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PHARMACOGNOSTIC EVALUATION OF INGREDIENTS OF DARBHAMOOLADI LEPA: A COMPREHENSIVE AUTHENTICATION STUDY

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ABSTRACT

Darbhamooladi Lepa is a classical Ayurvedic facial formulation described in the *Gandushadi Vidhi Adhyaya* of *Ashtanga Hridaya Sutrasthana*, recommended during *Vasanta Ritu* (spring season) for *Mukhaprasadana* (facial rejuvenation) by improving complexion and skin texture. Ensuring the authenticity of its herbal ingredients through Pharmacognostic evaluation is crucial for maintaining quality, safety, and therapeutic efficacy. The present study aimed to establish the Pharmacognostic profile of the six botanical ingredients of *Darbhamooladi Lepa* through macroscopic and microscopic evaluation for authentication and quality control. Six raw drugs—*Darbhamoola* [root of *Imperata cylindrica* (L.) Raeusch.], *Hima* [heartwood of *Santalum album* L.], *Ushira* [root of *Chrysopogon zizanioides* (L.) Roberty], *Sirisha* [bark of *Albizia lebbeck* (L.) Benth.], *Misi* [fruit of *Foeniculum vulgare* Mill.], and *Tandula* [fruit of *Oryza sativa* L.]—were analyzed at the Pharmacognosy Unit, Ayurveda Research Institute, Poojappura, Thiruvananthapuram. Microscopic examination of histological sections stained with safranin and observed under a digital microscope revealed distinctive diagnostic features, including an aerenchymatous cortex with silica deposits in *Imperata cylindrica*, uniseriate and biseriate medullary rays with golden-yellow contents in *Santalum album*, polyarch exarch xylem in *Chrysopogon zizanioides*, and abundant prismatic crystals and stone cells in *Albizia lebbeck*, while macroscopic evaluation demonstrated characteristic features in the fruits of *Foeniculum vulgare* and *Oryza sativa*. The study establishes Pharmacognostic reference standards for authentication and quality control of *Darbhamooladi Lepa* ingredients, supporting their use in pharmaceutical manufacturing and research.

KEYWORDS: *Darbhamooladi Lepa, Mukhalepa, Mukhaprasadana*, Pharmacognostic evaluation, Quality control.

1. INTRODUCTION

Pharmacognosy is the branch of pharmaceutical science that deals with the study of medicinal drugs derived from natural sources, including plants, animals, minerals, and microorganisms. According to the American Society of Pharmacognosy, pharmacognosy involves exploring the physical, chemical, biochemical, and biological characteristics of naturally derived drugs and drug substances, along with investigating natural sources for the discovery of new therapeutic agents.[1] Through macroscopic and microscopic evaluation, phytochemical screening, and quality control measures, pharmacognosy ensures the safe and effective use of natural products in healthcare.

The global resurgence of interest in traditional medicine systems has emphasized the critical need for standardization and quality assurance of herbal drugs. Pharmacognostic evaluation is a reliable method for identifying commercial varieties, detecting substitutes or adulterants, and ensuring the quality of crude drugs. It provides essential information about their physical and biochemical characteristics through techniques such as macroscopic and microscopic examination, histochemical tests, organoleptic assessment, fluorescence analysis, photomicrography, and measurements like pH, water solubility index, water absorption index, and acid value.[2] In Ayurvedic pharmaceutical practice, the authentication of raw

materials is paramount to ensure the safety, efficacy, and reproducibility of herbal formulations.

Darbhamooladi Lepa is a classical Ayurvedic topical formulation comprising six botanical ingredients, traditionally prescribed for *mukhaprasadana* (facial complexion enhancement) during *Vasanta ritu* (spring season). The formulation contains *Darbhamoola* (*Imperata cylindrica* root), *Hima* (*Santalum album* heartwood), *Ushira* (*Chrysopogon zizanioides* root), *Sirisha* (*Albizia lebbeck* bark), *Misi* (*Foeniculum vulgare* fruit), and *Tandula* (*Oryza sativa* fruit). Despite its therapeutic significance, comprehensive pharmacognostic documentation of all ingredients together has not been extensively reported.

The present study was undertaken to establish comprehensive pharmacognostic standards for all six ingredients of *Darbhamooladi Lepa* through systematic macroscopic and microscopic evaluation. These characteristics provide valuable diagnostic tools for authentication and quality control of herbal medicines. This research aims to provide reference standards that can facilitate accurate identification, prevent adulteration, and support quality assurance in the manufacture of this classical formulation.

