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Research Article

A Physicochemical and Chromatographic Fingerprint Study on Tilvak Ghrita: A Polyherbal Formulation

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Abstract

Background: *Tilvak Ghrita* is a classical Ayurvedic polyherbal formulation described in the Ayurvedic literature Ashtang Hridaya indicated in *Vata Vyadhi Chikitsa*. In view of its therapeutic utility and increasing clinical relevance, establishment of physiochemical analysis is essential.

Objective: To evaluate the physicochemical parameters and develop a chromatographic fingerprint of *Tilvak Ghrita* for quality standardization, as per Ayurvedic Pharmacopoeia of India (API) guidelines.

Materials and Methods: *Tilvak Ghrita* was prepared in accordance with classical references following Good Manufacturing Practices (GMP). *Tilvak Ghrita* formulations was evaluated for the physio-chemical parameters like rancidity, moisture, iodine value, refractive index, saponification value, specific gravity etc and finger printing by thin layer chromatography (TLC).

Results: Physicochemical analysis showed no rancidity, 226.90 saponification value, 37.87 iodine value, 1.4611 refractive index, 1.55 acid value and 0.16% moisture content. TLC analysis demonstrated distinct The Rf values for these spots were observed at 0.25, 0.31 and 0.80 at 365nm, indicating the presence of multiple phyto-constituents.

Conclusion: The physicochemical parameters may serve as reference quality control benchmarks for ensuring the identity, purity and consistency of the formulation, in accordance with API requirements. The chromatographic fingerprinting showed multiple phyto-constituents which validate this herbal formulation in today perspective.

Keywords: *Tilvak Ghrita, Vata Vyadhi,* Organoleptic Properties, Physicochemical Parameters, Thin-Layer Chromatography.

INTRODUCTION

Ayurveda, India's traditional system of medicine, promotes the use of polyherbal formulations to improve therapeutic efficacy through reciprocal interactions between medicinal herbs. Classical *Ayurvedic* pharmaceutics (*Bhaishajya Kalpana*) discusses several dosage forms, with *Ghrita Kalpana* (medicated ghee preparations) being considered superior due to its stability, extended shelf life and ability to extract and administer both lipid- and water-soluble active ingredients. *Ghrita* is defined as *Yogavahi*, which means it enhances the pharmacological activity of medications processed with it and allows for deeper tissue penetration (*Sukshma strotogami),* particularly into the brain system [1 2].

The formulation is explained in authoritative *Ayurvedic* books naming *Ashtanga Hridaya*, showing its clinical importance and long-term therapy[3]. From a modern pharmacological perspective, *ghrita*-based formulations are known for enhancing phyto-constituent bioavailability due to the lipid matrix, which helps intestine absorption and transport across biological membranes. Studies have shown that lipid-based herbal formulations can improve the distribution of active chemicals to target organs, including the brain, supporting the traditional use of *ghrita* in neurological and systemic illnesses[4]. Despite broad traditional use, rising global demand for *Ayurvedic* medicines has generated concerns about quality control, standardization, and batch-to-batch consistency of polyherbal formulations. Variations in raw ingredients, processing methods and storage conditions can have a considerable impact on the physicochemical and medicinal properties of such preparations. Thus, comprehensive analytical evaluation is required to assure identification, purity, safety and reproducibility in compliance with current pharmacopeial standards[5]. The current analytical study of *Tilvak Ghrita* was conducted to assess its physicochemical parameters and quality utilizing standard analytical techniques. Such research are critical for establishing reference

standards, scientifically confirming classical formulations and promoting their wider adoption in evidence-based *Ayurvedic* practice and integrative healthcare systems.

Aims and Objectives

Aim

To establish the analytical quality parameters of *Tilvak Ghrita,* a classical *Ayurvedic* formulation, with special reference to its potential role in the management of *Vata Vyadhi (Pakshaghat)*.

