The pharmacognostical and phytochemical analysis of *Adiantum lunulatum* Burm

Dr. Preethy Bhasimon, Dr. Priyalatha B and Dr. Vimala KS

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Abstract

**Objective:** The aim in the current research is the evaluation of the pharmacognostic properties, like the macroscopic, microscopic, physicochemical properties, phytochemical screening and HPTLC of whole plant of *Adiantum lunulatum*

**Methods:** The microscopic and macroscopic characteristics of dried samples were analyzed by standard methods, the organoleptic evaluations and physicochemical studies performed using WHO-recommended parameters. The extraction from the crude drug powder was done with various solvents like Aqueous, Chloroforms, Ethanol, Methanol, Petroleum ether with increasing polarity in a Soxhlet apparatus. The Physicochemical analysis, phytochemical analysis and HPTLC were done with methanolic extract. The phytochemical analysis was also done.

**Results:** The microscopic features of the rachis and fronds were taken. In microscopical features of fronds pinnule showed epidermal cells elongation with wavy margins. Anomocytic stomata were present on the lower surface only. The rachis microscopy showed an adaxial groove and abaxial convex outline. Hypoderms consist of 2-3 layer of sclerenchyma followed by parenchymatous ground tissue. Mono stele located at the notch proto stele was occupied by xylem and proto xylem metaxylem occupying center showing exarch condition. In physicochemical analysis foreign matter, moisture content, extractive values, ash content and pH were also determined. The phytochemical analysis revealed that the drug contained alkaloids, glycosides, phenols, steroids, triterpenoids and sugar.

**Conclusions:** The study will be helpful in the characterization of the crude drug. Further phytochemical research are needed to identity the active phytoconstituents of *Adiantum lunulatum*, this may serve as leads in the development of new pharmaceuticals.

**Keywords:** *Adiantum lunulatum*, alkaloids, glycosides, phenols, steroids, HPTLC, WHO

Introduction

The rich heritage of traditional medicine is found in India, constituting different components such as Ayurveda, Siddha, and Unani. The development of these traditional systems of medicines with the perspectives of safety, efficacy, and quality will help in the preservation of tradition in healthcare [1]. The Pharmacognostic study is a preliminary step in the standardization of a crude drug. An in-depth Pharmacognostic evaluation provides valuable information regarding the morphology, microscopic and physical characteristics of crude drugs. Plants have formed as one of the sophisticated traditional medicine systems and have been in existence for thousands of years [2-4], dating back to early humans [5]. They constitute an effective source of traditional and modern medicines and play an important role in health care system.

The medicinally important compounds which the pharmaceutical industry required are derived from the extractions of wild population raw materials. Due to ruinous harvesting practice and over-harvesting for production of medicines, with little or no regard to the future the genetic diversity of medicinal plants in the world are getting endangered at an alarming rate. The other causes for extensive destruction of the plant-rich habitat are forest degradation, agriculture encroachments, urbanization etc. In modern medicine, plants are used as sources of direct therapeutic agents, as model for new synthetic compounds and as a taxonomic marker for the elaboration of more complex semi synthetic chemical compounds [6]. Pharmacognosy is a simple and reliable tool by which complete information of the crude drug be obtained [7-10].

Today, with the current surge of interest in phytotherapeutics, the availability of genuine plant material is becoming scarce. Since crude plant drugs form the basis for the manufacture of numerous medicinal preparations, accurate determination of drug identity becomes an essential part of its study. It is extremely important to make an effort toward standardization of plant material as medicine [11]. The identification and authentication of the plant material through The Pharmacognostic studies help in the standardisation of single herbs.
One of the oldest and primitive vascular plant groups on earth are the Pteridophytes. They are represented by over 1200 taxa, belonging to 204 genera in the world. They make an important contribution to earth’s plant diversity and form a significant dominant component of many plant communities especially in the tropical and temperate regions. Pteridophytes have been poorly studied and considered economically less important group of plants in the plant kingdom. *Adiantum lunulatum* Burm. is a cosmopolitan fern belonging to the family Adiantaceae, and genus Adiantum. Authentication and standardization are prerequisite steps, especially for herbal drugs and their formulations in traditional systems of medicine \[^{[13]}\]. The present study is focused on the pharmacognostic standardization parameters such as organoleptic, microscopic, and macroscopic analysis, along with the determination of ash and moisture content, extractive values, foreign matter, and HPTLC characteristics of the Whole plant of *Adiantum lunulatum* as described in the World Health Organization guidelines.

