Phytochemical Screening, Isolation and Spectroscopic Characterization of Bioactive Compounds from leaves of *Bryophyllum pinnatum*

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Abstract
Aim: The present study was undertaken to evaluate the phytochemical constituents, isolate and characterize the bioactive compound, and assess the in vitro antioxidant activity of the hydroalcoholic leaf extract of *Bryophyllum pinnatum*.
Materials and Methods: Dried leaves of *Bryophyllum pinnatum* were extracted using 70% ethanol by Soxhlet extraction. Total phenolic content (TPC) and total flavonoid content (TFC) were carried out using standard methods. Thin-layer chromatography (TLC) and silica gel column chromatography were employed for the isolation of the bioactive compound, which was characterized by, FT-IR, ^1H NMR, and mass spectrometry. Antioxidant activity was evaluated using DPPH radical scavenging, reducing power assays and Hydrogen peroxide scavanging with ascorbic acid used as the reference standard.
Results: Phytochemical screening confirmed the presence of carbohydrates, alkaloids, flavonoids, terpenoids, glycosides, tannins, saponins, proteins, and phenolic compounds. The hydroalcoholic extract exhibited a total phenolic content of 63.44 mg GAE/g and a total flavonoid content of 51.10 mg RE/g. TLC analysis showed an Rf value of 0.50, indicating the presence of flavonoid constituents. The extract demonstrated notable antioxidant activity in the DPPH assay with an IC_{50} value of 34.168 µg/mL and also exhibited appreciable hydrogen peroxide scavenging activity with an IC_{50} value of 32.818 µg/mL, while moderate activity was observed in the reducing power assay.
Conclusion: The hydroalcoholic leaf extract of *Bryophyllum pinnatum* is rich in phenolic and flavonoid compounds and exhibits significant antioxidant activity. These findings support its traditional medicinal use and indicate its potential as a natural source of antioxidant bioactive compounds for pharmaceutical applications.
Keywords: *Bryophyllum pinnatum*; Flavonoids; DPPH, Hydrogen Peroxide scavenging and UV-Visible.
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1. Introduction

Medicinal plants have long served as an invaluable source of bioactive compounds with significant therapeutic potential and continue to play a crucial role in the discovery of novel pharmaceutical agents **(Al-Snafi et al., 2020).** These plants produce a wide range of secondary metabolites, including alkaloids, flavonoids, phenolic compounds, saponins, steroids, tannins, and terpenoids, which are responsible for their diverse biological and pharmacological activities. Among these, phenolic compounds and flavonoids have attracted considerable attention due to their potent antioxidant properties **(Doss et al., 2009).** Oxidative stress, resulting from the excessive production of reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals, and nitric oxide **(Kumar et al., 2010),** has been implicated in the pathogenesis of numerous chronic diseases, including diabetes mellitus, liver cirrhosis, nephrotoxicity, cardiovascular disorders, and neurodegenerative diseases. Therefore, the identification of plant-derived natural antioxidants capable of scavenging free radicals has become an important area of pharmaceutical and biomedical research **(Shirwaikar et al., 2006).**

Bryophyllum pinnatum, a succulent herbaceous plant native to Madagascar, is notably recognized for its extensive ethnopharmacological applications **(Sharma et al., 2024).** Traditional uses of *Bryophyllum pinnatum*, also known as the "life plant" or "air plant," include treatments for urinary tract infections in Ayurveda **(Fürer et al., 2016).** This perennial plant, native to Madagascar, propagates vegetatively through its 3–5 feet tall dark green leaves. It is also referred to as patharchatta and has trado medicinal applications in tropical regions.

