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PHARMACOGNOSTICAL AND PHARMACEUTICAL ANALYSIS OF *KOLAKULATTHADI LEPA* -AN AYURVEDIC FORMULATION FOR *AMAVATA* (RHEUMATOID ARTHRITIS)

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ABSTRACT

Background: *Kolakulatthadi Lepa* is mentioned in *Charaka Samhita* (*Aragvadhiya Adhyay*). So, for the initiation of standardisation and assurance of the quality of herbal compounds, pharmacognostical and pharmaceutical analysis should be done. **Methods:** *Kolakulatthadi Lepa* were subjected to microscopic evaluation for Pharmacognostical and physicochemical analysis, such as loss on drying, total ash, water-soluble extract, alcohol soluble extract and pH value. **Results:** Pharmacognostical study showed the presence of certain identifying characters of all the ingredients of *Kolakulatthadi Lepa Churna*. As per the preliminary physiochemical analysis, the loss on drying percentage of the *Kolakulatthadi Lepa* was 97.08% w/w, the pH value was 5, the total ash value was 35.90 % w/w, Water soluble extractive was 7.78% w/w, and Methanol soluble extractive was 7.52%w/w. **Conclusions:** Pharmacognostical and physico-chemical observations revealed the specific characteristics of all active constituents of *Kolakulatthadi Lepa Churna* confirmed the purity and genuinity of the drug.

KEYWORDS: *Kolakulatthadi Lepa*, *Amavata*, Rheumatoid Arthritis, Pharmacognosy, Pharmaceutical analysis.

INTRODUCTION

Most of ingredients of the *Kolakulatthadi Lepa* contain *Katu-Tikta Rasa, Ushna Virya* and

Katu Vipaka properties thus work as *Vata-Kaphahara*, *Sothahara*, *Shulahara* which helps to reduce *Sandhi Shula* (Joint-pain*), Sandhishotha* (Joint-swelling) and *Sandhistabdhata* (Joint-stiffness) locally over affected joints. It possesses *Shoshana* (absorption) and *Kapha-Amahara* (alleviation of vitiated *Kapha* and *Ama*) properties thus it assist in bringing back the *Vikrutha Vayu* to its normalcy and reducing *Ama* locally. Moreover, *Kolakulatthadi Lepa* has also provided highly significant relief in *Janu Sandhi Shula, Sandhishotha* and *Sandhistabdhata* in one of the clinical trials.[1]

Further, when the *Lepa* is applied with *Kanji,* the active principles of ingredients of *Lepa* are released in the base. This combination gets absorbed through the *Swedavaha Srotas* and thereafter, it is subjected for *Pachana* by *Bhrajaka pitta* present in skin which pacifies the provoked *Dosha* locally and thus breaks the *Samprapti* leading to alleviation of the symptoms.[2]

Ziziphus jujuba's anti-inflammatory activity comes from Flavonoids and Phenolic compounds that target inflammation-related prostaglandins.[3] Phaseolus mungo (black gram) shows anti-inflammatory and analgesic effects, proven to reduce paw oedema and pain in inflammation and Rheumatoid Arthritis animal models.[4] Saussurea lappa extracts show anti-inflammatory and anti-arthritic effects in rheumatoid arthritis by lowering markers like CRP, TNF-α, IL-1β, and IL-6. Active compounds, including phenolics and flavonoids, inhibit inflammatory pathways and protect joints.[5] Anethum graveolens may help manage Rheumatoid Arthritis symptoms due to its anti-inflammatory properties, reducing inflammation and paw volume in animal models through its essential oils and polyphenols.[6] Hordeum vulgare (barley) may aid in treating rheumatoid arthritis (RA) due to its anti-inflammatory properties from phytochemicals and antioxidants. It can help reduce inflammation and pro-inflammatory cytokines, making it a useful supplement alongside traditional RA treatments.[7]

In the case of external administration of a herbal drug, it should be safe, effective and free from adulteration, with appropriate quantity and ingredients. It is difficult to identify the herbal drug in dry or powdered form. So, there is a need for time to set proper parameters for the standardization of herbal drugs. Pharmacognostical studies reveal plant identification and set parameters for standardization which can be done in the case of herbal traditional medicine.

