# ANTIFUNGAL EFFECT OF *Bryophyllum pinnatum*(MIRACLE LEAF)


## PRESENTED BY:

## SALIMAN BALQEES TEMITOPE

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**SUPERVISED BY**

**MRS HAROON.T ABDULKADIR**

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# CERTIFICATION

This is to certify that this project research has been done and written by **SALIMAN BALQEES TEMITOPE** of Matric No: **HND/23/SLT/FT/0976** and submitted to the Department of Science Laboratory Technology, Microbiology Unit, Institute of Applied Sciences (IAS), Kwara State Polytechnic, Ilorin and has been read and approved in partial fulfillment for the award of Higher National Diploma (HND) in Science Laboratory Technology Department (Microbiology option).

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| **ABDULKADIR HAROON, H.T.**<br>**(PROJECT SUPERVISOR)** | **DATE** |
| **MR. OPEYEMI A.A.**<br>**(H.O.U. ENVIRONMENTAL BIOLOGYUNIT)** | **DATE** |
| **DR. USMAN ABDULKAREEM**<br>**(H.O.D. SLT)** | **DATE** |

## DEDICATION

I dedicate this project work to the Almighty God who has been my source of Wisdom, Knowledge and Understanding.

# ACKNOWLEDGEMENT

All glory be to God!, I am grateful to the all sufficient God, my Father for bestowing upon me the wisdom, strength, and perseverance to complete this project. His divine guidance and blessings have been my constant source of inspiration.

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I also appreciate my own dedication and perseverance which has enabled me to overcome challenges and complete this project, I am thankful for the opportunity to learn and grow from this experience. Thank you all for being part of my journey!

## TABLE OF CONTENT

## *Abstract*

*The study was carried out to evaluate the antifungal effect of Bryophyllum pinnatum against Aspergillus niger, Penicillum sp and Fusarium oxysporum. The ethanolic extract exhibit higher antifungal activity against Fusarium oxysporum (14mm at 2mm) and Aspergillus niger (14mm at 3mm), in contrast Penicillium spp demonstrated resistance against B.pinnatum". This study suggest that Bryophyllum pinnatum possesses significant antifungal properties and could serve as a source for developing alternative antifungal agents, especially in regions with limited access to conventional pharmaceuticals.*

# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1. Background of *Bryophyllum pinnatum*

Fungal infections pose a significant threat to public health, particularly with the rise of drug resistant strains such as; *Candida albican*, *Aspergillus spp* and dermatophytes (Perfect, J R. (2017). Medicinal herbs have been utilized since long ago for the treatment of different ailments in multiple traditional systems such as Traditional Chinese medicine and various indigenous healing systems. (Mekuria *et.al*., 2017).

Traditional herbal medicine has received great acceptance over the decades with about 80% of the world population using the system as the only form of treatment. The ease of access, availability, effectiveness and affordability of medicinal plant makes them acceptable for treating common ailments. (Dorine *et.al*., 2021).

Moreover, secondary metabolites of various plant origins have shown considerable against multiple microbes. This has been recognized by the World Health Organization (WHO) which considers traditional medicine a

fundamental component of health system with excellent results for users. (Palhares *et.al*., 2020).

*Bryophyllum pinnatum* is member of the family *Crassulaceae* also known as the family of sprouting leaves; it is bred as a decorative house plant on stones and in gardens. It hold various names like air plant, Cathedral bell, life plant, resurrection plant and miracle leaf, it is also known as Eru- odundun (Yoruba), Odaaopue (Igbo) and Abomoda (Hausa). The plant is native to Madagascar but has spread to tropical and subtropical regions around the world, including Africa, Asia, the Caribbean, and South America.(Quazi, et.al., 2018).

It is common worldwide due to its ease of cultivation and widespread use in herbal medicine. Deep green and scalloped edges, many trimmed in red, make up its lush foliage. It flowers from November to March and fruits in April (Khan *et.al.*, 2009).

B. pinnatum is recognized for its hemostatic and wound healing capabilities, Anti-inflammatory effect, Antifungal properties,Antioxidant properties and Renal (Kidney) protective effects.The leaves are often used to treat conditions such as kidney stones, ulcers, respiratory infections, skin diseases, and hypertension. (Akinmoladun, et.al., 2020). It is a good source of vitamin and

includes alkaloids, flavonoids, saponins, triterpenes, steroids, glycosides and tanins as bioactive substances (Uchegbu *et.al*., 2017).

