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Phytochemical Assessment and FTIR Analysis with UHPLC–MS-Based Characterization of Leaf and Root Extracts of Bryophyllumpinnatum

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ABSTRACT: Bryophyllum pinnatum is a medicinal plant that is renowned for its therapeutic potential and diverse phytochemical composition. The objective of the current investigation was to assess the phytochemical profile, quantify significant secondary metabolites, and characterize bufadienolide compounds in leaf and root extracts through FTIR analysis. The presence of flavonoids, alkaloids, tannins, terpenoids, cardiac glycosides, phenols, sterols, and saponins was identified through qualitative screening, with a greater abundance in the leaves. The leaf extract exhibited substantially higher levels of tannins (5.45 ± 0.22 mg/g GAE), alkaloids (2.98 ± 0.09 mg/g AE), phenolics (2.62 ± 0.10 mg/g FCAE), and flavonoids (2.54 ± 0.48 mg/g QE) than the root extract, as demonstrated by quantitative estimation. The presence of bufadienolide-type compounds was confirmed by the presence of a characteristic α, β-unsaturated lactone peak (~1731 cm^{-1}) in the leaf extract, which closely matched the bufalin standard (~1727 cm^{-1}) under FTIR investigation. Conversely, the root extract did not exhibit this characteristic peak. UHPLC–MS analysis further confirmed the presence of bufadienolide compounds, including bryophyllin A and related derivatives, based on retention time and mass spectral data. In general, the findings suggest that the leaves of Bryophyllum pinnatum are a more abundant source of bioactive metabolites, notably bufadienolides, which underscores their potential for pharmacological applications.

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1. INTRODUCTION

Plant-based medications are increasing in demand due to their biocompatibility, perceived safety, and minimal side effects. The pharmacological efficacy of plants is primarily attributed to a diverse array of secondary metabolites, which collectively contribute to their medicinal properties (García-Pérez et al. 2020). One such widely used medicinal plant is *Bryophyllum pinnatum* (Lam.) Oken, a member of the Crassulaceae family. The genus *Bryophyllum* is home to approximately 25 species, including *Bryophyllum pinnatum*, a perennial, succulent herb that is indigenous to Madagascar. It is a globous ornamental herb cultivated in gardens and homes. Its four-angled stems bear opposing, succulent leaves up to 20 cm long and grow 1–1.5 m tall(Kamboj and Saluja, 2009, 2010). The fruits are composed of membranous follicles that are enclosed within a persistent, papery calyx and corolla, while the blooms are pendulous, reddish-purple, and approximately 5 cm in length (Sharma et al. 2024).*Bryophyllum pinnatum* is abundant in a wide range of bioactive secondary metabolites, such as alkaloids, triterpenes, glycosides, flavonoids, cardenolides, steroids, bufadienolides, and lipids (Gaind and Gupta, 1972, 1974). Bufadienolides, a class of extremely bioactive compounds, are concentrated in the leaves, which is of special interest. Prominent bufadienolides, including bryotoxin A, B, and C, resemble the cardiac glycosides digoxin and digitoxin in terms of their structural and functional characteristics. Collectively, these metabolites underpin the extensive pharmaceutical and therapeutic applications of *Bryophyllum pinnatum*(Kamboj and Saluja, 2009)

Ethnomedicinal evidence has shown that *Bryophyllumpinnatum* is used to treat a variety of conditions such as skin discolorations, wounds, haemorrhoids, menorrhagia, diabetes mellitus(Akinpelu 2000), cancer, rheumatoid arthritis (Majaz et al. 2011), kidney stones, and inflammatory swellings (Lans, 2006; Kamboj and Saluja, 2009). The fresh leaf juice is used for wound healing and to reduce bleeding. The decoction of the roots is used in the traditional care of epilepsy. The herb is also used for dental discomfort, headaches, earaches, boils, eye infections, burns, and insect infestations (Kamboj and Saluja, 2009; Sharma et al. 2024).

The present study compares the phytochemical content and the amount of metabolites present in the leaf and root extracts of *Bryophyllumpinnatum*. The phytochemical profiles were evaluated qualitatively and quantitatively in a detailed manner with emphasis on the major secondary metabolites such saponins, phenolics, glycosides, terpenoids, tannins, flavonoids, bufadienolides, steroids and alkaloids. Total amounts of tannins, flavonoids, phenolics and alkaloids were quantified while bufadienolides were identified using Fourier Transform Infrared spectrometry (FTIR) and ultrahigh performance liquid chromatography-tandem mass spectrometry (UHPLC–MS). The results give insight on the tissue-specific metabolite distribution and provide support for future pharmacological studies and use of *Bryophyllumpinnatum* as a source of bioactive compounds.

2. MATERIAL AND METHODS

2.1 Plant material

The plantwas nurtured in the nursery at Guru Jambheshwar University of Science and Technology, (29.167°N, 75.737°E, 215m elevation) Hisar, Haryana, India. Healthy and juvenile leaves and root samples were procured qualitative and quantitative analysis.

2.2 Sample preparation

The cleaned plants were subjected to drying under a hot-air oven operated at a constant temperature range of 40°C to 70°C for 6 to 8 hours. This controlled drying process ensured the removal of moisture without degrading the phytochemical constituents. Once the plant material (leaf and root) is completely dried was pulverized with mechanical grinder, resulting in fine powder stored in dry conditions for later extraction and phytochemical analysis.

