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Exploring Variations in Phytochemical Constituents and Antioxidant Potential of Leaf and Stem Extracts of *Bryophyllum Pinnatum*

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ABSTRACT

Antioxidant capability and phytochemical components are important markers of plant extracts' potential for medicinal applications. The phytochemical constituents and in vitro antioxidant activity of Bryophyllum pinnatum (Lam.) leaf and stem extracts were compared in this study using standard methods. Qualitative phytochemical screening revealed that both leaf and stem extracts contained flavonoids, alkaloids, tannins, steroids, saponins, and phenolic compounds, whereas phlobatanins were absent in both extracts. Anthraquinones and cardiac glycosides were detected only in the stem extract, while phytosterols were absent from the stem extract. The leaf extract had higher amounts of total flavonoids (7.14 ± 0.21 mg/g), total phenolics (18.75 ± 1.21 mg/g), and total alkaloids (1.97 ± 1.04 mg/g) than the stem extract's 4.27 ± 0.25, 8.52 ± 1.10, and 0.91 ± 0.01 mg/g, respectively. All experiments were performed in triplicate (n = 3), and the data were presented as mean ± standard deviation (SD). These bioactive substances have been reported to possess anti-inflammatory, antibacterial, antioxidant, and anticancer properties. The higher phytochemical concentration in the leaf suggests stronger antioxidant potential, while the presence of unique compounds such as anthraquinones and cardiac glycosides in the stem highlights its additional medicinal importance. These findings support recent studies on the antioxidant properties of B. pinnatum leaf while offering more information about the stems comparatively unexplored phytochemical profile, with a general focus on B. pinnatum as a useful natural source of bioactive chemicals.

Keywords: Antioxidant, Anthraquinones, *Bryophyllum pinnatum*, Cardiac glycosides Phytochemicals

INTRODUCTION

Secondary metabolites including phenols, flavonoids, tannins, alkaloids, saponins, and glycosides, which have long been known as biologically active compounds, contribute significantly to the pharmacological activities of medicinal plants (Daniel *et al.*, 2020). Since differences in composition affect biological activity, recent studies have highlighted the need to investigate particular phytochemicals present in various plant tissues. Due to their pharmacological potential, safety, and accessibility, natural antioxidants derived from medicinal plants have garnered significant attention; because they can donate electrons and neutralize free radicals, phenolics and flavonoids in plant extracts are particularly significant (Daniel *et al.*, 2020; Shahidi & Ambigaipalan, 2015). Therefore, determining the therapeutic effect of plant extracts requires assessing antioxidant capacity utilizing assays such as the DPPH radical scavenging method (Bhatti *et al.*, 2012). Owing to their medicinal significance in cancer treatment, antibiotic activity, and cardiovascular modulation, anthraquinones and cardiac glycosides in particular have drawn a lot of attention (Qun *et al.*, 2023; Nabil *et al.*, 2024). *Bryophyllum pinnatum* (Lam.) Oken, a medicinal succulent plant in the Crassulaceae family, is one of these species of particular interest. It is well known for its numerous ethnomedical uses and rich phytochemical composition (Nguyen *et al.*, 2025). Often called "abamoda" by the Yoruba people of Nigeria, it is also referred to as the "wonder plant" or "life plant." Antioxidant, antibacterial, anti-inflammatory, and wound-healing activities of *B. pinnatum* have been reported (Selvakumar, 2022). *B. pinnatum* is utilized ethnomedically in South American, Asian, and African medicine to treat renal diseases, wounds, ulcers, and infections (Elufioye *et al.*, 2022).

B. pinnatum has a distinct advantage over many other plants due to its widespread availability, fast multiplication, versatile therapeutic benefits, and superior reduction potential (Okafor *et al.*, 2024). As a result of its rich phytochemical composition and broad range of bioactivities, *B. pinnatum* has emerged as a plant of great interest in a variety of modern study fields. Current research focuses on its applications in pharmacology for anti-inflammatory, antimicrobial, antioxidant, and wound healing purposes, as well as its potential roles in agriculture as a natural pesticide and environmental science for phytoremediation of contaminated soils (Okafor *et al.*, 2024; Akinterinwa *et al.*, 2025). Furthermore, its traditional medicinal value continues to spur research into novel therapeutic compounds derived from its bioactive constituents (Elufioye *et al.*, 2022). While several research have focused on leaf extracts, comparative evaluations of both leaf and stem sections of *B. pinnatum* are scarce. Hence, this study comparatively investigated the phytochemical composition and antioxidant potential of the *B. pinnatum* leaf and stem.

