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Ingredient Identification of Tiktashatpal Ghrita: A Pharmacognostical HPTLC Study

Ritika Mishra[1*], Anup B. Thakar[2], Ram PoojanVerma[3], Shruti D. Vinchhi[4], and Sandhya Barad[5]

[1]Research Officer, Regional Ayurveda Research Institute Patna–800007.
[2]Head, Department of Panchkarma, Institute of Teaching and Research in Ayurveda, Jamnagar, Gujrat–361008.
[3]Medical Officer, RAC, Basohari, Ambedkarnagar, Uttar Pradesh–224152.
[4]Assistant Professor, Department of Rognidan & Vikriti Vigyan, R. K. University, Rajkot, Gujrat–360020.
[5]Assistant Professor, Department of Rasa Shastra & Bhaishajya Kalpana, Bhargava Ayurved College, Dahemi, Gujrat–388560.

Abstract:

Tiktashatpal Ghrita is a classical polyherbal Ayurvedic formulation traditionally used in the management of various types of *Kustha* (skin disorders). The present study focuses on the pharmacognostical identification of its constituent ingredients and the physico-chemical evaluation of the formulation. Macroscopic and powder microscopy of the raw drugs confirmed the authenticity and quality of the ingredients used. Organoleptic assessment of the coarse powder prepared from crude drugs met the prescribed standards. The formulation was further analyzed for key physico-chemical parameters: the acid value was found to be 4.2815, the saponification value 0.227, refractive index 1.4710 (at room temperature), specific gravity 0.9071 (at room temperature), and loss on drying 1.6177%. High-Performance Thin Layer Chromatography (HPTLC) analysis was performed using an optimized solvent system, which revealed a maximum of five distinct spots. The similarity of R_f values in the alcoholic extract indicated the presence of certain consistent phytoconstituents across batches. All analytical procedures were carried out in accordance with standard protocols, contributing to the quality assessment and standardization of *Tiktashatpal Ghrita*.

Keywords: Tiktashatpal Ghrita, HPTLC, Pharmacognosy, Physico-chemical analysis, Microscopic analysis

1. Introduction:

Tiktashatpal Ghrita, as the name implies, is predominantly composed of herbs rich in *Tikta Rasa* (bitter taste). According to *Charaka*, *Tikta Rasa* possesses properties such as *Vishaghna* (antitoxic), *Krimighna* (antiparasitic), and *Kusthaghna* (anti-dermatological), along with *Twak-Mamsa Sthirikarana* (strengthening of skin and muscle tissues) [1]. Additionally, it exhibits *Kleda*, *Puya*, and *Shoshana* properties—useful in drying excess exudates, pus, and moisture—commonly seen in the pathogenesis of *Kustha* (skin diseases). This *Ghrita* also includes ingredients that are dominant in *Kashaya Rasa* (astringent taste), which, according to *Charaka*, have *Shamana* (pacifying), *Sangrahi* (absorbent), *Sandhankara* (tissue healing), *Pidan-Ropan* (analgesic and healing), *Shoshana* (drying), and *Rakta- Shleshma-Pitta Prashamana* (alleviation of vitiated blood, phlegm, and bile) actions. These pharmacological properties are aligned with the therapeutic needs of managing *Kustha*.

The base medium, *Go-Ghrita* (cow ghee), with its lipophilic nature, acts as an effective drug delivery system, enhancing the absorption and bioavailability of the active constituents. Furthermore, it naturally contains Vitamin A, Vitamin E, antioxidants, and phospholipids—all beneficial for skin health—supporting the *Varna Prasadana Karma* (complexion-enhancing action) described in the classical texts. Beyond dermatological conditions, *Tiktashatpal Ghrita* is also indicated in ailments such as *Gulma* (abdominal lumps), *Arsha* (piles), *Grahani* (malabsorption syndrome), *Shotha* (inflammation), and *Pama* (scabies), among others [2].

Adulteration of herbal formulations is a significant concern, often aimed at increasing bulk, enhancing appearance, mimicking potency, or substituting valuable ingredients. Identifying and tracing such impurities is essential to ensure the authenticity, efficacy, and safety of Ayurvedic formulations.