Generally, the physiochemical analytical study of drugs helps to interpret the

pharmacokinetics and pharmacodynamics involved. With the help of physiochemical analytical studies, it is possible to standardize the drug and differentiate the adulterants. It is a necessity of time in the field of Ayurveda to go for quality control of the raw drugs as well as final products using modern parameters, which provides credibility to Ayurvedic medicines and also helps in the globalization of Ayurveda. Hence, to evaluate the Authenticity of *Kolakulatthadi Lepa* through various Pharmacognostical procedures, and to develop the pharmacognostical and phytochemical profile of *Kolakulatthadi Lepa,* the present study was carried out.

MATERIALS AND METHODS

Collection, identification and authentication of raw drugs.

The raw materials were procured from the pharmacy of ITRA Jamnagar, an authentic source and the raw drugs were identified and authenticated in the pharmacognosy laboratory of the Institute of Teaching and Research in Ayurveda, Ministry of Ayush, Gov. of India, Jamnagar. The ingredients and parts used in *Kolakulatthadi Lepa Churna* are given in Table 1.

Table No. 1: Ingredients of *Kolakulatthadi Lepa Churna.*

Drug	Latin name	Family	Part Used	Proportion
Kola	*Ziziphus jujuba* Lam.	Rhamnaceae	Fruit	1 part
Kulattha	*Dolichos biflorus* Linn.	Fabaceae	Seed	1 part
Suradaru	*Cedrus deodara* (Roxb.) Loud.	Pinaceae	Heartwood	1 part
Rasna	*Pluchea lanceolata* Oliver & Hiern	Compositae	Root	1 part
Masha	*Phaseolus mungo* Linn.	Papilionaceae	Seed	1 part
Atasi	*Linum usitatissimum*	Linaceae	Seed	1 part
Eranda	*Ricinus communis* Linn.	Euphorbiaceae	Seed	1 part
Kushtha	*Saussurea lappa C* Linn.B. Clarke	Compositae	Root	1 part
Vacha	*Acorus calamus* Linn.	Araceae	Rhizome	1 part
Shatapushpa	*Anethum graveolens* Linn.	Apiaceae	Seed	1 part
Yava	*Hordeum vulgare* Linn.	Poaceae	Seed	1 part

Method of preparation of *Kolakulatthadi Lepa Churna*

All the raw materials for the drug under the trial were procured from the pharmacy of I.T.R.A. Jamnagar, and those that were not available in the pharmacy were procured from the authentic source. *Kolakulatthadi Lepa* was prepared by the Classical method in the pharmacy of I.T.R.A. Jamnagar.

The required quantity of *Kola* (*Ziziphus jujuba Lam.*) was collected by the scholar and made

into powder. Whereas *Atasi* (*Linum usitatissimum Linn.*), *Eranda* (*Ricinus communis Linn*.), and *Yava* (*Hordeum vulgare Linn*.) were then subjected to fine powder using sieve no. 80. The rest of the other ingredients (each 1 part) were made into powder form and were added to the mixture of *Atasi*, *Eranda* and *Yava*, which were stored in an airtight container.

Pharmacognostical study

The pharmacognostical study was divided into an organoleptic study and a microscopic study of the finished product.

Organoleptic study: The genuineness of the polyherbal formulation can be found with the organoleptic characters of the given sample. Organoleptic parameters comprise colour, odour, taste and touch of *Kolakulatthadi Lepa Churna* which was scientifically studied as per the standard references.

Microscopic study: *Kolakulatthadi Lepa Churna* ingredients were taken in powder form and dissolved with water, and microscopy of the sample was done without stain and after staining with phloroglucinol and HCl. Microphotographs of all ingredients, *Kolakulatthadi Lepa Churna*, were also taken under a Corl-Zeiss trinocular microscope.[8]

Physico-chemical analysis-With the help of various standard physico-chemical parameters, *Kolakulatthadi Lepa Churna* was analysed. The common parameters mentioned for *Kolakulatthadi Lepa Churna* in the Ayurvedic Pharmacopoeia of India and CCRAS guidelines are loss on drying, total ash, water-soluble extract, alcohol soluble extract and pH value.

