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Research Article

PHARMACOGNOSTIC AND ANALYTICAL PROFILING OF *TIMIRAHARA LAUHA* CAPSULE: AN AYURVEDIC HERBO-MINERAL FORMULATION

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ABSTRACT

Timirahara Lauha is a classical Ayurvedic polyherbomineral formulation primarily indicated in the management of *Timira*- a condition analogous to early-stage of visual impairment, including refractive errors and degenerative ocular changes. The formulation is composed of *Lauha Bhasma (Bhasma)* along with potent *Rasayana* (rejuvenative) herbs like *Amalaki* (*Emblica officinalis* Linn), *Bibhitaki* (*Terminalia bellirica* Roxb.), *Haritaki* (*Terminalia Chebula* Ritz.), *Yashtimadhu* (*Glycyrrhiza glabra* Linn.) and *Padma* (*Nelumbium speciosum* Willd.). This study aims to ensure its quality and efficacy. **Material and Methods:** The study included the authentication of raw ingredients and the preparation of *Timirahara Lauha* capsule. The prepared drug was evaluated for organoleptic, physico-chemical, heavy metal content, microbial analysis, mycotoxins and High-Performance Thin-Layer Chromatography (HPTLC) at Quality control laboratory of the All India Institute of Ayurveda (AIIA) and ARBRO pharmaceuticals Pvt. Ltd., New Delhi. **Results:** Physico-chemical parameters showed loss on drying (3.85%), total ash (47.65%), acid-insoluble ash (41.45%), water-soluble ash (1.00%), water-soluble extractive (22.31%) and alcohol-soluble extractive (12.87%). pH (5% aqueous solution) was 3.84, with a uniformity of weight of 249.8mg and disintegration time of 18 min. HPTLC fingerprinting showed distinct bands at 254nm, 366nm and after derivatization at 540nm with characteristic Rf values. Heavy metal content, aflatoxins, and microbial load were below permissible limits. Gallic acid content was 7.72% w/w. **Discussion:** The analytical findings confirm the formulation's compliance with pharmacopoeial standards. The absence of contaminants and defined HPTLC profile support its quality and authenticity. High ash content aligns with the presence of *Lauha Bhasma.*

INTRODUCTION

Visual disorders have surged globally due to increasing screen exposure, aging and lifestyle disorders. *Timira* (errors of refraction/partial blindness), as described in Ayurvedic texts, refers to a progressive blurring of vision often attributed to vitiation of *Doshas* (regulatory functional factors of the body) affecting the visual pathway. *Timira* is the *Drishtigata Roga* (diseases causing visual disturbance) which initially manifests as mild visual impairment (*Avyakta Darshana*) and if left untreated, can progress to complete vision loss (*Linganasha*). *Timirahara Lauha*[1] is an herbo-mineral formulation described in *Rasendra Sara Sangraha* as a therapeutic approach for *Timira*. Pharmacognostic and phytochemical analyses of these formulations are very helpful to ensure their quality, safety and efficacy. The World Health Organization (WHO) reports that 70–95% of the global population, especially in developing countries, relies on traditional, complementary, alternative or non-conventional medicines for their healthcare needs[2]. *Timirahara Lauha* Capsule contains *Triphala, Yashtimadhu, Padma* and *Lauha Bhasma*. This combination provides antioxidant, *Rasayana* and *Chakshushya* effects. Standardization of *Timirahara Lauha* capsule is essential to ensure quality, safety and efficacy. Physico-chemical and chromatographic

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profiling plays a crucial role in achieving this. The present study focuses on the evaluation of *Timirahara Lauha* capsule using standard methods to establish its identity, purity and strength. The analysis includes assessment of organoleptic features, ash values, extractive values, pH and chromatographic fingerprinting by HPTLC.

The ingredients of *Timirahara Lauha* were procured from Pharmacy of All India Institute of Ayurveda and from the market of New Delhi. All these were identified and authenticated at the pharmacognosy lab, RRDR (Regional Raw Drug Repository), Department of *Dravyaguna*, AIIA, New Delhi, with accession numbers RRDR/AIIA/phg./542.

