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FORMULATION AND STANDARDIZATION OF HARATALADI LEPA – AYURVEDIC DRUG TO COMBAT SWITRA (VITILIGO) IN CHILDREN

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ABSTRACT

Introduction: Harataladi lepa has been indicated in the management of Switra (Vitiligo) by the authentic book of Ayurveda, Baishajya ratnavali. There is no work on record on formulation and standardization aspect of compound formulation Harataladi lepa. This paper highlights the preparation, physico-chemical characterization, TLC and HPTLC densitogram profiling of Harataladi lepa which can be applied for authentication of this herbomineral formulation. **Methods:** The lepa was prepared by combining Chitraka (*Plumbago zeylanica* Linn.), Triphala *(Emblica officinalis* Linn., *Terminalia bellerica* (Gaertn.) Roxb., *Terminalia chebula* Retz.), Shodita Haratala (Orpiment (As_2S_3)), Shodita Kasisa (Green vitriol ($FeSO_4$)), Shodita Gandhaka (Sulphur (S)), Tila taila (Sesame oil), (Madhuchista Bee wax) were subjected for detailed physico-chemical and HPTLC analyses. **Results:** Set of standardization parameters were derived for the compounded Lepa by physico-chemical characterization. The tests proposed would serve as diagnostic parameters for the identity of this herbomineral formulation. HPTLC fingerprint profile which can serve as a fingerprint for the identification of the formulation has been obtained. **Conclusion:** The proposed method of making Lepa

will aid in yielding concentrated medicament with the same efficacy as per the classically proposed drug dosage at lower dose. Standards for the herbomineral formulation has been developed for the quality check of the formulation.

Keywords: Ayurveda, Harataladi lepa, Switra, Skin, Vitiligo, HPTLC densitogram

1. INTRODUCTION

Vitilgo is a progressive, idiopathic, depigmentation disorder of skin caused by the distruction of melanocytes which is responsible for the skin normal color **[1]**. This autoimmune disorder clinically manifests as well-demarcated white patches on the body **[1]**. There are different theories about the pathogenesis of vitiligo but exact etiology is still unknown. Childhood vitiligo deserves special attention as frequently 50 % of cases occurs before 20 years of age and in 25 % of the cases it start before the age of 10 years **[2]**. Vitiligo can be correlated with Shweta Kushta or Switra, which means a white patch occurred by vitiation of Pitta and Kapha Pradhana Tridosha **[3]**. Switra mainly affecting the physical, social and mental status of child becoming economical burden over parents. Due to adverse effect and limitations of modern contemporary medicine there is need of effective, safe and harmless treatment. In Ayurveda many such formulation and treatment modalities are explained for the management of Vitiligo.

Harataladi lepa has been indicated in the management of Switra (Vitiligo) by the authentic book of Ayurveda, Baishajya ratnavali. The Lepa contains Haratala, Kasisa, Gandaka, Chitrakamoola and Triphala churna in it **[4]**. When we observe the mechanism of manifestation of Switra, due to Ahitakara Ahara, Vihara and other Nidanas leading into vitiation of Kapha Dosha and vitiated Kapha Dosa does the Avarana of Bhrajaka Pitta i.e, Pittaruta Kapha and also decreases Bhrajaka Pitta **[5]**. In such cases, the pharmaco dynamic properties of Haratala, Gandaka, Kasisa and Chitraka which are of Katu, Tikta rasa, Ushna virya, Katu Vipaka and Laghu - Ruksha guna acts as good Srotoshodaka which helps in removing the Avruta kapha as well as increases the Pitta. The properties of Triphala such as Kashaya rasa, Sheeta guna and Madhura vipaka balances the Tridoshas **[6]**. Hence this Lepa has helped in the treatment of Switra. The present paper will be detailing the steps in the preparation of Harataladi lepa as well as its standardization.

