

Microbiological, Pharmacognostical and Pharmaceutical Analysis of Yashtimadhu Taila – A Compound Ayurvedic Formulation

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ABSTRACT

Aim and Objective: *Yashtimadhu Taila* is a choice of drug given in *Chakradatta* in the treatment of *khalitya* (Hair fall). *Ayurveda* texts describe hair fall under *Shirogata Rogas* (diseases of head) under the broad heading of *Khalitya*. In present study attempt has been made to evaluate microbiological and develop pharmacognostical and pharmaceutical standards as per *Ayurvedic Pharmacopoeia of India (API)* for *Yashtimadhu Taila*. **Methodology:** Preliminary Microbiological studies, Pharmacognostical and Pharmaceutical parameters, High-Performance Thin Layer Chromatography has been performed as per Standard method. **Result:** Microbiological study for *Yashtimadhu Taila* revealed absence of any microbial contamination under smear and culture study in aerobic atmosphere after 90 days of sample been prepared. The Pharmacognostical study of ingredients of *Yashtimadhu Taila* showed the Compound starch grains, Cork in surface, Pitted vessel, Rhomboidal crystal, Simple fiber, Scleroides and tannin of *Yashtimadhu* and Group of scleroides, Lignified scleroides, Parenchyma cells, Scleroides and Starch grains of *Amla* were found. Pharmaceutical analysis of *Yashtimadhu Taila* showed that saponification value (mg/g) was 178.7, acid value (%) was 0.488, iodine value (I₂ 100/g) was 82.91, specific gravity at room temperature was 0.9181 and refractive index was 1.482 respectively etc. and HPTLC at 254 nm resulted into 6 spots and at 366 nm resulted into 15 spots respectively. **Conclusion:** Standard value from this study can be used for further researches and evaluation of *Yashtimadhu Taila* helps in Quality control tool for its manufacturing or processing. Analysis through Pharmacognostical and Pharmaceutical shows suitability of drug for *Nasya* purpose.

Keywords: *Yashtimadhu Taila*, Microbiological, Pharmacognostical and Pharmaceutical, *Khalitya*.

INTRODUCTION

Hair is considered one of our most cherished treasures. Healthy, beautiful, long and attractive hairs add charm to the personality. In Ayurvedic approach, falling of hair is coined out as in term of “*Khalitya*” under the broad heading of *Shiroroga*.ⁱ *Khalitya* (hairfall) is a sign of early aging process.ⁱⁱ In this most advanced modernized era, the humans are gifted with lot of sophistication, luxuries but at the same time left with sedentary ways of life, stress induced hectic and unhealthy schedules. According to survey up to 40% of men and 25% of women in India are victims of hairfall.ⁱⁱⁱ

In *Charaka Samhita*, it is the only herb, which is considered at 11 places out of the 50 *Mahakashaya*^{iv} indicating broad spectrum medicinal use in Ayurvedic treatment. In texts *Nasya* is the accepted line of treatment for the *Khalitya* (Hair fall) as well as best therapy for the

UrdhvajatrugataRogas. Tailais the best Sneha for Nasya.^vIn *ChakradattaKshudrarogaChikitsaAdhyaya55- YashtimadhuTailaNasyais* quoted best for *Khalitya(Hairfall).*^{vi}

An attempt has been made to study *YashtimadhuTaila* by pharmacognostical, pharmaceutical, physico-chemical parameters and develop HPTLC fingerprints of the compound formulation.

MATERIALS AND METHOD

Collection of Raw drug:

Raw drug materials were collected from raw drug store of pharmacy of I.T.R.A., Jamnagar. The raw drugs were identified and authenticated and powder microscopy was done in the Pharmacognosy laboratory, I.T.R.A., Jamnagar. The study includes organoleptic evaluation and microscopic evaluation as per API standards for authentication. *YashtimadhuTaila* was stored in well filled closed glass containers away from the light. The ingredients & parts used in the preparation of the final product are listed in the Table 1.