MATERIALS AND METHODS

Plant Collection

Fresh leaves and stems of *B. pinnatum as shown* in figure 1, were collected from a garden in Ikangba, Ijebu-Ode, Ogun State, Nigeria. Samples were identified and authenticated by Professor O. O. Oyesiku in the Department of Plant Science, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria. The voucher specimens' EH/2024/3014 of collected parts were deposited in the herbarium of Department of Botany, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria. Methanol was obtained from Sigma-Aldrich (Germany) and used without further purification.

Figure 1: *Bryophyllum pinnatum* Plant

Extraction of Plant Samples

Air-dried and powdered samples of *B. pinnatum* were extracted using 70 % methanol with continuous agitation for 8 h, to maximize recovery of bioactive compounds. The different extracts were filtered separately with Whatman No.1 filter paper, concentrated by evaporation, and stored at 4 °C before use (Pawaskar & Sasangan, 2017).

Qualitative Phytochemical Analysis

The extracts were analyzed to detect major classes of secondary metabolites using standard qualitative method (Pawaskar & Sasangan, 2017).

Test for Alkaloids: To 2 mL of plant leaf and stem extracts respectively, 1.5 mL of 1 % HCl was added. After heating the solution in water bath, 6 drops of Mayors reagents and Wagner's reagent was added. Formation of orange precipitate indicates the presence of alkaloids.

Test for Anthraquinones: To 1 mL of plant leaf and stem extracts respectively, few drops of 10 % ammonia solution were added and shaken. The appearance of pink colour precipitate indicates the presence of anthraquinones.

Test for Phlobatannins: To 1 mL of plant leaf and stem extracts respectively, few drops of 2 % HCL were added appearance of red colour precipitate indicates the presence of phlobatannins.

Test for Cardiac Glycosides (Keller-Killani Test): 5 mL of the plant leaf and stem extracts respectively was treated with 2 mL of glacial acetic acid containing a drop of ferric chloride solution. Then it was underplayed with 1 mL concentrated sulphuric acid. A brown ring of the interface indicates a deoxy sugar characteristic of cardio glycosides. A violet ring may appear below the ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Test for Flavonoids: A portion of the plant leaf and stem extracts was separately heated with 10 mL of ethyl acetate in a water bath for 3 min. The mixture was filtered and 4 mL of each filtrate were shaken with 1 mL of dilute ammonia solution. A yellow colour observation indicates the presence of flavonoids.

Test for Tannins: 0.5 mL of the plant leaf and stem extracts respectively was boiled in 20 mL of water in a test tube and then filtered. A few drops of 0.1 % ferric chloride were added and observed for brownish green.

Test for Saponins: 5 mL of the plant leaf and stem extracts respectively was boiled in 5 mL of distilled water in a water bath and filtered. Filtrate was shaken vigorously for a stable persistent froth. The froth was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for Phenols: To 2 mL of the plant leaf and stem extracts respectively, 1 mL of 1 % ferric chloride solution was added. Blue or green colour indicates phenols.

Test for Steroids: 2 mL of acetic anhydride was added to 0.5 mL of the plant leaf and stem extracts respectively with 2 mL of H_2SO_4. The colour changes from violet to blue green in the sample indicated the presence of steroids and sterols.

Quantitative Phytochemical Analysis

Standard established procedures (Ohikhena *et al.,* 2018; Harborne, 1998) were employed to quantify total flavonoid content, total phenolic content and total alkaloid content respectively.

Flavonoid Content

The Aluminium chloride colorimetric assay described by Ohikhena *et al*. (2018) was used to determine the flavonoid content. Briefly, 0.5 mL aliquots each of the methanol extracts (1 mg/mL) and quercetin standards (10–100 μg/mL) where placed in a test tube. 2 mL of distilled water was added to each test tube followed by 5 % sodium nitrite (0.15 mL) and the mixture was left to stand for 6 minutes. 10 % $AlCl_3$ (0.15 mL) was added to the solution and left to stand for another 5 minutes and then 1 M sodium hydroxide (1 mL) was added and made up to 5 mL with distilled water. The absorbance was measured at 420 nm on a UV-Visible Spectrophotometer (JENWAY 6305). The total flavonoids content was expressed as milligram Quercetin equivalent per gram (mg QE/g) of the extracts. Data were reported as mean ± SD for three replicates.