The objective of the present study is to confirm the genuineness of the ingredients used in *Tiktashatpal Ghrita* and to verify the presence of pharmacopoeial components as prescribed in the Ayurvedic Pharmacopoeia of India (API) [3-5]. The study includes pharmacognostic and physico-chemical evaluations of the following ingredients: *1) Nimba*,

2) *Patola*, 3) *Darvi*, 4) *Duralabha*, 5) *Tiktarohini*, 6) *Haritaki*, 7) *Bibhitaki,* 8) *Amalaki*, 9) *Parpataka*, 10) *Chandana*, 11) *Kiratatikta*, 12) *Magadhi*, 13) *Triyaman*, 14) *Musta*, and 15) *Vatsakabija*.

2. Materials and Methods

2.1 Collection and Authentication of Raw Drugs

The ingredients of *Tiktashatpal Ghrita* [see Table 1 and 2] were procured from the Pharmacy of Gujarat Ayurved University, Jamnagar. All raw drugs were authenticated and identified in the Pharmacognosy Laboratory at the Institute for Postgraduate Teaching and Research in Ayurveda (IPGT & RA), Gujarat Ayurved University. *Go-Ghrita* (cow's ghee) was sourced from Khadi Gramodyog Bhandar, Jamnagar. The final formulation of *Tiktashatpal Ghrita* was prepared in the Pharmacy of Gujarat Ayurved University, Jamnagar.

2.2 Method of Preparation of Tiktashatpal Ghrita

Tiktashatpal Ghrita was prepared in the Department of Rasashastra and Bhaishajya Kalpana (RS & BK), IPGT & RA, Gujarat Ayurved University. All identified herbs were thoroughly washed and shade-dried before use. Ten *Kwatha Dravyas* (decoction drugs) were taken— each in a quantity of 2.5 kg (1 part)—and combined with approximately 400 liters of water (16 times the total weight of decoction drugs). This mixture was boiled on a low flame and reduced to 120 liters. The decoction was filtered and combined with 30 kg of plain *Go-Ghrita* and six *Kalka Dravyas* (06 drugs as 1 part/0.6kg each). The formulation followed the classical ratio of Kalka : Ghrita : Kwatha as 1 : 4 : 16 [6]. The mixture was then boiled on a moderate flame with continuous stirring. When *Madhyama Paka* (medium consistency) [7] was achieved, heating was stopped and the formulation was filtered using a clean, dry, white cotton cloth. The final yield was approximately 27 kg of *Tiktashatpal Ghrita*.

2.3 Pharmacognostical Evaluation of Ingredients

2.3.1 Organoleptic Study

The coarse powders of individual ingredients were evaluated for sensory characteristics such as color, taste, and odor. Observations were recorded for each sample.

2.3.2 Powder Microscopy

The powders of the relevant parts of each plant were examined microscopically without staining. Microphotographs were captured using a Carl Zeiss binocular microscope equipped with a camera [8,9].

2.3.3 Physico-Chemical Analysis

Tiktashatpal Ghrita was subjected to standard physicochemical testing including:

- **Acid Value**
- **Saponification Value**
- **Refractive Index**
- **Iodine Value**
- **Specific Gravity**
- **Loss on Drying**

These analyses were performed in accordance with standard pharmacopeial protocols at the Pharmaceutical Chemistry Laboratory, IPGT & RA, Jamnagar.

2.4 High-Performance Thin Layer Chromatography (HPTLC)

2.4.1 Sample Preparation

The *Ghrita* sample was first adsorbed onto silica gel and extracted with hexane. The hexane fraction was discarded. The remaining material was extracted three times with methanol. The methanol extracts were pooled, filtered, and evaporated to dryness. The dried residue was re-dissolved in methanol and used for Thin Layer Chromatography (TLC) analysis [10].

2.4.2 Chromatographic Conditions

- **Stationary Phase:** Silica gel GF 254(E. Merck) precoated TLC plates
- **Mobile Phase:**
- **Instrument:** Camag HPTLC system (Catalog No. 0276481, Switzerland)
- **Sample Application:** Camag Linomat V
- **Development Chamber:** Camag Twin Trough Chamber
- **Plates:** Pre-coated silica gel GF254 plates
- **Chamber Saturation Time:** 30 minutes
- **Development Time:** 30 minutes
- **Development Distance:** 7 cm

- **Scanner:** Camag scanner III
- **Detection:** Deuterium and Tungsten lamps
- **Data Acquisition:** WinCATS software

2.4.3 Procedure

Before use, TLC plates were prewashed with methanol and dried. Sample solutions were applied to the plate as narrow bands using the Camag Linomat V applicator. The mobile phase (20 ml) was poured into the development chamber, which was allowed to saturate for 30 minutes. The plate was then developed until the solvent front reached 80 mm from the base. After development, plates were dried under a stream of air. Detection and quantification of components were carried out using the Camag TLC Scanner III at wavelengths of 254 nm and 366 nm.