Table no. 1: *YashtimadhuTaila*: (Chakradatta55/111).^{vii}

No.	Sanskrit name	Latin name	Part used	Part
1	<i>Yashtimadhu</i>	<i>Glycerrhiza Glabra Linn.</i>	Root	1/4 Part
2	<i>Amalaki</i>	<i>Emblica Officinalis Gaertn.</i>	Fruit pulp juice	
3	<i>Krishna Taila</i>	<i>Sesamum Indicum Linn.</i>	Seed Oil	1 Part
4	<i>Godugdha</i>	Cow Milk		4 Parts

Method of preparation of *YashtimadhuTaila*

The *Snehapaka Kalpana* method of *Sharangdhara Samhita Madhyama Khanda*^{viii} was adopted for preparation of *YashtimadhuTaila*. The final product i.e. *YashtimadhuTaila* was prepared in the Pharmacy, I.T.R.A., Jamnagar.

Method

TilaTaila was taken in stainless still vessel and placed over mild fire when fumes started, *Taila* was taken from fire and *Kalka* of *Amalaki* and *Yashtimadhu* was added and fried. Soon after *Godugdha* were added to vessel and boiled further with frequent stirring maintaining on mild temperature. Continue the process on mild heating till the observation of *Snehapaka Siddhi Lakshana* appeared. After obtained *Snehapaka Lakshana*, it was filtered in warm condition through cotton cloth and allows cooling and then stored in a tightly closed containers to protect from light and moisture.

MICROBIOLOGICAL EVALUATION:

Microbiological investigation has been carried out of *YashtimadhuTaila* after 90 days from day of preparation at Microbiological laboratory of I.T.R.A., Jamnagar. Smear examination and Aerobic as well as fungal culture study has been carried out for *YashtimadhuTaila* under microscope. Smear examination: Gram's Stain and 10% KOH Preparation *YashtimadhuTaila* has been done. A sterile

sample smear collected under aseptic condition. Culture study: Aerobic and Fungal culture has been assessed for the sample of *YashtimadhuTaila*.^{ix}

PHARMACOGNOSTICAL EVALUATION:

A. MICROSCOPIC STUDY: Individual raw drugs identified and verified with API, Finished drug was identified and authenticated by the Pharmacognosy lab, I.T.R.A., Jamnagar. The identification was carried out based on organoleptic features and microscopy of the prepared drug. For Pharmacognostical evaluation, drugs studied under the Corlzeiss Trinocular microscope with staining and without staining. The microphotographs were also taken under the microscope.^x

B. ORGANOLEPTIC STUDY: The Organoleptic characters of Ayurvedic drugs are very important and give the general idea regarding the genuinity of the sample. It is done with the help of *PanchaGyanendriyaPariksha*. *YashtimadhuTaila* was evaluated for organoleptic characters ie. colour, odour, touch and taste.^{xi}

PHARMACEUTICAL EVALUATION:

Physico-chemical analysis: Physico-chemical Parameters of *YashtimadhuTaila* like loss on drying, water soluble extract, saponification and many more were determined as per the API guideline. *YashtimadhuTaila* was further subjected to High Performance Thin Layer Chromatography (HPTLC) study.^{xii}

HPTLC: Thin layer chromatography (HPTLC) study was carried out with dry methanol (MeOH) extract on pre-coated silica gel GF 6254 aluminium backed plate as 6mm bands, 8mm apart and 15cm from the edge of the plates, by means of a CamagLinomate V sample applicator fitted with a 100 µL Hamilton syringe. The mobile phase used was Toluene: Ethyl acetate (9:1v/v). The plates were developed in Camag twin trough chamber (20 x 10 cm²) and spots were detected in short U.V. (254 nm), Long U.V. (366nm). Video Densitometry rTLC shiny app was used for documentation.^{xiii}

RESULTS AND DISCUSSION:

Study of *YashtimadhuTaila* has been done to standardize the drug. For that Microbiological, Pharmacognostical and Pharmaceutical parameters were assessed.

1. MICROBIOLOGICAL EVALUATION: Microbiological study under microscopic examination till 90 days. It shows absence of any microorganisms on gram's stain and no organisms isolated after 48 hours of incubation at 37 degree Celsius under Aerobic Atmosphere. Similarly, for fungal culture, reveals absence of fungal filaments in 10% K.O.H preparation and on culture. No fungal pathogens found as shown in (Plate no. 1).

2. PHARMACOGNOSTICAL EVALUATION

2.1. MICROSCOPIC STUDY: Diagnostic microscopic characters of ingredients of *YashtimadhuTaila* showed the compound starch grains, Cork in surface, Pitted vessel, Rhomboidal crystal, Simple fiber, Scleroides and tannin of *Yashtimadhu* and Group of scleroides, Lignified scleroides, Parenchyma cells, scleroides and starch grains of amla were found. (Plate no. 2).

