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PRELIMINARY PHARMACEUTICAL AND ANALYTICAL EVALUATION OF SHRINGYADI LEHA

Karishma[1], Sharma Usha[2], Yadav Yadevendra[3], Shuchi Mitra[4] Sharma Khem Chand[5]

[1]MD Scholar, (P.G Department of Rasa Shastra & Bhaishjya Kalpana).
[2]Professor, (P.G Department of Rasa Shastra & Bhaishjya Kalpana)
[3]Assistant Professor, (P.G Department of Rasa Shastra & Bhaishjya Kalpana)
[4]Associate Professor, (P.G Department of Rasa Shastra & Bhaishjya Kalpana)
[5]Professor and H.O.D., (P.G Department of Rasa Shastra & Bhaishjya Kalpana)
Uttarakhand Ayurved University, Rishikul Campus, Haridwar, India

Corresponding Author: lovelykaletha1991@gmail.com

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ABSTRACT

Aims: *Shringyadi Leha* has been prepared and studied for its Physico-chemical parameters. The adopted formulation is *Shringyadi Leha* based on *Chakradatta*. *Shringyadi Leha* has significant efficacy in pediatric disorders. **Methods and Material**: The present study provides details of the preparation of *Shringyadi Leha*, and methods employed in Physico-chemical parameters. **Results:** Physico-chemical observations revealed the specific characters of the preparation. The preliminary HPTLC study of the compound revealed the components, eight spots in short UV 254nm, 09 spots in 366nm and 10 spots in long UV 550nm. **Conclusions:** Evaluation of Shringyadi lehyam can be used as a reference standard for further quality control research for the manufacturing and processing of Shringyadi Leah.

Keywords: *Shringyadi Leha*, Pharmaceutical parameters and HPTLC.

INTRODUCTION

Ayurveda, the Indian traditional system of medicine has been curing the illnesses of living beings for ages. The paediatric health in Ayurveda is dealt with under the discipline of Kaumarbhritya. A very few formulations have been mentioned in Ayurvedic texts with their specific use for children. Clinical Management of childhood illness is significantly at variance with that of an adult. Shringyadi Leha, along with honey, is one such emphatic substructure mentioned by Acharya Chakrapani in his treatise Chakradutt[1] in *Balarogadhikara* for the administration in Kasa in children. This Leha is one such important and widely practised formulation that works on respiratory disorders, fever, diarrhoea and vomiting of children. It is an Ayurvedic preparation containing four drugs namely Karkatashringi (Pistaciaintegerrima Stew.) Ativisha (Aconitum heterophyllumWall.) and Musta (Cyprus rotundusLinn.). with honey. There are no works carried out regarding the standardization of the compound formulation Shringyadi leha. Lack of standardization of poly-herbal formulations creates difficulty in validating the efficiency and preserving the quality of the product. It is significant to authorize the standard and quality right from the raw drugs to the finished product. Hence an attempt has been made to study Shringyadi leha by Physico-chemical parameters and to develop HPTLC (High-Performance Thin Layer chromatography study) fingerprints of this compound formulation.

MATERIALS AND METHODS:

COLLECTION OF RAW DRUGS

Raw drug materials were collected from the raw drug store of hans pharmacy, Haridwar. The ingredients and the part used are given in Table.No.1.

Table 1: Formulation composition of *Shringyadi Leha*[2]

S.No.	Ingredients	Latin Name	Part used
1.	***Karkatshringi***	Pistacia integerrima	*Shringakkarkosh*
2.	***Ativisa***	Aconitum hetrophyllum	*Mool*
3.	***Musta***	Cyperus rotundus	*rizome*
4.	***Madhu***	-	-

METHOD OF PREPARATION:

The rhizome of Cyprus rotundus Linn, roots of Aconitum heterophyllum Wall. and gall of Pistacia integerrima Stew. were collected. Authantication was carried out at dravyaguna department of Uttrakhand Ayurved University, Rishikul Campus, Haridwar. The collected drugs were dried in the shade and finely powdered by a pulverizer. Finally, Shringyadi leha was prepared by mixing the above three herbs in equal proportion and honey in double quantity which was stored in an air-tight container.

ANALYTICAL STUDY:

An analytical study was carried out at Vasu research centre, Vadodara, Gujarat. Organoleptic parameters were assessed. Physico-chemical analysis like pH value, Loss on drying, Total Ash, Acid Insoluble Ash, Total Solids and Total sugar were carried out by following standard procedures. High-Performance Thin-layer chromatography (HPTLC) studies were carried out with methanolic extract.

DETERMINATION OF pH VALUE[3]

The drug Shringyadi Leha was made into a 10% aqueous solution, and the pH of the liquid was determined with the help of a pH meter and electrode system. The pH of the drug was 5.20.