Table 1: Ingredients of *Tiktashatpal Ghrita* (Kwath Dravya)

Sr.no.	Drugs	Latin name	Parts used	Parts
1.	*Nimba*	*Azadirecta indica*	*Twak*	1
2.	*Patola*	*Trichosanthes dioica*	*Patra*	1
3.	*Darvi*	*Berberis species*	*Mula*	1
4.	*Duralabha*	*Fagonia Arabica*	*Panchang*	1
5.	*Tiktarohini*	*Picrorhiza kurroa*	*Mula*	1
6.	*Haritaki*	*Terminalia chebula*	*Phala*	1
7.	*Bibhitaki*	*Terminalia belerica*	*Phala*	1
8.	*Amalaki*	*Embelica officinalis*	*Phala*	1
9.	*Parpataka*	*Molluga stricta*	*Panchang*	1
10.	*Triyaman*	*Ficus heterophylla*	*Moola*	1

Table 2: Ingredients of *Tiktashatpal Ghrita* (six *Kalka Dravyas*)**

Sr. no.	Drugs	Latin name	Parts used	Parts
1.	*Chandan*	*Santalam album*	*Sara*	¼
2.	*Kiratatikta*	*Swertia chirayta*	*Panchang*	¼
3.	*Magadhi*	*Piper longum*	*Phala*	¼
4.	*Triyaman*	*Ficus heterophylla*	*Moola*	¼
5.	*Musta*	*Cyperus rotundus*	*Kanda*	¼
6.	*Vatsakbija*	*Holarrhenaantidysentrica*	*Bija*	¼

3. Observations and Results:

3.1 Pharmacognostical analysis: Pharmacognostical evaluation of *Tiktashatpal Ghrita* was conducted through organoleptic examination, powder microscopy, and physico-chemical analysis. The detailed observations are presented in the sections below.

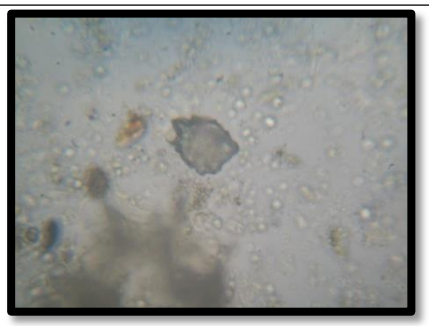
3.1.1 Organoleptic Analysis: Organoleptic evaluation of the powdered formulation was conducted to assess its sensory characteristics, which serve as preliminary indicators of quality and authenticity. As shown in Table 3, the powder exhibited a light brown color, characteristic aromatic odor, bitter taste, and a coarse texture. These features are consistent with the expected organoleptic properties of the constituent herbal ingredients and indicate proper preparation and preservation of the formulation.

Table 3: Organoleptic Characters of Tiktashatpal Ghrita Powder

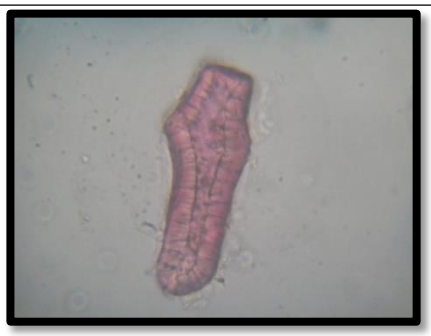
Sr. No.	Characters	Results
1	Color	Light brown
2	Odor	Aromatic
3	Taste	Bitter
4	Touch	Coarse

3.1.2 Microscopic Analysis:

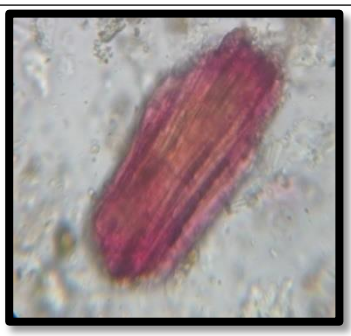
Powder microscopy of individual ingredients of *Tiktashatpal Ghrita* was carried out to observe characteristic diagnostic features. The observations were recorded, and relevant microphotographs were taken for reference and verification, as shown in Fig.1.