2. MATERIALS AND METHODS

2. 1. Collection and authentication of the raw drugs:

The mineral drugs like Haratala, Kasisa, Gandhaka and herbal drugs like Chitraka, Triphala, Tila Taila and animal product bee's wax were procured from teaching pharmacy, Department of

Rasashastra and Bhaishajya Kalpana, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan district, Karnataka state. The herbal drugs were authentified at Department of Dravyaguna Vijnana, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan district, Karnataka state. The procedures of standardization and HPTLC Photodocumentation, Rf values, densitometric scan, 3-D chromatogram were done at SDM Centre for Research in Ayurveda and Allied Sciences, (AYUSH Centre for Excellence and Recognized SIROs by DSIR), Laxminarayana Nagar, P.O., Kuthpady – 574 118, Udupi district, Karnataka state, India.

Table 1: Ingredients of Harataladi Lepa

S. No.	Ingredients	Latin name/ English name	Parts used	Quantity taken
1	Chitraka	*Plumbago zeylanica* Linn. [7]	Root	120gm
2	Triphala	*Emblica officinalis* Linn. [8] *Terminalia bellerica* (Gaertn.) Roxb. [9] *Terminalia chebula* Retz. [10-11]	Fruit	120gm
3	Shodita Haratala	Orpiment (As_2S_3) [12]	-	120gm
4	Shodita kasisa	Green vitriol ($FeSO_4$) [13]	-	120gm
5	Shodita gandhaka	Sulphur (S) [14]	-	120gm
6	Tila taila	Sesame oil [15]	-	1500ml
7	Madhuchista	Bee wax [16]	-	495gm

Haratala, Kasisa, Gandhaka - all these mineral drugs were subjected for Shodhana before taking them for lepa preparation.

2. 2. Haratala Shodhana: [17]

Two Kushmanda (pumpkin) fruit weighing 6.5 kg were taken, cut into pieces and the pulp was grinded, 3 litre Swarasa (juice) was obtained. 150gm of Haratala was taken, pounded to coarse powder, made into Pottali and hung in a Dola yantra (vessel) containing 2 litre 80 ml of Swarasa. The vessel is kept on a stove for boiling in Madhyama Agni (moderate fire) for about three hours. In between the process, after one and half hour of boiling, the vessel is added with 150 ml of Swarasa to maintain the Pottali immersed in Swarasa. After completion of three hours of boiling, Haratala collected from Pottali, dried and stored in airtight container.

2. 3. Kasisa Shodhana: [18]

Kasisa taken in a clean khalva yantra and triturated to powder. The required quantity of Nimbu Swarasa (lemon juice) required to wet Kasisa was added and triturated for 3 hours. Total three bhavana was given. After three Bhavana, the Kasisa was collected, dried and stored in airtight container.

2. 4. Gandhaka Shodhana: [19]

1 litre milk is taken and boiled. Coarsely powdered raw Gandhaka, taken in a pan smeared with Ghrita, and it is melted over a mild flame. Then melted Gandhaka is poured into a vessel containing warm milk,

through cloth. The solidified Gandhaka settled at bottom of vessel is collected, washed with warm water. This process was repeated for seven times, taking fresh milk every time.

2. 4. Preparation of Harataladi Lepa

120 gm of fine powder of each ingredient were taken. 1.5 litres of Tila Taila is taken in a vessel and it is added with 495 grams of bees wax, kept on a mild flame, till bees wax gets melted completely. After it melts, the content is filtered to get rid of physical impurities. To this filtrate the fine powder of the herbal medicine, such as Chitraka, Triphala are added, followed by fine powders of Haratala, Kasisa and Gandhaka are added one after the other and stirred homogenously till uniform mixture is obtained.

Ingredients of Harataladi Lepa

Figure 1: Shodhitha Haratala

Figure 2: Shodhitha Gandhaka

Figure 3: Shodhitha Kasisa

Figure 4: Triphala and Chitraka churna

Figure 5: Tila Taila

Figure 6: Madhuchista

Preparation of Harataladi Lepa

Figure 7: Melting bees wax in Tila Taila

Figure 8: Adding Haratala to Sikatha Taila

Figure 9: Melting bees wax in Tila Taila

Figure 10: Prepared Harataladi Lepa

2. 5. Instrumentation and techniques of drug analysis

Organoleptic characters

Color, odor and taste of sample are noted using sensory organs

Determination of pH

Preparation of buffer solutions:

Standard buffer solution: Dissolved one tablet of pH 4, 7 and 9.2 in 100 ml of distilled water.