Objectives

- To identify and authenticate the raw drugs used in the preparation of *Tilvak Ghrita* in accordance with Ayurvedic Pharmacopoeia of India (API) standards.
- To prepare *Tilvak Ghrita* in a Good Manufacturing Practices (GMP) certified pharmacy following classical Ayurvedic procedures.
- To evaluate the organoleptic characteristics of *Tilvak Ghrita*, including appearance, color, odor and taste.
- To assess the physicochemical parameters of *Tilvak Ghrita* as per API guidelines.
- To develop a thin-layer chromatographic (TLC) fingerprint of *Tilvak Ghrita* for quality control and standardization.

MATERIAL AND METHOD

Procurement of Raw Materials: - The raw drug was collected from the local market of Jaipur, Rajasthan and after being examined by the Department of *Dravya-guna*, after that *Tilvak Ghrita* was prepared under aseptic condition in the Nagarjuna pharmacy of PGIA, Dr. S. R. Rajasthan Ayurved University, Jodhpur under the supervision of competent authority.

Preparation of *Tilvak Ghrita: -*

All procedures adhered strictly to Standard Operating Procedures (SOPs) to ensure reproducibility, quality and consistency. Each ingredient was measured according to the classical prescription, cleaned, dried, pulverized, and sieved to obtain a course powder, *Murchhita Ghrita* was heated in a large-mouthed container until fumes disappeared, after partial cooling, the specified *Kwatha Dravya* was added, *Dadhi* (curd) was added after half of *Ghrita Paka* and cooking continued, the process was repeated for a day until *Ghrita* reached *Sidhhi Lakshana*[6]. After the preparation of medicine drug was packaged in a sterile, airtight container labelled with the date of manufacture (14/07/2025) and batch number (102/2025).

Table 1: Ingredients and Properties of *Tilvak Ghrita*

S. no	Ingredient	Latin name	Part used	Quantity (Grams)
1	Tilvak	Viburnum narvosum	Root bark	6000
2	Haritaki	Terminalia chebula Retz.	Fruit	4000
3	Vibhitaki	Terminalia belerica Roxb.	Fruit	4000
4	Amalaki	Embelica officinalis Gaertn.	Fruit	4000
5	Bilva	Aegle marmelos Corr.	Root	700
6	Agnimantha	Premna mucronate Roxb.	Root bark	700
7	Kashmari	Gmelina arborea Linn.	Root	700
8	Shyonak	Oroxylum indicum Vent.	Root bark	700
9	Patala	Stereospermum suaveolens DC	Root bark	700
10	Dadhi	Curd	-	55 Kg
11	Yavakshar	Potassium carbonate		5000
12	Ghrita	Ghee		10500
13	Jal	Water		164 litre

Parameters Studied in *Tilvak ghrita*

The analytical evaluation of *Tilvak Ghrita* was carried out following the guidelines outlined in the "Protocol for Testing of *Ayurvedic*, Siddha, and Unani Medicines", published by the National Institute of Science Communication and Information Resources (NISCAIR), CSIR, and issued by the Department of Ayurveda, Yoga, Naturopathy, Unani, Siddha & Homeopathy (AYUSH), Government of India[7].

Analytical study of *Tilvak Ghrita*

Place of work

Cultivator Phyto Lab Pvt. Ltd. Sonamukhi Nagar, Sangaria Fanta, Jodhpur. Sample registration no. CPL/O/25/12/02361. Sample sent to lab date and start of analysis 09/12/2025 and completed 15/12/25 in 7 days[8].

Analytical study was done under following headings-

1. Physio-chemical Parameters
2. Chromatographic fingerprint –TLC

Place of Work and Sample Details

The analyses were performed at Cultivator Phyto Lab Pvt. Ltd., Sonamukhi Nagar, Sangaria Fanta, Jodhpur. Sample Registration No. CPL/O/25/12/02361/2, bearing Sample Code CPL/O/25/12/02361, was sent to the laboratory on 09/12/2025. The analysis was initiated on 10/12/2025 and successfully completed on 15/12/2025. The total duration of the analysis was 7 days.

RESULT

Physiochemical parameters

Physicochemical parameters refer to the physical and chemical characteristics of *Ayurvedic* formulations. These parameters are crucial for assessing the quality, consistency, and stability of the formulations. Parameters include rancidity, saponification value, iodine value, refractive index, acid value, moisture etc.