2. MATERIALS AND METHODS

2.1 Study Setting

The pharmacognostic evaluation was conducted at the Pharmacognosy Unit, Ayurveda Research Institute, Poojappura, Thiruvananthapuram, Kerala, India.

2.2 Drugs to be Authenticated

Six botanical ingredients of *Darbhamooladi Lepa* were procured from authenticated sources and subjected to detailed evaluation (Table 1).

Table 1: Botanical Details of Darbhamooladi Lepa Ingredients.

Sanskrit Name	Botanical Name	Family	Part Used
Darbhamoola	*Imperata cylindrica* (L.) Raeusch.	Poaceae	Root
Hima	*Santalum album* L.	Santalaceae	Heartwood
Ushira	*Chrysopogon zizanioides* (L.) Roberty	Poaceae	Root
Sirisha	*Albizia lebbeck* (L.) Benth.	Leguminosae	Bark
Misi	*Foeniculum vulgare* Mill.	Umbelliferae	Fruit
Tandula	*Oryza sativa* L.	Poaceae	Fruit

2.3 Microscopic Evaluation

Histological examination was performed for four ingredients requiring microscopic authentication. *Imperata cylindrica* root, *Santalum album* heartwood, *Chrysopogon zizanioides* root, and *Albizia lebbeck* bark.

2.3.1 Materials Required

- Glass slides
- Watch glass
- Petri dishes
- cover slips
- Sharp-edged blades
- Fine brush
- Safranin stain
- Distilled water
- Digital microscope (Labomed Vision 2000 LED Binocular Microscope)

2.3.2 Procedure

Small pieces of plant material were soaked in water to facilitate sectioning. Thin transverse sections were prepared using sharp blades and collected in Petri dishes containing water. Selected sections were transferred to safranin stain using a fine brush and allowed to stain sufficiently. Excess stain was washed out in fresh water. Stained sections were mounted on clean glass slides with water and covered with cover slips. Microscopic examination was done at 4X, 10X, and 40X magnifications, and digital images were captured for documentation.

2.4 Macroscopic Evaluation

The two fruit samples subjected to macroscopic examination are *Foeniculum vulgare* and *Oryza sativa*. Macroscopy involves the systematic observation of the organoleptic features: color, odor, taste, size, shape, surface texture, and other distinguishing external features. Detailed documentation was done by taking digital photomicrographs using a digital microscope.

3. OBSERVATIONS

3.1 Microscopic Evaluation

3.1.1 *Imperata cylindrica* (L.) Raeusch.[3] - Root [*Darbhamoola*]

The transverse section of *Imperata cylindrica* root revealed characteristic features of monocotyledonous root anatomy (Figures 1-4). The outermost layer is a single-layered epidermis, which is composed of thin-walled, elongated cells. There was pronounced zonation in the cortical region. The outer cortex contained 3-4 layers of thick-walled Sclerenchymatous cells that provided mechanical support. This is followed by a well-pronounced Aerenchymatous zone made up of spherical longitudinal strips of tissues forming trabeculae separated by intercellular spaces. This zone is followed by a layer of compactly arranged endodermis, which is lined by an inner tangential wall that is thickened and black, likely due to silica deposition, which serves as a diagnostic feature for authentication. The vascular cylinder exhibited typical monocot root anatomy with radial arrangement of vascular elements. Xylem was polyarch (seven arms found) and exarch, with

protoxylem oriented toward the periphery. These features are in agreement with previous reported works of pharmacognostical importance and thus authenticate the identity of the sample.[4],[5]