OBSERVATION AND RESULTS

The initial purpose of the study was to confirm the authenticity of the drugs used in the preparation of *Kolakulatthadi Lepa Churna*. For this, all ingredients were subjected to organoleptic and microscopic evaluations to confirm the genuineness of all the raw drugs. Later, after the preparation of the formulation, Pharmacognostical evaluation was carried out. Organoleptic evaluation of organoleptic features like colour, odour, and taste of *Kolakulatthadi Lepa Churna was* recorded and is placed in Table 2.

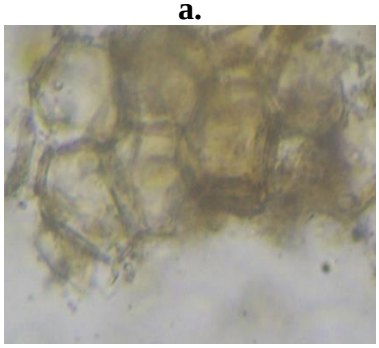
Table No. 2: Organoleptic characteristics of *Kolakulatthadi Lepa Churna.*

Sr. No.	Characters	Results
1	Color	Light brown
2	Odor	Slightly Aromatic
3	Taste	Astringent
4	Touch	Fine

Table No. 3: Microscopic Characteristics of *Kolakulatthadi Lepa Churna.*

a. Compound starch grain of *Rasna*	b. Mesocarp cells of *Eranda Beeja*
c. Oleoresin of *Vacha*	d. Lignified fibres of *Rasna*
e. Brown content and crystal of *Rasna*	f. Fibres of *Masha*
g. Endosperm cells of *Atasi*	h. Cork cells with brown content of *Rasna*
i. Epicarp cells of *Atasi*	j. Fibre with brown content of *Kushtha*
k. Crystal of *Eranda Beeja*	l. Annular vessels of *Vacha*
m. Fragment of palisade parenchyma cells of *Kulattha*	n. Parenchyma cells of cotyledon of *Kulattha*
o. Parenchyma cells with starch grain of *Kulattha*	

a.



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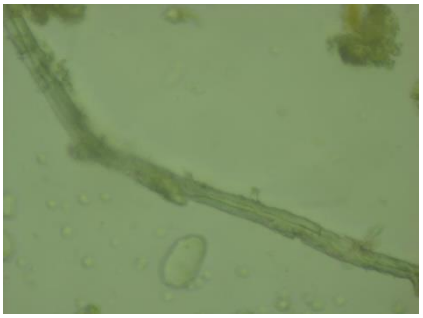
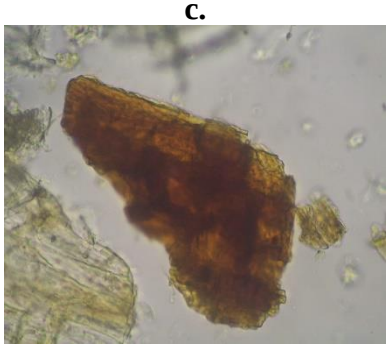


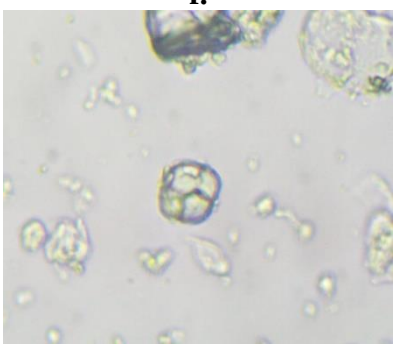
c.



d.

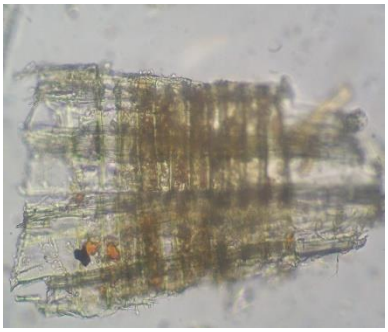
e.

f.