Total Phenolic Content

Total phenolic content was evaluated using Folin-Ciocalteu assay as previously described by Ohikhena *et al.* (2018). Briefly, 0.5 mL of the plant extracts (1 mg/mL) and gallic acid standards (10–100 μg/mL) were separately prepared and added to 2.5 mL of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) in a test tube. Thereafter, 7.5 % w/v sodium carbonate solution (2 mL) was added to the tube and the mixture was properly mixed and allowed to stand for 30 minutes at 40 ºC for colour development. Absorbance of the mixture was measured at 765 nm using a UV-Visible Spectrophotometer (JENWAY 6305). The phenolic content was calculated as gallic acid equivalent (GAE) by comparison with a calibration curve of gallic acid standards ($R^2 = 0.9971$). Results were expressed as mg gallic acid equivalent per gram (mg GAE /g) of dry extracts. Data were reported as mean ± SD for three replicates.

Determination of Alkaloid

Alkaloid content was determined by the method of Harborne (1998). Five (5 g) of the sample was weighed into a 250 mL beaker and 200 mL of 10 % acetic acid in ethanol was added and allowed to stand for 4 min. This was filtered and extracts was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wisely to the extracts until the precipitation was completed. The whole solution was allowed to settle; the precipitate was collected, washed with dilute ammonium hydroxide and filtered. The residue was dried and weighed. The weight difference was taken as the percentage alkaloid present in the sample.

DPPH Radical Scavenging Assay

The antioxidant capacity of the extract was evaluated spectrophotometrically based on its ability to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, following the procedure previously described by Álvarez-Casas *et al*. 2014. Briefly, an aliquot (100 µL) of the extract (20, 40, 60, 80 and 100 µg/mL) was mixed with 3.9 mL of 0.1 mM methanolic DPPH solution. The mixture was thoroughly mixed and allowed to stand in the dark for 30 min at room temperature. The absorbance was measured at 517 nm (Álvarez-Casas *et al.,* 2014). Results were expressed as percentage inhibition of DPPH free radical, which was calculated according to equation 1:

$$\%\ inhibition\ of\ DPPH = \frac{(Abs\ control - Abs\ sample)}{Abs\ control} \times 100 \quad (1)$$

Where Abs control is the absorbance of the DPPH without plant extracts, and Abs sample is the absorbance of the DPPH after adding extracts.

Statistical Analysis

All experiments were carried out in triplicates (n=3), and the results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using an independent samples Student's *t*-test to compare the mean values of the leaf and stem extracts. Differences between the mean values were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Qualitative Phytochemical Composition

Qualitative phytochemical screening of *Bryophyllum pinnatum* leaf and stem extracts was investigated and the results are presented in Table 1. Phytochemicals including alkaloids, flavonoids, phenols, steroids, saponins, and tannins were present in both leaf and stem samples of *B. pinnatum*. Some differences were noticed in the chemical profiles of the samples, with certain molecules exhibiting varying intensities. Phlobatanins were not detected in either extract. Phytosterols were absent in the stem extract, while the leaf extract lacked anthraquinones and cardiac glycosides, as shown in Table 1.

Table 1: Phytochemical Screening of Leaf and Stem Extract of *B. pinnatum*

S/N	Phytochemical	Test	Stem	Leaf
1.	Alkaloids	Mayer's test	+	+
		Wagner's test	+	+
2.	Anthraquinone	Anthraquinone test	+	-
3.	Cardiac Glycoside	Keller-Killani test	+	-
		Legal's test	+	-
4.	Flavonoids	Alkaline test	+	+
		Ammoniun test	+	+
5.	Phytosterols	Lieberman Buchard test	-	+
6.	Phenols	Ferric chloride test	+	+
7.	Phlobatanins	Phlobatanin test	-	-
8.	Saponin	Frothing test	+	+
9.	Steroids	Salkowski's test	+	+
10.	Tannins	Ferric chloride test	+	+
		Lead Sub Acetate test	+	+

+ = present - = absent

Quantitative Phytochemical Profile

The quantitative phytochemical analyses revealed the concentration of phytochemicals including flavonoids, phenols, and alkaloids, found in the leaf and stem extracts of *B. pinnatum* as presented in Table 2. The results highlight significant variations in the composition of the aforementioned phytochemicals in *B. pinnatum* leaf and stem extracts. Table 3 shows the results of the independent samples t-test done to compare the concentrations of selected bioactive compounds in the leaf and stem extracts of *Bryophyllum pinnatum.*

Table 2: Quantitative Phytochemical Analysis of B. pinnatum

Sample	Total flavonoid content (mg/g)	Total phenolic content (mg/g)	Total Alkaloid Content (mg/g)
leaf extracts	7.14±0.21	18.75±1.21	1.97 ±1.04
stem extracts	4.27±0.25	8.52±1.10	0.91 ±0.01

Values are expressed as mean ± standard deviation (n = 3). Total flavonoid content is expressed as mg quercetin equivalent per gram extract. Total phenolic content is expressed as mg gallic acid equivalent (GAE) per gram of dry extract.