Traditionally, it is used in Ayurveda for urinary tract infections and has various applications, including treatment for migraines, kidney stones, and bloody diarrhea **(Latif et al., 2019).** The plant's leaves, often consumed in decoctions or pastes, have diverse medicinal uses across different cultures, including skin injuries in India and various ailments like ulcers, headaches, and hypertension in West Africa **(Selvakumar et al., 2022).** Therefore, the present study was undertaken to investigate the phytochemical constituents and Isolation of compounds from *Bryophyllum pinnatum* leaf extract and evaluate its antioxidant activity, thereby providing scientific evidence for its traditional medicinal use.

2. Materials and Methods

2.1 Plant collection and Soxhlet extraction:

Dried powered of *Bryophyllum pinnatum (*Leaves) were successively defatted with 70% ethanol and then placed in a thimble of Soxhlet apparatus. The extraction was carried out using 70% ethanol solvent system at 40-60^{o}C temperature of the heating mantle for 8-12 hours **(Evans, 2009 and Alara *et al.*, 2019).**

2.2 Qualitative Phytochemical Estimation of Extracts

Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents in extracts of *Bryophyllum pinnatum* by using standard procedures **(Kokate *et al.*, 2006).** The extracts were subjected to tests for carbohydrates, alkaloids, flavonoids, glycosides, protein and amino acids, triterpenoids and steroids, tannin and phenolic compounds.

2.3 Quantitative Phytochemical estimation-

2.3.1 Spectrophotometric Quantification of Total Phenolic Content: -

The total phenolic content (TPC) of the plant extract was measured using the Folin-Ciocalteu Assay, where 0.2 mL of the stock solution was combined with 2.5 mL of the reagent. The estimation was performed in triplicate, and TPC was expressed as milligrams of gallic acid equivalents (GAE) per gram of dried sample **(Saeed *et al.*, 2012).**

2.3.2 Spectrophotometric Quantification of Total Flavonoid Content: -

The Flavonoid content was determined using Aluminium chloride method **(Chang *et al.*, 2002)**. A standard curve for total flavonoids was established using rutin standard solutions (20 to 100 µg/ml) following the same protocol. The total flavonoids were expressed as milligrams of rutin equivalents per g of dried fraction **(Senguttuvan *et al.*, 2014).**

2.4 Thin layer chromatography

Preliminary thin-layer chromatography (TLC) was carried out to optimize the solvent systems for *Bryophyllum pinnatum* leaves extracts. Various solvent combinations were tested, and the solvent systems showing the maximum number of well-resolved spots were selected. Based on these preliminary TLC results, the respective solvent systems Toluene: Ethyl acetate: Acetic acid (3:2:0.2) for leaves were selected as mobile phases for subsequent column chromatography **(Ozlem *et al.*, 2016).**

R_f Value $= \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$

2.5 Column chromatography

Isolation of flavonoids from the hydro-alcoholic extract of Bryophyllum pinnatum was achieved through silica gel column chromatography. The column, packed using silica gel (60-120) via wet packing, utilized slurry of toluene. One gram of the extract was added, followed by gradient elution with a mixture of Toluene: Ethyl acetate: Acetic acid (7: 2.5: 0.5). Various elutes were collected, concentrated, and analyzed using TLC to confirm the presence of the compounds **(Srivastava *et al.*, 2021 and Mukherjee *et al.*, 2024).**

2.6 Spectroscopic characterization: -

2.6.1 UV-visible Spectroscopy

The isolated fraction (H) of leaves extract *Bryophyllum pinnatum* was scanned from 200 to 800 nm wavelength using UV-Visible Spectrophotometer (Shimadzu UV-1700) and the characteristic peaks were detected and recorded **(Patel *et al.*, 2022)**.