2.2. ORGANOLEPTIC STUDY: *YashtimadhuTaila* was evaluated for organoleptic characters i.e. colour, odour, touch and taste as shown in (Table 2).

3. PHARMACEUTICAL EVALUATION:

3.1. PHYSICO-CHEMICAL TESTS: Comparative Physicochemical Analysis of *YashtimadhuTaila*.e. Refractive index, Specific gravity, Acid value, Iodine value, Saponification value were scientifically studied and results were detailed in respectively Table 3.

3.2. HPTLC STUDY RESULTS: Chromatographic study (HPTLC) was carried out under 254 and 366 nm UV to establish fingerprinting profile of which showed 6 spots at 254 nm and 15 spots at 366nm with R_f values were recorded which may be responsible for expression of *YashtimadhuTaila* pharmacological and clinical actions. (Table 4) (Plate no. 3).

A) Table 2: Organoleptic characters of *YashtimadhuTaila* (Chakradatta 55/111)

Sr. No	Parameters	Sample –Taila
1.	Colour	Dark golden yellow
2.	Touch	Oily liquid
3.	Odour	Slightly aromatic sweetish
4.	Taste	Oily astringent sweet

B) Table 3: Physio-chemical analysis of *YashtimadhuTaila*

Parameters	<i>YashtimadhuTaila</i>	API value
Specific Gravity	0.9181	0.899 to 0.925
Acid Value	0.4883	Not more than 3.0
Saponification	178.7	190 to 200
Iodine value	82.91	105 to 115
Refractive Index	1.4820	1.4470 to 1.4740

C) Table 4: High performance thin Layer Chromatography (HPTLC)

Sample	No. of spots	Observation	Max. R _f value
<i>YashtimadhuTaila</i>	6	Observed under short U.V. Light (254 nm)	0.01, 0.15, 0.19, 0.32, 0.59, 0.86
<i>YashtimadhuTaila</i>	15	Observed under Long U.V. Light (366 nm)	-0.05, 0, 0.1, 0.26, 0.29, 0.35, 0.42, 0.5, 0.57, 0.69,

			0.73, 0.77, 0.78, 0.85, 0.94
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PLATE 1: MICROBIOLOGICAL INVESTIGATIONS OF YASHTIMADHU TAILA

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CULTURE REPORT FOR MICROBIOLOGICAL INVESTIGATIONS

Date of Request	Drug Preparation Date	Drug Sample Detail	Aerobic Culture Report	Fungal Culture Report
31/08/2022	18/01/2022	Hillbreyograj tail oil Randomly selected from Bottle No. 62 Container No. 01	<p>Microscopic Examination:</p> <p>Green's Stain: Slides show absence of microorganisms.</p> <p>On Culture: No organisms isolated. After 48 hrs. of incubation at 37°C Under Aerobic Atmosphere</p>	<p>Microscopic Examination:</p> <p>10% K.O.H. Preparation: Structure resembling fungal filaments not seen.</p> <p>On Culture: No fungal pathogens isolated. After 15 days of incubation at 37°C Under Aerobic Atmosphere</p>