Fig. 1

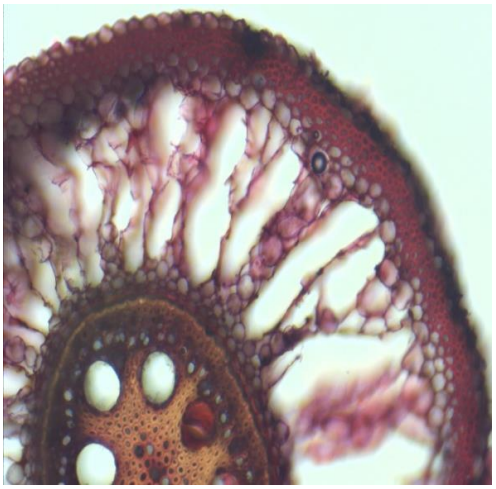

Fig. 2

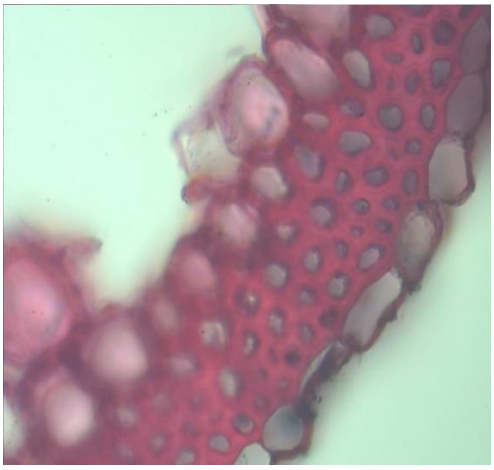

Fig. 3

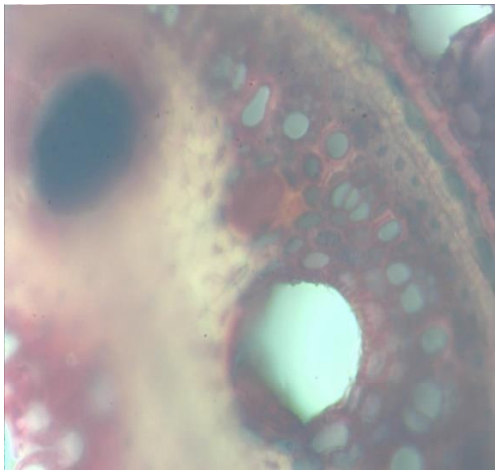

Fig. 4

- **Fig. 1 and Fig. 2** - T.S. of the root of *Imperata cylindrica* (L.) Raeusch.; magnification x 10.
- **Fig. 3 -** T.S. of the root of *Imperata cylindrica* (L.) Raeusch. showing the outer cortex of thick-walled Sclerenchymatous cells; magnification x 40.
- **Fig. 4 -** T.S. of root of *Imperata cylindrica* (L.) Raeusch. showing Endodermis, black inner tangential wall due to silica deposition; magnification x 40.

3.1.2 *Santalum album* L. - Heartwood [*Hima*][6],[7]

Microscopic examination showed *Santalum album* heartwood to possess characteristic anatomical features, important for authentication. The transverse section possesses numerous scattered vessels, which are mostly solitary, although sometimes in pairs. Vessels are round to oval in outline, consistent with diffuse-porous wood anatomy.

The wood structure consisted of tracheids, wood fibers, and xylem parenchyma, which were intersected by abundant medullary rays. Medullary rays occurred either as uniseriate or biseriate and were evident as elongated radial bands, set parallel to each other. A peculiar diagnostic feature was that medullary ray cells and xylem parenchyma contained golden-yellow to brownish contents due to essential oil deposits, responsible for the characteristic fragrance and therapeutic properties of sandalwood.

These microscopic features corroborate the standards set out in the Ayurvedic Pharmacopoeia of India, as well as with findings published in earlier studies.