Determination of pH: 1 ml of sample was taken and make up to 10 ml with distilled water, stirred well and filtered. The filtrate was used for the experiment. Instrument was switched on. 30 minutes time was given for warming pH meter. The pH 4 solution was first introduced and the pH adjusted by using the knob to 4.02 for room temperature 30°C. The pH 7 solution was introduced and the pH meter adjusted to 7 by using the knob. Introduced the pH 9.2 solution and checked the pH reading without adjusting the knob. Then the sample solution was introduced and reading was noted. Repeated the test four times and the average reading were taken as result.

Loss on drying at 105°C

10 g of sample (Harataladi Lepa) was placed in tared evaporating dish. It was dried at 105˚C for 5 hours in hot air oven and weighed. The drying was continued until difference between two successive weights was not more than 0.01 after cooling in desiccator. Percentage of moisture was calculated with reference to weight of the sample.

Total fat

1 g of the sample (Harataladi Lepa) mixed with 4gm of silica and was introduced into a thimble and placed it in a soxhlet fitted with a condenser. Taken 90 ml of petroleum ether (B.P. 40 - 60°C) in the 150 ml RB flask and boiled for 6 hours. The extract was taken in a pre-weighed conical flask and petroleum

ether was evaporated on a water bath. Removed the traces of petroleum ether in vacuum pump. Taken the weight of fat to constant weight.

Rancidity test:

1ml of melted fat was mixed with 1ml of conc. HCl and 1ml of 1% solution of phloroglucinol in diethyl ether and then mixed thoroughly with the fat acid mixture. A pink color indicates that the fat is slightly oxidized while a red color indicates that the fat is definitely oxidized.

Parallel plate method for determining the spreadability of semisolid preparations

The spreadability of bases were determined by keeping the sample between two Plexiglas's at 37^0C, it is based on linearity and spreading diameter measurements. Viscosity and spreading diameter is independent of derivative used. 1gm of the gel was placed between the Plexiglas plate and known wt was kept upon it and was then measured the diameter of spread and was calculated using the formula,

S =m * l/t

S is the spreadability of the gel formulation, m is the weight (g) tied on the upper plate, l is the length (cm) of the glass plates, and t is the time taken (s) for the plates to slide the entire length.

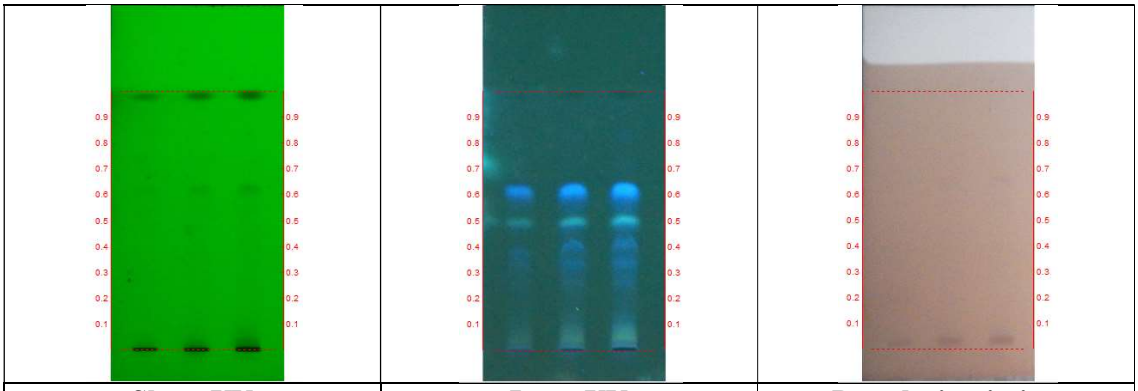
HPTLC for Harataladi Lepa: 1g of sample was triturated or mixed with 10 ml of *methanol* and filtered. 4, 8, 12µl of each of the above extract was applied on a pre-coated silica gel F_{254} on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl Acetate (9:1). The developed plates were visualized in under short UV, long UV and then derivatised with vanillin sulphuric acid followed by heating the plate at 105^0C until the spots appeared. Plate was scanned under UV 254nm, 366 nm. R_f, colour of the spots and densitometric scan were recorded.