Table 4: Physicochemical Parameters of *Tilvak Ghrita*

S. No.	Test Parameters	Unit	Result	Reference (API)
1.	Rancidity	_	Absent	API Part II, Vol. IV, 2017
2.	Saponification value	_	226.90	API Part II, Vol. IV, 2017
3.	Iodine value	–	37.87	API Part II, Vol. IV, 2017
4.	Refractive index	_	1.4611	API Part II, Vol. IV, 2017
5.	Acid value	_	1.55	API Part II, Vol. IV, 2017
6.	Moisture	_	0.16	API Part II, Vol. IV, 2017
7.	Specific gravity	_	0.9173	API Part II, Vol. IV, 2017
8.	Thin-Layer Chromatography	–	Major RF Value: 0.25,0.31,0.80,0.98	API Part II, Vol. IV, 2017

DISCUSSION

Organoleptic evaluation is a sensory assessment of a material based on its physical properties, such as appearance, texture, color, odor, and taste. The organoleptic examination serves different functions in the manufacture of *Tilvak Ghrita*. It is critical to ensure that the preparation has a consistent appearance, texture, and taste when evaluating quality. Variations in these factors could indicate batch-to-batch discrepancies or a departure from standard *Ayurvedic* practices[10]. Rancidity in it, which is predominantly induced by *Ghrita* oxidation and hydrolysis, has a substantial impact on its sensory properties and medicinal efficacy. Proper storage, packaging, and quality control techniques are critical for preventing rancidity and maintaining the formulation's potency and effectiveness. Regular organoleptic and chemical testing can assist identify early symptoms of spoiling and guarantee that *Tilvak Ghrita* is safe and good for consumption[11]. The saponification value of long-chain fatty acids, which are found in fat, is low, whereas that of short-chain fatty acids (SCFAs) is high[12]. It has been demonstrated that short-chain fatty acids are a vital source of energy for colonocytes, especially those in the distal colon[13]. The histological, endoscopic, and metabolic similarities between diversion colitis and ulcerative colitis indicate that a dietary SCFA shortage may play a role in the etiology of both diseases. Short chain fatty acids are easily absorbed; there may be a

