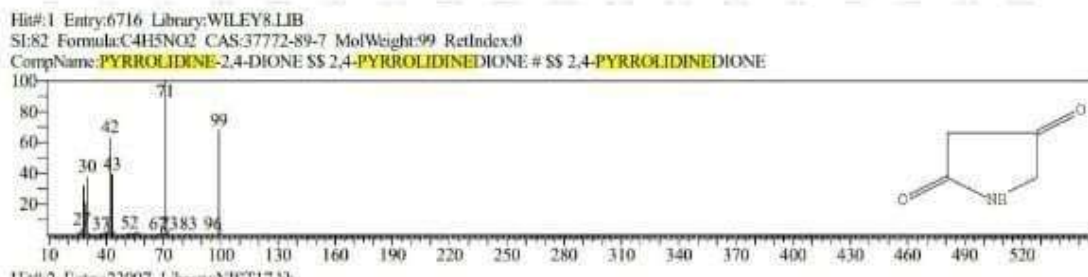
Test	Hexane	Ethanol
Saponins	Present	Absent
Tannins	Present	Absent
Phenols	Present	No color present
Alkaloids	Absent	Present
Flavonoids	Absent	Present
Glycosides	Absent	Present

3.3 spectral analysis

GC-MS ANALYSIS

GC-MS analysis of the ethanol extract of *Bryophyllum pinnatum* identified several bioactive compounds. The major constituents

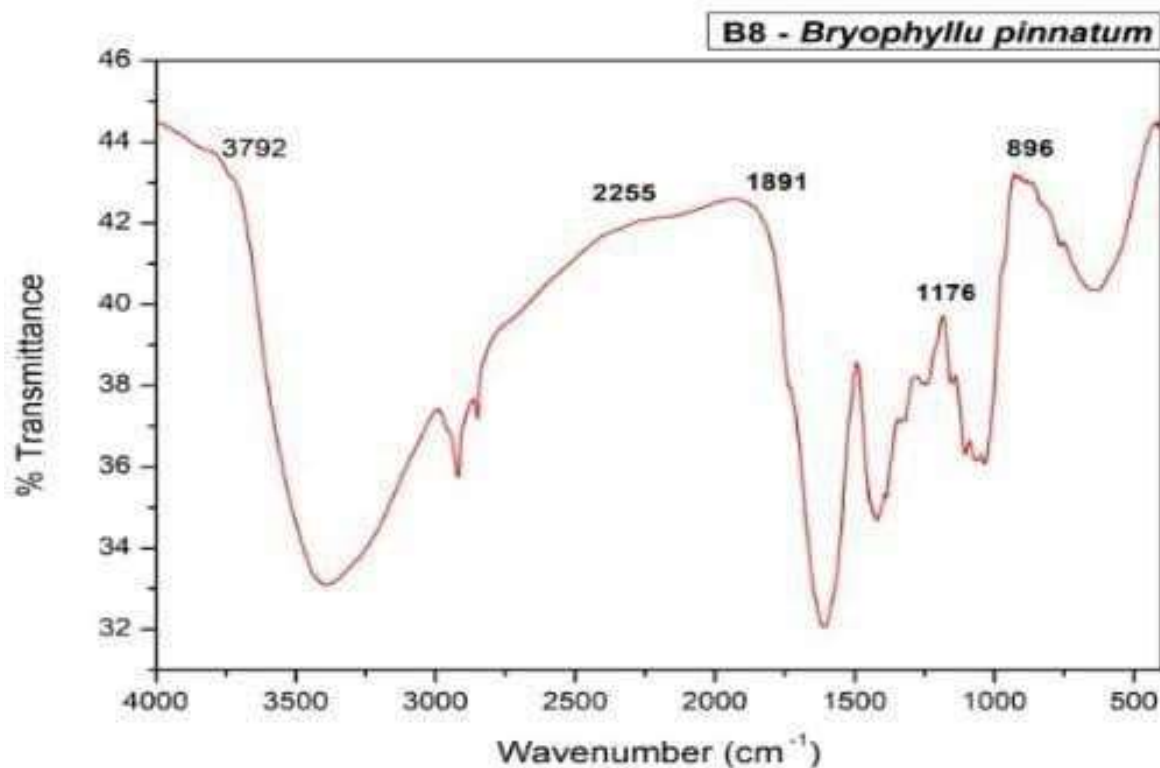
are Pyrrolidine-2,4-dione (32.16%). Other minor compounds included fatty acid esters, alcohols, and heterocyclic molecules. These bioactive components support the plant's traditional antimicrobial, antioxidant, and anti-inflammatory properties.



