



Research Article

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ANALYTICAL VALIDATION OF HRASHVA PANCHMOOLA TAILA: INTEGRATING TRADITIONAL FORMULATION PRINCIPLES WITH MODERN QUALITY ASSESSMENT

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ABSTRACT

Background: Hrashva Panchmoola Taila is a traditional Ayurvedic polyherbal compound described in the Ayurvedic text Charaka Samhita and mentioned in Vata Vyadhi Vikara. Given its therapeutic efficacy and rising clinical relevance, pharmacognostic and analytical quality standards have to be established in fulfillment of AYUSH and API recommendations. Aims and Objectives: To evaluate the pharmacognostic and physicochemical aspects of Hrashva Panchmoola Taila and create a chromatographic fingerprint for quality standardization. Materials and Methods: Hrashva Panchmoola Taila formulation contains medicinal compounds such as Laghupanchmoola, a collection of five roots widely used in Ayurveda for Vata-shamaka, Shothahara (anti-inflammatory), Vedanasthapana (analgesic), and tila pinyaka for Balya (strength-promoting) characteristics. Laghu Panchmoola and tila pinyaka are commonly used in inflammatory disorders, neuromuscular ailments, and pediatric conditions where Vata Dosha predominates. The above formulations were assessed using Thin Layer Chromatography (TLC), organoleptic characteristics and physicochemical properties. Results: Organoleptic testing indicated brick red formulation with a characteristic odor. Physicochemical investigation revealed that rancidity was absent, the saponification value was 199.10, the iodine value was 115.31, the refractive index was 1.4720, the acid value was 2.22, and the moisture content was 0.12%. TLC analysis has R_f values of 0.05, 0.10, 0.31, 0.40, 0.56, 0.75, 0.87, and 0.96 at 365 nm, showing the presence of several phyto-constituents. Conclusion: The current study establishes pharmacognostic, physicochemical, and chromatographic standards for Hrashva Panchmoola Taila. These parameters may be used as reference quality control standards to ensure the formulation's identity, purity, and consistency in line with AYUSH and API regulations.

Keywords: Analytical Validation, Thin Layer Chromatography, Quality Control, Drug Standardization.

INTRODUCTION

Drug development begins with the identification or synthesis of a molecule that demonstrates potential therapeutic efficacy in the prevention, control, or management of disease¹. Analytical studies form a crucial component of this process, involving the identification, authentication, and quantification of substances and their constituents, along with structural elucidation of chemical compounds². The chemical constitution of a drug, particularly the presence and arrangement of specific bioactive components, directly influences its pharmacological activity and therapeutic outcome. Standardization encompasses the systematic measures undertaken during manufacturing and quality control to ensure consistent quality, safety, and efficacy of a formulation. Quality control testing is performed at every stage, from raw material procurement to the finished product. In the context of Ayurveda, standardization is especially important to ensure stability, reproducibility, and global acceptance of classical formulations amid rising international demand³.

Hrashva Panchmoola Taila is a classical medicated oil indicated for Basti therapy, particularly in neurological and neuromuscular disorders such as cerebral palsy. The analytical evaluation of Hrashva Panchmoola Taila (Matra Basti) was undertaken under the clinical trial entitled "Clinical Study to Evaluate the Efficacy of Dashmooladi Ghrita and Hrashva Panchmoola Taila Matra Basti in the Management of Spastic Cerebral Palsy in Children –

A Single Arm Open Label Prospective Study," registered with the Clinical Trials Registry of India (CTRI/2024/05/067955). The formulation, described in Charaka Samhita, Chikitsa Sthana, Chapter 28 (Vata Vyadhi Chikitsa), was selected for pharmaceutical and analytical evaluation prior to clinical application. The analytical study was conducted using standard parameters, including organoleptic evaluation (colour, odour, taste, and consistency), physicochemical tests such as moisture content, saponification value, iodine value, refractive index, specific gravity, acid value, and rancidity assessment, along with Thin Layer Chromatography (TLC) for determination of R_f values and establishment of a chromatographic fingerprint profile.