**Material and Methods**

**Identification & collection of study drug**

The study drug *Adiantum lunulatum* Burm. was identified at its natural habitat at Kottayam with the help of various vegetative parts explained in different flora available in the month of August 2019. The identity was confirmed by a taxonomist before collection. A voucher specimen was deposited at herbarium of Dravyaguna Department of Amrita School of Ayurveda on 28th August 2019. After the confirmation of identity, fresh specimens of whole plant of *Adiantum lunulatum* Burm were collected in the month of September from Kottayam district, Kerala India.

**Preparation of study drug (Adiantum lunulatum Burm)**

The collected samples of study drug from Kottayam district of Kerala from its natural habitat were cleaned off mud and washed thoroughly under tap water. The whole plant was allowed to dry under shade. After attaining proper dryness, required amount of the samples were made into coarse powder and kept in a clean transparent air tight containers and labeled properly for Pharmacognostical and Physico-chemical analysis.

**Organoleptic evaluation**

Organoleptic evaluation were performed according to the color, size, odor, and taste parameters

**Pharmacognostical and physico chemical analysis**

**Pharmacognostical analysis of Adiantum lunulatum Burm**

Pharmacognosy is one of the main tools to assess the quality of the drug through morphological and histological peculiarities of the plant and embrace a vital role in standardization procedures of the drug. Keeping the above views in mind the pharmacognostical study of *Adiantum lunulatum* BURM, was carried out in Quality control lab and Department of Dravyaguna, Amrita School of Ayurveda.

**Macroscopic evaluation**

**Materials**

Magnifying lens and dissecting microscope were used for this study.

**Methodology of Macroscopic Evaluation**

The collected sample of *Adiantum lunulatum* Burm were subjected to identification with naked eyes and by tactile and other sensory inspections. The macroscopic features of the collected sample drug were then compared with that of the description of *Adiantum lunulatum* Burm available in Ayurveda Pharmacopoeia of India and Quality standards of medicinal plants.

**Microscopic evaluation**

**Materials required**

- Razor or safety razor blade, dissecting needles, watch glasses, microscope glass slides, cover slips, camel hair brush (Medium size), dropper, safranine stain, glycerin, compound microscope, digital camera.

**Methodology of Microscopic evaluation**

After macroscopic evaluation, microscopy evaluation of the fine hand transverse section of pinnule and stipe of *Adiantum lunulatum* Burm were made separately with the help of razor blade in the Department of Dravyaguna Vijnana. The cut sections were then suspended in water in a watch glass. After that a few drops of Safranine stain was added to the water containing thin section. When the section was sufficiently stained, it was transferred on a clean glass slide with the help of a hair brush. The stained section was mounted at the center of the slide and a drop of glycerin was added on the section. Then it was covered with a cover slip without getting air bubble between the slide and cover. The prepared stained slide was placed on the stage of the compound microscope and fixed it with the clips and proper illumination was given. After this the lens was adjusted, to a magnification of 10X for visualizing. Then the power was adjusted to 40X magnification. Photographs of the sections were taken using a digital camera at 10X and 40X powers. The histological features of the collected sample were then compared with that of the histological description of *Adiantum lunulatum* Burm available in Ayurveda Pharmacopoeia of India and other authentic text books.

**Powder microscopy**

The powder of whole plant of *Adiantum lunulatum* Burm had been prepared in CARRe KERALAM, Koratty.

**Materials required**

- Whole plant of *Adiantum lunulatum* Burm, Pestle & mortar, Sieve of mesh size 80.

