Sahel Journal of Life Sciences FUDMA (SAJOLS)
March 2026 Vol. 4(1): 207-214
ISSN: 3027-0456 (Print)
ISSN: 1595-5915 (Online)
DOI: *https://doi.org/10.33003/sajols-2026-0401-24*

Research Article

Preliminary Phytochemical Characterization, *In vitro* Antioxidant and Gastric Acid–neutralizing Properties of Aqueous Leaf Extract of *Bryophyllum pinnatum*

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ABSTRACT

Peptic ulcer disease (PUD) remains a significant global health challenge, driven largely by *Helicobacter pylori* infection, non-steroidal anti-inflammatory drug use, and oxidative stress–mediated mucosal injury. In this study, the phytochemical composition, antioxidant capacity, and in vitro gastric acid–neutralizing potential f the aqueous leaf extract of *Bryophyllum pinnatum* and its solvent fractions were evaluated as preliminary indicators of gastroprotective potential, rather than confirmed anti-ulcer efficacy. Qualitative phytochemical screening indicated the presence of flavonoids, alkaloids, tannins, saponins, glycosides, triterpenoids, phenolics, and steroids. Quantitative assays, expressed relative to extract dry weight, revealed triterpenoids as the most abundant class. Antioxidant activity was assessed using DPPH, ABTS, nitric oxide scavenging, and ferric reducing antioxidant power (FRAP) assays, with results expressed as mean ± SEM (n = 3). Several solvent fractions demonstrated concentration-dependent antioxidant activity; however, comparisons with butylated hydroxytoluene (BHT) were descriptive and not subjected to inferential statistical equivalence testing. Gastric acid neutralization was evaluated using an *in vitro* titration model, where extracts increased solution pH, particularly in the presence of sodium carbonate. These findings indicate that *B. pinnatum* contains bioactive constituents with antioxidant activity and acid-buffering capacity that may contribute to gastroprotective effects, but do not constitute direct evidence of anti-ulcerogenic efficacy. Further *in vivo* and mechanistic studies, including mucosal protection and enzymatic models, are required to substantiate therapeutic relevance.

Keywords: Antioxidant activity; *Bryophyllum pinnatum*; Gastric acid neutralization; Gastroprotection; *In vitro* studies; Phytochemicals

Citation: Abdullahi, Z.L., AbdulGaniyu, O.A., AbdulGaniyu, B.B., Jaji, A.B., AbdulRahman, R.A., & Adekunle, J. (2026). Preliminary Phytochemical Characterization, *In vitro* Antioxidant and Gastric Acid–neutralizing Properties of Aqueous Leaf Extract of *Bryophyllum pinnatum*. *Sahel Journal of Life Sciences FUDMA*, 4(1): 207-214. DOI: *https://doi.org/10.33003/sajols-2026-0401-24*

INTRODUCTION

Peptic ulcer disease (PUD) is a chronic gastrointestinal disorder characterized by mucosal erosion in the stomach or proximal duodenum, primarily resulting from an imbalance between aggressive factors (gastric acid, pepsin, reactive oxygen species, and *Helicobacter pylori*) and mucosal defensive mechanisms (mucus–bicarbonate barrier, prostaglandins, nitric oxide, and antioxidant systems) (Lanas & Chan, 2017; Malfertheiner *et al.,* 2017). Contemporary gastroenterological classification recognizes gastric and duodenal ulcers as acid-related disorders with multifactorial pathogenesis rather than purely hypersecretory conditions (Sung *et al.,* 2009).

Oxidative stress has been implicated in ulcerogenesis through lipid peroxidation, protein oxidation, and impairment of mucosal microcirculation, thereby

exacerbating tissue injury and delaying healing (Bhattacharyya *et al.,* 2014). Consequently, antioxidants capable of scavenging reactive oxygen and nitrogen species may contribute indirectly to gastroprotection by preserving mucosal integrity (Sumbul *et al.,* 2011). However, antioxidant activity alone does not equate to anti-ulcer efficacy, which requires demonstration of mucosal protection, modulation of acid secretion, or enhancement of repair mechanisms in vivo (Wallace & Granger, 2013). *Bryophyllum pinnatum* (Crassulaceae) is widely used in traditional medicine for the management of inflammatory and gastrointestinal disorders and has been reported to possess antioxidant, anti-inflammatory, and cytoprotective properties (Ojewole, 2002; Kamboj & Saluja, 2009). Despite extensive prior studies on its gastrointestinal effects, gaps remain regarding standardized extraction approaches, quantitative phytochemical profiling, and mechanistic interpretation of in vitro findings (Kamboj & Saluja, 2009).