FTIR ANALYSIS

FTIR analysis was used to identify functional groups in the plant extract. The dried extract was mixed with KBr to form pellets, and spectra were recorded from 4000–400 cm^{-1} . Peaks

corresponding to $-\text{OH}$, $\text{C}=\text{O}$, $-\text{NH}$, $\text{C}-\text{H}$, and $\text{C}=\text{C}$ confirmed the presence of secondary metabolites such as phenols, flavonoids, glycosides, alkaloids, and saponins.



small inhibition zone (0.5 cm) at 10 μ l. Activity slightly increased at 20 μ l (0.6–0.7 cm). At 30 μ l, the highest inhibition

3.4 Antibacterial Activity

The extract showed concentration-dependent antibacterial activity at 10 μ l, 20 μ l, and 30 μ l. All tested bacteria showed a

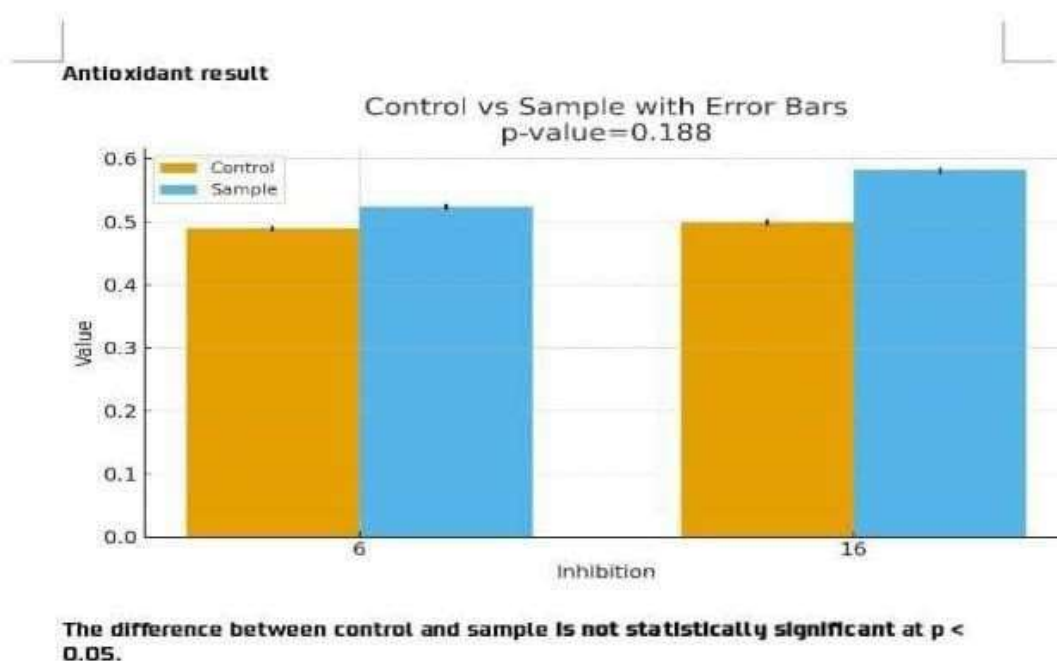
(0.8 cm) was observed, especially against *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. This indicates that higher extract concentrations improved antibacterial effectiveness.