Table 3: T-Test Results for Bioactive Compounds in Leaf and Stem Extract of B. pinnatum (n = 3)

Parameter (mg/g)	Leaf Extract (Mean ±SD)	Stem Extract (Mean ±SD)	t-value	p-value
Flavonoid content	7.14 ± 0.21	4.27 ± 0.25	15.23	0.0001
Phenolic content	18.75 ± 1.21	8.52 ± 1.10	10.84	0.0004
Alkaloid content	1.97 ± 1.04	0.91 ± 0.01	1.77	0.1523

Antioxidant Activity

Antioxidant potential in both extracts was concentration-dependent, according to DPPH radical scavenging activity as indicated in Table 4. The results demonstrated that the extract's capacity to scavenge free radicals increased with concentration. Compared to the stem extract (96.38 µg/mL) and ascorbic acid standard (98.71 µg/mL), the methanol leaf extract showed the lowest IC_{50} value of 67.20 µg/mL, indicating greater scavenging activity. Meanwhile, Table 5 shows the results of the independent samples t-test done to compare the DPPH free radical scavenging activity of leaf and stem extracts of *Bryophyllum pinnatum*.

Table 4: DPPH Scavenging Activity of B. pinnatum Extracts

Sample	Percentage Inhibition of DPPH (µg/mL)					IC_{50} (µg/mL)
	20	40	60	80	100	
Leaf Extract	19.12	27.57	49.44	61.43	68.54	67.20
Stem Extract	11.04	19.87	33.90	40.55	51.76	96.38
Ascorbic Acid	10.87	19.06	32.99	39.89	50.44	98.71

Table 5: T-Test Results for DPPH Scavenging Activity of *B. Pinnatum* Extracts

Concentration (µg/mL)	Leaf Extract (Mean ±SD)	Stem Extract (Mean ±SD)	t-value	p-value
20	19.12±1.15	11.04±0.24	11.91	0.00028
40	27.57±1.01	19.87±1.09	8.98	0.00085
60	49.44±1.10	33.90±1.04	17.78	<0.0001
80	61.43±1.21	40.55±1.24	20.87	<0.0001
100	68.54±1.25	51.76±1.21	16.71	<0.0001
IC_{50} (µg/mL)	67.20±1.21	96.38±0.25	-40.91	<0.0001

Discussion

Qualitative and Quantitative Phytochemical analysis of B. pinnatum

The present findings are consistent with a similar trend reported by Igiebor *et al.* (2024), who also noted the absence of anthraquinones and phlobatanins in their analysis of *B. pinnatum* extracts.

The pharmacological significance of this comparatively unexplored plant organ is highlighted by the discovery of anthraquinones and cardiac glycosides in the stem of *B. pinnatum* but not in the leaf. Because of their capacity to control oxidative stress and cellular signaling pathways, anthraquinones are recognized for a variety of biological activities, including antioxidant, antibacterial, anti-inflammatory, and anticancer effects (Zhao & Zheng, 2023). By inhibiting the Na+/K+-ATPase pump and enhancing heart contractility, cardiac glycosides are also beneficial phytochemicals with proven cardiotonic effects (Ponce *et al.,* 2025). Other studies have documented their possible anti-inflammatory, antiviral, and anticancer properties (Nabil *et al.,* 2024; Akinwumi & Ambali, 2024). Due to its many ethnomedical uses, the leaf has historically been the subject of most research, but new data indicates that the stem may be a distinctive source of specialized bioactive chemicals with unique pharmacological significance. Therefore, their presence in the stem extract implies a further therapeutic dimension beyond the leaf's widely known antioxidant qualities (Qun *et al.,* 2023).