The present study therefore aimed to generate methodologically transparent, preliminary data on the antioxidant activity and gastric acid–neutralizing capacity of *B. pinnatum* aqueous leaf extract and its solvent fractions, while clearly delineating the limitations of in vitro assays in predicting anti-ulcer activity. The aim of this study was to investigate antioxidant activity and gastric acid–neutralizing potential of aqueous leaf extract of *Bryophyllum pinnatum* in vitro.

MATERIALS AND METHODS

Collection and Authentication of Plant Sample

The leaves of *Bryophyllum pinnatum* were harvested from "Basin", Ilorin, Kwara State, Nigeria between November and December 2021 and authenticated by a taxonomist at the herbarium section of the Department of Plant Biology, University of Ilorin. A voucher specimen (UILH/003/1225/2021) was deposited in the herbarium of the Department of Plant Biology, Faculty of Life Sciences, University of Ilorin, for future reference. The leaves were dried (Air drying) inside the biochemistry laboratory and milled using grinding machine at tipper garage tanke, ilorin, Nigeria.

Preparation of Aqueous Leaf Extract of *Bryophyllum pinnatum*

Fresh leaves of *Bryophyllum pinnatum* were collected, chopped, washed with tap water and air-dried. After drying, the leaves were macerated and a known weight (500g) of the sample was soaked in 5litres of distilled water for 72hrs with occasional stirring.

The aqueous solution was filtered and the solvent was evaporated under reduced pressure and controlled temperature in a Freeze Dryer. Greenish black (leaves) extract was obtained. The lyophilized product was partitioned using three solvents; 50% Ethyl acetate, 25% methanol and 25% butanol percentage yields respectively.

Determination of Secondary Metabolites in Aqueous *Bryophyllum pinnatum* Leaf Extract

Classical qualitative phytochemical tests were conducted for major metabolite groups. Given their non-specific nature, results were interpreted as preliminary indicators only. Quantitative assays were performed using established spectrophotometric methods with appropriate analytical standards (gallic acid for phenolics, quercetin for flavonoids, atropine for alkaloids, camphor for terpenoids). Results were normalized to extract dry weight (mg/ml dry extract). All measurements were conducted in triplicate (n = 3).

In vitro Antioxidant Investigation of Aqueous Leaf Extract of *Bryophyllum pinnatum* and its Solvent fractions

DPPH (α, α-diphenyl-β-picrylhydrazyl) scavenging assay

The DPPH assay was used to evaluate the antioxidant activity of the extracts with slight modification of previously described methods. Different concentrations of the extracts (250–1000 µg/mL) were mixed with 5 mL of 0.1 mM ethanolic DPPH solution and incubated in the dark for 30 minutes. A control without extract was prepared, and ethanol served as the blank. Absorbance was measured at 518 nm using a UV–visible spectrophotometer (Systronics AU-2700, India). All experiments were performed in triplicate, and butylated hydroxytoluene (BHT) was used as the reference standard. Antioxidant activity was expressed as percentage inhibition.

ABTS (2,2-Azinobis (3-ethylbenzothiazoline sulphonic acid)) Scavenging assay

The ABTS radical cation ($ABTS^{\bullet+}$) was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate and incubating the mixture in the dark at

room temperature for 16 hours. The solution was diluted with methanol to obtain an absorbance of 0.70 ± 0.05 at 734 nm. In a 96-well plate, 25 µL of each extract was added to 200 µL of the working solution and incubated at room temperature for 30 minutes in the dark. Absorbance was then measured at 734 nm using a Multiskan™ microplate spectrophotometer. Antioxidant activity was expressed as percentage inhibition of ABTS radicals.

Nitric Oxide Scavenging Assay

Nitric oxide scavenging activity was determined using sodium nitroprusside, which generates nitric oxide that reacts with oxygen to produce nitrite ions detectable by the Griess reaction. Extracts (250–1000 µg/mL) were incubated with 0.5 mL sodium nitroprusside (5 mM) at 27°C for 2 hours. An aliquot of the incubated solution was mixed with Griess reagent and the absorbance measured at 540 nm. BHT was used as the positive control. The experiment was conducted in triplicate, and nitric oxide scavenging activity was expressed as percentage inhibition.

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay, based on the method of Benzie and Strain, was used to determine the reducing power of the extracts. The assay measures the ability of antioxidants to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), forming a blue Fe^{2+}–TPTZ complex with absorbance at 593 nm. The FRAP reagent was mixed with distilled water and incubated at 37°C, after which plant extracts were added and further incubated for 10 minutes. Absorbance was then measured at 593 nm. A calibration curve was prepared using different concentrations of $FeSO_4 \cdot 7H_2O$.