Rosset of *Vibhitak*



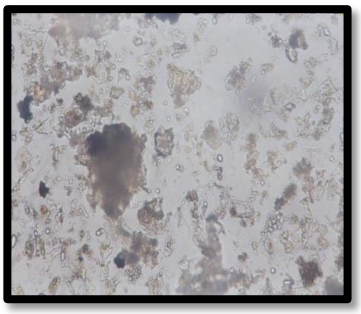
Sclerid of *Vibhitaki*



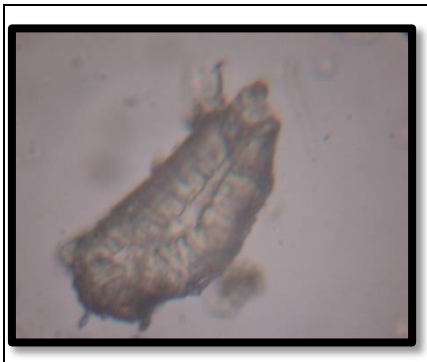
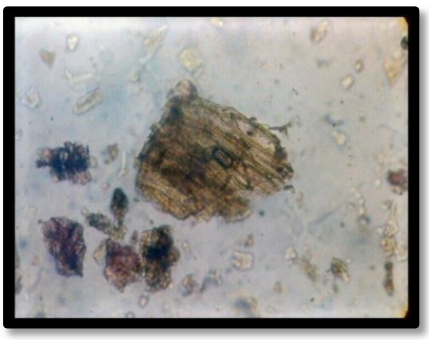
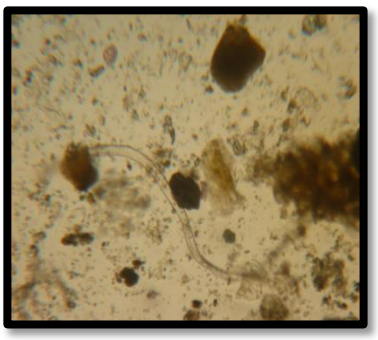
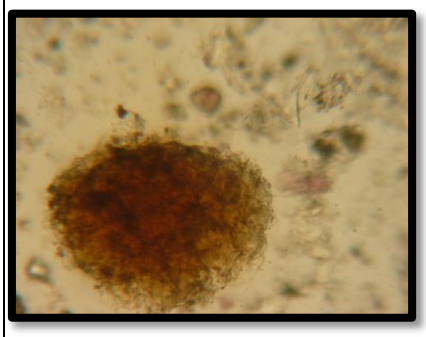
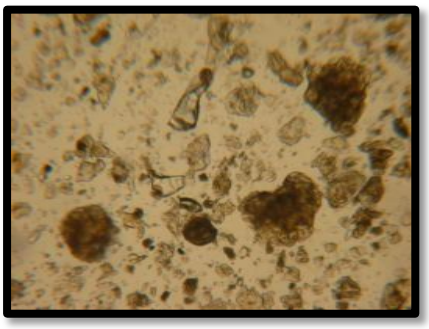
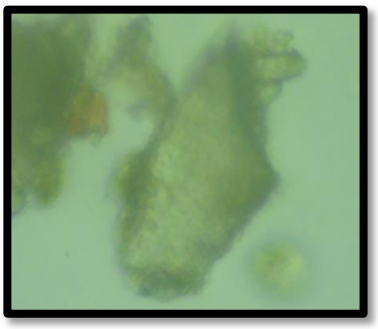
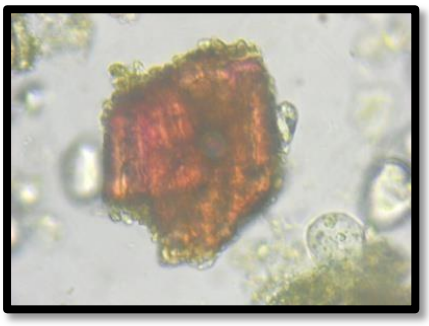
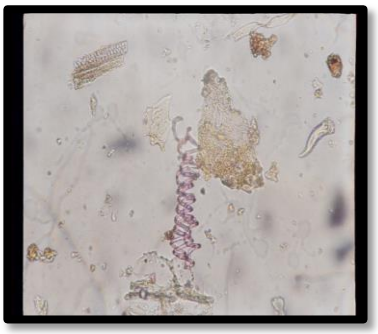
Sclerides of *Amalaki*