The plant has shown anti-leishmanial activity, hepato and nephro-protective effects and analgesics, Anti-hypertensive is one suggested action of the leaf extract and anti-allergic activity. (Parra *et.al*.,2019).

Fungal infections pose significant challenges in healthcare and agriculture, particularly in regions with scarce commercial antifungal drugs. In circumstance such as Somoto state Nigeria, where high temperature and humidity foster the prevalence of fungal infections; the problems are worsening due to antifungal resistance. *Bryophyllum pinnatum* has been traditionally used for wound healing and anti-microbial treatments, yet it's antifungal potential remains underexplored. (Datani, M, *et.al*.,2025).

## 1.2 AIMS AND OBJECTIVES OF THE STUDY

### AIMS

To evaluate the antifungal properties of *Bryophyllum pinnatum* extract against selected pathogenic fungi and explore its efficacy as a natural remedy.

### OBJECTIVES

- To collect and identify healthy *Bryophyllum pinnatum* leaves for

extraction and to prepare *Bryophyllum pinnatum* leaves extract using standard extraction techniques.

- To test the antifungal activity of the extracts against selected fungal isolates.

## 1.3 PROBLEM STATEMENT OF THE STUDY

The rise of antifungal resistance and side effect of conventional drugs highlight the need for alternative treatments. *Bryophyllum pinnatum* known for its traditional medicinal uses has shown potential antifungal activity. However, there is limited scientific data validating its efficacy and spectrum of antifungal activity against clinically relevant fungal isolates.

# CHAPTER TWO

## 2.0. LITERATURE REVIEW

### 2.1 Botanical Description: Taxonomy, Morphology And Native Habitat of *Bryophyllum pinnatum*

### DESCRIPTION

*Bryophyllum pinnatum* is a succulent perennial herbs of genus Bryophyllum belonging to family *Crassulaceae*. The family comprises of 25 genera and 450 species. It is erect and grows about 1.5m height (Nagaratna and Hedge (2019). It is a native of Madagascar but it is now widely naturalized in tropical and sub-tropical regions around the world. It grows between 0.3-2 meters with erect, fleshy and glabrous stem that may have a reddish tinge in young growth. The stems are cylindrical and thick and stores water to survive dry conditions, the leaves are opposite and can be simple with 3-5 leaflets. The plant produces erect, terminal inflorescences that are paniculate or cymose, bearing numerous tubular pendulous flowers.

The fruit is follicle containing numerous tiny seeds but mainly propagates vegetatively through the leaf plantlets. Overall, *Bryophyllum pinnatum* is

robust, succulent and fast growing plant with a unique reproductive strategy, making it both an ornamental and medicinally valuable specie in tropical and sub tropical area. (The Green Institute (2017).

## TAXONOMY

Taxonomic classification of Bryophyllum pinnatum:

| RANK | CLASSIFICATION |
|---|---|
| Kingdom | Plantae |
| Phylum | Tracheophytes |
| Class | Magnoliopsida |
| Order | Saxifragales |
| Family | Crassulaceae |
| Genus | Bryophyllum |
| Specie | *Bryophyllum pinnatum* |

*Bryophyllum* is now generally considered a section within the genus *Kalanchoe* based on molecular phylogenetic studies. (Royal Botanic Garden, Kew. (2024).

## MORPHOLOGY

- **PLANT:** *B. pinnatum* is a short succulent herb measuring about 0.3-1.5m in height.
- **PROPAGATION:** Adventitious buds emerging from the fissures along leaf margins give rise to new plants once they take root. *B. pinnatum* is predominantly propagated through vivaparous seedlings, which exhibit both somatic embryogenesis and organogenesis making it exemplary model for studying asexual reproduction (Du *et.al*., 2023).
- **STEM:** The plant stem is dimly quadrangular, the older the stem, the lighter the colour, whereas, the younger ones are reddish with a dispersed white sprinkles.
- **LEAVES:** The leaves of the plant are fickle and decussate; the lower ones are generally simple/compound, while the upper ones are 3-7 leaflets with a long petiole. A ridge formed by union of the petiole can be observed around the stem. The leaflets are ovate along with aerated margins. Moreover, small plantlets can be observed arising from the serated margins of the leaves (Rahman *et.al*., 2019).

