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Pharmacognostic And Phytochemical Evaluation Of Bryophyllum Pinnatum Leaves As A Standardized Herbal Source

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Bryophyllum pinnatum (Lam.) Pers. is a succulent plant often used in traditional medicine. It has great healing potential, especially for wounds and stopping bleeding. This study aimed to create a detailed profile of the leaves. It focused on pharmacognostic, phytochemical, and analytical aspects. This work supports standardization and the development of medicinal formulations. Morphological observations showed key features. These include bright green, ovate-oblong leaves. The leaves have notches along the edges that bear plantlets. Microscopic and powder analyses showed key elements. These include paracytic stomata, calcium oxalate crystals, lignified xylem, and starch granules. The proximate values, like extractive yields and ash content, met pharmacopeial standards. This confirms the quality and purity of the plant material. The ethanolic extract had bioactive groups. These included flavonoids, glycosides, tannins, alkaloids, saponins, and sterols. We used High-Performance Liquid Chromatography (HPLC) for standardization. It confirmed bufadienolides were present at 0.84% w/w. The retention time was 7.91 minutes, and the calibration curve R^2 was 0.9985. These findings support traditional uses and provide scientific proof for herbal medicine. It can help with coagulation and tissue regeneration.

Keywords: Bryophyllum pinnatum, Pharmacognosy, Phytochemical screening, Bufadienolides, HPLC analysis.

INTRODUCTION

Herbal medicines are becoming more popular worldwide. This shows a strong trust in traditional plant-based treatments, despite modern medicine being available. In many places, especially in Asia, Africa, and South America, medicinal plants are key to primary healthcare. They are easy to find, affordable, and have a long history of trust. In recent decades, modern pharmacology has shifted. Many now explore phytotherapy as a real, evidence-based alternative to synthetic drugs. This is especially true for chronic conditions and lifestyle disorders [1].

Bryophyllum pinnatum, or the "Miracle Leaf," is unique in the world of medicinal plants. It offers wide-ranging health benefits and holds cultural importance. This succulent plant is valued in many cultures. It helps treat wounds, reduce bleeding, and promote tissue healing. In Indian Ayurveda, it helps balance doshas and boosts wound healing. In Africa and the

Caribbean, it plays a key role in childbirth and postpartum care rituals [2]. The plant can regenerate well. It forms new shoots from its leaf edges. This ability strengthens its link to healing and renewal in many indigenous traditions [3].

Even though Bryophyllum pinnatum is widely used, it lacks pharmacognostic standardization. This makes it hard for doctors to accept and use it in their practice. For any herbal medicine to be deemed reliable, reproducible, and safe, it must undergo rigorous quality control. Parameters like plant identity, purity, chemical profile, and microscopic traits need to be set and confirmed. The inconsistency in phytochemical content is influenced by environmental and processing factors. So, we need a systematic approach to standardization. This ensures therapy works well and meets global herbal drug rules [4].

Interest in herbal medicine is rising. So, it's important to connect traditional knowledge with modern science. Plants like Bryophyllum pinnatum have a rich history and strong bioactive properties. This makes them great candidates for integrative research.

Pharmacognostic Profile of Bryophyllum pinnatum

Biological Source

Bryophyllum pinnatum (Lam.) Oken, also known synonymously as Kalanchoe pinnata, belongs to the family Crassulaceae. It is a juicy, long-lasting herb used mainly for its fresh or dried leaves. These leaves are the key source of active compounds that have medicinal benefits. The plant has several names. People call it “Miracle Leaf,” “Patharchatta” in Hindi, and “Goethe Plant” in different parts of the world. This is because of its special ability to regenerate.

Figure 1: Bryophyllum pinnatum leaf

Geographical Distribution

Bryophyllum pinnatum, native to Madagascar, now grows in many tropical and subtropical areas. You can find it in India, Bangladesh, Africa, the Caribbean, and parts of South America. In India, it grows abundantly in Maharashtra, West Bengal, and South India, particularly in semi-arid and rocky terrains. Its ability to grow in many environments makes it useful for medicine and ethnobotany [6].