Trichome of *Vibhitaki*

Pitted Sclerites of *Haritaki*



Rosset of *Kiratikta*

		
Scleroids of *Haritaki*	**Fragments of fiber of *Katuki***	**Fibers of *Amalaki***
		
Mesocarp of *Amalaki*	**Silica deposition with tannin of *Amalaki***	**Black deberis of *Pippali***
		
Bottle neck stone cells of *Pippali*	**Stone cells of *Pippali***	**Trichome,spiral pitted vessels of *Katuki***

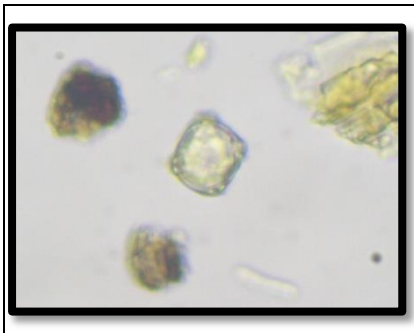
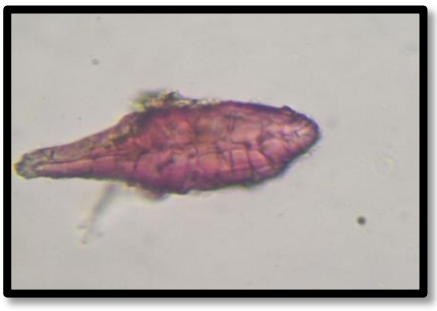
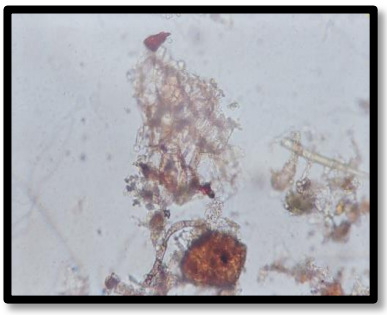
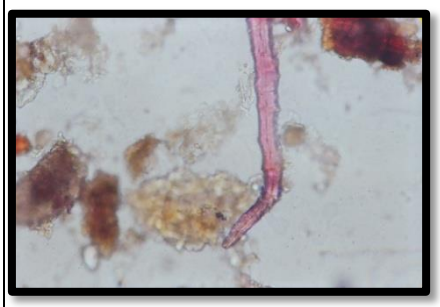
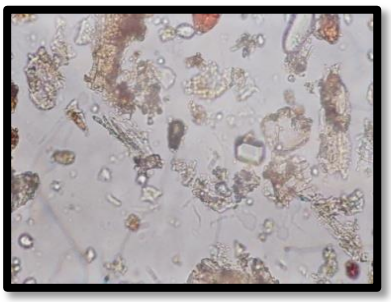
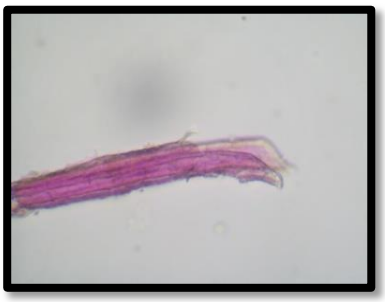
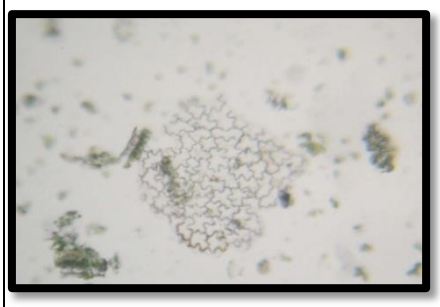
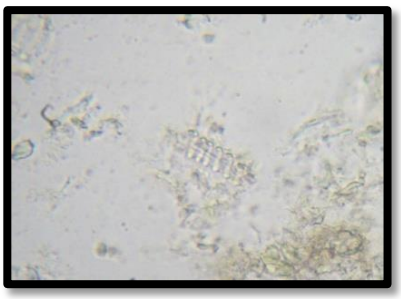
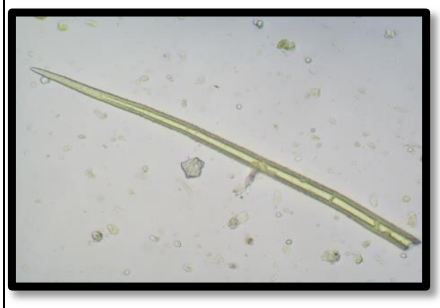
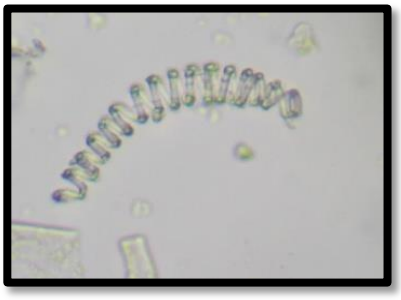
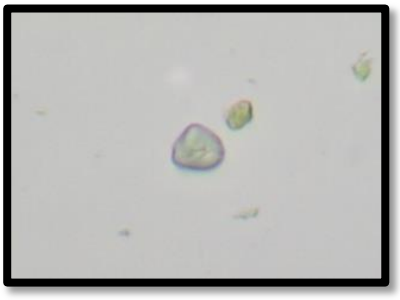
 **Pollen grain of *Kiratikta***	 **Stone cells of *Kiratikta***	 **Cork in surface of Nimba**
 **Lignified fiber of *Nimba***	 **Prismatic starch of *Nimba***	 **Lignified fibres of *Patola***
 **Epidermal cells of *Parpat***	 **Spiral vessels of *Parpat***	 **Unicellular Simple Trichome of *Parpat***
 **Simple fibres of *Patola***	 **Spiral vessels of *Patola***	 **Starch grains of *Patola***