- **FLOWER:** The plant possesses pendulous flowers, which are about 7cm in length, and the stalk possessing the flower measure about 10-25mm in length along with prominent, extravagant yellowish green to pastel green sepals. The sepals under the flowers are partially fused, forming a tube like structure speckled with reddish-green spots. Petals are reddish-purple colored and measures around 3-6cm in length. They are swollen or octagonal at the base with triangular lobes. The anther is pinkish hastate and black with green filaments with green style. Flowering in the plant occurs mostly in winter and spring (Rahman *et.al*., 2019).
- **FRUITS:** The plant's fruit is flimsy and membranous, having four cylindrical carpels, which generally remains encased in older parts of the flowers. The fruit is a follicle containing numerous seeds.
- **SEEDS:** *B. pinnatum* seeds are small, smooth, oblong, slightly striate and brown (Quazi majaz *et.al*., 2022

**Fig 1 Description of *Bryophyllum pinnatum* flower and leaves**


**(Source: International Press Telecommunications Council)**

## NATIVE HABITAT

*Bryophyllum pinnatum* is native of Madagascar. However, it has become naturalized in many tropical and sub tropical regions around the world, including: Tropical Africa, South and Southeast Asia e.t.c. It grows well in well drained soil, sunny or partially shaded areas, and disturbed habitats such as roadsides, open wood lands and rocky hill sides. It can also be grown as ornamental plants in gardens.

It is also a succulent plant, making it well adapted to arid or semi-arid climates, although thrives in warm, moist environment as well. Its ability to reproduce vegetatively from leaf margins allows it spread rapidly (Global Biodiversity Information Facility (GBIF) (2023).

## 2.2. Phytochemical Constituents of *Bryophyllum pinnatum*

Numerous important chemical constituents and secondary metabolites of the plant have been documented in which the most significant are bufadienolides and flavonoids (Furler *et.al*., 2020). From leaves, bryophyllin B and A have been isolated which are major bufadienolides (Potterat *et.al*., 2021).

In leaves and their extract various flavonoids are separated, included: quercitin, kapinnatoside, 8-methoxyquercetin-3, 7-di-orhamnopyranoside and 3', 4'-dimethoxy quercetin. Other flavonoids compounds i.e Afzelin and a-rhamnoisirobin were also found. In ethanol extract of the plant, fatty acids such as stearic acid, palmitic acid and traces of the arachidic and behenic were also spotted (Milad *et.al.*, 2018). The presence of alkaloids, saponins, glycosides and tannins has been confirmed in the plant (Telep *et.al*., 2021).

It has also been found that the presence of different flavonoids, polyphenols, triterpernoids and other chemical constituents in the plant are responsible for its various therapeutic activities such as anti- nociceptive, anti-inflammatory, antibacterial and anti-diabetic effects (Ferreira *et.al.*, 2022). The herb is an excellent reserve of vitamins such as ascorbic acid, niacin acid and thiamine acid and also minerals i.e Ca, Mg, Na, Fe, P, K, and Zn ( Milad *et.al*., 2018). It contains essential oils and about twenty four compounds were isolated out of nonanal and (E)-geraxylacetone are the most abundant. Bryophyllum pinnatum is enriched with variety of the pharmacological active chemicals and therefore, it is necessary for carrying

out further scientific research in order to confirm the justification behind its use in folk therapeutics (Hamburger *et.al*., 2024).

## FLAVONOIDS

Several flavonoids have been identified from different parts of B. pinnatum (Ousta, S.S., Muizitano, M.F (2019).