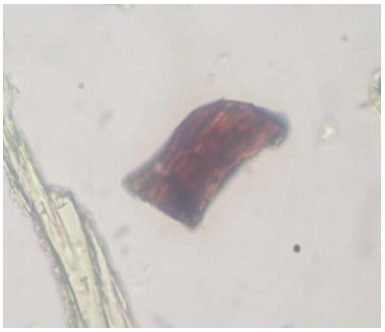


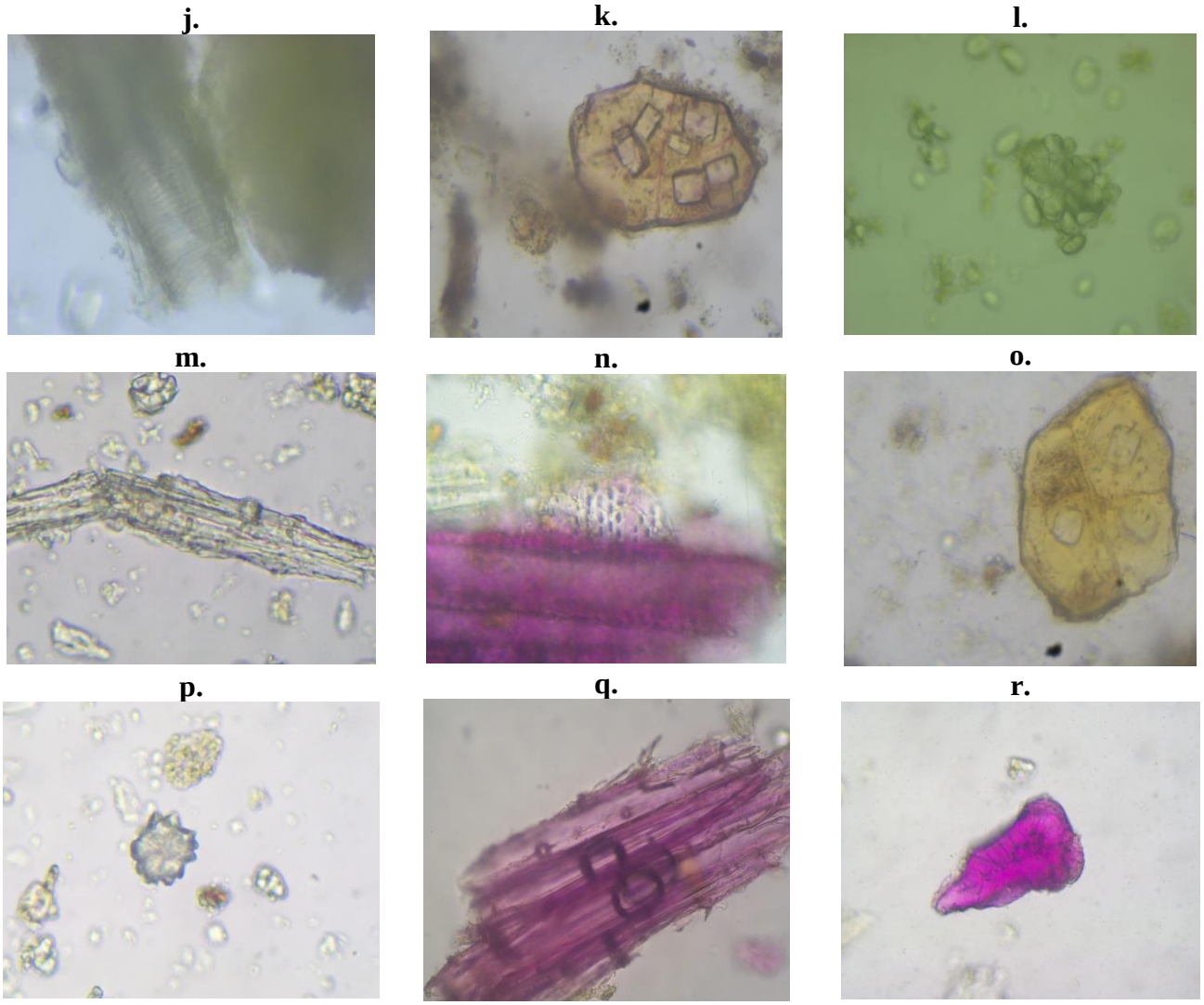
g.

h.



i.