2.3 Extraction

Leaf and root materials were individually pulverised, to a homogeneous powder, 10 g of each sample was placed into clean extraction flasks. Each pulverised sample was treated with continuous magnetic stirring for 24 hours at room temperature and extracted with 100 mL of ethanol for efficient solubilisation of the phytoconstituents. The mixtures obtained after extraction were filtered with Whatman filter paper (Grade 3) to remove insoluble plant debris and to achieve a clean filtrate. The filtrates were then allowed to evaporate at room temperature until a desiccated crude residue was produced. The dried crude extracts

were carefully collected, placed into sterile containers and stored at 4°C for further qualitative and quantitative phytochemical analysis. Ethanolic extract of crude samples were prepared and put through preliminary bioactive plant compound analysis using standard qualitative procedures.

2.4 Qualitative analysis of phytochemicals

2.4.1 Test for alkaloids

Mayer's test: A small volume of leaf and root extracts was transferred into distinct test tubes, and 1–2 drops of Mayer's reagent (potassium mercuric iodide, K_2HgI_4) were carefully included along the side of each tube. The presence of alkaloids in the plant preparations was indicated by the formation of a cream or pale-yellow precipitate. This test was carried out on both leaf and root samples to validate the presence of alkaloid in distinct tissues (Narnaware and Dhabarde, 2025).

Wagner's examination: In clean test tubes, around 2 mL of each plant extract was added, along with a few drops of Wagner's reagent (iodine-potassium iodide solution). The presence of alkaloids in the samples was verified by the development of a reddish-brown precipitate(Narnaware and Dhabarde, 2025).

2.4.2 Test for tannins

Ferric chloride test: A few droplets of a 1% ferric chloride ($FeCl_3$) solution were added to 5 mL of the plant extract. The presence of hydrolysable tannins was suggested by the development of a blue-black coloration, whereas the presence of condensed tannins was suggested by a brownish-green precipitate (Farhat et al. 2011).

2.4.3 Test for saponin

Approximately 50 mg of the leaf and root crude extracts were diluted in 5 mL of distilled water and vigorously shaken for 5-7 minutes to produce froth. The presence of saponins was indicated by the creation of a stable and continuous froth that remained following light heat (Ujah and Onyishi, 2023).

2.4.4 Test for cardiac glycoside

Keller-Killiani test: In separate test tubes, 5 mL of the leaf and root extracts were combined with 2 mL of glacial acetic acid. One drop of ferric chloride ($FeCl_3$) solution was subsequently introduced, followed by the careful addition of 1 mL of concentrated sulfuric acid (H_2SO_4) along the side of the test tube to establish a distinct lower layer. A prominent brown ring at the interface revealed the presence of deoxysugars, which are characteristic of cardiac glycosides. In certain instances, a violet ring was observed beneath the brown layer, and the upper acetic acid layer developed a greenish coloration, which further substantiated a positive reaction (Narnaware and Dhabarde, 2025).

2.4.5 Test for phenol

Gelatine test: To 5 mL of plant extracts (leaf and root), add 2 mL of 1% gelatin solution containing 10% sodium chloride (NaCl) and well mix. The production of a white precipitate suggested the existence of phenolic compounds as a result of gelatin-phenolic ingredient interaction (Ajuru et al. 2017).

2.4.6 Test for terpenoids

Salkowski test: To 1 mL of each plant extract, add 10 mL of chloroform and stir thoroughly. Subsequently, 10 mL of concentrated sulfuric acid (H_2SO_4) was gently added down the side of the test tube, forming two separate layers. The appearance of a red coloration in the top chloroform layer, as well as a yellow coloration and green fluorescence in the acid layer, showed the existence of steroidal chemicals inside the extract. This assay was used to qualitatively detect steroids in plant materials (Narnaware and Dhabarde, 2025).

2.4.7 Test for glycoside

Borntrager's test: To 2 mL of plant extract in separate test tubes, 3 mL of chloroform was added, and the mixture was thoroughly agitated before being allowed to stand for phase separation. The chloroform layer was carefully extracted and treated with an equivalent volume of dilute ammonium solution. The pink to crimson tint in the ammoniacal layer showed the presence of anthraquinone glycosides. In a confirmation stage, the chloroform extract was extracted and combined with

an equal volume of 10% ammonia solution. The appearance of a pink or crimson tint in the ammoniacal phase revealed the existence of anthraquinone molecules in plant extracts (Narnaware and Dhabarde, 2025).

2.4.8 Test for steroids

Liebermann–Burchard test: Approximately 2 mL of the plant extracts was mixed with 2 mL of chloroform in a clean, dry test tube. To this mixture, add 1-2 mL of acetic anhydride and gently stir. Afterward, a few droplets of concentrated sulfuric acid (H_2SO_4) were carefully added to the side of the test tube to create a distinct layer. The appearance of blue-green color confirms the presence of steroids (Narnaware and Dhabarde, 2025).

2.4.9 Test for flavonoids

Alkaline reagent test: A portion of the aqueous crude extracts from the leaf and root samples were placed in separate test tubes and treated with 5 mL of dilute ammonia solution. Following that, concentrated sulfuric acid (H_2SO_4) was carefully added. The appearance of a yellow tint in the extracts showed the presence of flavonoids. The slow fading of the yellow colour after standing confirmed the favourable result for flavonoids (Narnaware and Dhabarde, 2025).

2.5 Quantitative Analysis

2.5.1 Estimation of total tannin content: To measure total tannin concentration, 0.5 ml of plant extract was mixed with 0.25 ml of Folin-Denis reagent, sodium carbonate (Na_2CO_3), and 3.5 ml of distilled water. The mixture was incubated at room temperature for 30 minutes, after which the absorbance at 700 nm was measured using a spectrophotometer. The tannin concentration was determined using a standard tannic acid calibration curve and represented in mg tannic acid equivalents (TAE) per gram (Blainski et al. 2013; Padma et al. 2013).