Figure 1: Microphotographs of *Tiktashatpal Ghrita* ingredients.

3.1.3 Physico-Chemical Analysis: The physico-chemical evaluation of *Tiktashatpal Ghrita* was carried out to determine its quality, stability, and compliance with standard parameters. As shown in Table 4, the formulation exhibited a Loss on Drying value of 1.6177%,

indicating low moisture content and suggesting good shelf stability. The Specific gravity was found to be 0.9071, which is within the typical range for ghee-based formulations. The Acid value was 4.2815, reflecting the extent of free fatty acids present, and the Saponification value was 0.227, indicative of the fatty acid composition of the *Ghrita*. The Refractive index measured at room temperature was 1.4710, consistent with values expected for medicated ghee. These results confirm the formulation's acceptable quality and adherence to standard pharmaceutical properties.

Table 4: Physico-Chemical Analysis of *Tiktashatpal Ghrita***.**

Sr. No.	Analytical Parameters	Results
1.	Loss On Drying	1.6177 %
2.	Specific Gravity	0.9071
3.	Acid Value	4.2815
4.	Saponification Value	0.227
5.	Refractive Index	1.4710

3.2 HPTLC Analysis of *Tiktashatpal Ghrita*:

The HPTLC analysis of the methanolic extract of *Tiktashatpal Ghrita* was carried out to assess the presence and distribution of phytochemical constituents at different wavelengths. The chromatographic profiles obtained at 254 nm and 366 nm are illustrated in Figures 2 and 3, respectively, and the corresponding R_f values are summarized in Table 5. At 254 nm (short-wave UV), the densitogram revealed five distinct peaks with R_f values of 0.03, 0.05, 0.64, 0.68, and 0.93, indicating the presence of multiple UV-absorbing compounds. In contrast, at 366 nm (long-wave UV), three peaks were observed at R_f values of 0.04, 0.68, and 0.97, which likely correspond to fluorescent or near-visible absorbing phytochemicals. The R_f value of 0.68, common to both wavelengths, may indicate the presence of a chemically stable or prominent marker compound within the formulation. The complexity of the chromatographic pattern reflects the polyherbal nature of *Tiktashatpal Ghrita* and confirms the presence of a diverse array of phytoconstituents.

Additionally, this pattern is more clearly represented in the three-dimensional (3D) densitogram at 254 nm, which provides a comparative visualization of the R_f values of the sample in relation to the standard [see Figures 4 and 5]. These findings further substantiate the qualitative richness and authenticity of the formulation.