protective advantage if SCFA production is raised and SCFAs, particularly butyrate, are delivered more effectively[14] to the distal colon. It has a greater saponification value. *Ghrita's,* which are esters, hydrolyze in the presence of an alkali (due to the alkaline nature of *Kalka Dravya* or the other *Drava Dravya* used in the *Snehapaka* procedure), resulting in the production of fatty acid (short chain). This shows that *Tilvak Ghrita* contains higher short-chain fatty acids. As a result, easily absorbed and digested, has a preventative effect, and improves intestine and overall health. The amount of unsaturated fatty substance in the *Ghrita* is determined by the iodine levels. The amount of unsaturated bonds in the fat increases with the iodine numbers. Unsaturated fat supplementation increases overall dietary energy intake to the necessary amounts without negatively affecting blood lipid levels. It also improves nutritional status and lowers systemic inflammation. Polyunsaturated fatty acids, which provide health benefits like controlling blood cholesterol levels, are abundant in lipids with high Iodine levels determine the quantity of unsaturated fat in *Ghrita.* The amount of unsaturated bonds in the fat increases as the iodine concentration rises. Unsaturated fat supplementation raises overall dietary energy intake to the required levels while having no deleterious effects on blood lipid levels. It also enhances nutritional status and reduces systemic inflammation. Polyunsaturated fatty acids, which give health benefits such as lowering blood cholesterol levels, are prevalent in lipids with a high iodine value[15]. The increased iodine value suggests that it contains more unsaturated fatty acids. This analytical value demonstrates that *Tilvak Ghrita* improves nutritional status and lowers systemic inflammation without having a negative effect on blood lipids despite the fatty acid. The increasing unsaturation of the *Ghrita* can be the result of the *Snehapaka* process. The refractive index is an important optical property that measures how light is distorted as it passes through a substance. The refractive index of *Tilvak Ghrita* can provide useful information about the formulation's composition, purity and quality. This feature can be especially useful in determining the consistency of substances, such as *Ghrita's* lipid matrix. The role of the refractive index in quality control is an important consideration. Studies have indicated that the refractive index of oils and fats, such as ghee, can be used as an indicator of purity and adulteration[16]. Variations in the refractive index may indicate the presence of impurities like too much water, artificial additives or inferior raw ingredients. Therefore, maintaining the formulation's intended therapeutic qualities would depend in large part on the refractive index being consistent throughout batches. The acid value, which is related to the stability of the *Ghrita*, indicates the amount of free fatty acid (FFA) in the *Ghrita*. For the *Ghrita*, the production of free fatty acids may be a crucial indicator of rancidity. Triglycerides hydrolyse to produce FFA, which can be accelerated by the *Ghrita's* contact with moisture[17]. The *Ghrita's* stability, flavour, and shelf life are all impacted by its fatty acid composition. The presence of FFA in the *Ghrita* signifies its purity or individuality[18]. *Tilvak Ghrita* contains a higher acid value. This shows that *Ghrita* undergoes hydrolysis during the *Snehapaka* process, which may be facilitated by the reactivity of *Ghrita's* triglycerides with the active components of *Tilvak Ghrita,* resulting in glyserol and free fatty acids. Excessive free fatty acid levels (Acid Value) promote a reduction in *Ghrita* quality. This shows that its nutritional value, stability and shelf life are inferior to those of *Go Ghrita.* Its moisture content influences its shelf life, stability, and medicinal efficacy. *Tilvak Ghrita* is commonly made with *Ghrita,* which has a low moisture content (less than 0.2%). The presence of moisture can promote the growth of microbiological organisms, lowering the product's shelf life. Research on other *Ayurvedic* formulations has established a link between moisture content and spoilage and microbial growth[19]. Excess moisture can alter the balance of ingredients and possibly affect the pharmacological properties of *Tilvak Ghrita*. Moisture could potentially compromise the bioavailability of these compounds. Specific gravity is defined as the density of one substance divided by the density of another, usually water. The specific gravity of liquids and semi-solid preparations indicates the formulation's relative density and purity. Because *Tilvak*, *Trifala* and *Guru pachamoola* extracts play a role in the composition of *Ghrita,* changes in specific gravity could suggest alterations in their concentrations or the presence of impurities, which may interfere with therapeutic efficacy[20]. TLC can be used to separate and identify these compounds using their Rf (retention factor) values, which are unique to each one. Ghosal et al. (2000) confirmed the presence of these chemicals in the herbal extract by comparing their Rf values to recognized standards. TLC can be used to evaluate batch-to-batch consistency and predict the existence of bioactive chemicals. According to a study by Kaur et al. (2018) demonstrated that TLC was an effective method for analysing *Chyawanprash* (another Ayurvedic formulation) for its active principles, ensuring its consistency. By applying similar methods to *Tilvak Ghrita*, TLC can help confirm that each batch meets the desired specification for active compounds, ensuring therapeutic efficacy[21]. TLC can be used to detect unwanted substances such as microbial contamination, solvent residues, or adulterants that may affect the quality and safety. In contrast to the target compounds expected Rf values, impurities may show up on the TLC plate as extra spots. In a study by Gawande et al. (2017), TLC was employed to monitor the purity of Ayurvedic *Ghrita* based formulations, helping to identify potential contaminants and adulterants[22]. A similar approach can be applied to *Tilvak Ghrita* to ensure that it remains free of foreign substances that could compromise its safety or effectiveness. The 'fingerprint' refers to the distinctive pattern of spots produced by the compounds present in the sample when subjected to TLC. This fingerprint can be used to compare samples of *Tilvak Ghrita* from various batches or even from

different manufacturers to ensure authenticity and consistency[23].

CONCLUSION

The present analytical study concludes that *Tilvak Ghrita* exhibits well-defined organoleptic characteristics along with a comprehensive range of physicochemical parameters, including rancidity, moisture content, iodine value, refractive index, saponification value, and specific gravity. These parameters provide essential evidence regarding the quality, safety and efficacy of the formulation. Furthermore, chromatographic fingerprinting through Thin Layer Chromatography (TLC) confirmed the presence of multiple phytoconstituents. The study also establishes specific analytical markers that can serve as reference standards for the identification, quality control, and consistent production of this formulation.

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