2.6.2 FT-IR

To establish the presence of the functional groups in the isolated fraction (G) of BP (hydro-alcoholic) extract, FT-IR spectroscopy was performed using Perkin Spectrum 95763 spectrophotometer **(Luciene *et al.*, 2008).**

2.6.3 NMR Spectroscopy

NMR spectroscopy was performed for the isolated fraction (G) of PG (hydro-alcoholic) extract to identify the structure of the compound present in the isolated fraction. JNM EC-500 NMR spectroscopy for this purpose was Nuclear Magnetic Resonance spectroscopy **(Zia *et al.*, 2019 and Marion *et al.*, 2013).**

2.6.4 Mass Spectroscopy

The mass spectrometer used for the identification of the molecular weight of isolated fraction (G) of PG (hydro-alcoholic) extract was recorded on mass spectrometer instrument micrOTOF-Q 228888.10348 **(Wiley *et al.*, 1995).**

2.7 Activity (*In-vitro* Anti-oxidant Activity)

2.7.1 DPPH Radical Scavenging Activity

A 0.1mM solution of DPPH in methanol was prepared, along with a 1 mg/ml solution of extracts/standard. Various volumes (20-100 µl) of the extracts/standard were mixed with methanol to reach

1 ml. After adding 2 ml of DPPH reagent, the mixture was incubated in the dark for 30 minutes, and absorbance was measured at 517 nm. A control of 3 ml DPPH solution was also incubated similarly, with absorbance recorded against methanol as a blank at 517 nm **(Sagar, 2011).**

Percentage antioxidant activity of sample/standard was calculated by using formula:

% Inhibition = [(Ab of control- Ab of sample/ Ab of control x 100]

2.7.2 Reducing power assay

3 mg of ascorbic acid was dissolved in 3 ml of distilled water, creating dilutions of 20, 40, 60, 80, and 100 µg/ml. Stock solutions of extracts were made by dissolving 1 mg of dried extracts in 1 ml of methanol. Aliquots of ascorbic acid and extracts were mixed with phosphate buffer and potassium ferricyanide, incubated at 50°C for 20 minutes, and then treated with trichloroacetic acid and centrifuged. The absorbance of the resulting solution was measured at 700 nm using a UV spectrometer, with a blank prepared for comparison **(Quisumbing, 1978).**

2.7.3 Hydrogen peroxide scavenging activity

The ability of the extract to scavenge hydrogen peroxide (H_2O_2) was determined according to the method of Ruch et al. **(Ruch et al., 1989).** Aliquot of 0.1 mL of extracts (20–100 µg/mL) was transferred into the eppendorf tubes and their volume was made up to 0.4 mL with 50 mM phosphate buffer (pH 7.4) followed by the addition of 0.6 mL of H_2O_2 solution (2 mM). The reaction mixture was vortexed and after 10 min of reaction time, its absorbance was measured at 230 nm. Ascorbic acid was used as the positive control. The ability of the extracts to scavenge the H_2O_2 was calculated using the following equation:

% Inhibition = [(Ab of control- Ab of sample/ Ab of control x 100]

3. Results

3.1 Percentage yield

Table 1: Percentage yield of extracts

S. No.	Plant name and Part	Solvent	Color of extract	Theoretical weight (gm)	Yield (gm)	% Yield
1.	*Bryophyllum pinnatum*	Whole plant	40 gm	1.	*Bryophyllum pinnatum*	Whole plant

3.3 Qualitative Phytochemical Analysis of different extracts

3.4

Table 2: Phytochemical analysis of *Bryophyllum pinnatum* Extracts

S. No.	Tests	Hydroalcoholic
1.	Test for Carbohydrates	+
2.	Test for Alkaloids	+
3.	Test for Terpenoids	-
4.	Test for Flavonoids	+
5.	Test for Tannins and Phenolic Compounds	+
6.	Test for Saponins	+
7.	Test for Protein and Amino acids	-
8.	Test for Glycosides	+

3.5 Quantitative Phytochemical analysis Leaves of *Bryophyllum pinnatum* -

3.5.1 Total Phenolic Content (TPC) Estimation

Figure 1: Represent standard curve of Gallic acid

Table 3: Standard table for Gallic acid

Concentration (µg/ml)	Absorbance (nm)
20	0.111
40	0.233
60	0.369
80	0.467
100	0.574