3. RESULTS

The Physico-chemical parameters of Harataladi Lepa is detailed in table 2. The HPTLC Photo documentation of Methanolic extract of Harataladi Lepa is shown in **Figure 11**. The R_f value of Methanolic extract of Harataladi Lepa is detailed in **Table 3**. The densitometric scan of Methanolic extract of Harataladi Lepa is shown in **Figure 12**.

Table 2: showing physico-chemical parameters of Harataladi Lepa

Parameters	Results n=3 %w/w Harataladi Lepa
pH	6.0
Color	Green
Odour	Characteristic
Rancidity	Fat is not oxidised
Total fat (%)	83.62
Loss on drying	0.0
Spreadability (g.cm/sec)	11.23



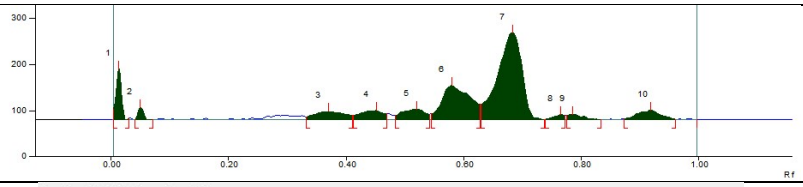
Short UV	Long UV	Post derivatiation

Track 1 - Harataladi Lepa – 4µl
Track 2 - Harataladi Lepa – 8µl
Track 3- Harataladi Lepa – 12µl
Solvent system – Toluene: Ethyl Acetate (9:1)
Figure 11: showing HPTLC photo documentation of Methanolic extract of Harataladi Lepa

Table 3: showing R_f value of Methanolic extract of Harataladi Lepa

Short UV	Long UV	Post derivatisation
-	0.26 (F. blue)	-
-	0.34 (F. blue)	-
-	0.41 (F. blue)	-
-	0.50 (F. blue)	-
0.62 (D. green)	0.62 (F. blue)	-
-	-	0.66 (Purple)

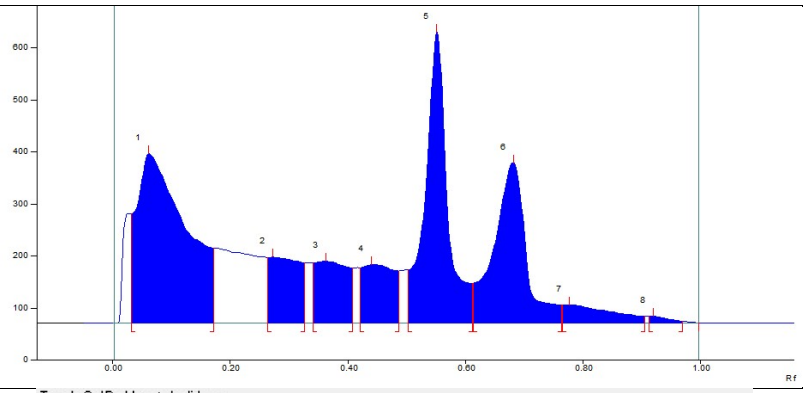
***f- fluorescent**



Track 3, ID: Harataladi lepa

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	21.5 AU	0.01 Rf	110.1 AU	21.92 %	0.03 Rf	3.5 AU	744.1 AU	6.32 %
2	0.04 Rf	0.3 AU	0.05 Rf	25.7 AU	5.12 %	0.07 Rf	0.0 AU	173.5 AU	1.47 %
3	0.33 Rf	5.9 AU	0.37 Rf	17.8 AU	3.53 %	0.41 Rf	9.4 AU	647.7 AU	5.50 %
4	0.41 Rf	9.6 AU	0.45 Rf	19.7 AU	3.92 %	0.47 Rf	12.2 AU	550.0 AU	4.67 %
5	0.48 Rf	9.2 AU	0.52 Rf	22.5 AU	4.49 %	0.54 Rf	12.0 AU	652.8 AU	5.54 %
6	0.55 Rf	12.3 AU	0.58 Rf	73.8 AU	14.68 %	0.63 Rf	33.2 AU	2554.6 AU	21.69 %
7	0.63 Rf	33.6 AU	0.68 Rf	188.6 AU	37.53 %	0.74 Rf	0.0 AU	5522.6 AU	46.89 %
8	0.74 Rf	0.4 AU	0.77 Rf	11.0 AU	2.20 %	0.77 Rf	9.5 AU	144.4 AU	1.23 %
9	0.78 Rf	9.6 AU	0.79 Rf	11.9 AU	2.37 %	0.84 Rf	0.3 AU	218.4 AU	1.85 %
10	0.87 Rf	1.4 AU	0.92 Rf	21.4 AU	4.25 %	0.96 Rf	0.3 AU	569.4 AU	4.83 %

