

Phytochemical Screening and Antifungal Activity of *Bryophyllum pinnatum* (Crassulaceae) Leaf Extract and Fractions Against *Aspergillus fumigatus*

***Patricia I. Kobo, Hadiza Aliyu-Amoo, Mohammed Sulaimon, Emmanuel C. Fred, Sakeena F. Yahaya, and Abdallah M. Tauheed**

Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria

Article info: Volume 15, Issue 2, June 2026; Received: May 15, 2026; Reviewed: May 23, 2026, Accepted: June 6, 2026; Published: June 15, 2026; DOI: 10.60787/nijophasr-v14-i2-651

ABSTRACT

Background: *Bryophyllum pinnatum* (*B. pinnatum*) is a succulent plant widely used in traditional medicine for its therapeutic properties. Recently, studies have focused on its potential antifungal properties, driven by the search for newer and safer alternative treatments due to the emergence of drug-resistant strains and toxicity of available agents. This study investigated the phytochemical constituents and antifungal activity of the extract and fractions of the leaves of *B. pinnatum* against *Aspergillus fumigatus* (*A. fumigatus*).

Methods: The crude methanol extract of *B. pinnatum* was serially partitioned with hexane, ethyl acetate, and butanol; both the extract and its fractions were then evaluated for phytochemical constituents and antifungal efficacy against *Aspergillus fumigatus* at concentrations ranging from 25 mg/mL to 800 mg/mL.

Results: Phytochemical screening of the methanol extract revealed the presence of tannins, saponins, flavonoids, terpenoids, carbohydrates, alkaloids, and cardiac glycosides, while steroids, phenols, and anthraquinones were absent. The hexane, ethyl acetate and butanol fractions were devoid of steroids, phenols, and anthraquinones; additionally, the hexane and butanol fractions lacked glycosides and terpenoids, respectively. Neither the crude extract nor any of the fractions showed visible zones of inhibition at any of the tested concentrations.

Conclusion: The study revealed that *B. pinnatum* extract and fractions showed no antifungal activity against *A. fumigatus*, suggesting its limited potential as an antifungal agent against this fungal species.

Keywords: Antifungal activity, *Aspergillus fumigatus*, *Bryophyllum pinnatum*, methanol extract, phytoconstituents.

1. INTRODUCTION

Bryophyllum pinnatum is a succulent plant belonging to the family Crassulaceae. The plant is commonly referred to as the air plant, cathedral bells, life plant, or miracle leaf, this perennial herb is renowned for its medicinal and ecological versatility [1]. It is widely used across parts of Africa, Asia, and South America to treat a range of ailments in animals [2]. The leaves of *B. pinnatum* contain bioactive compounds such as flavonoids, tannins, alkaloids, glycosides, and saponins, which are observed to be associated with their antimicrobial, anti-inflammatory, and antioxidant activities [3]. Its antimicrobial, anti-inflammatory, and haemostatic properties make it effective in the treatment of wounds and skin infections in animals [4]. The leaf extract has been administered to manage diarrhea, wounds, retained placenta, mastitis, and coughs in livestock such as goats, sheep, and cattle [2,5]. Studies have shown that methanol extracts of *B. pinnatum* promote wound healing in animals by enhancing fibroblast proliferation, collagen synthesis, and reducing microbial contamination at the wound site [6]. The plant's phytochemicals are assumed to act synergistically to modulate oxidative stress, inhibit pathogenic organisms, and accelerate tissue repair, making it a valuable natural resource in animal health management [7]. Fungal infection caused by pathogens such as *Candida albicans*, *Aspergillus niger*, and *Trichophyton spp.*, are of significant veterinary and public health concern, particularly in animals with compromised immune systems [8]. The overuse of synthetic antifungal drugs has led to the emergence of resistant strains, reducing treatment efficacy

*corresponding author: Email: dorobyeing@gmail.com; Phone: +2348063549004

This is an open-access article distributed under the Creative Commons Attribution License, (<http://creativecommons.org/licenses/by/4.0/>) which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

Kobo et al: Phytochemical Screening and Antifungal Activity of *Bryophyllum pinnatum* (Crassulaceae) Leaf Extract and Fractions Against *Apergillus fumigatus*

and necessitating the search for novel therapeutic agents [9]. With increasing global burden of fungal infections and the rising resistance to conventional antifungal agents, there is a pressing need to explore natural alternatives, making *B. pinnatum* a promising candidate for investigation. Therefore, this study aimed at evaluating the antifungal effect of *B. pinnatum* methanol leaf extract and fractions against *A. fumigatus*.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Biological Materials

Leaves of *B. pinnatum*, and *A. fumigatus*.