Compared to the stem extract (8.52 ± 1.10 mg/g), the leaf extract had a substantially greater phenolic content (18.75 ± 1.21 mg/g). Also, the leaf had a higher flavonoid concentration (7.14 ± 0.21 mg/g) than the stem (4.27 ± 0.25 mg/g). The same pattern was seen in the alkaloid content, which was 1.97 ± 1.04 mg/g in the leaf and 0.91 ± 0.01 mg/g in the stem. This suggests that the leaves have higher concentrations of these secondary metabolites, which are known to have pharmacological actions, including antioxidant qualities. The independent Student's *t*-test (n = 3) (Table 3) showed that the leaf extract contained significantly higher flavonoid ($t = 15.23$, $p < 0.05$) and phenolic contents ($t = 10.84$, $p < 0.05$) than the stem extract. The significantly higher flavonoid and phenolic contents in the leaf extract indicates that the leaf may possess superior therapeutic potential and stronger antioxidant activity than the stem. The leaf extract may therefore be more suitable for medicinal applications especially where antioxidant activity is desired. However, although the mean alkaloid content of the leaf extract was higher than that of the stem extract, the difference was not statistically significant ($t = 1.77$, $p = 0.152$). This suggests that any observed variation in the alkaloid contents of the leaf and stem extracts may be due to random experimental variation rather than a true biological difference. The findings are consistent with previous studies which demonstrated that leaves frequently accumulate more bioactive phytochemicals than stems or roots (Ogidi *et al*., 2019).

Antioxidant Profiling of B. pinnatum extracts

Radical scavenging activities seen in the DPPH experiment are closely linked to the predominance of phenolic compounds, which are recognized for their ability to donate electrons. Through radical scavenging, metal ion chelation, and hydrogen atom donation, phenolics and flavonoids are known to have potent antioxidant effects (Mittal *et al.,* 2022). The larger content and/or better structural arrangement of phenolic and flavonoid chemicals, which are known to function as hydrogen and electron donors in radical scavenging reactions, account for its superior efficacy (Mittal *et al.,* 2022; Ogidi *et al.,* 2019). Such antioxidant activity supports the ethnomedical use of *B. pinnatum* and has implications for treating illnesses linked to oxidative stress (Riaz *et al*., 2023). On the other hand, the stem extract's considerably lesser antioxidant efficacy is shown by its relatively higher IC_{50} value. The stem may have lower quantities of total phenolics and flavonoids, which are responsible for rapid radical scavenging, even though it contains special chemicals such anthraquinones and cardiac glycosides. The independent Student's *t*-test (n = 3) (Table 5) showed that the DPPH radical scavenging activity of the leaf extract was significantly higher than that of the stem extract at all tested concentrations (20–100 µg/mL; $p < 0.05$). Furthermore, the IC_{50} value of the leaf extract was

significantly lower than that of the stem extract ($t = -40.91$, $p < 0.001$). This suggests that the leaf extract possesses significantly higher antioxidant capacity than the stem extract likely owing to its higher flavonoid and phenolic contents. This could be the result of variations in phytochemical composition in different plant tissues. However, since these phytochemical components are well known for their many biological activities, especially their antibacterial and medicinal properties, their presence is still important (Zhao & Zheng, 2023). Additionally, by boosting antioxidant capacity and promoting electron transfer mechanisms, these substances may work in concert within intricate phytochemical matrices to support redox-related processes (Qun *et al.*, 2023; Riaz *et al.*, 2023). Interestingly, both plant extracts exhibited antioxidant activity comparable to that of ascorbic acid, suggesting that *B. pinnatum* contains a diverse array of phytochemicals that may collectively contribute to its antioxidant potential (Okafor *et al.*, 2024). According to a previous study by Onoja *et al.* (2018), these results suggest that *B. pinnatum* leaf and stem extract have antioxidant activity because of their ability to scavenges free radicals.

CONCLUSION

The results of this investigation through phytochemical analyses revealed that both the leaf and stem of *B. pinnatum* have varied important bioactive constituents. The leaf extract, however, exhibited better radical scavenging activity. This highlights its usefulness as a natural antioxidant source for combating oxidative stress and its suitability as an effective reducing and stabilizing agent in green nanoparticle synthesis. Notably, anthraquinones and cardiac glycosides were discovered in the stem extract but were less prominent in the leaf extract. Anthraquinones possess antimicrobial and anticancer properties, while cardiac glycosides possess cardiotonic effects, enhancing heart function. Investigating less-studied plant tissues is crucial because they may serve as large reservoirs of bioactive chemicals and greatly increase the therapeutic value of *B. pinnatum*, according to a comparative analysis with the body of existing research. By proving that the stem of *B. pinnatum* has unique phytochemical and pharmacological characteristics, this study helps close the current research gap. To fully realize the promise of this neglected plant component, future research needs to focus on comparative evaluations of various plant parts, isolation of bioactive chemicals specific to stems, and validation of their therapeutic efficacy.

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