Antacid Analysis Assay

The acid-neutralizing capacity of the extracts was evaluated using a modified Fordtran's model. Each extract sample (0.666 g or 1.332 g), with or without 0.4 g Na_2CO_3, was dissolved in 90 mL of water, warmed to 37°C, and stirred continuously to simulate gastric conditions. The mixture was titrated with artificial gastric acid containing pepsin in NaCl solution (pH 1.2) until a pH endpoint of 3 was reached. Acid was added at a rate of approximately 3 mL/min, and pH was recorded every minute using a pH meter. The experiment was performed in triplicate, with Na_2CO_3 used as the standard.

RESULTS

Qualitative Phytochemical Analysis Result

Table 1 shows qualitative phytochemical profile of the aqueous leaves extract of *Bryophyllum pinnatum.* The phytochemicals present are saponins, flavonoids, steroiods, glycosides, triterpenes, phenol, tannin, alkaloid and protein; whereas, reducing sugar was absent.

Quantitative Phytochemical Analysis Result

The quantitative phytochemical profile of the aqueous leaves extract of *Bryophyllum pinnatum*. Triterpenoid (62.3566mg ± 0.0727), has the highest concentration, followed by glycoside at 20.1689mg ± 0.0358, while tannin concentration is the lowest (1.7452mg ±0.0003). The qualitative result shows that saponin, flavonoid, tannin, phenol, steroids, protein and triterpenoid were present while reducing sugar was absent.

Table 1: Quantitative Phytochemical Analysis of Aqueous *Bryophyllum pinnatum* Leaves Extract

Phytochemicals	Concentration (mg/ml)
Total Flavonoid Content	9.1546 ± 0.0782
Total Phenol Content	4.4158 ± 0.0016
Total Saponin Content	10.8319 ±0.0014
Total Tannin Content	1.7452 ±0.0003
Total Steroid Content	2.3721 ± 0.0039
Total Triterpenoid Content	62.3566 ± 0.0727
Total Glycoside Content	20.1689 ± 0.0358
Total Alkaloid Content	5.1275 ± 0.0012

Values show the mean of 3 determinations ± SEM ($p<0.05$)

***In vitro* Antioxidant Activity of Aqueous Leaf Extract of *Bryophyllum Pinnatum* and Its Solvent Fractions**

The antioxidant activity of phytochemicals is mainly due to their redox properties which allows them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. Thus, aqueous leaves extract of *Bryophyllum pinnatum* and its solvent fractions was investigated for antioxidant potential using different free radical scavenging assays. Butylated hydroxyl toluene (BHT) was used as a reference standard.

DPPH Scavenging Activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to evaluate the free radical scavenging activity of *Bryophyllum pinnatum* aqueous leaf extract and its solvent fractions (Figure 4 A–D), with butylated hydroxytoluene (BHT) as the standard. The ethyl acetate and butanolic fractions showed no significant difference ($P > 0.005$) compared with the standard. Among the fractions, the butanolic and ethyl acetate fractions exhibited the highest antioxidant activity, indicating strong radical scavenging potential.

ABTS Scavenging Activity

The ABTS assay was employed to assess the antioxidant capacity of the solvent fractions of *Bryophyllum pinnatum* aqueous leaf extract. As shown in Figure 5 (A–D), the methanolic and ethyl acetate fractions demonstrated the highest activity. All extracts scavenged $ABTS^{+}$ radicals, leading to a reduction in absorbance at 734 nm. The antioxidant activity increased with concentration, with optimum activity observed at 1000 µg/mL, similar to the standard (BHT).

Nitric Oxide Scavenging Activity

Nitric oxide scavenging activity was determined by measuring the decrease in the intensity of the pink chromophore at 540 nm. The extracts exhibited concentration-dependent scavenging activity by competing with oxygen and reducing nitric oxide production. The activity of each extract was compared with that of BHT, as illustrated in Figure 6 (A–D).

FRAP Scavenging Activity

The ferric reducing antioxidant power (FRAP) assay evaluates antioxidant capacity based on the reduction of ferric ions to ferrous ions, resulting in increased absorbance at 593 nm. As shown in Figure 7 (A–D), the antioxidant activities of the extracts were compared with BHT. The butanolic and methanolic fractions displayed the highest activity, with antioxidant power increasing in a concentration-dependent manner. At 1000 µg/mL, the butanolic fraction showed the strongest reducing effect. Values are means of 3 determinants ± SEM. Bars with different superscripts are significantly different ($p<0.05>$) from one another.

Anti-Ulcer Activity of Aqueous Leaf Extract of *Bryophyllum pinnatum* And Its Solvent Fractions

In table 3, Gastric acid was prepared and titrated against Na2CO3 and the pH reading was taken and it served as the standard value. The acid neutralizing ability (antacid) of aqueous leaves extract of *Bryophyllum pinnatum* and its solvent fractions was carried out in vitro as shown in table 3 below. It was observed that the PH of solutions containing a mixture of sodium carbonate and aqueous leaves extract of *Bryophyllum pinnatum* or its solvent fractions increased compared with when only aqueous leaves extract of *Bryophyllum pinnatum* or its solvent fractions and sodium carbonate were used independently.