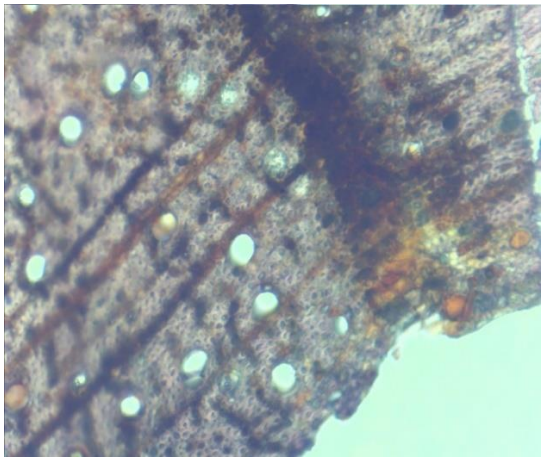

Fig. 5

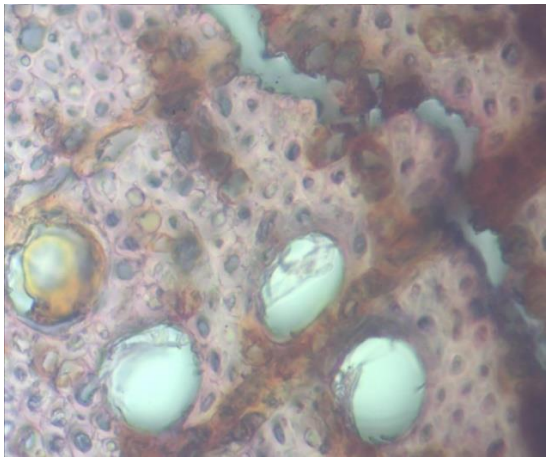

Fig. 6

- **Fig. 5** - T.S. of heartwood of *Santalum album* L. showing uniseriate and biseriate medullary rays; magnification x 10.
- **Fig. 6** - T.S. of heartwood of *Santalum album* L. shows golden yellow to brownish content.

3.1.3 *Chrysopogon zizanioides* (L.) Roberty - Root [*Ushira*][8]

The transverse section of *Chrysopogon zizanioides* root showed a circular to slightly wavy outline of typical of monocotyledonous roots. The epidermis was composed of a single layer of thin-walled cells. The cortex showed a distinct differentiation into an outer and inner cortex, with the outer cortex being made up of 3-5 layers of tightly compacted cells that provide structural support. This region is followed by a broader Aerenchymatous zone, which consists of longitudinal strips of parenchymatous tissue separated by wide intercellular spaces. The Aerenchymatous cortex was followed by a uniseriate endodermis consisting of barrel-shaped cells. The stelar region was delimited by 2-3 layers of thick-walled Sclerenchymatous pericycle. The vascular arrangement followed the usual monocot radial pattern with polyarch exarch xylem. Among the grasses, these diagnostic features distinguish *Chrysopogon zizanioides* root from other grasses and confirm its botanical identity.

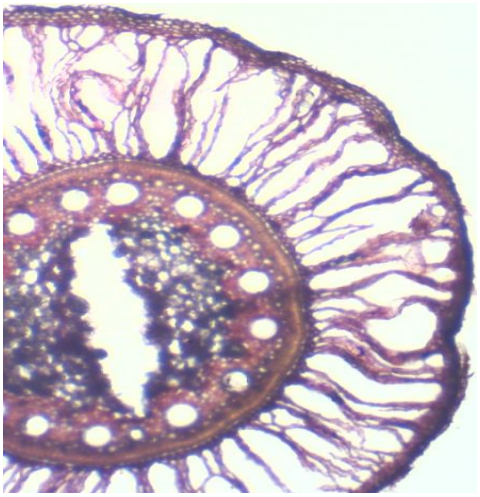

Fig. 7

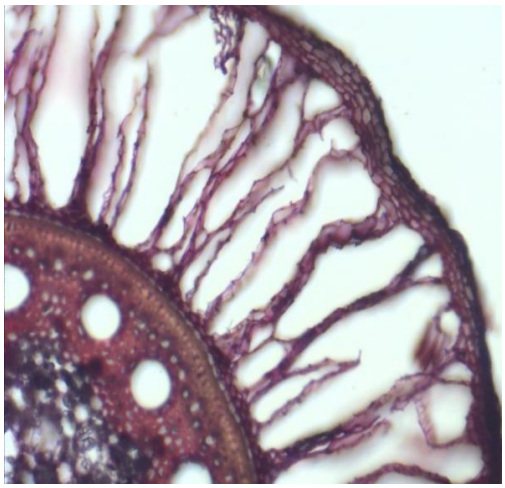

Fig. 8

- **Fig. 7** - T.S. of the root of *Chrysopogon zizanioides* (L.) Roberty; magnification x 4
- **Fig. 8** - T.S. of root of *Chrysopogon zizanioides* (L.) Roberty ; magnification x 10

3.1.4 *Albizia lebbeck* (L.) Benth. - Bark [*Sirisha*][9]

A microscopic study of *Albizia lebbeck* bark shows that there is an outermost layer of phellem or cork with several layers of thick-walled, tangentially elongated, and compactly arranged cells. These cork cells provide protection against desiccation and mechanical injury.