Active Chemical Constituents

Bryophyllum pinnatum holds great healing potential because of its many secondary metabolites, which include:

- Flavonoids: Quercetin, Kaempferol – known for antioxidant and anti-inflammatory actions.
- Triterpenoids: Bryophollone, Bryophillin A and C – contribute to antimicrobial and hemostatic effects.
- Alkaloids: Involved in vasoconstriction and analgesic activity.
- Bufadienolides are cardiac-active glycosides like bryophyllin A and B. They support blood vessel function and aid in blood clotting.
- Phenolic compounds: Gallic acid, Ferulic acid – promote wound healing and provide antioxidative protection.
- Saponins and Glycosides: Enhance bioavailability and exhibit antimicrobial activity [7,8].

Therapeutic Uses

Bryophyllum pinnatum is traditionally and pharmacologically used to manage many conditions:

- Hemostatic: Helps blood clot and is used for wounds and postpartum bleeding [7].
- Wound healing: Accelerates tissue regeneration by stimulating fibroblast and collagen activity.
- Anti-inflammatory: Used in treating ulcers, arthritis, and skin inflammations.
- Antimicrobial: Effective against various bacterial and fungal strains due to triterpenoids and flavonoids.
- Renal support: Historically used to manage kidney stones ("Patharchatta" implies "stone-breaker").
- Gynecological uses: Helps control heavy menstrual bleeding and postpartum hemorrhage.
- Antioxidant and hepatoprotective: Prevents oxidative stress and supports liver function [8,9].

MATERIALS AND METHOD

Materials

Plant Material

We picked fresh, healthy leaves of Bryophyllum pinnatum from gardens in Chopda, Maharashtra. The Botanical Survey of India in Pune verified the plant specimen. A voucher

specimen was also kept for future reference. The leaves were cleaned well, dried in the shade at room temperature, and then ground into a coarse powder with a mechanical grinder.

Chemicals and Reagents

All chemicals and reagents used in the study were of analytical grade and procured from reputed suppliers. The details are summarized in Table 6.1.

Table 1: List of Chemicals and Reagents

S.N.	Name of Chemical/Reagent	Supplier
1	Ethanol or Hydroalcoholic solution	Research Lab, Mumbai
2	Chitosan (Natural polymer for nanoparticle formulation)	Research Lab, Mumbai
3	Sodium Tripolyphosphate (TPP) – Crosslinking agent	Loba Chemie Pvt. Ltd., Mumbai
4	Acetic acid	Merck Life Science Pvt. Ltd.
5	Phosphate-buffered saline (PBS)	Himedia Laboratories Pvt. Ltd.
6	Tween 80 – Surfactant/stabilizer	Loba Chemie Pvt. Ltd., Mumbai
7	Dimethyl sulfoxide (DMSO) – Solubilizing agent	Research Lab, Mumbai
8	Calcium chloride ($CaCl_2$) – For coagulation activity	Merck Life Science Pvt. Ltd.
9	Citrated human blood / Commercial blood plasma	Approved Blood Bank / Himedia
10	Ethylenediaminetetraacetic acid (EDTA) – Anticoagulant	Loba Chemie Pvt. Ltd., Mumbai

Fresh leaves of Bryophyllum pinnatum came from trusted botanical sources. Experts at the Botanical Survey of India verified them. The samples were cleaned, shade-dried, and coarsely powdered with a grinder. This prepared them for pharmacognostic and phytochemical analysis [10].

We recorded the leaves' morphological traits. These include shape, size, margin, apex, base, surface texture, and color. We also checked the organoleptic properties, such as odor and taste. We used standard botanical identification methods for this. These observations provide essential diagnostic criteria for crude drug identification and authentication [11].

We analyzed fresh leaves under a microscope. This included looking at hand-cut slices of the midrib. The pith method was employed to support the sample during sectioning. Sections were treated with chloral hydrate. They stained the samples with phloroglucinol and strong hydrochloric acid. This process revealed the lignified tissues. Finally, they were mounted in glycerin. We observed the prepared slides with a compound microscope. This helped us learn about anatomical structures. We studied vascular bundles, epidermal layers, and mesophyll arrangement [12].