**Procedure**

The collected whole plant which was cleaned, dried and preserved was taken. Then it was powdered with the help of Pestle and Mortar and then it was sieved with a sieve of mesh size 80. A pinch of powder from this sample was taken on slide. This was spread well on the slide and the microscopic characters were observed after mounting in glycerin.

**Physico chemical analysis of Adiantum lunulatum burm**

The physico chemical analysis of whole plant of *Adiantum lunulatum* Burm had been done in Quality Control Lab, Amrita School of Ayurveda as per the WHO guideline of herbal drug standardization. It includes Foreign matter, Loss on drying, pH value, Ash value, Acid insoluble ash, Water soluble extractive value and Alcohol soluble extractive value

**Phyto-chemical study:** Phyto-chemical analysis was done at in CARRe KERALAM, Koratty, extract of whole plant of *Adiantum lunulatum* is used.
Detection of terpenoids and steroids

**Liebmann Burchard test:** 2 ml of the test extract was mixed with 1 ml CHCl₃ and 1 ml acetic anhydride and one drop of concentrated sulphuric acid was added. Presence of blue green to red-orange colour indicated the presence of terpenoids and steroids.

**Alkaloids**

**Dragendorff’s test:** 2 ml of reagent is mixed with 2 ml filtrate of plant drug extract. Absence of colour change indicated alkaloid is absent in the methanolic extract.

**Preparation of Dragendorff’s reagent**

**Preparation of solution A:** 0.21g Bismuth nitrate is taken in a beaker, add 2.5 ml Glacial acetic acid And 10 ml distill water. Mixed properly.

**Preparation of solution B:** 2.6 g potassium iodide is dissolved in 10 ml distill water. Properly mix the solution A & B together in a beaker.

**Flavonoids:** *(Shinoda test)*: 2 ml of sample solution was taken and added with magnesium powder and a few drops of concentrated Sulphuric acid or concentrated Hydrochloric acid. No change indicated the extract does not have flavonoids.

**Saponins**

**Foam test:** Aqueous solution of the sample was taken in a test tube and shaken well for one minute, appearance of foam which is stable for more than 15 seconds shows presence of saponin. Absence of any foam indicated the absence of saponins.

**Carbohydrates**

**Benedict’s test:** Mixed 2 ml of Benedict’s reagent with 2 ml test solution. Boiled in a water bath. Formation of red, yellow or green colour or precipitate depending on the sugar concentration indicated the presence of Carbohydrate.

**Tannins**

**Ferric chloride (5%):** Mixed 2 ml of test solution with ferric chloride solution. Presence of Blue, blue black or blue green colour change indicated the presence of tannin. As there was no colour change in the given test solution tannins was absent. Preparation of Ferric chloride solution Taken 0.5 g of Ferric chloride anhydrous and added with 10 ml alcohol, then mixed well.

**Phenol**

**Folinis ciocault reagent test:** 2 ml sample was taken in a test tube, added sodium carbonate solution in to it, and then added few drops of Phenol reagent. Dark green colour is observed, indicated phenolic content in the sample

**Preparation of sodium carbonate solution**

Sodium carbonate anhydrous was dissolved in distill water.

**Glycosides:** 2 ml sample was taken in a test tube, added 2 ml of picric acid in to it. Presence of yellowish orange colour indicated glycosides presence.

**High power thin layer chromatography (HPTLC)**

HPTLC was done at in CARe KERALAM, Koratty. Spotting of HPTLC plate: The prepared methanolic extract of the sample drug was applied in a dose of 10.0µl at a position of 12.5mm, 25.0mm and 37.5mm in the HPTLC silica gel plate by Hamilton syringe at a speed of 150nl/s in CAMAG Linomat 5.

**Development glass chamber:** The chamber type used is twin trough chamber of 20*10 cm. Mobile phase: Toluene: Chloroform: methanol (8:3:1)

**Solvent front:** 80.0 mm

**Volume:** 10 ml

Drying device: oven (105 °C)

Post chromatographic derivatization: The post chromatographic derivatization was done in chromatographic sprayer. The derivatization agent used was Anisaldehyde sulphuric acid. The volume applied is 100 ml. The plate was oven dried. CAMAG TLC scanner was used for the visualization of derivatized plate.