AIMS AND OBJECTIVES

Aim: To undertake a comprehensive pharmaceutical and analytical evaluation of Hrashva Panchmoola Taila in accordance with standard quality control parameters.

Objectives

- To evaluate the organoleptic characteristics of Hrashva Panchmoola Taila.
- To assess the physicochemical parameters in accordance with the standards prescribed in the Ayurvedic Pharmacopoeia of India⁴ (API).

- To perform Thin Layer Chromatography (TLC) for establishing the chromatographic fingerprint profile of the formulation.

MATERIALS AND METHODS

Procurement of raw materials

The herbal ingredients required for the preparation of Hrashva Panchmoola Taila along with Tila Taila (sesame oil) were procured from the local market of Jodhpur, Rajasthan. Fresh Godugdha (cow’s milk) was obtained from a nearby village in Karwar, Jodhpur. The Murchana (purificatory processing) of Tila

Taila Murchana⁵ was performed in accordance with classical Ayurvedic guidelines prior to its use in the formulation.

Identification and authentication of raw drugs

All raw herbal drugs were identified and authenticated by experts from the Department of Dravyaguna, Post Graduate Institute of Ayurveda (PGIA), Kadwar, Jodhpur. The authentication was carried out based on classical Ayurvedic parameters and standard pharmacognostical characteristics to ensure the genuineness and quality of the raw materials.

Table 1: Ingredients of Hrashva Panchmoola Taila

Ingredient	Latin Name	Part Used	Quantity
Shalparni	<i>Desmodium gangeticum</i> DC	Panchang	1 part
Prishniparni	<i>Uria picta</i> Desv	Panchang	1 part
Kantakari	<i>Solanum surattense</i>	Panchang	1 part
Brihati	<i>Solanum indicum</i> Linn.	Panchang	1 part
Gokshura	<i>Tribulus terrestris</i> Linn	Fruit /Root	1 part
TilaPinyaka	<i>Sesamum indicum</i>	Seed (Oil)	1 part
Taila	Oil		1 Prastha
Payas	Milk		8 Prastha

Method of preparation of hrashva panchmoola taila

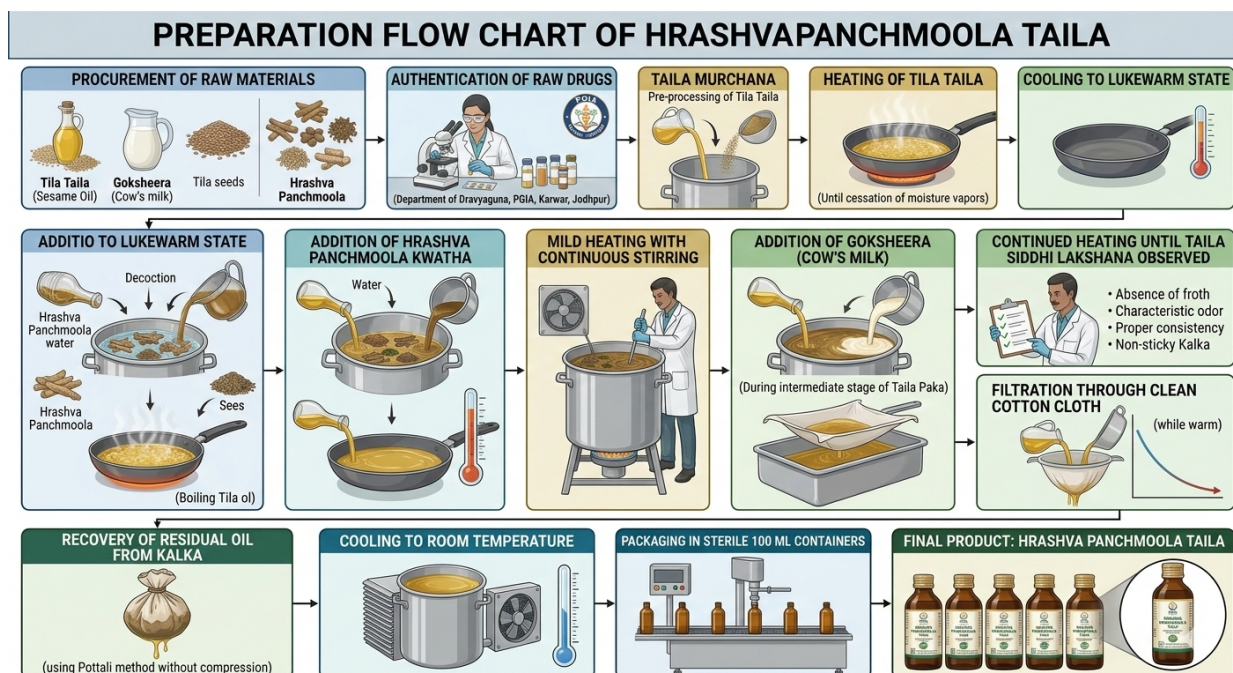
The preparation of Hrashva Panchmoola Taila was carried out following the classical procedure of Sneha Kalpana. Initially, Taila Murchana was performed, a pre-processing method in which raw Tila Taila is subjected to specific heating and herbal processing to enhance its therapeutic efficacy, remove Ama Dosha, and eliminate undesirable odor. For Murchana, Tila Taila was heated in a wide-mouthed vessel until the cessation of visible vapors, indicating the removal of moisture content. The oil was then removed from direct heat and allowed to cool slightly. Subsequently, the prescribed quantity of Kwatha Dravya (decoction of Hrashva Panchmoola) was added to the oil. The mixture was heated uniformly over mild fire.