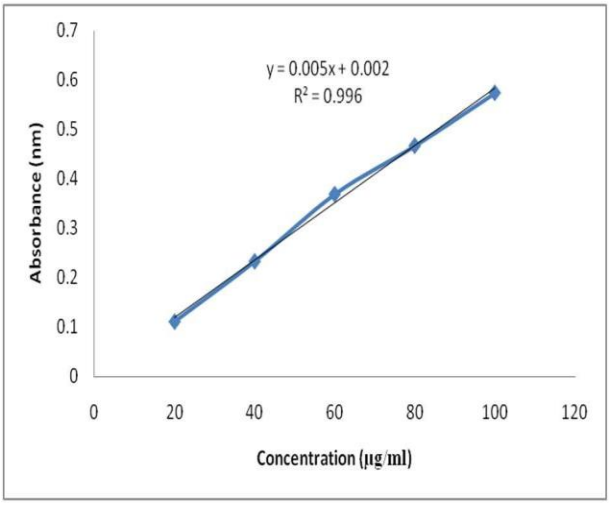


Table 4: Total Phenolic Content in *Bryophyllum pinnatum* extracts-

Total Phenolic content (mg/gm equivalent to Gallic acid)	
Extracts	***Leaves***

Absorbance Mean±SD	0.319±0.003
TPC	63.44

3.4.2. Total Flavonoid Content (TFC) Estimation:

Table 5: Standard table for Rutin

Figure 2: Graph represent standard curve of Rutin

Concentration (µg/ml)	Absorbance (nm)
20	0.087
40	0.163
60	0.232
80	0.307
100	0.403

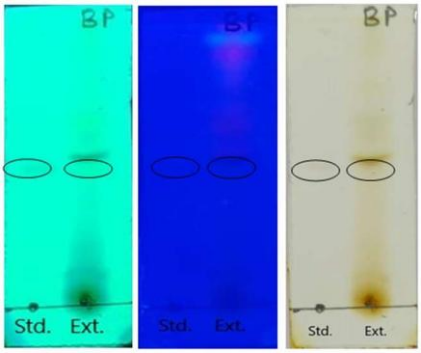
Table 6: Total Flavonoid Content in *Bryophyllum pinnatum* extracts -

Total Flavonoid content (mg/gm equivalent to Rutin)	
Extracts	***Bryophyllum pinnatum***
Absorbance Mean±SD	0.158±0.002
TPC	51.10

3.6 Preliminary TLC preparation for the estimation of active constitutes

1. Flavonoid for hydro-alcoholic extract of *Bryophyllum pinnatum*

Mobile Phase- Toluene: Ethyl acetate: Acetic acid (7: 2.5: 0.5))



Long-UV (365 nm) Visible Light Short-UV (254nm)

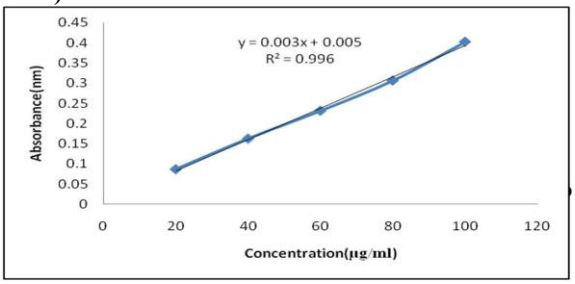


Figure 3: TLC estimation by UV lamp for BP with Std. flavonoid

(Std. = Standard, BP= *Bryophyllum pinnatum*)

TLC of BP extract was performed on different solvent systems (solvent system was selected on the basis of literature survey). TLC performed in Toluene: Ethyl acetate: Acetic acid (7: 2.5: 0.5) for BP hydro-alcoholic was clearly visible bands of extracts with Std. flavonoid. The Rf values of hydro-alcoholic extract of BP with Std. flavonoid were found to be 0.50 and 0.50.