**Table 1 Flavanoids of *B. pinnatum***

| CLASSIFICATION | COMPOUNDS NAME | PARTS |
|---|---|---|
| Flavanoids | 3,8-dimethyl-4,5,-trihydroxyflavone | Leaves |
| | 4,5,7-Trihydroxy-3',8-dimethoxyflavone-7-0β-D-glucosepyrano-side(ii) | Leaves |
| | Afzelin (5) | Leaves |
| | Astragalin (16) | Leaves |
| | Luteolin (1) | Leaves |
| | α-Rhamnivisorobin (iv) | Leaves |
| | Quercitin (3) | Leaves |
| | Quercitrin (4) | Leaves |

## 2.3 Traditional Use of *Bryophyllum pinnatum*

Different parts of *Bryophyllum pinnatum* such as the leaves, bark, and the juice have found application in the management of various diseases. The leaves have been used both internally as a tonic, carminative and as an astringent (Fernandes, J.M., Cunha, L.M., (2021). It has been used against many bacterial viral and fungi infections related diseases such as diarrhea and vomiting, upper respiratory infection and flu. It has also been used to treat fever, leishmaniasis (Da silva, S.A., Ousta, S.S., (2022). Ulcer and hypertension (Ojewole, J.A et.al, 2023). The potent anti-histamine and anti-allergic effects of the plant have further been reported (Pali, S., Sen, T. (2019).

**Table 2.0 Traditional uses of different parts of *B. pinnatum***

| PARTS OF PLANT | USES | PREPARATION |
|---|---|---|
| Leaf | For swelling | Application of raw leaf topically over affected area |
| Leaf | For skin problems (Scabies) | Decoction and juice |
| Stems, Leaf | Anti-tumor activity | Extract |
| Leaf | High Blood pressure | Decoction |
| Leaf | Injury due to numbness of limbs, Pain in bones | Rubbing and massaging of leave paste |
| Leaf , flower | Wound ulcer, diabetes, analgesic | Flower and leave juice |
| Seeds | Sight disease | The eye is treated with crushed seed juice (1-2 drops daily, 3 times a |

| | | |
|---|---|---|
| | | day) |
| Leaf | Inflammation in lungs, cough | Syrup, juice with milk |
| Leaf | Kidney problem, high cholesterol | Ingestion of raw leaf |

(Selvakumar, P., Devi, K. 2023).

## 2.4. MEDICAL RELEVANCE AND CHALLENGES IN TREATMENT

### MEDICAL RELEVANCE

- **Anti-inflammatory and Analgesic Activity:** *Bryophyllum pinnatum* exhibits significant anti-inflammatory and pain-relieving effects. These are primarily attributed to the presence of flavonoids, bufadienolides, and other phenolic compounds.
- **Antimicrobial and Antifungal Activity:** Extracts from the plant have shown broad-spectrum antimicrobial activity against bacteria and fungi, including Staphylococcus aureus and Candida albicans. (Akinmoladun, F.O., et.al., 2020).

- **Wound Healing**: *Bryophyllum pinnatum* promotes faster wound contraction and tissue regeneration due to its antimicrobial and anti-inflammatory properties; this makes it effective in treating skin swelling and damage.
- **Antioxidant Properties:** The plant contains compounds such as flavonoids and polyphenols that scavenge free radicals, indicating strong antioxidant activity.
- **Antihypertensive and Anti-ulcer effect:** Aqueous extracts of the leaves have demonstrated blood pressure-lowering effects in human, suggesting a possible role in cardiovascular health. *Bryophyllum pinnatum* extracts protect the gastric mucosa and reduce ulcer formation , possibly through cytoprotective and antioxidant mechanisms (Ojewole, J.A.O 2022).
- **Urinary Issues:***Bryophyllum pinnatum* is used to treat urinary problems such as stones and infections, due to its therapeutic characteristics. (Sridhar, S. (2019).

**Challenges in treatment with *Bryophyllum pinnatum***

- **Lack of Standardized Dosage and Formulation:** Most studies involving *Bryophyllumpinnatum* use crude extracts, and there is limited data on standardized dosing and proper dosage and preparation methods are crucial to avoid adverse effects.
- **Challenges in Standardization and Quality Control:** The lack of standardized extraction methods and quality control measures presents a significant challenge in the therapeutic use of *Bryophyllum pinnatum*. Variations in phytochemical content due to differences in growing conditions and extraction processes can lead to inconsistent therapeutic outcomes, potentially compromising the efficacy and safety of its treatment (Kalio , I. S., Chidinma , E. V., & Aleru-Chuku , M. D. (2023).
- **Side Effects:** Consuming *Bryophyllum pinnatum* in large can cause stomach pain, heart burn, nausea and diarrhea.
- **Regulatory Barriers and Limited Research:***Bryophyllum pinnatum* is not regulated by most national drug authorities, and herbal supplements often lack Good Manufacturing Practice (GMP)

standard.This hinders its adoption in modern therapeutic systems and creates concerns about product safety and consistency. More research is also needed to fully understand the plant's effects and potential Interaction (Zurfluh, L., et.al., 2023).