2.1.2 Chemicals and Reagents

Methanol, n-hexane, ethyl acetate, and n-butanol (analytical grade), Sabouraud Dextrose Agar (SDA) and Dimethyl sulfoxide (DMSO).

2.1.3 Equipment and other materials

Incubator, autoclave, rotary evaporator, analytical balance, conical flasks, Petri dishes, pipettes, micropipettes, beakers, water bath, filter papers and Whatman No.1 filter paper.

2.2 Methods

2.2.1 Collection, identification and preparation of plant leaves

The leaves of *B. pinnatum* were obtained from a garden at Zango, Zaria, Kaduna State, Nigeria and taken for identification at the herbarium, Department of Botany, Faculty of Life sciences, Ahmadu Bello University Zaria, Nigeria. A specimen voucher number of ABUHO1838 was assigned. The leaves were then washed and left to dry in the laboratory. The leaves were then pulverized into powder form using a mortar and pestle.

2.2.2 Extraction and fractionation

The powdered leaves of *B. pinnatum* (280 g) was transferred into a maceration bottle and 840 mL of absolute methanol was added, this was allowed to stand for 72 hours. Thereafter, the mixture was filtered to obtain the methanol extract and concentrated by air drying in the laboratory to obtain the solid extract.

The crude methanol extract (100 g) was serially partitioned using hexane (100 mL), ethyl acetate (90 mL) and butanol (85 mL) in increasing polarity. Each procedure was exhaustively carried. All fractions obtained were concentrated and labeled appropriately.

2.2.3 Qualitative phytochemical screening

Qualitative phytochemical screening of the methanol extract (ME) and the fractions [hexane fraction (HF), ethyl acetate fraction (EAF) and butanol fraction (BF)] were carried out according to the methods of Evans [10].

2.2.4 Test organism

The fungal strain used was *A. fumigatus*, identified by macroscopic and microscopic characteristics. The culture was maintained on Sabouraud Dextrose Agar (SDA) slants and sub-cultured prior to the experimental assays to ensure viability.

2.2.5 Antifungal susceptibility testing

The antifungal activity of the plant extracts was assessed using the agar well diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI), with slight modifications.

2.3 Data Analysis

The results were presented descriptively. Due to the absence of antifungal activity (i.e., no zones of inhibition), no statistical comparison was made between the test groups.

3. RESULTS

3.1 Phytochemical screening

Phytochemical screening of *B. pinnatum* leaf extracts revealed a diverse profile of phytoconstituents (Table 1). The methanol crude extract exhibited the most comprehensive range of constituents, including tannins, saponins, flavonoids, terpenoids, alkaloids, cardiac glycosides, and carbohydrates. Notably, steroids, phenols, and anthraquinones were absent from all tested fractions. Solvent – specific variations were observed, with the hexane and butanol fractions lacking both cardiac glycosides and terpenoids, while the ethyl acetate fraction was devoid of steroids and phenols.



Table 1: Phytoconstituents of the extract and fractions of the leaves of *B. pinnatum*

Inferences				
Phytoconstituents	ME	HF	EAF	BF
Anthraquinones	-	-	-	-
Tannins	+	+	+	+
Saponins	+	+	+	+
Flavonoids	+	+	+	+
Terpenoids	+	+	+	-
Steroids	-	-	-	-
Carbohydrates	+	+	+	+
Phenols	-	-	-	-
Alkaloids	+	+	+	+
Cardiac glycosides	+	-	+	-

KEY: ME = methanol extract; HF = Hexane fraction; EAF = ethyl acetate fraction; BF = Butanol Fraction.

+ = Presence; - = Absence

3.2 Antifungal susceptibility testing

The extract and fractions of *B. pinnatum* showed no antifungal activity against *A. fumigatus* at concentrations of 100, 50, and 25 mg/mL. Even at higher concentrations (800, 400, and 200 mg/mL), no activity was observed, similar to the negative control (DMSO) (Table 2), confirming the lack of activity of the fractions under the experimental conditions.

Table 2: Antifungal activity of *B. pinnatum* methanol leaf extract and fractions on *A. fumigatus*

E/F	Concentration (mg/mL)					
	800	400	200	100	50	25
MCE	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm
HF	“	“	“	“	“	“
EF	“	“	“	“	“	“
BF	“	“	“	“	“	“
DSMO	“	“	“	“	“	“

KEY: E/F = extract/fractions; MCE = methanol crude extract; HF = hexane fraction; EAF = ethyl acetate fraction; BF = butanol fraction; DSMO (negative control) = dimethyl sulfoxide.