3.7 Column Chromatography

The fractions/elutes obtained from silica gel column chromatography of hydro-alcoholic extract of *Bryophyllum pinnatum* was tested for the detection of various phyto compounds using TLC.

3.8 Spectroscopic characterization: -

3.8.1 Active constitutes estimation by UV-Spectroscopy-

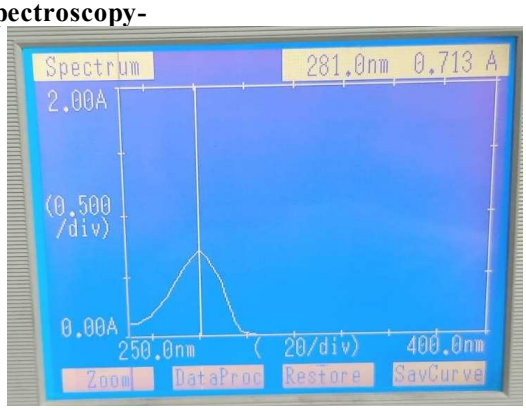


Figure 3: Active constitutes estimation by UV-Spectra of isolated fraction (G) of BP (hydro-alcoholic) extract after column chromatography

3.8.2 Active constitutes estimation by FTIR – Spectroscopy

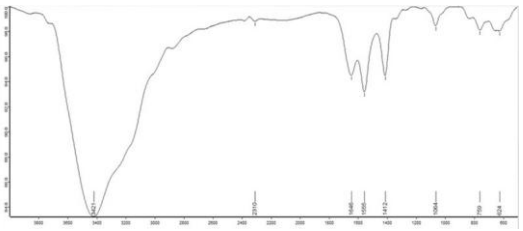


Figure 5: FTIR spectra of the isolated fraction (G) of BP (Hydro-alcoholic) extract

3.8.3 ^{1}H NMR - Spectroscopy

H NMR spectra of the isolated fraction (H) of *Bryophyllum pinnatum* leaves extract

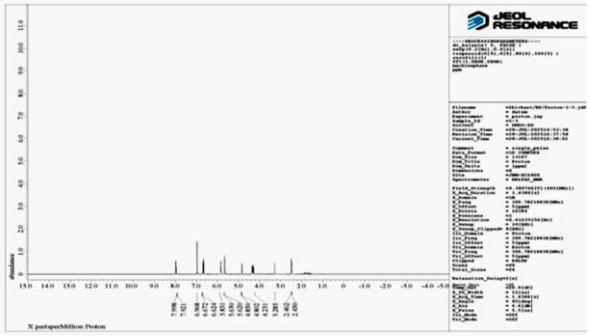


Figure 6: ^{1}H-NMR spectra of the isolated fraction (G) of BP (hydro-alcoholic)

3.8.4 Mass – Spectroscopy

(A) Mass spectra of the isolated fraction (H) of *Bryophyllum pinnatum leave* extract

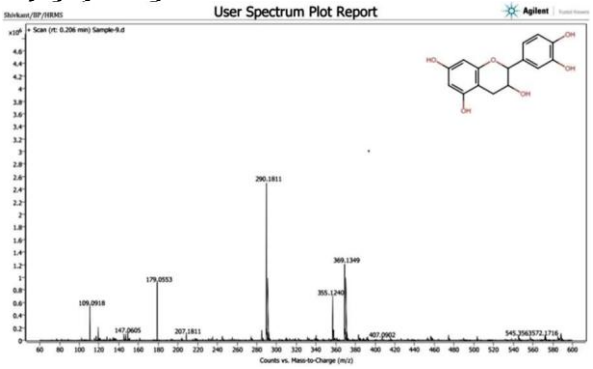
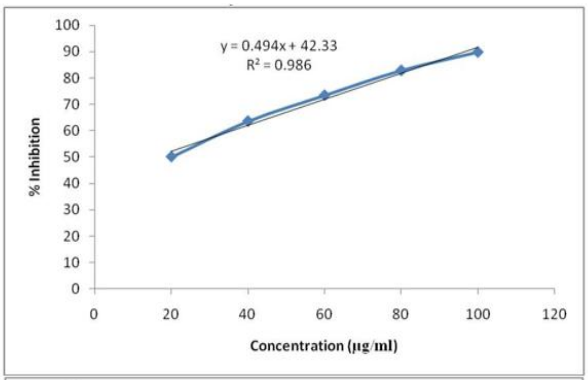