MB - No. 543, 544, 545 Date: $18/01/2022$ R - FC - 03/11/2003




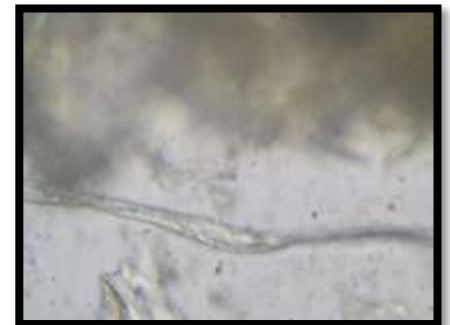
PLATE 2: MICROSCOPIC STUDY OF YASHTIMADHU TAILA



Brown content of *Yashti*



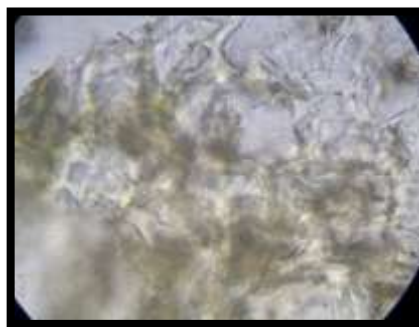
Cork cell of *Yashti*



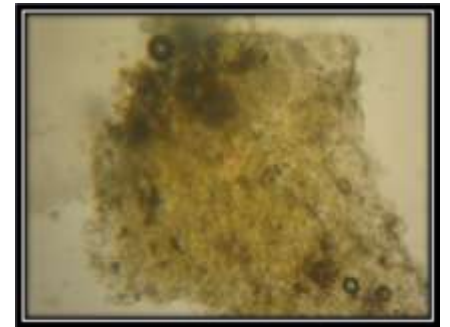
Fibers of *Amalaki*



Lignified crystal fibre *Yashti*



Mesocarp cells of *Amalaki*



Mesocarp cells of *Amalaki*



Pitted vessel of *Yashti*

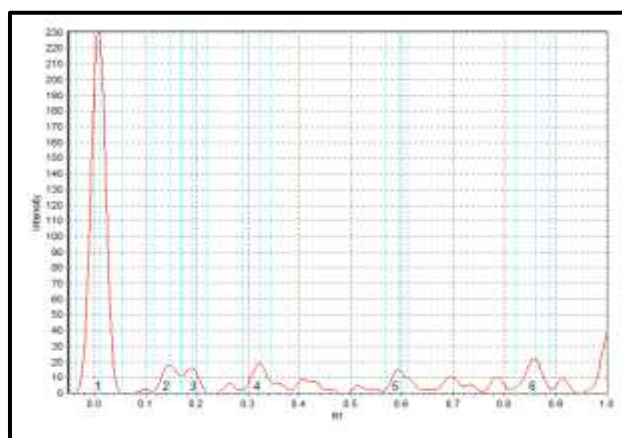


Rhomboidal crystal of *Yashti*

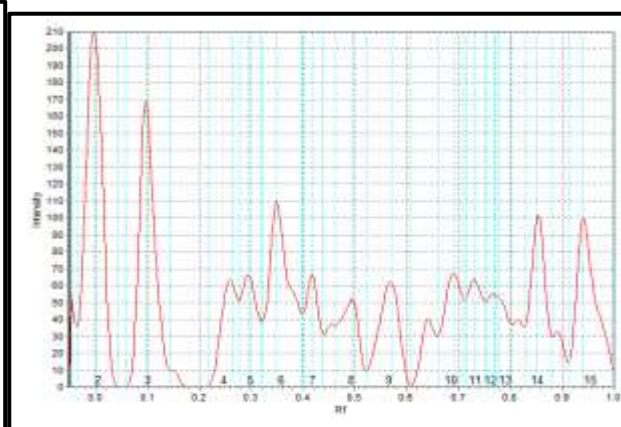


Scleroid of *Amalaki*

PLATE 3:HPTLC STUDY: HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC STUDY (HPTLC)



Observed under short U.V. light (254nm)
Yashtimadhu Taila



Observed under long U.V. light (366nm)
Yashtimadhu Taila

DISCUSSION:

Study of *YashtimadhuTaila* has been done to standardize the drug formulation. No any microbial growth found in final preparation till 90 days of preparation. Despite different uses of medicinal plants in traditional culture of each country, people are still worried about efficacy and nonhazardous effects of these drugs. So many investigations have been focused on various dimensions of safety, toxicity, quality, efficacy and rational use of medicinal plants.

Organoleptic evaluation was performed for coarse powder of *Yashtimadhu* and *Amalaki*. They were authenticated and analyzed before processing of *Taila* because good quality products mainly dependent upon genuine raw materials. The diagnostic characters of microscopic analysis of *YashtimadhuTaila* ingredients showed the Compound starch grains, Cork in surface, Pitted vessel, Rhomboidal crystal, Simple fiber, Scleroides and tannin of *Yashtimadhu* and Group of scleroids, Lignified scleroids, Parenchyma cells, Scleroids and Starch grains of *Amla* were found. These Microphotographs indicates there is no any substitution and adulteration in raw material. These microphotographs are similar to Ayurvedic Pharmacopoeia of India.^{xiv}