$y= 0.0102x+0.029$ $R^2 = 0.9782$

2.5.2 Estimation of total alkaloid content: To measure total alkaloid content, 0.5 ml of the plant extract was mixed with 5 ml of phosphate buffer (pH 4.7) and 5 ml of bromocresol green (BCG) reagent in a separating funnel. The mixture was vigorously agitated with 5 mL of chloroform, and the chloroform layer separated. This layer's absorbance was measured at 470 nm with a spectrophotometer, and a blank (without plant extract) was used to compensate for baseline. The intensity of the yellow color in the chloroform layer was related to the concentration of alkaloids. The total alkaloid content was determined using an atropine standard calibration curve and represented in mg atropine equivalents (AE) per gram (Narnaware and Dhabarde, 2025).

$y= 0.0134x+0.7788$ $R^2 = 0.9735$

2.5.3 Estimation of total phenolic content: To evaluate total phenolic content, 0.5 ml of plant extract was mixed with 5 ml of 10% Folin-Ciocalteu reagent and 4 ml of 1 M Na_2CO_3 in a 50 ml volumetric flask. The volume was adjusted using distilled water. A 0.5 ml sample of the reaction mixture was obtained, and absorbance at 760 nm was measured using a UV-Visible spectrophotometer, with a blank (without plant extract) used for baseline calibration. The intensity of the blue hue produced was related to its phenolic content. The total phenolic content was determined using a standard gallic acid calibration curve and represented as mg gallic acid equivalents (GAE) per gram of extract (Narnaware and Dhabarde, 2025).

$y= 0.0146x+0.0528$ $R^2 = 0.9286$

2.5.4 Estimation of total flavonoid content: To evaluate the total flavonoid content, 0.5 ml of plant extract was mixed with 2 ml of distilled water and 0.15 ml of 5% $NaNO_2$ solution. After a few minutes, add 0.15 mL of 10% $AlCl_3$ solution, followed by 2 mL of 1 M NaOH. The final volume was adjusted with distilled water, and the combination was kept at room temperature. After 15 minutes, absorbance was measured at 510 nm with a spectrophotometer. The intensity of the pinkish-red color correlated directly with flavonoid content. The total flavonoid content was determined using a standard quercetin calibration curve and represented as mg rutin equivalents (RQ) per gram(Blainski et al. 2013).

$y= 0.0182x+0.7694$ $R^2 = 0.9812$

2.6 Detecting the presence of bufadienolide

2.6.1 Fourier Transform Infrared spectroscopy: Crude extracts of leaf, and root of *Bryophyllum pinnatum*, in powder form, were mixed with potassium bromide (KBr). The mixtures were compacted into translucent pellets and subjected to FT-IR spectroscopy (PerkinElmer Spectrum BX II). The spectra were recorded within the 4000–400 cm^{-1} range and subsequently processed and compared to the spectrum of the bufalin standard to identify characteristic functional groups (Rani et al., 2021).The FTIR spectra were processed and baseline-corrected, and graphs were generated using Origin software (OriginLab Corporation, USA).

2.6.2 Ultrahigh performance liquid chromatography-mass spectroscopy assay:

Qualitative analysis of bufadienolide compounds was conducted using a high-resolution quadrupole time-of-flight mass spectrometer (TripleTOF 5600/5600+, SCIEX) in conjunction with an ultra-high-performance liquid chromatography system (Nexera 2, Shimadzu). Chromatographic separation was accomplished using a Phenomenex Synergi C18 column (Fusion RP 80 Å, 4 μm particle size, 50 × 2 mm internal diameter). In order to guarantee optimal peak resolution and reproducible retention behavior, the column temperature was maintained at 55°C by a thermostatically controlled column oven. The mobile phase contained (A) 10 mM ammonium formate in water with 0.05% formic acid, and (B) 0.05% formic acid in acetonitrile. The gradient elution program was established as follows: 5% B (0–1 min), linearly increased to 32% B (1–4 min), maintained at 32% B (4–11 min), gradually increased to 100% B (11–11.1 min), held at 100% B (11.1–13 min), and subsequently returned to the initial conditions (5% B) for re-equilibration (13–13.9 min). The total cycle time was 14 minutes, and the flow rate was maintained at 0.4 mL/min. A 5 μL injection volume was employed, and the autosampler temperature was maintained at 25°C. The samples were prepared as mentioned by (Oufir et al. 2015).

Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) sources were employed in both positive and negative ionization modes for mass spectrometric detection. The system enabled the selection of precursor ions within the m/z 5–1250 range using the quadrupole analyzer, which was subsequently followed by fragmentation in the collision cell and precise mass detection using the time-of-flight analyzer with a mass range of up to 40 kDa. Analyst TF was employed to acquire data, while PeakView was employed to conduct data processing and spectral analysis.

2.7 Statistical Analysis:

All the quantitative estimation of phytochemicals were conducted in triplicates in independent experiments and reported as the mean ± standard error. Microsoft excel and was employed to conduct statistical analysis and graphical representations. One-way ANOVA was employed to assess the differences between the means, and a significance level of $P \leq 0.05$ was regarded as statistically significant. Additionally, the coefficient of determination (R^2) was computed to evaluate the strength and direction of relationship between variables.

3. RESULTS

3.1 Phytochemical screening

Both the leaf and root extracts of *Bryophyllum pinnatum* include a number of bioactive substances, such as flavonoids, sterols, terpenoids, tannins, cardiac glycosides, alkaloids, phenols, and saponins, according to the phytochemical screening results (Table 1). Nevertheless, these phytochemicals were more abundant in the leaf extract than in the root extract.

Table1 Results of the phytochemical screening of leaf and root extract of*Bryophyllum pinnatum*.