stirring. The process was maintained until the appearance of Taila Siddhi Lakshana (classical signs of completion), such as absence of froth, characteristic odor, proper consistency, and formation of soft, non-sticky Kalka.

After attaining Siddhi Lakshana, the prepared oil was filtered through a clean cotton cloth while still warm and collected in a sterile container. The residual oil entrapped in the Kalka (herbal residue) was recovered by suspending it in a Pottali (cloth pouch) and allowing the oil to drip naturally into a clean vessel without manual compression.

During the intermediate stage of Taila Paka, Goksheera (cow’s milk) was incorporated, and heating was continued with constant

The final product, Hrashva Panchmoola Taila, was allowed to cool to room temperature and subsequently packed in sterile 100 mL containers for further analytical evaluation.



Place of work: Cultivator Phyto Lab Pvt. Ltd. Sonamukhi Nagar, Sangaria Fanta, Jodhpur, Rajasthan, India.

Sample registration no.: CPL/O/25/08/01682

Date of sample sent to lab and sample registration: 23/08/2025

Date of start of analysis: 25/08/2025

Date of completion of analysis: 27/08/2025

Analytical study was conducted under the following headings

- Organoleptic Characters
- Physiochemical Parameters
- Chromatographic Fingerprint -TLC

Organoleptic Characters

Organoleptic evaluation refers to the examination of a substance through the sense organs sight, smell, touch, and sometimes taste to determine its physical appearance, consistency, and sensory quality.

Table 2: Organoleptic Properties of Hrashva Panchmoola Taila

Macroscopic study	Hrashva panchmoola taila
Appearance	Liquid
Color	Brick red
Odor	Characteristic

Physiochemical Parameters

The physio-chemical examination of Ayurvedic formulations is an essential part of quality control and standardization. It provides objective, measurable evaluations of a drug's stability, effectiveness, and purity. These traits demonstrate the oil's physical consistency and chemical integrity in the case of Taila Kalpana (oil preparation), ensuring that it retains the Gūṇas (qualities) necessary for medicinal action. Parameters including acid value, saponification value, iodine value, refractive index, specific gravity, moisture content and rancidity test are examined using the Ayurvedic Pharmacopoeia of India.

Rancidity Test (Kries Test) - This qualitative test detects oxidative rancidity caused by aldehydes and peroxides. Chemical stability is indicated by the absence of pink in the oil. If so, it implies rancidity. Rancid oils produce an unpleasant odor and taste, indicating chemical breakdown and a lack of medicinal effectiveness. Pink or red coloration indicates rancidity (oxidized oil).