Table 3: *In vitro* Gastric- Acid neutralizing pH of different *Bryophyllum pinnatum* Extracts

Extract	pH at 1.332g of Extract + Na2CO3	pH at 0.666g of Extract + Na2CO3	pH at1.332g Extract only	pH at 0.666g Extract only
Aqueous	7.08 ± 0.002	6.02 ± 0.003	5.00 ± 0.004	3.40 ± 0.002
Methanol	7.0 ± 0.01	5.85 ± 0.002	3.50 ± 0.03	3.37 ± 0.001
Butanol	7.29 ± 0.01	6.32 ± 0.01	3.75 ± 0.02	3.50 ± 0.001
Ethyl Acetate	7.15 ± 0.003	6.25 ± 0.002	3.45 ± 0.003	3.47 ± 0.0032

Values are mean of 4 replicates ± SEM($p<0.05$)

DISCUSSION

Medicinal plants require thorough investigation before therapeutic use because their efficacy largely depends on the quality and chemical composition of the plant material. Phytochemical analysis therefore plays a critical role in validating their medicinal value. In this study, phytochemical screening of the aqueous leaf extract of *Bryophyllum pinnatum* revealed the presence of several bioactive constituents, including flavonoids, alkaloids, tannins, saponins, glycosides,

proteins, and triterpenoids, while reducing sugars were absent. Quantitative analysis further indicated that triterpenoids were the most abundant, followed by glycosides, saponins, and flavonoids. These findings are consistent with earlier reports that also documented similar phytochemical constituents in *B. pinnatum* leaves (Quazi Majaz *et al*., 2011). Similarly, previous investigations confirmed the presence of comparable phytochemicals and highlighted the antioxidant potential of the plant (Benzie & Strain, 1996). Further studies using different solvents are recommended, as solvent polarity may influence the extraction of various phytochemicals and enhance the medicinal applications of the plant.

The antioxidant potential of the aqueous leaf extract and its solvent fractions was evaluated using standard antioxidant assays with butylated hydroxytoluene as the reference compound. The results demonstrated that both the crude extract and its fractions possess notable antioxidant activity, an important property associated with antiulcer agents. Among the fractions, the butanolic, methanolic, and ethyl acetate fractions exhibited stronger antioxidant activity than the crude aqueous extract, likely due to the differential solubility of phytochemicals in various solvents. All fractions displayed concentration-dependent antioxidant activity, with the highest activity observed at 1000 µg/mL. These findings are consistent with previous studies showing that solvent fractions of *B. pinnatum* effectively scavenge free radicals in DPPH and ABTS assays (Alves Júnior *et al*., 2020). Similarly, the methanolic extract of the plant has been reported to exhibit strong DPPH radical scavenging activity (Benzie & Strain, 1996). The antioxidant activity observed in the DPPH assay is attributed to the hydrogen-donating ability of the phytochemicals present in the extract. When DPPH radicals accept hydrogen or electrons from antioxidants, the violet-colored solution becomes pale yellow, and the degree of discoloration reflects the free-radical scavenging capacity of the extract (Adesanwo *et al*., 2007; Akah *et al*., 2020).

The acid-neutralizing potential of the aqueous extract and its solvent fractions was also investigated by measuring the pH of the extracts following titration with artificial gastric acid in the presence and absence of sodium carbonate (Na_2CO_3). The results indicated that combining the extracts with Na_2CO_3 significantly increased the pH compared with the extracts alone or the standard alone, demonstrating improved acid-neutralizing capacity. Among the fractions tested, the butanolic and ethyl acetate fractions showed greater neutralizing effects, with the butanolic fraction exhibiting slightly higher activity. Additionally, the acid-neutralizing efficiency increased with higher extract concentrations, although excessively high concentrations may pose potential toxicity risks. These findings suggest that combining the extract with suitable basic compounds could enhance its antacid efficacy. The results obtained in this study are comparable with earlier findings that reported similar acid-neutralizing activity of *B. pinnatum* extracts when used with calcium carbonate (Quazi Majaz *et al*., 2011). Overall, these observations support the potential of *Bryophyllum pinnatum* as a natural source of antioxidant and antacid agents relevant to ulcer management.

CONCLUSION

The results obtained in the study and available literature reports, showed that *Bryophyllum pinnatum* aqueous leaves extracts possess a rich source of bioactive molecules with antioxidant, some phytochemicals and has acid-neutralising properties potential.

Competing Interests

The authors have declared that no competing interest exists.

Acknowledgements

We will like to express our profound gratitude to the management of Kwara State Polytechnic for finding us worthy to be granted this research grant through Institution Based Research (IBR) under the aegis of TETFUND.

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