Figure 12a. At 254nm



Track 3, ID: Harataladi lepa

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	209.6 AU	0.06 Rf	325.3 AU	20.35 %	0.17 Rf	43.8 AU	19696.8 AU	31.00 %
2	0.26 Rf	125.5 AU	0.27 Rf	126.5 AU	7.92 %	0.33 Rf	15.6 AU	4887.9 AU	7.69 %
3	0.34 Rf	115.3 AU	0.36 Rf	118.9 AU	7.44 %	0.41 Rf	06.1 AU	4787.7 AU	7.54 %
4	0.42 Rf	106.4 AU	0.44 Rf	112.4 AU	7.03 %	0.49 Rf	00.0 AU	4518.4 AU	7.11 %
5	0.50 Rf	102.2 AU	0.55 Rf	559.0 AU	34.98 %	0.61 Rf	76.5 AU	15125.4 AU	23.81 %
6	0.61 Rf	76.7 AU	0.68 Rf	308.0 AU	19.27 %	0.76 Rf	33.9 AU	12078.2 AU	19.01 %
7	0.77 Rf	34.1 AU	0.78 Rf	35.0 AU	2.19 %	0.91 Rf	12.6 AU	2125.5 AU	3.35 %
8	0.91 Rf	12.5 AU	0.92 Rf	13.3 AU	0.83 %	0.97 Rf	2.8 AU	316.6 AU	0.50 %

Figure 12b. At 366nm

Figure 12: showing densitometric scan of Methanolic extract of Harataladi Lepa

4. DISCUSSION

Lepa as preparation applied on to skin must get into adipose tissue fat and then get absorbed into systemic circulation, so absorption across skin depends on blood supply, partition coefficient, solubility of drug in lipid, thickness of bilayer and pH. pH was formulation Harataladi Lepa was found to be 6.0, Skin pH is mildly acidic formulation also being on acidic pH will not cause any irritation and adverse effect etc. Preparation has characteristic odour, color being greenish yellow since it contains Haratala. Suphur has anti bacterial property so could be use for variety of skin conditions burns, wound infection, septicemia etc. The vehicle here in the Lepa being fat, rancidity test was done to prove its stability in adverse environmental conditions extremes of weather hot and cold, the formulation was not rancid, no chemical signs of deterioration was observed.

Total fat was 83.62% showing predominantly the fat was carrier for drug or vehicle for drug absorption, so duration of action could be longer and absorption could be predictably better. Moisture content was 0.0% which could be commendable because presence of moisture could lead to deterioration of formulation, making it unfit for application of skin. Further moisture could lead to physical instability leading tom phase separation. Spreadability test was done to ensure uniform film forming capability of formulation on skin surface which in turn has influence on absorption.

HPTLC plate when observed under short UV showed one band (Green), Long UV 5 bands (all fluorescent blue), Post derivatisation with Vanillin sulphuric acid spraying reagent it could show one band (Purple). Densitometric scan of the plate at 254nm, it showed 10peaks notable are 0.58 (21.69%), 0.68 (46.89%). Scan at 366nm was evident with 8peak major peaks were being 0.55 (23.81%), 0.68 (19.01%). These two major peaks in both 254nm and 366nm could be attributing factors for therapeutic benefits.

5. CONCLUSION

The unique R_f values, densitometric scan and densitogram obtained at different wavelengths pre- and post-derivatisation can be used as fingerprint to identify the herbomineral formulation, Harataladi Lepa and can be used as reference while setting the pharmacopoeial standards to ensure the quality. Hence, efforts have been made to provide scientific data on formulation and standardization of Harataladi Lepa. The novel concentrated compound formulation Harataladi Lepa thus developed yield better acceptability in terms of palatability, increased shelf life and as compared to prescribed forms in a lesser dose.

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