Saponification Value - The saponification value is the number of milligrams of potassium hydroxide (KOH) required to saponify 1gram of oil. This assay determines the average molecular weight of the oil's fatty acids. Oils with shorter-chain fatty acids have higher saponification levels compared to oils with longer chains.

RESULTS

Table 3: Physiochemical Parameters of Hrashva Panchmoola Taila

Test Parameters	Unit	Result	Reference (API)
Rancidity, Saponification value, Iodine value, Refractive index, Acid value, Moisture, Specific gravity	% by weight	Absent, 267.74, 30.37, 1.4543, 2.12, 0.11%, 0.9120	API Part II, Vol. IV, 2017

Iodine Value - The iodine value specifies how much iodine is absorbed by 100 grams of oil or fat. It is an indication of the degree of unsaturation (number of double bonds) in the fatty acid chain of triglycerides.

Refractive Index- The refractive index (RI) of oil is the ratio of the velocity of light in air to the velocity of light in the oil.

Acid Value- The acid value is the milligrams of potassium hydroxide (KOH) required neutralizing the free fatty acids in one gram of oil. It is an essential marker of oil's quality and freshness.

Moisture - The moisture content test evaluates the quantity of water or volatile components in an oil sample. Moisture is an important factor that influences the stability, purity, and shelf life of oils.

Specific Gravity - The specific gravity of oil is the weight of a particular volume of oil divided by the weight of an identical volume of water at a given temperature (often 25°C). It is a dimensionless physical feature used in the identification and standardization of oils.

Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) is a simple, fast, and efficient analytical technique for identifying, purity testing, and fingerprint profiling of herbal compositions. It utilizes the adsorption chromatography principle to separate a sample's constituent parts based on their varied affinities for the mobile phase (solvent mixture) and stationary phase (adsorbent). On the TLC plate, each molecule in the mixture travels a specific distance and generates unique spots at varying retention factor (Rf) values, which are determined as follows:

$$R_f = \frac{\text{Distance traveled by the compound}}{\text{Distance traveled by the solvent front}}$$

To produce a test solution, dissolve a small amount of Ayurvedic oil in an acceptable solvent, such as ethanol or hexane. Coat a TLC plate with a thin layer of silica gel. Mark a baseline about 1 cm from the bottom of the plate with a pencil. Use a micropipette or capillary tube to apply a little drop of the test solution to the baseline. To concentrate the sample, repeat after allowing the area to dry. Place the TLC plate in a developing chamber containing a trace amount of the solvent solution, or mobile phase. Common solvent systems include ethyl acetate and toluene (7:3). Make that the solvent concentration is lower than the baseline. By using capillary action, let the solvent raise the plate until it is about 1 cm from the top. Mark the solvent front as soon as you remove the plate. Use a little stream of air or let the plate air dry. To see the separated components, examine the plate under a UV lamp or apply an appropriate spray reagent (such as Anisaldehyde and Sulfuric acid). Calculate how far the solvent front and each spot have travelled.

The physicochemical analysis of Hrashva Panchmoola Taila revealed various criteria that confirm its purity, stability, and therapeutic efficacy. The oil's moisture level was exceptionally low (0.12%), suggesting minimal water activity and ensuring good microbiological stability and shelf life. The absence of rancidity (Kries Test: negative) demonstrated that the oil had not been oxidatively degraded and preserved its chemical freshness. The saponification value of 199.10 indicates the presence of primarily medium-chain fatty acids, which contributes to improved absorption and bioavailability during Basti administration. The iodine value was 115.31, indicating a moderate level of unsaturation and a balanced combination of saturated and unsaturated fatty acids, which prevents fast oxidation while preserving therapeutic efficacy. Similarly, an acid value of 2.22 indicated low free fatty acid concentration and limited hydrolytic breakdown. The refractive index of 1.4720 and specific gravity of 0.9150 were within the standard values defined by the Ayurvedic Pharmacopoeia of India, verifying the oil's consistency, density, and purity. These physicochemical properties confirm that Hrashva Panchmoola Taila is a stable and authentic formulation appropriate for therapeutic usage.