Powder microscopy looked at the shade-dried leaf powder. This was done after treating it with chloral hydrate and phloroglucinol-HCl stain. Microscopic evaluation showed several components: calcium oxalate crystals, lignified xylem vessels, phloem fibers, starch grains, and paracytic stomata. These elements help identify powdered herbal materials [13].

The stomatal index was determined using lower epidermal peels of the leaves. Samples were cleared with chloral hydrate, stained, and mounted for microscopic observation. A square of known dimensions was traced using a camera lucida. We counted the number of stomata and epidermal cells, including trichomes, in this area. Then, we calculated the stomatal index using this formula:

Stomatal Index (S.I.) = [S / (E + S)] × 100,

Where S = number of stomata, and E = number of epidermal cells [14].

Proximate analysis was conducted to assess the quality of the powdered drug. Extractive values in ethanol, water, and ether were measured by macerating 4 g of the drug in 100 mL of the respective solvent. The samples were shaken every so often for 6 hours. Then, they stood for 18 hours and were filtered. Next, 25 mL of the filtrate was evaporated in a shallow dish at 105°C. Finally, the residues were weighed to find the percentage extractive values [15].

Moisture content was found by weighing a specific amount of leaf powder. It was dried in a hot air oven at 105°C until it reached a constant weight. The weight loss was then shown as a percentage of the starting weight [16].

We measured ash values by burning 2 g of powdered drug in a silica crucible at 450°C. We calculated total ash, acid-insoluble ash, water-soluble ash, and sulphated ash through gravimetric analysis. To find acid-insoluble ash, treat total ash with dilute hydrochloric acid. Then, filter it, ignite the residue, and weigh it. Water-soluble ash was derived by subtracting the weight of the residue left after boiling total ash with water. For sulphated ash, samples were treated with sulfuric acid and incinerated at about 800°C until a constant white residue was obtained [17].

Pressurized Liquid Extraction (PLE) used 10–15 g of dried, powdered leaves. These were packed in extraction cells with filter paper at each end. A hydroalcoholic mixture of ethanol and water (70:30 v/v) served as the extraction solvent. Conditions included a temperature of 100°C, pressure of 1500 psi, and two static extraction cycles of 10 minutes each. The flush volume was set at 60%, followed by a nitrogen purge for 60 seconds. Extracts were pooled, evaporated under reduced pressure using a rotary evaporator below 45°C, and stored in amber glass bottles at 4°C [18].

We did a preliminary phytochemical screening. We used standard colorimetric and precipitation methods to find different phytoconstituents. Carbohydrates were tested using Molisch's, Benedict's, and Fehling's tests. Proteins and amino acids were identified by Millon's and ninhydrin tests. Sterols were confirmed using Salkowski and Liebermann-Burchard reactions. We detected glycosides and cardiac glycosides using two methods. First, we performed acid hydrolysis, then used Fehling's test for glycosides. For cardiac glycosides, we applied Keller-Killani and Legal's tests. Anthraquinone glycosides were revealed through Borntrager's reaction. Saponins were confirmed by persistent froth and haemolysis tests. Alkaloids were identified by Mayer's, Dragendorff's, Wagner's, and Hager's reagents. Phenolic compounds and flavonoids were confirmed by ferric chloride, Shinoda, and zinc-HCl tests. Tannins were verified using gelatin and vanillin-HCl tests [19].

Standardization of the ethanolic extract was performed using High-Performance Liquid Chromatography (HPLC). The sample was dissolved in methanol and filtered through a 0.45 µm membrane. We analyzed samples using a C18 reversed-phase column. The mobile phase included acetonitrile and water, with 0.1% formic acid. We performed this under gradient conditions. The flow rate was maintained at 1.0 mL/min, the injection volume was 20 µL, and

the detection wavelength was 220–240 nm. We found bufadienolides by checking their retention time and UV spectra against known standards. Results were expressed as a percentage of dry extract weight [20].