Phytochemicals	Test	Leaf	Root
Alkaloid	Mayer's test	+++	+
	Wanger's test	+++	+
Tannin	Ferric chloride	+++	++
Saponin	Foam index	++	+

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Cardiac glycoside	Keller-Killani test	+++	+
Phenols	Gelatine test	+	++
Terpenoids	Salkowski test	+++	+
Glycoside	Borntrager's test	++	+
Steroids	Liebermann-Burchard test	++	+
Flavonoids	Alkaline reagent test	++	+

3.2Qualitative analysis

3.2.1 Total tannin content (TTC)

From the results in Table 2, The TTC of *Bryophyllum pinnatum* varied notably between the two plant extracts analysed. The leaf extract exhibited a substantially higher tannin content (5.45 ± 0.22 mg/g GAE) compared with the root extract (1.28 ± 0.10 mg/g GAE), indicating greater accumulation of tannins in the leaves. This implies that *Bryophyllum pinnatum*'s leaves contain more tannins than its roots (Fig. 1).

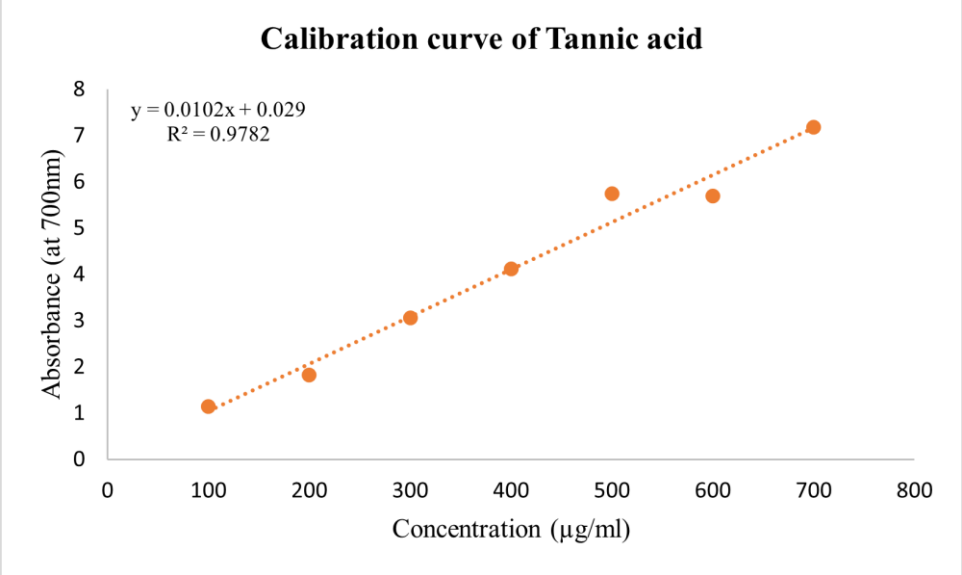


Fig.1 Calibration curve of tannic standard solutions showing the linear relationship between concentration (100–700 µg/mL) and absorbance measured at 700 nm. The regression equation was y = 0.0102x + 0.029 with a correlation coefficient of R^2 = 0.9782.

Table 2 Quantitative estimation of major secondary metabolites in leaf and root extract of *Bryophyllum pinnatum.*

Secondary metabolites	Leaf extract	Root extract
Tannin	5.45±0.22 mg/gm GAE	1.28±0.10 mg/gm GAE
Alkaloid	2.98±0.09 mg/gm AE	0.79±0.10 mg/gm AE
Phenolics	2.62±0.10 mg/gm FCAE	0.83±0.05 mg/gm FCAE
Flavonoids	2.54±0.48 mg/gm QE	1.05±0.07 mg/gm QE

3.2.2 Total alkaloid content (TAC)

The total alkaloid content of *Bryophyllum pinnatum* exhibited a comparable trend to that of tannins, with a higher accumulation in the leaf extract than in the root extract. While the root extract demonstrated a substantially lower value of 0.79 ± 0.10 mg/g AE, the leaf extract exhibited an alkaloid content of 2.98 ± 0.09 mg/g AE. These findings suggest that the foliage is a more abundant source of alkaloids than the roots (Fig. 2.).

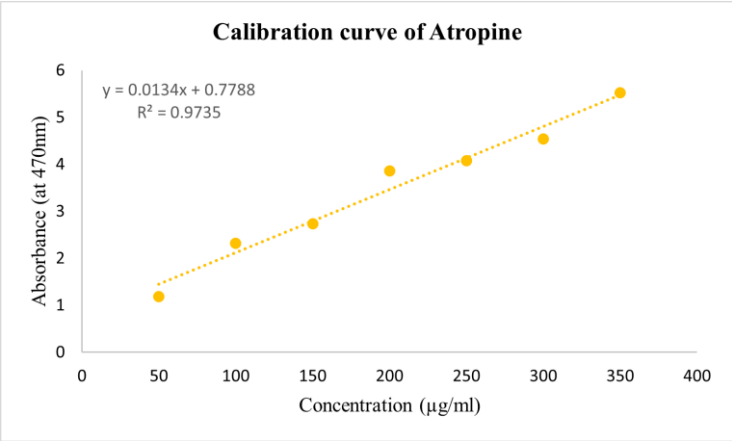


Fig.2 Calibration curve of atropine standard solutions showing the linear relationship between concentration (50–350 µg/mL) and absorbance measured at 470 nm. The regression equation was y = 0.0134x + 0.7788 with a correlation coefficient of R^2 = 0.9735.

3.2.3 Total phenolic content (TPC)

Compared to the root extract (0.83 ± 0.05 mg/g FCAE), the leaf extract of *Bryophyllum pinnatum* exhibited a higher total phenolic content (2.62 ± 0.10 mg/g FCAE), suggesting that the leaves accumulate more phenolic compounds. This implies that the leaves may be a more abundant source of phenolic metabolites than the roots (Fig.3.).