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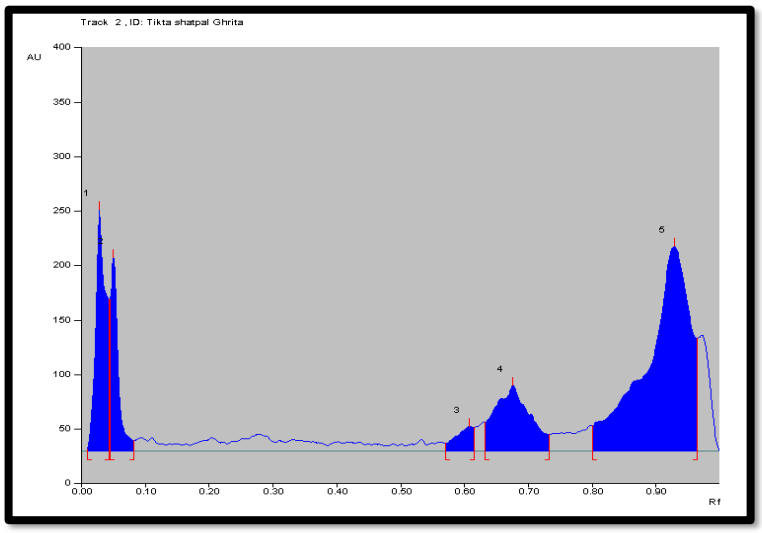

Figure 2: Densitogram of *Tiktashatpal Ghrita* at wavelength 254 nm

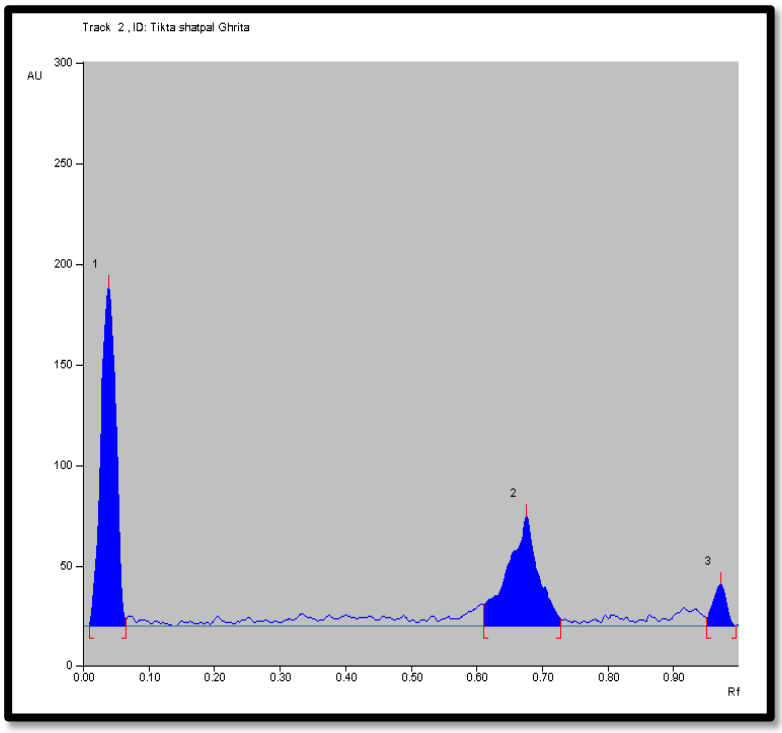

Figure 3: Densitogram of *Tiktashatpal Ghrita* at wavelength 366 nm

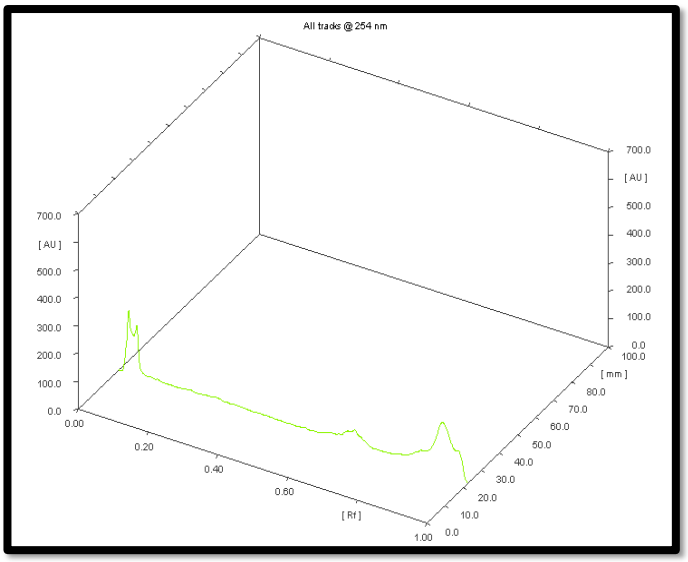

Figure 4: Three-Dimensional HPTLC (3D) Densitogram of *Tiktashatpal Ghrita* at 254 nm (Short-Wave UV)