4. DISCUSSION

Phytochemical screening of *B. pinnatum* methanol leaf extract and its fractions revealed a rich composition of bioactive compounds, including tannins, saponins, flavonoids, terpenoids, alkaloids, carbohydrates, and cardiac glycosides, while anthraquinones, phenols, and steroids were not detected. This diverse phytochemical profile demonstrates the plant's significant biochemical richness, which supports its ethnomedicinal applications. These results are consistent with Nnaebue *et al.* [11], who identified flavonoids, alkaloids, tannins and saponins in the methanol leaf extract, confirming methanol as an effective solvent for extracting a wide range of these compounds. However, the findings differ from those of Sodipo *et al.* [12], who reported the presence of steroids and phenols but the absence of alkaloids. This difference could be attributed to geographical location, as their work was carried out in the arid environment of Maiduguri, whereas this study was conducted in Zaria, a tropical savannah climate. Methanol proved to be a more effective extraction solvent compared to fractions because it was directly applied to the plant material first, facilitating higher recovery of the available phytoconstituents. Furthermore, the efficiency of subsequent extraction fractions varies based on the chemical nature of the target compounds, solubility, and polarity of the solvent used [13]. A polar solvent is highly effective at extracting polar compounds. This principle aligns with findings by Okoye *et al.* [14], which indicated that *B. pinnatum* methanol extract contained the richest phytochemical composition, while the ethyl acetate fraction demonstrated greater compound yield than the butanol fraction, a trend that is similar to our findings. The findings obtained from this study confirmed that *B. pinnatum* is a rich source of bioactive secondary metabolites, with methanol proving to be an effective solvent for extraction. The presence of compounds such as flavonoids, tannins, terpenoids, and alkaloids provides a biochemical basis for the plant's traditional medicinal applications, including its use in treating wounds, inflammation, microbial infections, and gastrointestinal disorders. The absence of steroids and anthraquinones in



Kobo et al: Phytochemical Screening and Antifungal Activity of *Bryophyllum pinnatum* (Crassulaceae) Leaf Extract and Fractions Against *Aspergillus fumigatus*

the methanol extract and other fractions does not diminish the plant's pharmacological potential but rather reflects the solvent's selectivity [15]. The complete lack of antifungal activity against *A. fumigatus* observed at concentrations up to 800 mg/mL in this study contrasts with the reports of Dantani *et al.* [16] and Shivani *et al.* [17] who demonstrated notable *in vitro* antifungal activity of *B. pinnatum* extract against *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans*. Furthermore, Ogungbemile *et al.* [18] established that *B. pinnatum* requires a high concentration of up to 1000 mg/ml to fully inhibit the growth of *A. fumigatus*. At lower concentrations, the plant extract has significantly reduced efficacy. Therefore, our findings do not imply a total absence of antifungal activity but rather indicate that the concentration used is below the threshold required to disrupt the highly resilient cell wall of *A. fumigatus*. This low concentration, coupled with the tendency of the Agar well diffusion method to limit the movement of low solubility or high molecular weight molecules [19], likely explains the lack of observable zone of inhibition. Natural variations in plant composition and regional extraction profiles [20] may have further contributed to this outcome.

5. CONCLUSION

Consequently, the results indicated that *B. pinnatum* leaf extract and fractions exhibited no detectable *in vitro* antifungal activity against *A. fumigatus* under the specific conditions of this laboratory test.

DECLARATION

We declare that this is an original work and is not being considered for publication anywhere else.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgement

The authors are grateful to the technical staff of Departments of Veterinary Pharmacology and Toxicology and Veterinary Microbiology, Ahmadu Bello University, Zaria for their technical assistance during the course of this research.

Contribution of the authors

Patricia Ishaku Kobo and Hadiza Alyu-Amoo conceptualized, design the work, revised and finalized the manuscript. Emmanuel Chingoe'en Fred and Mohammed Sulaimon conducted the experiment, Sakeena Falilu Yahaya and Abdallah Mohammad Tauheed drafted the manuscript.

Ethics and approval

Ethical approval was sought from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC), Ahmadu Bello University, Zaria and was obtained with approval number U17VM1007. The authors declare that Artificial Intelligence (AI) or language models were not used in the conceptualization, data generation, or writing of this manuscript.