RESULT

Table 2: Morphology of the Leaves of Bryophyllum pinnatum (Lam.) Pers

Sl. No.	Features	Observation
1.	Color	Bright green
2.	Taste	Slightly sour
3.	Odour	Odourless
4.	Size	5–12 × 2–5 cm (varies with maturity and position on stem)
5.	Texture	Fleshy, smooth
6.	Petiole	1–3 cm long, cylindrical to slightly flattened
7.	Leaf base	Decurrent
8.	Lamina	Ovate to oblong, thick and succulent, margins with notches bearing plantlets
9.	Apex	Acute to rounded

Figure 2: Photograph of T.S of Bryophyllum pinnatum Leaves

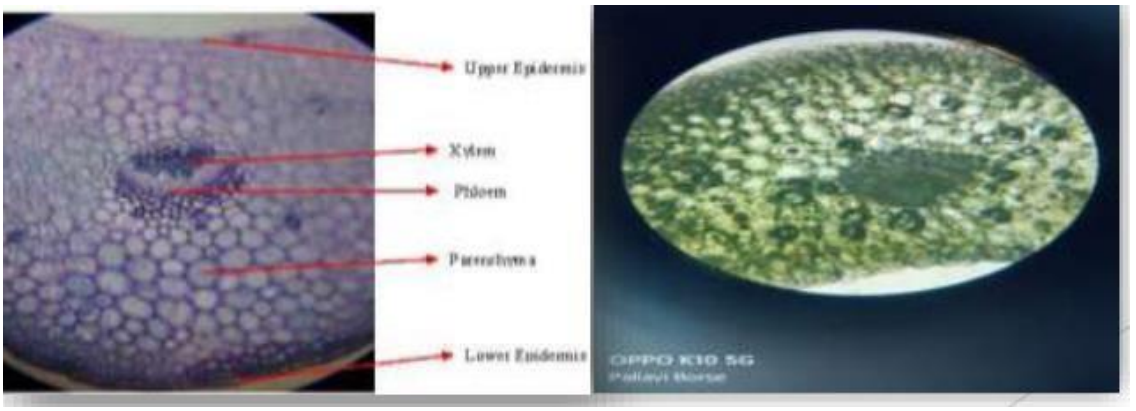

Table 3: Powder Characteristics of Bryophyllum pinnatum Dried Leaves

Sl. No.	Features	Observation
1.	Nature	Coarse to moderately fine powder
2.	Color	Green to yellowish-green powder
3.	Odour	Characteristic but faint
4.	Taste	Slightly sour and mucilaginous

Table 4: Microscopic Powder Characteristics of Bryophyllum pinnatum

Sl. No.	Features	Observations
1.	Xylem	Lignified xylem vessels and fibers are present
2.	Stomata	Paracytic stomata commonly observed on the lower epidermis
3.	Epidermal cells	Polygonal epidermal cells with straight to slightly curved anticlinal walls
4.	Trichomes	Unicellular and multicellular glandular trichomes present
5.	Starch granules	Abundant simple and compound starch grains observed
6.	Palisade	Fragments of palisade parenchyma visible
7.	Calcium oxalate crystals	Large rosette and cluster crystals of calcium oxalate present in ground tissue