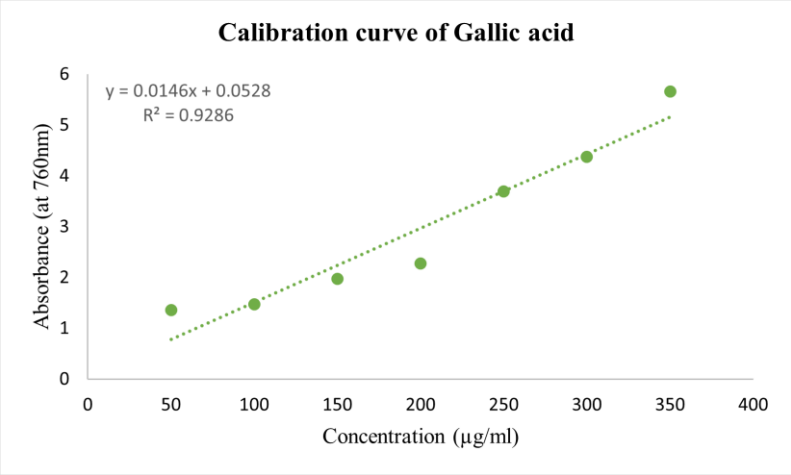


Fig.3 Calibration curve of atropine standard solutions showing the linear relationship between concentration (50–350 µg/mL) and absorbance measured at 760 nm. The regression equation was y = 0.0146x + 0.0528 with a correlation coefficient of R^2 = 0.9286.

3.2.4 Total flavonoids content (TFC)

The leaf extract of *Bryophyllum pinnatum* exhibited a higher flavonoid content (2.54 ± 0.48 mg/g QE) than the root extract (1.05 ± 0.07 mg/g QE), indicating a marked difference in flavonoid content among plant parts. This suggests that flavonoids are more prominently concentrated in the leaves of the plant (Fig. 4.).

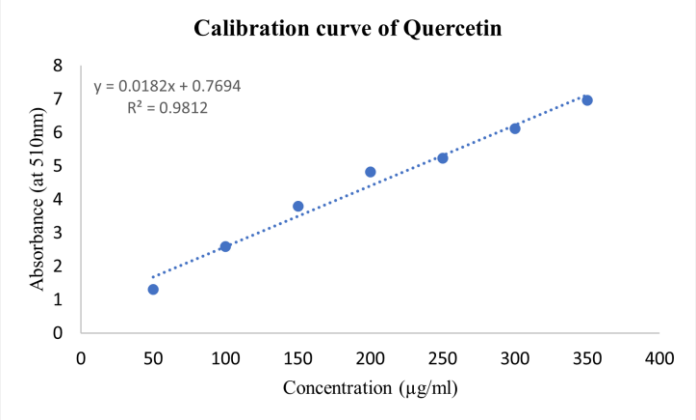


Fig. 4 Calibration curve of atropine standard solutions showing the linear relationship between concentration (50–350 µg/mL) and absorbance measured at 510 nm. The regression equation was y = 0.0182x + 0.7694 with a correlation coefficient of $R^2 = 0.9812$.

3.3 Characterization of bufadienolides in *Bryophyllum pinnatum* by FTIR

The FTIR spectrum of the bufadienolide standard (bufalin) exhibited characteristic bands at 3446.52 cm^{-1} (O-H stretching), 2941.66 cm^{-1} (aliphatic C-H stretching), 1727.22 cm^{-1} (C=O stretching of the α,β-unsaturated lactone ring), 1635.42 cm^{-1} (C=C stretching), 1384.50 cm^{-1} (C-H bending), and 1249.40/1033.17 cm^{-1} (C-O stretching) (Langat et al. 2021). The leaf extract of *Bryophyllum pinnatum* exhibited a similar pattern with matching peaks at 3437.48, 2922.52, 1731.04, 1632.78, 1384.61, 1236.62, and 1043.64 cm^{-1}. These peaks closely resembled the bufalin standard and supported the presence of compounds of the bufadienolide type. The root extract, on the other hand, lacked the characteristic lactone carbonyl band near 1730 cm^{-1} and displayed fewer similar diagnostic bands, with peaks primarily at 3437.08, 2924.65, 1632.55, 1384.58, 1232.23, and 1049.24 cm^{-1}. The leaf extract demonstrated a higher degree of spectral similarity to the bufadienolide standard than the root extract, which implies that bufadienolide-like compounds are more abundant in the leaves of *Bryophyllum pinnatum*. The FTIR spectra are shown in Fig. 5, and the corresponding interpretations are summarized in Table 3.

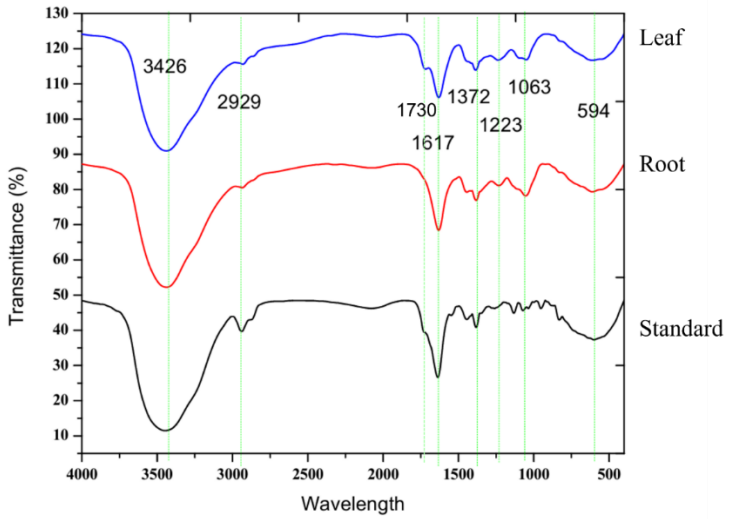


Fig. 5 FTIR spectra of leaf and root extracts of *Bryophyllum pinnatum* compared with the bufadienolide standard. Characteristic absorption peaks corresponding to functional groups, including O–H (~3426 cm^{-1}), C–H (~2929 cm^{-1}), C=O (lactone, ~1730 cm^{-1}), C=C (~1617 cm^{-1}), and C–O (~1223–1063 cm^{-1}), are indicated, confirming the presence of bufadienolide-related compounds in the leaf extract.