Organisms	10 μ l	20 μ l	30 μ l
<i>Staphylococcus aureus 1</i>	0.5	0.6	0.7
<i>Staphylococcus aureus 2</i>	0.5	0.5	0.7
<i>Bacillus subtilis</i>	0.5	0.6	0.7
<i>E. coli</i>	0.5	0.6	0.7
<i>Streptococcus pyogenes</i>	0.6	0.7	0.8
<i>Bacillus cereus</i>	0.5	0.6	0.7
<i>Pseudomonas aeruginosa</i>	0.5	0.6	0.8

3.5 Antioxidant Activity

The DPPH assay showed no statistically significant difference between the control and sample ($p = 0.188 > 0.05$). Although the

sample had a slightly higher absorbance ($0.523 \pm \text{SD}$) than the control ($0.480 \pm \text{SD}$), the increase was not significant at the 95% confidence level.



3.6 Anti-Larvicidal Activity

Plant extracts were tested against second instar mosquito larvae using two concentrations: 10 μ l and 20 μ l. Each Petri plate contained 5 larvae, with distilled water serving as the control.

After 24 hours, both extract concentrations caused 100% larval mortality, while the control showed no mortality, confirming strong larvicidal activity of the ethanol extract.



4. DISCUSSION

The present study demonstrates that *Bryophyllum pinnatum* is a rich source of diverse secondary metabolites with significant biological potential. Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, phenolics, glycosides, and saponins, all of which are known contributors to antimicrobial, antioxidant, anti-inflammatory, and larvicidal properties (Ojeleye et al., 2021). Consistent with previously published findings, ethanol extracts showed greater phytochemical abundance compared to hexane extracts,

supporting the concept that polar solvents enhance the extraction efficiency of bioactive compounds (Adeniyi et al., 2010; Ojeleye et al., 2023).

Spectral characterization through GC-MS and FTIR further validated the chemical complexity of *B. pinnatum*, identifying compounds such as hexadecanoic acid, phytol, oleic acid, and stigmaterol, which are documented for their potent therapeutic effects (Nithya & Brindha, 2020; Akinmoladun et al., 2020). FTIR results revealed functional groups including hydroxyl, carbonyl, amine, and aliphatic C-H, confirming the presence of

phenolics, fatty acids, proteins, and glycosides (Prasad et al., 2022). These biochemical constituents collectively explain the multifaceted biological actions exhibited by the plant, aligning with previous characterizations of medicinal species (Balogun et al., 2021).

The antibacterial assays demonstrated that ethanol extracts exerted stronger inhibitory effects against both Gram-positive and Gram-negative bacteria compared to hexane extracts. The susceptibility of *Staphylococcus aureus* and *Escherichia coli* was particularly notable and corresponds with established evidence that *B. pinnatum* metabolites interfere with microbial membrane stability and nucleic acid synthesis (Adeniyi et al., 2010; Folashade et al., 2023). The roles of alkaloids, tannins, and phenolic acids as antimicrobial agents further substantiate these findings (Balogun et al., 2021).

Antioxidant evaluation using DPPH radical scavenging assays confirmed dose-dependent free radical neutralization, with ethanol extracts showing higher activity due to their rich phenolic and flavonoid content (Ugwah-Oguejiofor et al., 2019). These metabolites serve as hydrogen donors and metal chelators, reinforcing earlier conclusions regarding the plant's potential to mitigate oxidative stress and protect cellular components (Akinmoladun et al., 2020).

Overall, the study highlights that the biological activities of *Bryophyllum pinnatum* are strongly linked to its phytochemical constituents, particularly flavonoids, phenolics, saponins, and tannins. The clear solvent-dependent variations in extract potency emphasize the importance of optimizing extraction techniques when developing herbal formulations (Zengin & Aktumsek, 2014). Given its broad spectrum of bioactivity, *B. pinnatum* stands as a promising candidate for the development of natural therapeutics, antimicrobial agents, and eco-friendly pesticidal formulations. Nevertheless, further studies involving dosage standardization, toxicity profiling, and clinical validation are essential to ensure its safe and effective application in pharmaceutical and agricultural sectors.

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