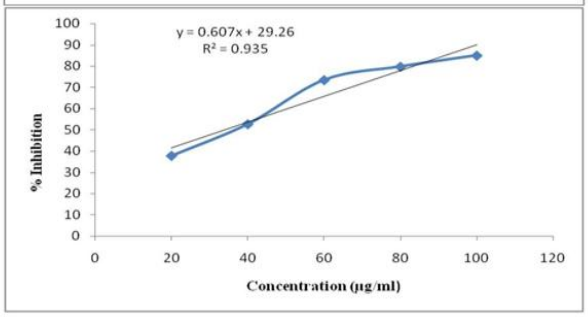
Figure 7: Mass spectra of the isolated fraction (H) of *Bryophyllum pinnatum* leaves extract

3.9 Anti-oxidant activity

3.9.1 DPPH Assay



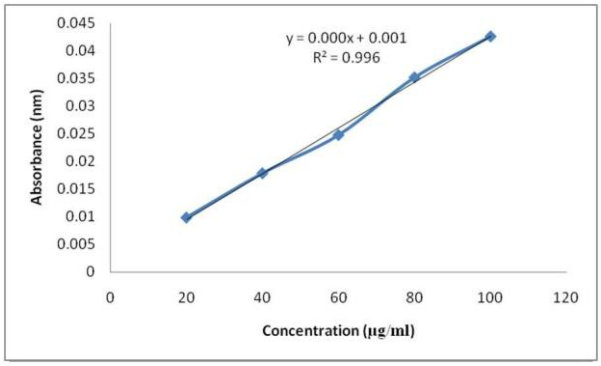
Graph 7.1 Graph represents the % Inhibition *Vs* conc. of Ascorbic acid



Graph 7.2 Graph represents the % Inhibition *Vs* conc. of *B. pinnatum*

The extract showed better activity in quenching DPPH radicals with an IC50 value of 34.168 µg/ml. The activity was moderate in remaining antioxidant models.

3.9.2 Reducing power scavenging activity



Graph 7.3 Graph represents the Absorbance *Vs* conc. of Ascorbic acid Absorbance Vs *conc.* of Ascorbic acid conc. of Hydroalcoholic extract of

B.pinnatum

3.9.3 Hydrogen peroxide scavenging activity

Table 7: Hydrogen peroxide scavenging activity

Sample	% inhibition at 100 µg/ml	IC50
Ascorbic acid	89.494	18.318
Bryophyllum pinnatum	88.120	32.818

4. Discussion

The hydroalcoholic extract of Bryophyllum pinnatum leaves was found to be a rich source of biologically active phytochemicals. Preliminary phytochemical screening confirmed the presence of flavonoids, alkaloids, terpenoids, glycosides, tannins, saponins, carbohydrates, and phenolic compounds, indicating that the hydroalcoholic solvent efficiently extracted a broad range of polar and moderately polar constituents. The high total phenolic content (63.44 mg GAE/g) and total flavonoid content (51.10 mg rutin equivalent/g) further support the phytochemical richness of the extract and suggest that these compounds are responsible for its biological activity. Thin-layer chromatography was initially employed to optimize the solvent system for the separation of phytoconstituents. Among the different solvent combinations tested, toluene acetate acid (7:2.5:0.5)