Physio-chemical parameters: Physio-chemical parameters like Loss on drying, total ash, water-soluble extract, alcohol soluble extract and pH value were found within the normal range. Details are shown in Table 4.

Table No. 4: Physio-Chemical Parameters of *Kolakulatthadi Lepa Churna.*

S.N.	Parameters	*Shadanga Guggulu*
1	Loss on drying	97.08%
2	Ash value	35.90%
3	Water-soluble extract	7.78%
4	Methanol-soluble extract	7.52%
5	pH value	5

- No heavy metal in this formulation.
- **Microbiological Study**

- Samples of *Kolakulatthadi Lepa Churna* were studied to check microbial contamination at different climatic conditions. The study was conducted at the Microbiology Laboratory, ITRA, Jamnagar, India.

- **Preparation Time**
  - Drug was prepared under SOP with the utmost care to avoid any sort of contamination.
  - Date of preparation: *Kolakulatthadi Lepa Churna* - 16/07/2024

- **Microbial profile:** Microbial contamination was assessed by two methods to check for any mycological and bacteriological findings.

1. Smear Examination

a. Wet mount / 10% K.O.H. Preparation

b. Gram's stain

2. Culture Study

a. Fungal culture

b. Aerobic culture

Table No. 3.1.2.46: Showing observations of sample *Kolakulatthadi Lepa Churna*. Preserved at room temperature.

Sr. No.	Date of Sample given	Observations of the sample			
		Gram's Stain	Aerobic culture	Wet mount/ 10% KOH Preparation	Fungal culture
1.	14/10/2024	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
2.	11/11/2024	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
3.	11/12/2024	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
4.	16/01/2025	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
5.	06/02/2025	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
6.	17/03/2025	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
7.	24/04/2025	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
8.	28/05/2025	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
9.	30/06/2025	Microorganisms	No organisms	Fungal filaments	No Fungal

		Not Seen	isolated	not seen.	Pathogen Isolated
10.	**30/07/2025**	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated
11.	**28/08/2025**	Microorganisms Not Seen	No organisms isolated	Fungal filaments not seen.	No Fungal Pathogen Isolated

DISCUSSION

Study on *Kolakulatthadi Lepa Churna* was a step towards Pharmacognostical and pharmaceutical standardisation of the drug. The Pharmacognostical study revealed the presence of diagnostic characters of *Kolakulatthadi Lepa Churna -like*. The diagnostic characters are *Rasna-like compound* starch grain, Brown content and crystal, lignified fibre, Cork cells with brown content. *Vacha*-like oleoresin, Annular vessels. *Kulattha* like Fragment of palisade parenchyma cells, Parenchyma cells with starch grain, Parenchyma cells of cotyledon. *Kushth* like Fibers with brown content. *Erand Beeja* like Crystal, Mesocarp cells. *Atasi* like Endosperm cells, Epicarp cells.

This confirms the presence of all ingredients of raw drugs in the final product, and there is no major change in the microscopic structure of raw drug during the pharmaceutical process of preparation of the final product. This shows the genuineness of the final product. All the physico-chemical parameters loss on drying percentage of the *Kolakulatthadi Lepa Churna* was 97.08% w/w, pH value of 5, total ash value was 35.9 % w/w, water-soluble extractive was 7.78% w/w, and methanol-soluble extractive was 7.52%w/w. Were analysed and found to be in the normal reference range.

CONCLUSION

The pharmacognostical and physico-chemical analysis of *Kolakulatthadi Lepa* confirmed the purity and genuinity of the drug. As no standard fingerprint is available for this formulation, an attempt has been made to evolve pharmacognostical and physico-chemical profiles of *Kolakulatthadi Lepa.* Information acquired from this study may be beneficial for further research work and can be used as a reference standard for quality control researches.

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Conflict of interest: None.

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