Below the phellem, a clear zone of thin-walled phellogen could be seen as cork cambium. The phelloderm was well-developed and extensive, with its cellular content featuring thick-walled cells that had conspicuous reddish-orange contents, possibly representing phenolic compounds or tannins. A number of stone cells (Sclerids) were scattered in the phelloderm, showing thick-walled, compactly placed, large cells with narrow lumen. A diagnostic feature was the abundance of prismatic calcium oxalate crystals distributed throughout cortical tissues. These crystals were rectangular to quadrangular

in outline. The medullary rays could be seen through the secondary phloem as radial bands. Mechanical strength was further imparted by patches of sclerenchymatous fibers. In a collective way, all these anatomical features may serve as reliable markers for authentication and differentiation of *Albizia lebbeck* bark from its potential adulterants.

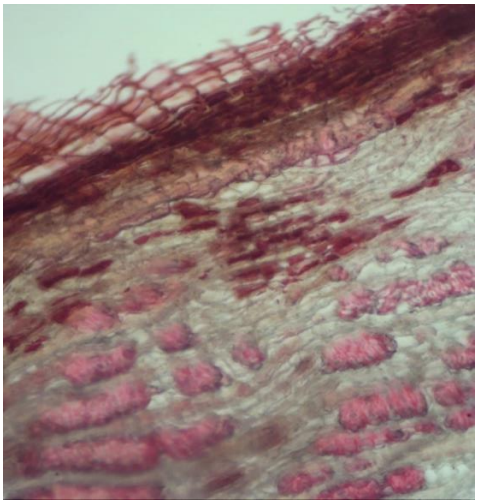

Fig. 9

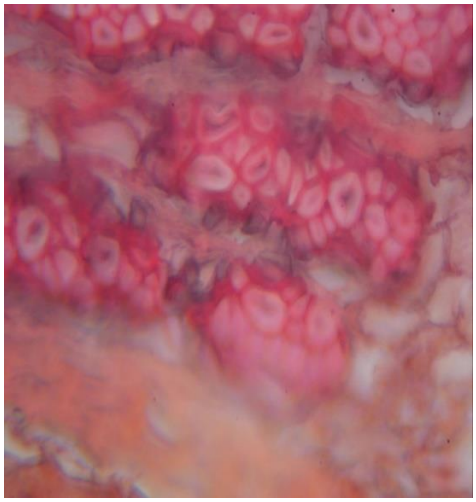

Fig. 10

- **Fig. 9** - T.S. of bark of *Albizzia lebbeck* (L.) Benth, shows phellem, phellogen and phelloderm, and cells with reddish orange contents; magnification x 10.
- **Fig. 10**- T.S of bark of *Albizzia lebbeck* (L.) Benth shows patches of Schlerenchymatous, prismatic crystals and medullary rays; magnification x 40.

3.2 Macroscopic Evaluation

3.2.1 *Foeniculum vulgare* Mill. - Fruit [*Misi*][10]

Foeniculum vulgare (Fennel) fruits had typical morphological characteristics for their identification. The color of the drug was greenish to yellowish-brown. The odor of the fruits was strong and aromatic, which is characteristic and due to the presence of volatile oil, mainly trans-anethole, which makes up about 80-90% of its essential oil content. The taste was distinctly sweet, and slightly pungent. The surface had a smooth, dry, and firm to touch texture. The fruits were typically entire, consisting of mericarps up to about 10 mm long and 4 mm wide. They were oblong to ovoid in shape, marked by five prominent, paler longitudinal primary ridges. Both base and apex showed slight tapering, with the apex topped by a small conical stylopod.

All these characteristic features distinguish the fruits of Foeniculum vulgare from other members of the Umbelliferae and confirm their authenticity for pharmaceutical use. These macroscopic parameters are in accordance with pharmacopeial standards and therefore also constitute a reliable diagnostic criterion.

Fig. 11.

- **Fig. 11** - Macroscopic photography of *Foeniculum vulgare* Mill.

3.2.2 *Oryza sativa* L. - Fruit [*Tandula*][11]

Fruits of *Oryza sativa* varieties with red-husked grains, showed typical macroscopic features. Usually, grains containing deep reddish-brown color due to anthocyanin pigments deposited in the bran layer are endowed with high antioxidant properties. Grains were hard and dense with a firm texture. On touch, the surface was rough. In the raw form, they emit a mild, earthy aroma. Individual grains were elongated, measuring approximately 5-6 mm in length and 2-3 mm in width. It had a cylindrical shape with rounded ends. The characteristic red pigmentation, unpolished surface, and size parameters are the key identifiers for this variant of *Oryza sativa*. These

macroscopic features allow for reliable markers to be established for authentication and quality assessment.