Supporting these findings, Thin Layer Chromatography (TLC) research revealed an extensive variety of phytoconstituents, as shown by the existence of eight different Rf values of 0.05, 0.10, 0.31, 0.40, 0.56, 0.75, 0.87, and 0.96. Multiple well-defined bands indicate the presence of a wide range of bioactive molecules, including as terpenoids, phenolic compounds, flavonoids, and other lipid-soluble substances. TLC fingerprinting is a confirmatory analytical method for standardization that ensures the identity, purity, and reproducibility of herbal compounds.

DISCUSSION

Prior to clinical utilization, analytical standardization constitutes a fundamental pillar of Ayurvedic pharmaceuticals, as it ensures the quality, safety, stability, authenticity, and batch-to-batch reproducibility of classical formulations. Contemporary Ayurvedic pharmacopoeial guidelines emphasize organoleptic, physicochemical, and chromatographic evaluation as essential parameters for validating Sneha Kalpana preparations before therapeutic application⁷. Such analytical assessment bridges classical textual descriptions with modern quality control benchmarks, thereby strengthening the scientific credibility of Ayurvedic formulations in integrative healthcare settings⁸.

The organoleptic evaluation of Hrashva Panchmoola Taila demonstrated a viscous, homogeneous, brick-red oil with a pleasant characteristic odor, reflecting a properly processed Sneha Kalpana (oil preparation). These features correspond to the classical attributes of Snigdha, Mridu, and Guru Gunas, which are described to pacify aggravated Vata Dosha and enhance tissue lubrication and neuromuscular stability⁹. The uniform consistency and absence of rancid odor further indicate appropriate Murchhana and Sneha Paka procedures, ensuring optimal extraction of lipid-soluble phytoconstituents into the oil base.

Physicochemical analysis revealed parameters well within the limits prescribed in the Ayurvedic Pharmacopoeia of India (API), confirming purity and stability. The saponification value of 199.10 suggests the predominance of medium-chain fatty acids, which are known to facilitate enhanced absorption and bioavailability due to easier enzymatic hydrolysis and intestinal transport¹⁰. The iodine value of 115.31 indicates a moderate degree of unsaturation, contributing to optimal fluidity and effective transmembrane diffusion of lipophilic active principles. The refractive index (1.4720) and specific gravity (0.9150 at

25°C) fall within acceptable ranges for medicated oils, signifying consistency and absence of adulteration. Low moisture content (0.12%) minimizes the risk of microbial growth and oxidative deterioration, while an acid value of 2.22 reflects minimal free fatty acid formation, indicating negligible hydrolytic degradation and satisfactory storage stability. Collectively, these parameters substantiate that the formulation is chemically stable, non-rancid, and pharmaceutically sound for clinical use¹¹.

Thin Layer Chromatography (TLC) profiling revealed multiple distinct spots with Rf values of 0.05, 0.10, 0.31, 0.40, 0.56, 0.75, 0.87, and 0.96, demonstrating the presence of diverse phytochemical fractions. Such a spectrum typically corresponds to alkaloids, terpenoids, flavonoids, and volatile constituents extracted into the lipid matrix during Sneha Paka^{12, 13}. These bioactive compounds are reported to possess anti-inflammatory, antioxidant, neuroprotective, and myorelaxant properties, thereby supporting the classical indications of Hrashva Panchmoola Taila in Vata-dominant disorders. The TLC profile thus serves as a characteristic chemical fingerprint, ensuring authenticity, reproducibility, and quality assurance of the formulation. Analytical validation in this manner not only upholds traditional standards but also aligns Ayurvedic pharmaceuticals with contemporary regulatory and scientific expectations.

CONCLUSION

The physicochemical examination of Hrashva Panchmoola Taila revealed information indicative of high level of pharmaceutical quality, compositional consistency, and formulation stability. Therapeutic compound claims that Hrashva Panchmoola Taila is a Vata-shamaka, Shothahara (anti-inflammatory), Vedanasthapana (analgesic). This formulation appropriate for therapeutic use, specifically in Basti therapies for neurodevelopmental disorder such as VataVyadhi (Cerebral Palsy Disorder).

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