The plates were visualized under visible light, UV short (254nm) and UV long (366nm) and post derivatization. Rf value calculation.

The Rf values were calculated from the obtained Densitogram and Densitograph.

**Chromatogram evaluation**
The wave length distribution of all spots of 4 samples obtained was monitored using software win CATS planar chromatography.

**Results**

**Organoleptic characteristics**
The dried powder of the herb *Adiantum lunulatum* is dark greenish brown with a characteristic bitter odour and taste. The organoleptic characteristics of the plant are summarized in Table 1.

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Organoleptic characteristics</th>
<th>Nature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Colour</td>
<td>Pale green</td>
</tr>
<tr>
<td>2.</td>
<td>Sand &amp; Silica</td>
<td>Absent</td>
</tr>
<tr>
<td>3.</td>
<td>Odour</td>
<td>Pungent</td>
</tr>
<tr>
<td>4.</td>
<td>Taste</td>
<td>Bitter</td>
</tr>
<tr>
<td>5.</td>
<td>Insect infestation</td>
<td>Absent</td>
</tr>
<tr>
<td>6.</td>
<td>Rodent contamination</td>
<td>Absent</td>
</tr>
</tbody>
</table>

**Macroscopic and Microscopic analysis**
The macroscopic evaluation showed the Fronds is 10-28 cm long, stipes erect, long glabrous, lamina broad, rachis zigzag, pinnules fan shaped, petiole short, sori elliptic in roundish sinus of the crenation, sporangia globose. The pictures are shown in 1 and 2

Microscopical study was done for the fronds and rachis of *Adiantum lunulatum* Burm.

In microscopical features of fronds pinnule showed epidermal cells elongated with wavy margins. Stomata were present on the lower surface only, anomocytic stomata were seen. The result is depicted in PIC NO: 3

The rachis microscopy showed an adaxial groove and abaxial convex outline. Hypodermis consist of 2-3 layer of sclerenchyma followed by parenchymatous ground tissue. Mono stele located at the notch proto stele was occupied by xylem and proto xylem metaxylem occupying center showing exarch condition. The result shown in pic No: 4
Observation in powder microscopy of whole plant of *Adiantum lunulatum*

Powder showed the presence of tracheids, epidermal cells of leaves, leaf fragrant, fibers, spores, fragments of mesophyll and annulus cells. The results are depicted in PIC NO: 5, 6, 7, 8, 9, 10

Result of physio-chemical analysis

The table number: 2 shows the result of parameters like foreign matter, loss on drying (LOD), pH, total ash, acid insoluble ash, water insoluble ash, water soluble ash, water extractives and alcohol extractive

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Parameters</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Foreign matter</td>
<td>1.12%</td>
</tr>
<tr>
<td>2</td>
<td>LOD(at 110⁰C)</td>
<td>12.1%</td>
</tr>
<tr>
<td>3</td>
<td>pH</td>
<td>6.0</td>
</tr>
<tr>
<td>4</td>
<td>Total ash</td>
<td>15%</td>
</tr>
<tr>
<td>5</td>
<td>Acid Insoluble ash</td>
<td>6.8%</td>
</tr>
<tr>
<td>6</td>
<td>Water insoluble ash</td>
<td>11.7%</td>
</tr>
<tr>
<td>7</td>
<td>Water soluble ash</td>
<td>2.9%</td>
</tr>
<tr>
<td>8</td>
<td>Water extractives</td>
<td>8.4%</td>
</tr>
<tr>
<td>9</td>
<td>Alcohol extractives</td>
<td>4.8%</td>
</tr>
</tbody>
</table>

Result of phyto-chemical analysis

The phytochemical results of alkaloids, flavonoids, tannins, phenols, glycoside, carbohydrate, steroid, triterpenoids, saponins and carbohydrates are shown in Table No: 3