produced a well-resolved spot with an Rf value of 0.50, which corresponded with the flavonoid standard. This result confirmed the suitability of the selected mobile phase for the separation of flavonoid constituents. The same solvent system was therefore used for silica gel column chromatography, which successfully separated the hydroalcoholic extract into ten fractions (A–J). TLC analysis of the isolated fraction (G) showed an Rf value identical to that of the standard flavonoid, indicating that this fraction contained the target bioactive compound. The isolated fraction (G) was further characterized using spectroscopic techniques. UV–Visible spectroscopy showed a characteristic absorption maximum (λmax) at 281 nm, which is commonly associated with phenolic and flavonoid compounds. FTIR analysis revealed the presence of hydroxyl, alkane, alkene, and alcohol functional groups, as indicated by absorption bands at 3421, 2310, 1646, 1555, 1412, 1064, 759, and 624 cm^{-1}. These functional groups are commonly reported in flavonoid molecules and support the phytochemical nature of the isolated compound. Further structural confirmation was obtained using ^1H NMR spectroscopy. The observed proton signals in the aromatic and aliphatic regions were consistent with the presence of a flavonoid skeleton containing multiple hydroxyl substitutions. Mass spectrometric analysis showed a molecular ion peak at m/z 290.1811, corresponding to the molecular formula $C_{15}H_{14}O_6$. The fragmentation pattern further supported the identification of the isolated compound as (2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol, a flavonoid compound commonly known as catechin. The combined chromatographic and spectroscopic analyses confirmed the successful isolation and structural characterization of the major antioxidant constituent from the hydroalcoholic extract of *Bryophyllum pinnatum*. The antioxidant activity of the extract was evaluated using DPPH radical scavenging and reducing power assays. In the DPPH assay, the extract exhibited concentration-dependent radical scavenging activity, with inhibition increasing from 37.75% at 20 µg/mL to 84.97% at 100 µg/mL. Although the activity was lower than that of ascorbic acid, the IC_{50} value of 34.168 µg/mL indicates good free radical scavenging ability. Similarly, the reducing power assay showed a gradual increase in absorbance with increasing concentration, demonstrating the electron-donating capacity of the extract and the extract also demonstrated effective hydrogen peroxide scavenging activity with an IC_{50} value of 32.818 µg/mL. Hydrogen peroxide, although relatively less reactive than other oxygen radicals, readily penetrates biological membranes and can generate highly reactive hydroxyl radicals in the presence of transition metal ions.. These findings indicate that the antioxidant activity of *B. pinnatum* is closely associated with its phenolic and flavonoid constituents, particularly the isolated catechin-rich fraction. Overall, the results demonstrate that the hydroalcoholic leaf extract of Bryophyllum pinnatum contains significant amounts of phenolic and flavonoid compounds and exhibits considerable antioxidant activity. The successful isolation and spectroscopic characterization of catechin provide scientific evidence for the antioxidant potential of this medicinal plant. These findings support the traditional use of B. pinnatum and suggest that it may serve as a valuable natural source of antioxidant compounds for future pharmaceutical and nutraceutical applications. Further studies involving in vivo evaluation and mechanistic investigations are required to confirm its therapeutic potential.

5. Conclusion

The present study demonstrated that the hydroalcoholic leaf extract of *Bryophyllum pinnatum* is a rich source of bioactive phytochemicals with significant antioxidant potential. Preliminary phytochemical screening confirmed the presence of flavonoids, alkaloids, terpenoids, glycosides, saponins, carbohydrates, and phenolic compounds, indicating the chemical complexity of the extract. Quantitative analysis indicated significant total phenolic and flavonoid contents in the plant, contributing to its biological activity. Techniques like TLC and column chromatography were used to isolate a major fraction, while UV–Visible, FT-IR, ^1H NMR, and mass spectrometry confirmed a flavonoid-rich compound's characteristics. The coherence between chromatographic and spectroscopic methods underscores the isolation's reliability for further structure investigation. Additionally, the extract demonstrated concentration-dependent antioxidant activity in various assays, suggesting the antioxidant effects are linked to its high phenolic and flavonoid content.

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