LOSS ON DRYING[4]

Accurately weigh two grams of medicine and place it in a porcelain crucible. Heat on a hot plate at 110°C for 3 hours. After heating sufficiently, allow the crucible to cool in a desiccator and then weigh. Continue heating, cooling and weighing until a stable weight was reached. Calculate the change in the weight of the ceramic crucible to calculate the drying loss. The drying loss of the sample was 16.45%.

TOTAL ASH[5]

Place five grams of air-dried ground material into a heavy, pre-lit crucible (usually platinum or silica). The material was evenly layered and ignited by gradually increasing the heat to 500-600 ° C until it turns white, indicating no charcoal. Repeat heating, cooling, and weighing until the crucible weight was stable. Compared to air-dried materials, the total ash content was mg / g. Therefore, the total ash content was found to be 2.89%.

ACID INSOLUBLE ASH[6]

The collected total ash lasts 5 minutes and was boiled with 25 ml of dilute hydrochloric acid. The solution was filtered with Whatman filter paper (# 40). The filter paper was burned in a Gucci crucible along with the insoluble ash. The crucible was heated, cooled, and weighed until the crucible weight was stable. The acid-insoluble ash was 1.12%, in the calculated 5 g air-dried drug.

TOTAL SOLID[7]

A 10gm of sample was placed in a previously washed and dried beaker. Then the beaker was placed on a water bath at a low temperature, to remove the maximum moisture content. After that, we placed the beaker in an oven at 40°C till constant weight was reached. The percentage of Total solids was 83.54.

TOTAL SUGAR CONTENT (%W/W)[8]

Preparation of Reagent:

Fehling's Solution:

A) Dissolve 69.278 g of copper sulphate in water and make the volume up to one litre.

B) Dissolve 100 g of sodium hydroxide and 340 g sodium potassium tartarated in purified water and make the volume 1 litre.

Mix equal volumes of A and B solution before the experiment.

Clarifying Reagent:

Solution 1: Dissolve 21.9 g of zinc acetate and 3 ml of glacial acetic acid in purified water and make the volume 100 ml.

Solution II: Dissolve 10.6 g of potassium ferrocyanide in water and makeup to 100 ml. A suitable amount of the sample was taken and neutralized with sodium hydroxide solution (10% in water). The neutralized solution was evaporated to half the volume on a water bath at 50ºC to remove the alcohol. After cooling the solution 10 ml of the clarifying solution I was added followed by 10 ml of the clarifying solution II. All the solutions were mixed well then filtered through a dry filter paper. After that 15 ml of 0.1 N hydrochloric acid was added to the filtrate. Now the solution was covered with a stopper and heated to boiling for two minutes. Then phenolphthalein was added and neutralized with sodium hydroxide solution (10%). This solution was transferred in a 100 ml volumetric flask and volume was made up to 100 ml and the titration was performed as done for the reducing sugars. In this way percentage of the total sugars was calculated.

LIMIT TESTS FOR HEAVY METALS

The heavy metal limit is shown in the monograph in the number of heavy metal parts per million (by weight) of the substance.

LIMIT TEST FOR ARSENIC[9]

The glass tube is lightly filled with cotton wool, moistened with a solution of lead acetate and dried beforehand so that the top surface of the cotton is not less than 25 mm from the top of the tube. Then insert the upper end of the tube into the narrow end of one of the pairs of rubber plugs to a depth of approximately 10 mm. Put a flat piece of mercury chloride paper on top of the stopper, and put another stopper on top and fix it with an elastic band, so that the perforations of the two stoppers (or upper stopper and glass tube) come together to form a real tube with a diameter It is 6.5 mm, separated by a mercury chloride paper diaphragm The test solution was prepared according to specifications and placed in a wide-mouth bottle. Then 1 g of potassium iodide AsT and 10 g of zinc AsT are added, and the prepared glass tube is quickly put in place. Allow this action for 40 minutes. Compare the yellow spots produced on the mercury chloride paper in daylight with the standard spots produced in a similar manner using a known amount of AsT diluted arsenic solution. Compare stains immediately after testing is completed. Determine the proportion of arsenic in the substance by matching the colour depth to the colour depth of the standard dye. The stain equivalent to 1 ml

of standard stain produced by handling 10 g of a substance indicates that the proportion of arsenic is one part per million. Here in this test, comparing the drug with the standard 3 ppm strain, we found that the arsenic content was less than the normal value (<3 ppm).

LIMIT TEST FOR CADMIUM[10]

First NH4OH was added to the sample solution then potassium ferrocyanide was added. White ppt observed excess cadmium hydroxide soluble in NH4OH. The presence of CD was inferred. Here, in this trial, the drug was studied in comparison to the 0.30 ppm standard strain, and we found that the cadmium content was lower than the normal value (< 0.30 ppm).

HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY[11]

Sample Preparation

Preparation of Test Solutions: Weigh 2.5 g of sample in a conical flask and add 25 mL of water to it. Reflux for 30 Minutes, and filter with Whatman filter paper No. 1. Transfer the filtrate to a separating funnel and partition with 20 mL Ethyl Acetate. Repeat the procedure twice with 15 mL Ethyl Acetate. Collect all Ethyl acetate layer and evaporate to dryness. Reconstitute the sample with 2 mL Ethyl Acetate and filter with 0.22 µm syringe filter. Use the Test solution thus obtained for HPTLC fingerprinting.

Stationary Phase (Application)

The prepared sample was applied over the pre-coated silica gel 60 F_{254} on Aluminium sheets.

Development (Mobile Phase)

The sample was developed with the help of the mobile phase, i.e., Toluene: Ethyl Acetate: Formic acid: methanol: (6:3:0.1:1v/v).

Visualization (Scanning)

For visualization, the plate was dried at 100°C and scanned at 254 nm UV, 366nm and 540nm.

Steps involved in HPTLC:

1) Selection of chromatographic layer.
2) Sample and standard preparation.
3) Layer pre-washing, layer pre-conditioning.
4) Application of sample and standard.
5) Chromatographic development.
6) Detection of spots.
7) Scanning.
8) Documentation of chromatic plate.

Chromatographic Conditions:

Application Mode	CAMAG Linomat 5 - Applicator
Filtering System	Whatman filter paper No. 1
Stationary Phase	MERCK - TLC / HPTLC Silica gel 60 F254 on Aluminum sheets
Application (Y-axis) Start Position	10 mm
Development End Position	80 mm from plate base
Sample Application Volume	15.0 µL
Development Mode	CAMAG TLC Twin Trough Chamber
Chamber Saturation Time	30 minutes
Mobile Phase (MP)	Toluene: Ethyl Acetate: Formic acid: Methanol (6 : 3 : 0.1 : 1 v/v)
Visualization	@ 254 nm, @ 366 nm and @ 540 nm (after derivatization)
Spray reagent	Vanillin Sulphuric acid reagent
Derivatization mode	CAMAG – Dip tank for about 1 minute
Drying Mode, Temp. & Time	TLC Plate Heater Preheated at 100± 5^0C for 3 minutes

RESULTS:

PHARMACEUTICAL STUDY OF SHRINGYADI LEHA:

Table 2: Result & Observations of *Shringyadi Leha*

S.No.	Observations	
1.	Date of Starting	11/02/2020
2.	Date of Completion	13/02/2020
3	Weight of crude drugs (g)	3200 g
4.	Weight of fine powder (g) (85 no. Mesh)	2558 g
5.	Honey	5116 g
6.	Colour	Brownish

PHARMACOGNOSTICAL STUDY OF SHRINGYADI LEHA:

Organoleptic evaluation: The sample was light brown with a characteristic spicy pungent smell and predominant *Kashaya Tikta Rasa*.

Table 3: Organoleptic characters of samples of *Shringyadi Leha*

Sample	Colour	Taste	Touch	Texture
SL	Brown	Bitter taste with later pungent	Sticky	Semi-Solid

ANALYTICAL STUDY OF SHRINGYADI LEHA:

Physico-chemical parameters like pH, Total Ash, Loss on Drying, Acid Insoluble Ash, Total Solids, Total sugar etc were carried out and the results are depicted in (Table 4).

Table 4: Physico-chemical characters of samples of *Shringyadi Leha*

S. No	Parameters	Shringyadi Leha
1.	pH	5.20
2.	Total Ash (% w/w)	2.89%
3.	Loss on Drying (% w/w) at 105ºC	16.45%
4.	Acid Insoluble Ash (% w/w)	1.12%
7.	Total Solids	83.54%
8.	Total sugar (%w/w)	53.58%
9.	Reducing sugar	47.26%
10.	Non-Reducing sugar	6.32%

Table 5: Heavy metal analysis of samples of *Shringyadi Leha*

S. No	Heavy metals	***Shringyadi Leha***
1.	Lead	ND
2.	Cadmium	0.29 ppm
3.	Mercury	ND
4.	Arsenic	0.63ppm

Table 6: HPTLC Study of samples of *Shringyadi Leha*

Rf Values observed of the three samples			
	254 nm	**366 nm**	**540**
SL	0.13, 0.21, 0.36, 0.43, 0.50, 0.59, 0.68, and 0.88.	0.13, 0.17, 0.26, 0.39, 0.43, 0.50, 0.59, 0.68 and, 0.88.	0.11, 0.13, 0.21, 0.32, 0.36, 0.43, 0.56, 0.71 and 0.88.