Despite its rich Traditional history and promising pharmacological properties, Bryophyllum pinnatum faces significant challenges including toxicity risks, lack of clinical data, and poor standardization which limits its widespread adoption.

## 2.5. ANTIFUNGAL ACTIVITY (EFFECT) OF *Bryophyllum pinnatum*

*Bryophyllum pinnatum* has shown promising antifungal activities against various pathogenic fungi and this includes:

- **Inhibition of fungal growth:** *B. pinnatum* extracts (especially ethanol) have shown inhibitory effects on *Candida albicans* growth which is a common cause of Oral and Vaginal thrush.
- **Mechanisms of Action:** *B. pinnatum* potential mechanisms include:Disruption of fungal cell membranes (via saponins), Inhibition

of spore germination, Oxidative stress induction via phenolics and flavonoids (Okpoho et.al., 2020).

- **Active Compounds**: The presence of variety of bio-active compound in *Bryophyllumpinnatum* e.g., Flavanoids, Terpenoids, Alkaloids e.t.c. helps in disrupting fungal cell membrane and interfere with the metabolic pathways in fungi.
- **Minimum Inhibitory Concentration (MIC):** The MIC of ethanolic extracts has been reported to be as low as 60mg/ml against *F. oxysporum* and *Aspergillus niger* (Okpoho et.al., 2020).

# CHAPTER THREE

## 3.0. MATERIALS AND METHODS

### 3.1 Collection and Identification of Plant Extract and Fungal Isolates.

*Bryophyllum pinnatum* leaves were obtained from Alfa Yahya, Kwara State,Ilorin, Nigeria.

Clinical isolates: *Aspergillus niger*, *Fusarium oxysporum* and *Penicillium spp* obtained from the Microbiology Laboratory of Kwara State Polytechnic, Ilorin.

**Materials Used**

These are the materials used for the extraction and isolation:

- Leaf (Bryophyllum pinnatum).
- Mortar and Pestle.
- Distilled water.
- Ethanol.
- Beaker.
- Inoculating loop.
- Conical Flask.
- Measuring cylinder.

- Potato Dextrose Agar (PDA).
- Petri dishes.
- Cork borer (5mm).
- Bursen burner.
- Pressure pot.
- Gas.
- Divider.
- Ruler.
- Fungal Isolates (Aspergillus niger, Fusarium oxysporum and Penicillium spp).
- Weighing balance
- Cotton wool.
- Spatula.
- Foil.
- Hand gloves.
- Nose mask.

### 3.2 Preparation of *Bryophyllum pinnatum* extracts.

Extraction was done by washing the leaves thoroughly with distilled water to

remove any visible impurities. 35g of the leaves was weighed and grounded using mortar and pestle to extract the juice from the leaves, then 70ml of ethanol was added to the paste and thoroughly mixed. The mixture was then filtered and the filtrate was collected and left to thicken for 2-3 days.

### 3.3 Preparation of Media

Potato Dextrose Agar (PDA) was prepared by dissolving 11.7g of PDA powder in 300ml of distilled water. It was then mixed thoroughly while heating till the powder dissolved and was then sterilized using pressure pot at 121°c for 30 minutes. After it was cooled at room temperature, the agar was dispensed into sterile Petri dishes.

### 3.4 Antifungal Activity Test.

The test was carried out using the Agar well diffusion technique. The potato dextrose agar in the petri dishes was allowed to solidify. The isolates were inoculated into the plate aseptically using a sterile inoculating loop. After each wells were bored on the surface of the inoculated agar plates using 5mm cork borer. The ethanolic extract of *Bryophyllum pinnatum* was poured into the well at different concentration using a syringe, one hole was left as control. The wells were well as spaced to prevent the zones of inhibition from

overlapping. The plates were kept at room temperature for 24 hours.