Diagonastic Charachters of Powdered Leaves of Bryophyllum pinnatum

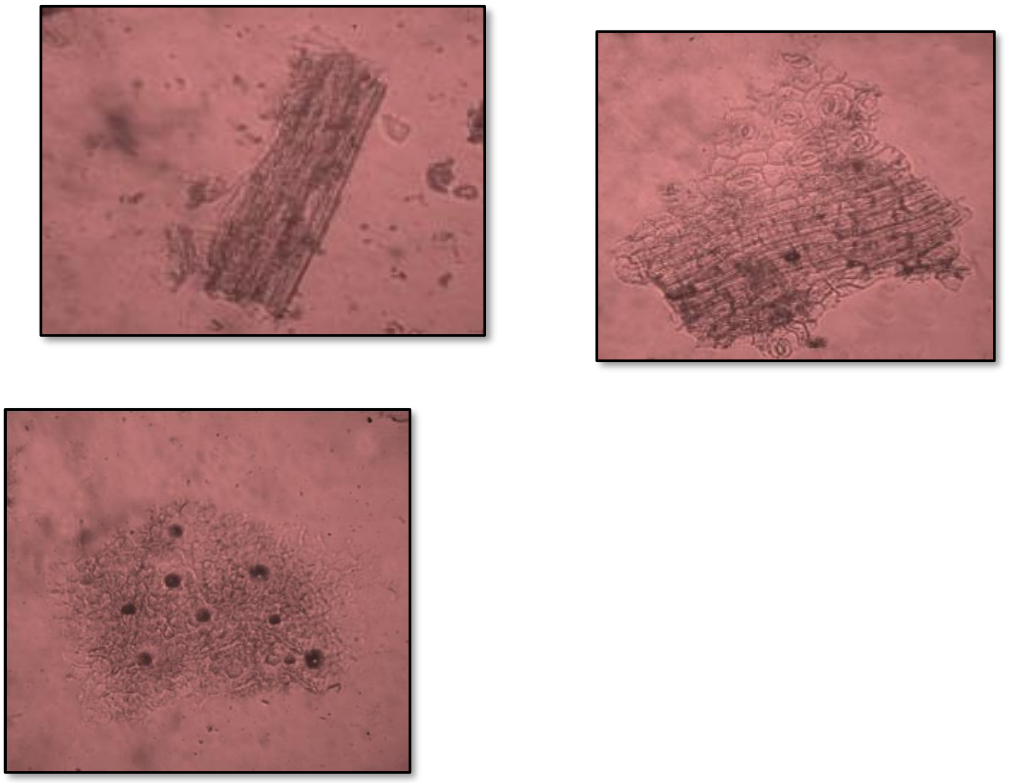

Figure 3: Powdered Characteristics of Leaves of Bryophyllumpinnatum

Table 5: Proximate Values of Bryophyllum pinnatum Dried Leaves

Sl. No.	Parameter	Determined Value (% w/w)
(A) EXTRACTIVE VALUES		
1	Alcohol soluble extractive value	11.2 %
2	Water soluble extractive value	13.7 %
3	Chloroform soluble extractive value	3.6 %
4	Petroleum ether soluble extractive value	1.5 %
(B) MOISTURE CONTENT		
1	Total moisture content	7.45 %
(C) ASH VALUES		
1	Total ash	6.89 %
2	Acid insoluble ash	1.32 %
3	Water soluble ash	0.88 %
4	Sulphated ash	9.46 %

Table 6: Physical Characteristics and Phytochemical Screening of Ethanolic Extract of Bryophyllum pinnatum

Sl. No.	Parameter	Observation / Result
A. Physical Characteristics		
1	Extract Type	Ethanolic Extract
2	% Dry Weight (g)	16.79
3	Colour	Dark Brown

4	Odour	Characteristic
5	Consistency	Non-sticky
B. Phytochemical Tests		
6	**Carbohydrates**	Present (Molisch's, Fehling's, Benedict's positive)
7	**Proteins and Amino Acids**	Present (Ninhydrin and Millon's tests positive)
8	**Sterols**	Present (Liebermann-Burchard and Salkowski tests positive)
9	**Glycosides**	Present (General test for glycosides positive)
10	**Cardiac Glycosides**	Present (Keller-Killani and Legal's tests positive)
11	**Anthraquinone Glycosides**	Absent (Borntrager's test negative)
12	**Saponins**	Present (Foam and Haemolysis tests positive)
13	**Alkaloids**	Present (Mayer's, Dragendorff's, Wagner's, and Hager's tests positive)
14	**Phenolic Compounds**	Present (Ferric chloride, Shinoda, and Zinc-HCl tests positive)
15	**Flavonoids**	Present (Shinoda, Zinc-HCl, and Alkaline reagent tests positive)

16	**Tannins**	Present (Gelatin, Ferric chloride, Vanillin-HCl, and Alkaline reagent tests positive)

Table 7: HPLC Standardization Results of Bufadienolides from Bryophyllum pinnatum

Sl. No.	**Parameter**	**Result**
1	Extract used	Ethanolic extract
2	HPLC column	C18 (250 × 4.6 mm, 5 µm)
3	Mobile phase	Acetonitrile : Water (with 0.1% formic acid) – Gradient
4	Flow rate	1.0 mL/min
5	Detection wavelength	220 nm
6	Column temperature	30°C
7	Injection volume	20 µL
8	Retention time of standard bufadienolide	7.92 minutes
9	Retention time of sample	7.91 minutes
10	Calibration curve (R^2 value)	0.9985
11	Concentration of bufadienolide in extract	0.84% w/w (of dry extract)
12	Peak purity confirmation	No interfering peaks; UV overlay matched