6. REFERENCES

- [1] Elufioye TO, Oyedeji AO, Habtemariam S. A review of the traditional uses, phytochemistry and pharmacology of *Bryophyllum pinnatum* (Lam) (Crassulaceae). *J Biol Active Prod Nature*. 2022;12. Doi: 10.1080/22311866.2021.1988706.
- [2] Nwosu MO, Ejikeme CM, Ukaoma AA. Traditional uses of *Bryophyllum pinnatum* in animal health management among Nigerian livestock farmers. *Afr J Tradit Complement Altern Med*. 2017;14(5):59-65.
- [3] Yadav P, Mishra AK, Singh H. A brief review on chemistry and biological activities of *Bryophyllum pinnatum* (Lam.) Oken family: Crassulaceae. *Orient J Chem*. 2021;37(2):269-280.
- [4] Daniel IE, Akpan EI, Utam EC. Phytochemical evaluation, antioxidant and antimicrobial activities of various extracts from leaves and stems of *Bryophyllum pinnatum*. *Nepal J Biotechnol*. 2020;8(1):17-28.
- [5] Akinmoladun OF, Muchenje V, Fon FN, Mpendulo CT. Ethnoveterinary practices and plant-based remedies for livestock health management among smallholder farmers. *J Anim Plant Sci*, 2021;32(2):1-9.



- [6] Pandit PD, Rode A, Jadhav A. Pharmacological activity of *Bryophyllum pinnatum*. *Int J Pharm Res Appl*. 2025;10(1):8-15.
- [7] Onwubuya EI, Ogbunugafor HA, Okafor CS, Oladejo AA. Acute and chronic anti-inflammatory effect of *Bryophyllum pinnatum* leaf extract on Wistar rats. *EJMP*. 2021;32(6):6-14.
- [8] Seyedmousavi S, Bosco SMG, De Hoog S, Ebel F, Elad D, Gomes RR, Jacobsen ID, Jensen HE, Martel A, Mignon B, Pasmans F, Pieckova E, Rodrigues AM, Sing K, Vicente VA, Wibbelt G, Wiederhold NP, Guillot J. Fungal infections in animals: a patchwork of different situations. *Med Mycol*. 2018;56:S165-S187.
- [9] Denning DW, Bromley MJ. How to manage chronic fungal diseases and antifungal resistance. *Sci*. 2015;347(6229):1414-1416.
- [10] Evans WC. *Trease and Evans' Pharmacognosy* (16th ed.). Saunders Elsevier. 2009.
- [11] Nnaebue ND, Anaukwu CG, Anyaoha VI, Soludo OC, Isiaka AB, Ajogwu TM, Onuorah SC. Comparative phytochemical constituents of extracts of *Bryophyllum pinnatum* grown in Anambra State, Nigeria. *Int J Appl Sci Biotechnol*. 2024;12(1):1-7.
- [12] Sodipo OA, Usman F, Yakubu J, Wampana B. Ethnopharmacological benefit of Nigerian indigenous *Bryophyllum pinnatum* leaf on convulsive rats. *JPAM*. 2023;1(2):98-105.
- [13] Kanti S, Jain S, Shrivastava S. Influence of solvent polarity and chemical nature on successive efficiency of bioactive fractions. *J Pharmacogn Phytochem*. 2015;4(2):201-206.
- [14] Okoye KF, Anowi FC, Ejiofor II, Ololade OM. Pharmacognostic study and sedative activity of *Bryophyllum pinnatum* stem methanol extract and fractions. *Sci Phytochem*. 2023;2(2):226-234.
- [15] Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. *Afr J Biotechnol*. 2005;4(7):685-688.
- [16] Dantani M, Musa AR, Dantani A. Antifungal activity of aqueous and ethanol extracts of *Bryophyllum pinnatum* against pathogenic fungi in Sokoto State, Nigeria. *USci*. 2025;4(1):200-206.
- [17] Shivani S, Avadhesh K, Dipali D, Ayush J, Ayush T, Bhanu PV, Bittu KS, Chouhan SR, Rathi JC. Phytochemical screening and antimicrobial activity of *Bryophyllum pinnatum* leaf extract. *WJBPHS*. 2025;22(1):482-486.
- [18] Ogundbemile OA, Etaware PM, Odebode AC. Application of plant-base fungicides to control aflatoxigenic fungi producing mycotoxins in stored cowpea seeds. *J Biotechnol Biomed*. 2020;3(1):1-9.
- [19] Pinto A, Botelho MJ, Churro C, Asselman J, Pereira P, Pereira JL. (2023). Methods and limitations in antimicrobial diffusion assays: overcoming molecular weight and solubility restrictions. *J Microbiol Methods*. 2023;205,Article 106684.doi.org.
- [20] Eloff JN. (2019). Avoiding pitfalls in determining antimicrobial activity of plant extracts and publishing results. *BMC Complement Altern Med*. 2019;19(1):158.