The colour of *YashtimadhuTaila* was dark golden yellow because of *Yashtimadhu*. The odour was *Tilasmell* due to *Tila Tail* was used as a *Sneha Dravya*. Taste is slight astringent sweetish and oily touch found. All values of the physico-chemical parameters were– Acid value (0.4883), Saponification (178.7), Iodine value (82.91), specific gravity (0.9181) and refractive index (1.4820). All results were found to be within the normal reference range according Ayurvedic Pharmacopoeia of India.^{xv} The obtained values of these tests were found within normal limit which indicate good quality of product and better results in the diseases. HPTLC is a powerful analytical tool in the field of analysis. An *Rf* value is characteristic for any given compound (provided that the same stationary and mobile phases are used). It can provide corroborative evidence as to the identity of a compound. HPTLC profile of the methanolic extract of the drug showed 6 spot at 254 nm and 15 spots at 366 nm. This HPTLC study indicates maximum active chemical compound were found at each peak level of densitogram.

CONCLUSION:

Microscopic evaluation is mandatory to avoid adulteration and contamination in *YashtimadhuTaila*. Analysis through Pharmacognostical and Pharmaceutical shows suitability of drug for *nasya* purpose.

The results of these studies can be used for the reference standard, authentication and further researches.

REFERENCE:

ⁱParadkar H.S.S., *Astanga Hridaya of Vagbhata, Sarvangasundara of Ārunadatta and Āyurvedarasayana of Hemadri*, Reprint Ed., Varanasi: Chaukhamba Surbharati Prakashana, *Uttarasthana*, 2002;23-20.

ⁱⁱParadkar H.S.S., *Astanga Hridaya of Vagbhata, Sarvangasundara of Ārunadatta and Āyurvedarasayana of Hemadri*, Reprint Ed., Varanasi: Chaukhamba Surbharati Prakashana, *Uttrasthana*, 2002;23-26.

ⁱⁱⁱ<http://www.southIndiatourtravel.com/baldness-hair-loss.html>

^{iv}Acharya Agnivesha, *Charaka Smahita*, Edited by Brahmananda Tripathi, *Sutrasthan - Chaukhamba Surbharti Prakashan*, Varanasi, 1994; 3: 4-41.

^vParadkar H.S.S., *Astanga Hridaya of Vagbhata, Sarvangasundara of Ārunadatta & Āyurvedarasayana of Hemadri*, Reprint Ed., Varanasi: Chaukhamba Surbharati Prakashana, *Sutrasthan*, 2002;20-1.

^{vi}Dr. Indradeva Tripathi; *Chakradatta – Vaidyaprabhav, Hindi Commentary of Chakrapanidatta*. Chaukhamba Sanskrit Sansthan Varanasi; Edition: reprint, *Kshudraroga Chikitsashadyaya*, 2018;55-111.

^{vii}Dr. Indradeva Tripathi; Chakradatta – Vaidyaprabhava, Hindi Commentary of Chakrapanidatta. Chaukhamba Sanskrit Sansthana Varanasi; Edition: reprint, Kshudraroga Chikitshadhyaya, 2018;55-111.

^{viii}Pandit Parshuramshastri, Sharagdhara samhita, Madhyama Khanda; published by Chaukhamba Krishnadas Academy, 2013; 212: 9,1-2.

^{ix}Dr. P. Gunasekaran, Laboratory Manual in Microbiology, reprint 2005, New Age International (P) Limited, Publishers, New Delhi

^xAnonymous, the Ayurvedic Pharmacopoeia of India, Part-I, Vol. 1-4, Govt. of India, Ministry of Health & FW, Dept. of ISM and H. New Delhi; Dept. of Ayush, 1999; 155-56

^{xi}Trease and Evans, Pharmacognosy, W.B. Saunders Company Ltd, 1996; 15:569-570.

^{xii}Ayurvedic Pharmacopoeia of India PDF-1, Govt. of India, Ministry of health and family welfare, Delhi, appendix, 2007; 5, 2, 2, 9;214.

^{xiii}Anonymous, Planner Chromatography, Modern Thin layer Chromatography, Switzerland,1999;2-1

^{xiv}Atlas of Macroscopic and Microscopic Characters of Ayurvedic Pharmacopoeial Drugs, Ayurvedic Pharmacopoeia of India, addition, Ministry of Ayurveda and Family Welfare (AYUSH), New Delhi, 127:1.

^{xv}The Ayurvedic Pharmacopoeia of India, Part-2(Formulations), Ministry of Ayurveda and Family Welfare (AYUSH), New Delhi, 103;1.