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameters</th>
<th>Test methods</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Dragendorff’s reagent test</td>
<td>Present</td>
</tr>
<tr>
<td>2</td>
<td>Tannins</td>
<td>Ferric chloride test</td>
<td>Absent</td>
</tr>
<tr>
<td>3</td>
<td>Flavonoids</td>
<td>Shinoda’s test</td>
<td>Absent</td>
</tr>
<tr>
<td>4</td>
<td>Phenols</td>
<td>Folin ciocalteu reagent test</td>
<td>Present</td>
</tr>
<tr>
<td>5</td>
<td>Triterpenoids</td>
<td>Liebermann- burchard’s reagent test</td>
<td>Present</td>
</tr>
<tr>
<td>6</td>
<td>Saponins</td>
<td>Foam test</td>
<td>Absent</td>
</tr>
<tr>
<td>7</td>
<td>Sugars</td>
<td>Picric acid test</td>
<td>Present</td>
</tr>
<tr>
<td>8</td>
<td>Carbohydrates</td>
<td>Benedict’s reagent test</td>
<td>Present</td>
</tr>
<tr>
<td>9</td>
<td>Steroids</td>
<td>Salkowski test</td>
<td>Present</td>
</tr>
</tbody>
</table>

High performance thin layer chromatography

Stationary phase

The Material used is HPTLC PLATE 60 F 254.the size of the plate is 5cmx10cm. The Sample application is done with CAMAG Linomat 5. The Spray gas used was Inert gas. The Sample solvent type was Methanol, Dosage speed: 150 nL/s, Predosage volume: 0.2 ul

Syringe size: 100 μl, Application position Y: 10.0 mm, Band length: 8.0 mm, Development Chamber type - Glass tank Twin Trough Chamber 20x10cm, Mobile phase Toluene: Chloroform: Methanol(8:3:1), Solvent front position 80.0 mm, Volume 10.0 ml, Drying device Oven, Temperature 105 °C, Time 5 Minutes

Post-Chromatographic Derivatization

Instrument Chromatographic sprayer, Solution Anisaldehyde sulphuric acid, Volume 100.0 mL, drying device Oven, Temperature 105 °C, Time 20 Minutes

The plate was developed in Toluene: Chloroform: Methanol (8:3:1). The developed plates were visualized under UV 254nm and 366 nm and scanned under UV 254nm and 366 nm The Rp values and densitographs and Densitogram were recorded. The picture no: 24-31 shows the densitographs and Densitogram. The Rp values are tabulated in following Tables (4-5).

Discussion

Discussion about pharmacognostical analysis

To establish the quality and reliability of the drug, various Pharmacognostical screening measures were adopted. The macroscopic and microscopic diagnostic features were established, which will allow to detect its quality, safety, and efficacy and to avoid adulteration, misuse in the process of medicines preparations on its basis. The macroscopic and microscopic features of the collected samples were compared with that of the description of Hamsapadi (*Adiantum lunulatum*. Burm.) available in Ayurvedic Pharmacopoeia of India (API) and Quality standards of medicinal plants for its authentication. While considering the macroscopical features of the drug, it was found that the stipes erect, long glabrous, lamina broad, rachis zigzag, pinnules fan shaped, petiole short, sori elliptic in roundish sinus of the crenation, sporangia globose. In microscopical features of fronds pinnule showed epidermal cells elongated with wavy margins. Stomata were present on the lower surface only, anomocytic stomata were seen. The rachis microscopy showed an adaxial groove and abaxial convex outline. Hypodermis consist of 2-3 layer of sclerenchyma followed by parenchymatous ground tissue. Mono stele located at the notch proto stele was occupied by xylem and proto xylem metaxylem occupying center showing exarch condition. These results obtained from pharmacognostical analysis of *Adiantum lunulatum*. Burm assure the genuinity of the drug.