Table 3 Comparative FTIR analysis of bufadienolide standard and *Bryophyllum pinnatum* extracts.

Functional group	Bufadienolide standard	Leaf	Root	Assignment
O-H	3446	~3437	~3437	Presence of hydroxyl groups in leaf and root
C-H	2941	~2922	~2924	Steroidal backbone present in both samples
C=O (lactone)	1727	~1730	Ab	α, β- unsaturated lactone present in leaf sample
C=C	1635	~1632	~1632	Conjugated double bond in both samples
C-H	1384	~1384	~1384	Methyl groups present in both samples
C-O	1249, 1033	~1236/~1043	~1232/~1049	Alcohol/glycosidic linkage present in both samples

3.4 UHPLC-MS

The leaf extract was subjected to UHPLC–MS analysis, which identified numerous bufadienolide compounds based on their retention times and characteristic precursor ions (m/z). The identification was accomplished by comparing the mass spectral data that was acquired with the values that had been previously reported in the literature (Table 4). Bersaldegenin-1-acetate and Bersaldegenin-3-acetate were detected at retention periods of 4.6 min and 5.0 min, respectively, and both exhibited a precursor ion at m/z 475.31. Bryophyllin A was detected at a retention time of 6.3 minutes, with a corresponding precursor ion at m/z 472.31. Bersaldegenin-1,3,5-orthoacetate was identified at a retention time of 9.124 min with a precursor ion at m/z 457.27. Furthermore, the retention time of bufalin, which was employed as an internal standard, was 10.6 minutes, and a characteristic precursor ion was detected at m/z 388.12 (Oufir et al. 2015). The total ion chromatogram (TIC) of leaf crude extract with bufalin as internal standard are represented as (Fig.6-10).

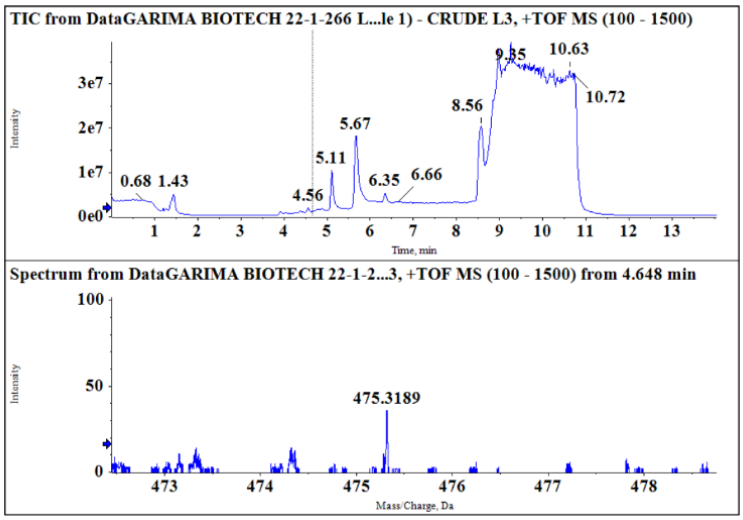


Fig.6 Total ion chromatogram (TIC) and corresponding mass spectrum of the leaf crude extract of *Bryophyllum pinnatum* obtained by TOF–MS analysis. The chromatogram shows multiple resolved peaks, with a prominent signal observed around 4.6 min. The mass spectrum recorded at this retention time exhibits a prominent ion at *m/z* 475.31, indicating the presence of a bersaldegenin-1-acetate compound, tentatively based on reported literature values

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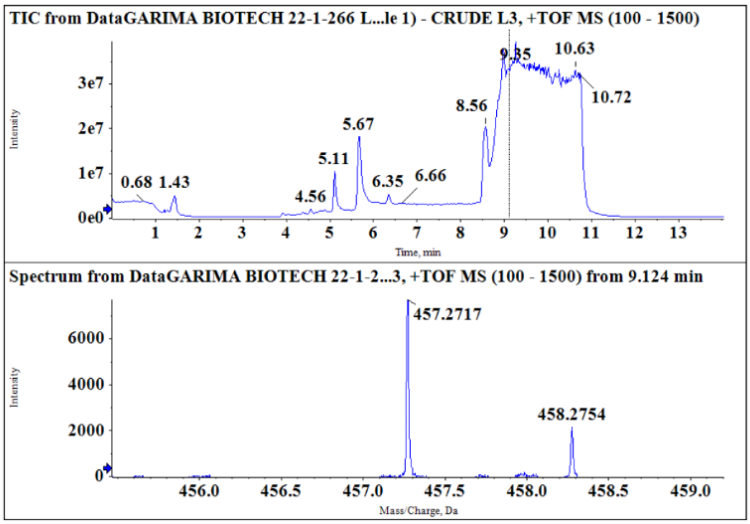


Fig.7 TIC and corresponding mass spectrum of the leaf crude extract of *Bryophyllum pinnatum* obtained by TOF–MS analysis. The chromatogram shows multiple resolved peaks, with a prominent signal observed around 9.12 min. The mass spectrum recorded at this retention time exhibits a prominent ion at m/z 457.27, indicating the presence of a bersaldegenin-1,3,5-orthoacetate compound, tentatively based on reported literature values