DISCUSSION

The current pharmacognostic and phytochemical evaluation of Bryophyllum pinnatum (Lam.) Pers. It highlights important diagnostic and treatment features. This supports its role in herbal medicine and drug development. The leaves of B. pinnatum are bright green and smooth. They have a fleshy texture and an ovate to oblong shape. The margins are notched, which helps them grow plantlets. These visual traits, a slightly sour taste, and no smell match what traditional medicine describes. They confirm the identity of the plant material used in this study.

The microscope showed clear anatomical features in the cross-section. It included lignified xylem vessels and polygonal epidermal cells. Powder analysis helped confirm the plant's identity. It had key features like paracytic stomata, unicellular and multicellular glandular trichomes, and many starch granules. It also showed bits of palisade parenchyma, along with big rosette and cluster crystals of calcium oxalate. These features give a clear microscopic fingerprint of the species. This fingerprint is vital for quality control in crude drug standardization.

A close look at the dried leaves showed a good extractive profile. Water had the highest yield at 13.7%, while ethanol gave 11.2%. This indicates strong hydrophilic and moderately polar compounds. Chloroform and petroleum ether extracts were lower, at 3.6% and 1.5%. This shows there are few non-polar components. The moisture content was 7.45%. This is within the acceptable limits. It helps keep the drug stable during storage. Ash value analysis showed total ash content of 6.89%. Acid-insoluble ash was 1.32%. This indicates low contamination with siliceous materials. Sulphated ash was notably high at 9.46%, which may reflect the presence of thermally stable inorganic constituents.

Phytochemical screening of the ethanolic extract revealed a rich spectrum of bioactive constituents. Tests found carbohydrates, proteins, sterols, glycosides, and cardiac glycosides. Saponins, alkaloids, phenolic compounds, flavonoids, and tannins were also present. However, anthraquinone glycosides were not found. These findings match what we know about Bryophyllum pinnatum. It's traditionally used for wound healing and blood clotting. This is mainly due to saponins, flavonoids, and glycosides. These compounds have anti-inflammatory, haemostatic, and healing properties.

HPLC standardization confirmed that bufadienolides are the main bioactive marker. These compounds are a type of cardiac-active glycoside. We analyzed the ethanolic extract using a C18 column. The mobile phase had a gradient of acetonitrile and water, with 0.1% formic acid. We detected the extract at 220 nm. The standard bufadienolide had a retention time of 7.92 minutes. This closely matched the sample's peak at 7.91 minutes. This shows great compound identity and purity. The calibration curve exhibited high linearity ($R^2 = 0.9985$), and the bufadienolide content was quantified at 0.84% w/w of the dry extract. The peak purity analysis showed the marker's specificity. No interfering peaks appeared in the chromatogram.

CONCLUSION

The present investigation into Bryophyllum pinnatum (Lam.) Pers. provides a complete profile of the plant’s pharmacognostic, phytochemical, and analytical properties. This shows its promise as a valuable herbal resource. The detailed analysis showed clear markers. These markers are key for standardizing and identifying the plant material in both crude and processed forms. The analysis showed that the extractive values, moisture content, and ash levels met the pharmacopoeia's acceptable limits. This confirms the purity and quality of the dried leaves.

Phytochemical screening showed important compounds in the plant. These include flavonoids, saponins, glycosides, phenolics, and tannins. They play a key role in the plant's traditional use for treating wounds, stopping bleeding, and helping tissue repair. The HPLC standardization of the ethanolic extract showed bufadienolides at 0.84% w/w. It had high accuracy and peak purity. This supports the pharmacological claims for this species.

The findings back the traditional claims about Bryophyllum pinnatum. They also highlight the need for systematic standardization in herbal medicine. This study shows the plant's healing power and supports its use in making standardized herbal products. This is especially true for wound healing and blood clotting treatments.

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