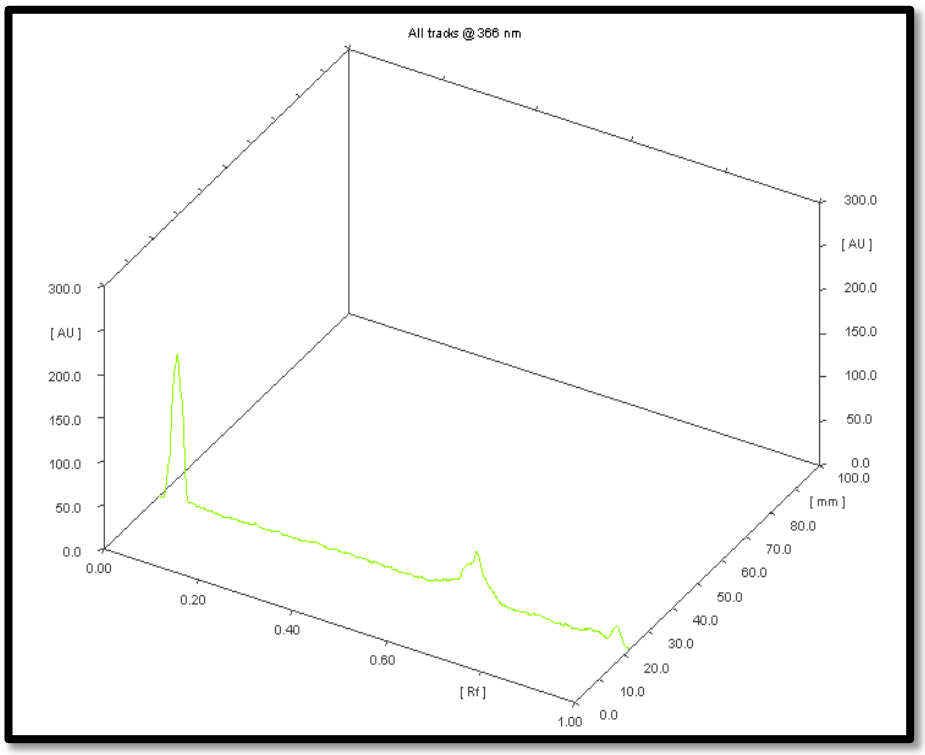

Figure 5: Three-Dimensional HPTLC (3D) Densitogram of *Tiktashatpal Ghrita* at 366 nm (Long-Wave UV).

Table 5: Three-Dimensional HPTLC (3D) Densitogram Analysis of *Tiktashatpal Ghrita*

Wavelength	**No. of Spots**	***R f* values**
Short UV (254 nm)	05	0.03, 0.05, 0.64, 0.68, 0.93
Long UV 366nm	03	0.04, 0.68, 0.97

4. Discussion:

A pharmacognostical study confirmed the authentication of the individual raw drugs used in the preparation of *Tiktashatpal Ghrita*, and the identification was further cross- verified with standard references [11-14]. Microscopic examination of the ingredients revealed characteristic features such as oleoresins, pitted vessels, tannins, prismatic crystals, stomata, and fibers, validating the identity of each component.

Physico-chemical parameters—including acid value, saponification value, refractive index (RI), and specific gravity—were found to be within the standard reference range [15]. These results indicate that the formulation remains chemically stable and free from rancidity, even after ten months of storage. Overall, the findings affirm the standard quality and integrity of the prepared *Ghrita* formulation.

5. Conclusion:

The pharmacognostical analysis confirmed the identity and authenticity of the raw ingredients used in the preparation of *Tiktashatpal Ghrita*. The presence of specific phytochemical components aligns with the intended therapeutic actions of the formulation. HPTLC analysis revealed three distinct peaks at 254 nm and five peaks at 366 nm, reflecting the presence of multiple bioactive compounds. These findings suggest that the formulation adheres to key qualitative standards and demonstrates good chemical stability. The data generated in this study can serve as a valuable reference for future research and quality control of similar Ayurvedic preparations.

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