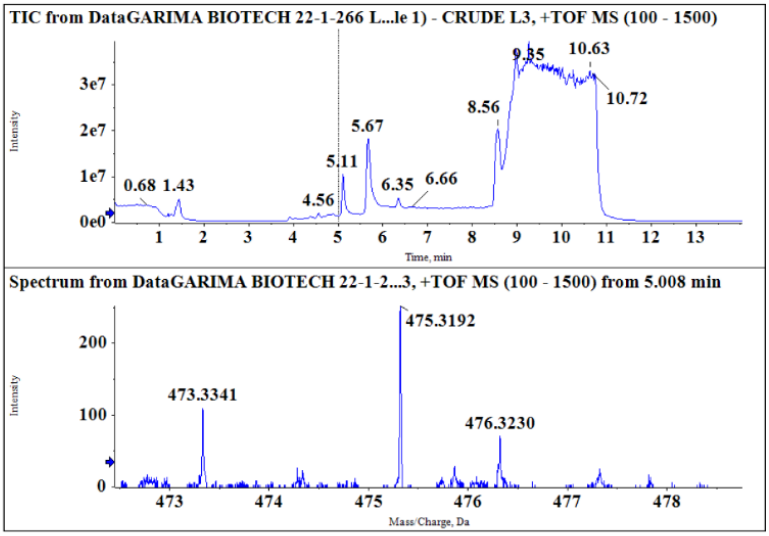


Fig.8 TIC and corresponding mass spectrum of the leaf crude extract of *Bryophyllum pinnatum* obtained by TOF–MS analysis. The chromatogram shows multiple resolved peaks, with a prominent signal observed around 5.0 min. The mass spectrum recorded at this retention time exhibits a prominent ion at m/z 475.31, indicating the presence of a bersaldegenin-3-acetate compound, tentatively based on reported literature values

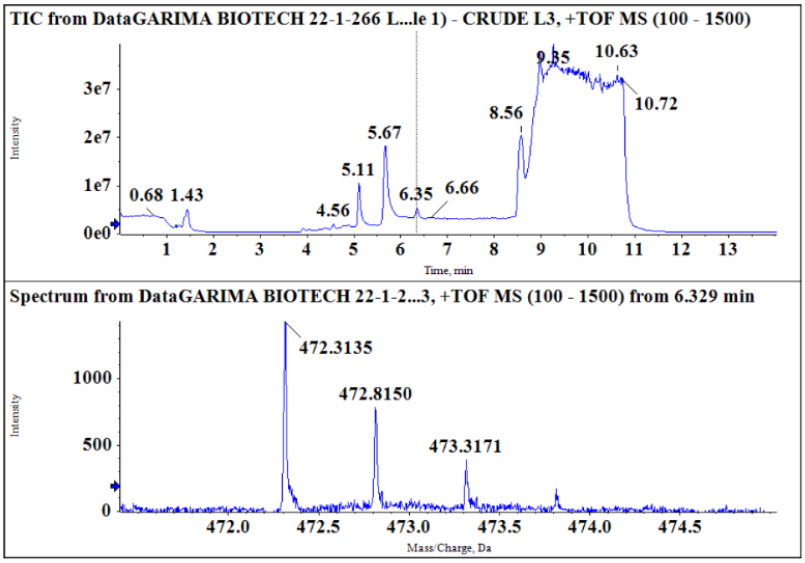


Fig.9TIC and corresponding mass spectrum of the leaf crude extract of *Bryophyllum pinnatum* obtained by TOF–MS analysis. The chromatogram shows multiple resolved peaks, with a prominent signal observed around 6.3 min. The mass spectrum recorded at this retention time exhibits a prominent ion at *m/z* 472.31, indicating the presence of a bryophyllin A compound, tentatively based on reported literature values

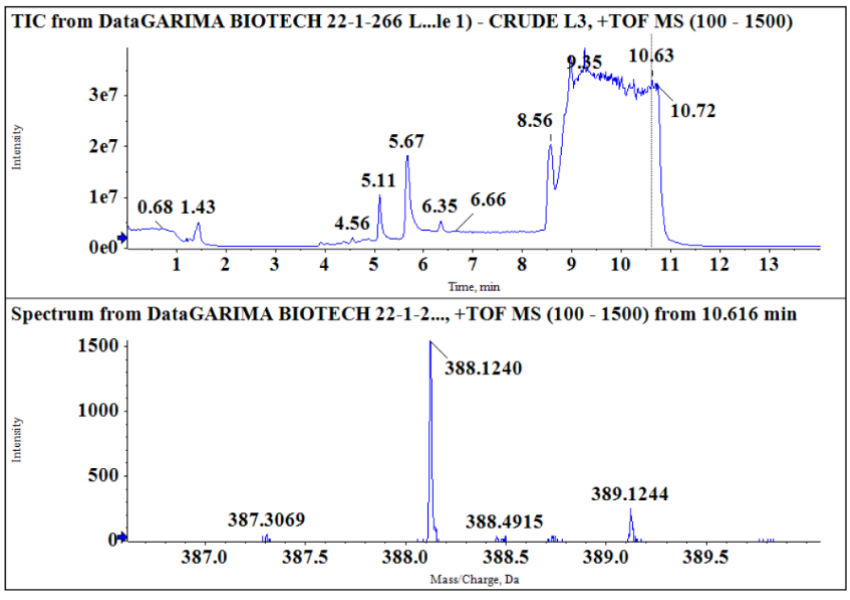


Fig.10 TIC and corresponding mass spectrum of the leaf crude extract of *Bryophyllum pinnatum* obtained by TOF–MS analysis. The chromatogram shows multiple resolved peaks, with a prominent signal observed around 10.6 min. The mass spectrum recorded at this retention time exhibits a prominent ion at *m/z* 388.12, indicating the presence of a bersaldegenin-1-acetate compound, tentatively based on reported literature values

Indicating effective separation under the optimized UHPLC conditions, the chromatographic peaks were well resolved. The presence of bufadienolide constituents in the leaf extract of *Bryophyllum pinnatum* was confirmed by the observed retention durations and mass-to-charge ratios of all detected compounds, which were consistent with previously reported literature.