# CHAPTER FOUR

## 4.0. RESULTS

## 4.1. PHYSICAL APPEARANCE OF THE EXTRACT RECOVERED

After measuring 70ml of ethanol and adding it to the 35g of the *B. pinnatum* leaves, the extract has a light green color.

## 4.2 ANTIFUNGAL ACTIVITY ON SOME FUNGI ISOLATE

The antifungal activity of *Bryophyllum pinnatum* synthesized with plant extract was tested on some fungi isolate; *Penicillium spp*, *Aspergillus niger* and *Fusarium oxysporum*. The zones of inhibition in (mm) as shown in Table 3.0 below:

**Table 3 Antifungal activity (Zone of inhibition) of B. pinnatum leaf extract**

| **FUNGAL ISOLATE** | **2mm** | **3mm** | **4mm** |
|---|---|---|---|
| ***Penicillium sp.*** | 12mm | _ | _ |
| ***Aspergillus niger*** | _ | 14mm | 12mm |
| ***Fusarium Oxysporum*** | 14mm | 10mm | _ |

This table shows the effects of Bryophyllum pinnatum on different fungi isolates. Aspergillus niger showed 14mm inhibition at 3mm and 12mm inhibition at 4mm, Fusarium oxysporum showed 14mm inhibition at 2mm and 10mm at 3mm which indicates high susceptibility to B.pinnatum while Penicillium spp showed only 10mm at 2mm making it resistant to B.pinnatum.These inhibition zones confirm that the Bryophyllum pinnatum leaf extract was effective in suppressing the growth of these fungi, especially Aspergillus niger and Fusarium oxysporum, which showed substantial inhibition.

### 4.3 Phythochemical Screening of *Bryophyllum pinnatum*

The leaf extract of *Bryophyllum pinnatum* is rich in various

Phytochemicals including:

- Flavonoids.
- Saponins.
- Tannins.
- Alkaloids compounds.
- Glycosides.

These compounds have been reported to posses reducing properties

which contribute to the plants therapeutic effects.

# CHAPTER FIVE

## 5.1. DISCUSSION

Fungal infection pose a significant threat to public health, with the rise of drug resistant strains as major concern, development of new drugs could be useful in treating them.

From the results obtained, *Fusarium oxysporum* and *Aspergillus niger* are more susceptible to the ethanolic extract of *Bryophyllum pinnatum* than *Penicillium spp* which is a bit resistant to the extract.

These results are consistent with previous studies that have reported the presence of bioactive compounds in *B. pinnatum*, including flavonoids, alkaloids, saponins, tannins, and glycosides, many of which have recognized antifungal activity. The mechanism of action of flavonoids and saponins are known to disrupt fungal cell membranes, while tannins may inhibit fungal enzymes or interfere with cell wall synthesis (Akinmoladun, F.O, et al., 2020).

Limited research has been done on identification and quantification of specific antifungal compounds, absence of in vivo testing, and no assessment of potential cytotoxicity.

Further studies are recommended to isolate the active components, clarify their mechanisms of action, and assess the therapeutic index through animal models and clinical trials.

## 5.2 CONCLUSION

The study shows that *Bryophyllum pinnatum* ethanol extracts have moderate antifungal activity. The ethanolic extract from the leaves demonstrate significant Inhibitory effects against common pathogenic fungi, including *Aspergillus niger, Fusarium oxysporum* and *Penicilliumspp*.These effects are attributed to the plant's rich phytochemical content, particularly flavonoids, alkaloids, tannins, and saponins, which are known to exert antimicrobial properties by disrupting fungal cell membranes and inhibiting fungal growth. The findings support the traditional use of B. pinnatum in the treatment of fungal infections and suggest its potential as a source of natural antifungal agents. Compared to synthetic antifungals, plant-based treatments like B. pinnatum offer the advantages of lower toxicity, reduced resistance development, and cost-effectiveness, particularly in low-resource settings.

However, further research, including phytochemical isolation, mechanism of action studies, and in vivo evaluations is essential to fully establish its therapeutic potential and develop standardized formulations for clinical use.

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