Fig. 12

- **Fig. 12** - Macroscopic photography of *Oryza sativa* L.

4. DISCUSSION

4.1 Significance of Pharmacognostic Evaluation

The pharmacognostic evaluation of *Darbhamooladi Lepa* ingredients gives reference standards for authentication and quality control of this classical formulation. Such standardization is crucial to ensure the identity, purity, and safety of herbal medicines in the wake of growing global demand and the risk of increased adulteration. Pharmacognostic studies are systematic investigations of the macroscopic, microscopic, phytochemical, and physicochemical nature of crude drugs, which help in detecting substitution or impurities, thereby assuring their therapeutic efficacy. A major focus of pharmacognostic research is distinguishing closely related or controversial plant species and confirming the identity of commonly used traditional medicinal plants through morphological, phytochemical, and physicochemical analyses.[12] Thus, these established standards have become reliable tools for pharmaceutical industries and research laboratories and help in the production of quality herbal formulations that have gained wider acceptance in integrating traditional medicinal systems into modern healthcare.

The microscopic examination of the four ingredients- *Darbhamoola* [root of *Imperata cylindrica* (L.) Raeusch.], *Hima* [heartwood of *Santalum album* L.], *Ushira* [root of *Chrysopogon zizanioides* (L.) Roberty], *Sirisha* [bark of *Albizia lebbeck* (L.) Benth.] - showed distinctive anatomical features, and the macroscopic examination of *Misi* [fruit of *Foeniculum vulgare* Mill.], and *Tandula* [fruit of *Oryza sativa* L.] presented characteristics that could be reliably employed for authentication.

4.2 Comparative Analysis with Pharmacopoeial Standards

The observed microscopic and macroscopic features align with the standards prescribed in the Ayurvedic Pharmacopoeia of India for individual drugs and on the previous pharmacognostic research on these medicinal plants, validating the authenticity of the samples used. Such comprehensive characterization is essential for maintaining pharmaceutical quality standards in Ayurvedic drug manufacturing.

4.3 Future Research Directions

Pharmacognostic evaluation covers various diagnostic features, thus supporting many objectives in quality control. Macroscopic evaluation allows for the rapid, preliminary authentication suitable for routine quality checks, while microscopic examination offers confirmatory identification down to the cellular level. This study lays down comprehensive macroscopic and microscopic standards, but future studies should be done with the aid of advanced techniques such as powder microscopy, HPTLC, photomicrography, and measurements of pH, water solubility index, water absorption index, and acid value. The inclusion of various analytical techniques provides a multi-tier authentication strategy that offers the highest form of quality assurance. The phytochemical characterization and anatomical features correlation with therapeutic bioactive compounds would further strengthen the pharmacognostic profile.[13]

5. CONCLUSION

This comprehensive pharmacognostic evaluation successfully established the detailed macroscopic and microscopic diagnostic parameters for all the six botanical ingredients of *Darbhamooladi Lepa.*

The study documented characteristic anatomical features such as an outer cortex with thick-walled Sclerenchymatous cells, an inner Aerenchymatous cortex, and a compactly arranged endodermis with silica deposits in *Imperata cylindrica* (L.) Raeusch.; uniseriate or biseriate medullary rays and golden-yellow to brownish contents in *Santalum album* L.; a broader Aerenchymatous zone consisting of longitudinal strips of tissue separated by wide intercellular spaces and typical monocot root anatomy in *Chrysopogon zizanioides* (L.) Roberty; an abundance of stone cells and prismatic crystals of calcium oxalate in *Albizia lebbeck* (L.) Benth.; and distinctive morphological features of *Foeniculum vulgare* Mill. and *Oryza sativa* L. fruits.

These findings scientifically validate the reference criteria for proper identification, ensuring quality and preventing adulteration in the manufacture of *Darbhamooladi Lepa*. The defined analytical parameters further improve regulatory compliance, ensure therapeutic efficacy, and merge the traditional knowledge of *Ayurveda* with modern pharmaceutical practices. Overall, this study plays an important role in standardizing classical *Ayurvedic* formulations and lays a solid foundation for future studies on this clinically useful topical preparation.

6. ACKNOWLEDGEMENT

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