Fig 1: HPTLC at 254nm

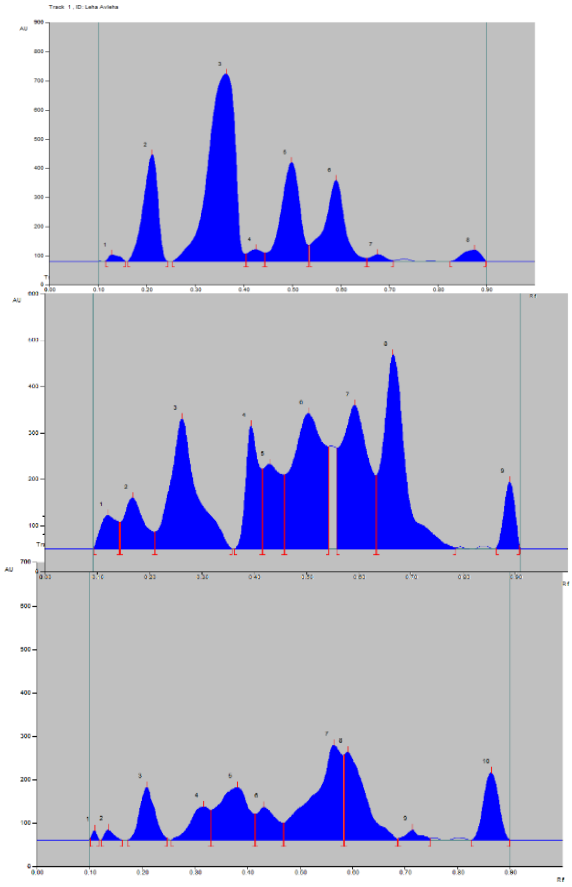
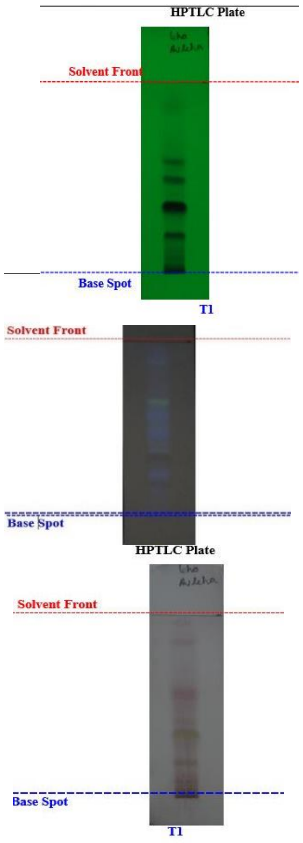


Fig 2: HPTLC at 366nm



DISCUSSION

A study on *Shringyadi Leha* is a step towards pharmacognostic and pharmaceutical standardization of the drug. The existence of all contents of raw drugs in the final product shows the genuinity of the final product. In the pharmaceutical study, 2558g powder was found from 3200g of crude drug, the percentage of loss was twenty, which was found due to the presence of fibres in *Musta* and *Ativisha* in table no. 2. All the pharmaceutical parameters analysed showed values permissible for the *Leha*. It also shows the presence of a slightly acidic nature of *Leha* which may help in augmenting the *Jatharaagni* (digestive fire). Pharmaceutical study reveals pH, Total ash value, Acid insoluble Ash, Loss on drying, Total solids and Total sugar of *Shringydi Leha* were 5.2, 2.89%w/w, 1.12%w/w, 16.45%w/w 83.54%w/w and 53.58%w/w respectively. For *leha* total sugar was 53.58%w/w out of which reducing sugar was 47.26%w/w and non-reducing sugar was 6.32%w/w in Table No.4. The preliminary HPTLC study of the compound revealed the components, eight spots in short UV 254nm in Fig No.1, 09 spots in 366nm in Fig No.2 and 10 spots in long UV 550nm in Fig No. 3. The solvent system shows good separation of components so it can be used for further analysis. Only 2 heavy metals were present in *Shringyadi Leha* which was within permissible limits in Table No.5. All the pharmaceutical parameters analysed showed values permissible for the *leha*.

CONCLUSION

The *leha* dosage form is more acceptable, palatable and also have a good shelf life as compared to Churna. Thus, *Shringyadi Leha* was considered for the present study. The study was pointed to prove the genuinity of the drugs used and to assess the pharmaceutical parameters. The results were found satisfactory. The parameters of this study can be used for authentication and further research. It can be concluded that the complete

and accurate physicochemical values of the present study are useful in the identification and authentication of Shringyadi leha.

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