Table 4 MS-parameters for the detection of bufadienolides and internal standard (bufalin) in the positive ion mode (ESI).

Compound	Retention time (min)	Precursor (m/z)	Reference
Beesaldegenin-1-acetate	4.6	475.31	(Oufir et al. 2015)
Beesaldegenin-3-acetate	5.0	475.31	(Oufir et al. 2015)
Bryophyllin A	6.3	472.31	(Oufir et al. 2015)
Bersaldegenin-1,3,5-orthoacetate	9.124	457.27	(Oufir et al. 2015)
Bufalin	10.6	388.12	(Oufir et al. 2015)

4. DISCUSSION

The present work emphasises the importance of *Bryophyllumpinnatum* as a reservoir of secondary metabolites with significant difference between root and leaf extracts. Qualitative phytochemical screening showed the presence of flavonoids, alkaloids, tannins, terpenoids, cardiac glycosides, phenols, sterols and saponins in both segments of the plant, with the leaves having a somewhat higher quantity. The discovery is in agreement with previous reports which showed that active metabolic and photosynthetic roles of aerial sections of medicinal plants especially leaves are the major sites for biosynthesis and accumulation of secondary (Kolodziejczyk-Czepas and Stochmal, 2017).

Quantitative research further confirmed this and showed that the leaf extract had much higher quantities of tannins, alkaloids, phenolics and flavonoids compared to the root extract. The higher tannin content in leaves implies that the defensive ability and antioxidant potential of the plant has been strengthened as tannins are known to protect the plants from biotic stress. Similarly, the greater alkaloid contents may increase the pharmacological action of leaves, which may include antibacterial and anti-inflammatory properties. The increased antioxidant capacity of leaf extract can be attributed to the higher phenolic and flavonoid contents which are well known to scavenge free radicals and prevent oxidative damage. These findings confirm the earlier reports of increased accumulation of phenolic and flavonoid chemicals in the leaves of *Bryophyllumpinnatum*(Oufir et al. 2015)

Crucial evidence for the existence of bufadienolide-type compounds in B was provided by FTIR analysis. The bufalin standard exhibited characteristic absorption bands, particularly the α,β-unsaturated lactone carbonyl peak at approximately 1727 cm^{-1}, which is a critical diagnostic feature of bufadienolides. The leaf extract exhibited a spectral pattern that was nearly identical, which included a prominent peak at approximately 1731 cm^{-1}, as well as bands that corresponded to hydroxyl groups, conjugated C=C bonds, and C–O stretching vibrations. These results indicate that the leaf extract contains bufadienolide constituents. Conversely, the root extract lacked the distinctive lactone carbonyl peak, suggesting the absence or minimal presence of such compounds. Bufadienolides, which include bryophyllin derivatives, are steroidal cardiac glycosides that are distinguished by a six-membered α-pyrone (lactone) ring at the C-17 position (SteynVan Heerden, 1998). Previous reports have identified bufadienolides as the primary bioactive constituents of B, and the detection of this functional group in the leaf extract is consistent with this. pinnatum, particularly in aerial tissues (Oufir*et al.* 2015). The tissue-specific distribution of these compounds is further substantiated by the absence of this essential peak in the root extract

Further, the existence of bufadienolide metabolites in the *Bryophyllumpinnatum* leaf extract was confirmed by UHPLC–MS analysis. A number of compounds were identified based on their characteristic retention times and precursor ions, which closely matched previously published values, including bryophyllin A and bersaldegenin derivatives. The identification of these compounds, along with the internal standard bufalin, suggested the effectiveness of chromatographic separation and confirmed the presence of structurally diverse bufadienolides in the leaf extract. These findings are in agreement with recent reports on the selective storage of the bufadienolide compounds in aerial parts of *Bryophyllumpinnatum*.

Overall, the integrated phytochemical screening, quantitative estimation, FTIR and UHPLC-MS analysis clearly reveal that leaves of *Bryophyllumpinnatum* are a more abundant source of bioactive secondary metabolites, especially bufadienolide-type

compounds than the roots. Thus, the findings not only confirm the traditional medical usage of the leaves but also provide a scientific basis for its possible use in pharmaceuticals and therapeutic formulations.

5. CONCLUSION

The current study shows that *Bryophyllumpinnatum* is a good source of bioactive secondary metabolites, with a clear differentiation between leaf and root extracts. Flavonoids, alkaloids, tannins, phenolics, and other phytoconstituents are substantially more abundant in the leaf extract than in the root, according to both qualitative and quantitative tests, indicating that the leaves are the location of metabolite accumulation. By identifying the distinctive α,β-unsaturated lactone functional group, which was lacking in the root extract, FTIR analysis offered crucial proof that bufadienolide-type chemicals were present in the leaf extract.

Overall, the combination of phytochemical screening, quantitative estimate, FTIR, and advanced mass spectrometry identifies *Bryophyllumpinnatum* leaves as a rich source of pharmacologically significant chemicals. These findings demonstrate the plant's potential for use in medication development and therapeutic formulations in the future and offer a solid scientific foundation for its traditional use. Further studies focusing on isolation, structural characterization, and biological evaluation of individual compounds are recommended to fully explore its medicinal potential.

DECLARATIONS

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare that no conflict of interest exist with respect to this study and its publication.

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Author's contributions

G.S. conceptualized the study and performed the experiments. S.Y. contributed to writing the manuscript. A.J. and R.K. assisted with data curation. V.C. provided overall supervision, critically reviewed the manuscript, and contributed to its finalization.

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