Discussion on physicochemical analysis

To establish the quality and purity of the drug, physico chemical parameters of *Adiantum lunulatum* were analysed and compared with standards mentioned in Ayurvedic Pharmacopoeia of India. All the physico chemical parameters like foreign matter, total ash, acid insoluble ash, water extractives and alcohol extractives comply with API standards. In addition to this, the pH and loss on drying were also analysed. The pH value of this drug was 6 which indicates that the drug can be u. The LOD value determines the amount of volatile matter of any kind including water. The foreign matter of the drug can include contamination by stone, sands, excreta etc. from the value of foreign matter it indicates the the foreign material value was in accordance with the API standards. The ash value is the residue remaining after incineration of the drug. It represents the presence of inorganic salts of carbonates, phosphates, silicates of sodium, potassium, calcium and magnesium. The total ash value of this drug was in tally with the ash value of this drug given in API. The acid insoluble ash indicates the presences of metals and minerals.

The extractive values are useful for the evaluation of a drug especially when the constituents of the drugs cannot be readily estimated by other methods. It also indicates the nature of chemical constituents’ present. Also help in the identification of adulteration. The water-soluble extractive values indicate the presence of water-soluble constituents like tannins, sugars, plant acids and mucilage. The water- soluble extractive value proved to be higher than alcohol soluble extractive. This indicates that the constituents of the drug are more extracted and soluble in water rather than in acid. The alcohol soluble extractive value is applied for the drugs which contain alcohol soluble constituent like phenol, glycoside,
alkaloids and steroids. The extractive values of the drug was in compliance with that of the standard mentioned in API.

**Discussion on phytochemical analysis**

The phytochemical analysis revealed the presence of alkaloids, phenols, triterpenoids, glycosides, steroids and carbohydrate in the sample of *Adiantum lunulatum* Burm. The alkaloids present in this drug has antimicrobial activity. The phenolic compounds, the triterpenoids, the glycosides and the steroids present have been proved to have antibacterial effect.

**Discussion on high performance thin layer chromatography**

The various colours obtained in HPTLC plates indicated the presence of various active phytoconstituents in the drug. The various bands obtained in HPTLC were red, violet, faint fluorescent blue, orange zone brownish grey and reddish orange zones which indicated the presence of steroids, chlorophyll derivatives, triterpenoids, glycoside, phenolic compound, alkaloids respectively.
Table 4: Table showing Rf value at 254 nm

![Table showing Rf value at 254 nm]

Pic 5: Densitogram of *Adiantum lunulatum* under UV long 366nm

![Pic 5: Densitogram of *Adiantum lunulatum* under UV long 366nm]

Pic 6: Densitograph of sample one of *Adiantum lunulatum* under 366nm

Table 5: Table showing the Rf value of sample 1 of *Adiantum lunulatum* under 366nm

![Table 5: Table showing the Rf value of sample 1 of *Adiantum lunulatum* under 366nm]
Pic 7: Microscopy of TS of frond of *Adiantum lunulatum* Burm

Pic 8: Microscopy of TS of Rachis of *Adiantum lunulatum* Burm

Pic 9: Tracheids

Pic 10: Epidermal cells of leaves

Pic 11: Spore

Pic 12: Fragments of mesophyll

Pic 13: Annulus cell

Pic 14: Spore
Conclusion
Microscopical study of Adiantum lunulatum revealed that the sample taken for the study was genuine. Preliminary phytochemical study of Adiantum lunulatum with various chemical tests (qualitative and quantitative) revealed the presence of various chemical constituents in methanolic extract such as alkaloids, phenol, carbohydrate, glycosides which helps to ensure the quality of trial drug. In conclusion, screening of phytochemicals, physiochemical analysis and HPTLC in A. lunulatum clearly reveals that the maximum classes of phytoconstituents are present in it. Hence, the above plant extracts could be explored for its highest therapeutic efficacy by pharmaceutical companies in order to develop safe drugs for various ailments. The quantitative analyses of these phytocompounds will be an interesting area for further study. Efforts should be geared up to exploit the biomedical applications of these screened plant due to the presence of certain class of phytocompounds for their full utilization.

Conflict of interest statement
We declare that we have no conflict of interest.

Acknowledgement
The present work had been carried out in the Department of Dravyaguna, Amrita school of Ayurveda, and phytochemistry Department of Amrita school of Biotechnology Amritapuri Kollam and Care Keralam Korraty. We thank the college authorities for providing the facilities.

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