It was prepared as per the standard method described in Ayurvedic Formulary of India at the pharmacy of All India Institute of Ayurveda, New Delhi. As per literature, all ingredients were shade-dried, individually powdered, sieved through a #85 mesh and then mixed together in the necessary proportions to produce a uniformly blended *Churna.* After that 250 mg size of capsule were made from this powder in the pharmacy attached to AIIA, New Delhi[3]. [Table 1]

Table 1: Ingredients of *Timirahara Lauha*

S.No.	Sanskrit Name	Botanical Name	Part used	Ratio
1	*Haritaki*	*Terminalia chebula* Retz.	Fruit	1
2	*Bibhitaki*	*Terminalia belerica* Roxb.	Fruit	1
3	*Amalaki*	*Phyllanthus emblica* Linn.	Fruit	1
4	*Yashtimadhu*	*Glycyrrhiza glabra* Linn.	Root	1
5	*Kamal*	*Nelumbium speciosum* Willd.	Flower	1
6	*Lauha*	Calcined iron	*Bhasma*	5

RESULT

Analytical study

Organoleptic Parameters

Organoleptic evaluation revealed the formulation as a fine, light brown, bitter powder with characteristic odour.

Table 2: Organoleptic properties of *Timirahara Lauha*

Test	Result
Appearance	Fine powder
Colour	Light Brown
Odour	Characteristics
Taste	Bitter

Physico-chemical parameters

Physicochemical parameters were determined to assess the quality and purity of the formulation according to API part-II, volume-3 guidelines[4]. The loss on drying was found to be 3.85%. Total ash and acid-insoluble ash values were 47.65% and 41.45% respectively. Water-soluble ash and extractive values (water-soluble:22.31%, alcohol-soluble:12.87%) provide insight into the solubility and extraction potential of the active constituents. The pH of the 5% aqueous solution was 3.84. [Table 3]

Table 3: Physico-Chemical Parameters of *Timirahara Lauha*

Test	Result
Loss on drying (%)	3.85
Total ash (%)	47.65
Acid-insoluble ash (%)	41.45
Water –soluble ash	1.00
Water-soluble extractive (%)	22.31
Alcohol-soluble extractive (%)	12.87
pH (5% aq. solution)	3.84
Uniformity of weight (gm)	249.8
Disintegration time (min)	18 min

Uniformity of weight/ weight variation test

The consistency in capsule weight was checked by selecting 20 tablets at random from a batch, weighing them individually, and comparing each weight to the mean weight to ensure uniformity[5].

Heavy metal, Mycotoxin and Microbial testing

Performed at ARBRO pharmaceuticals Pvt. Ltd. Using ICP-MS for heavy metals, LC-MS/MS for aflatoxins, and standard microbial culture methods. (Table 4, 5, 6)

Table 4: Heavy Metals

Heavy metals	Results
Lead (as Pb)	Below quantification limit
Cadmium (as Cd)	Below quantification limit
Mercury (as Hg)	Below quantification limit
Arsenic (as As)	Below quantification limit
Iron	

Table 5: Mycotoxins

Mycotoxins	Results
Total Aflatoxin	Below quantification limit
Aflatoxin B1	Below quantification limit
Aflatoxin B2	Below quantification limit
Aflatoxin G1	Below quantification limit
Aflatoxin G2	Below quantification limit

Table 6: Microbial Analysis

Microbial analysis	Results
Total microbial plate count (cfu/g)	5900
Total yeast & mold count (cfu/g)	360
Escherichia coli/g	Absent
Pseudomonas aeruginosa/g	Absent
Salmonella/g	Absent
Staphylococcus aureus/g	Absent
Enterobacteriaceae (cfu/g)	Less than 10

Gallic Acid

Gallic acid content was 7.72% w/w, which acts as an excellent antioxidant and is responsible for many other important biological activities such as anti-inflammatory, anti-cancer, anti-bacterial, anti-viral and anti-mutagenic effects[6,7]. In oxidative stress conditions, lens epithelial cells undergo apoptosis, which leads to cataract. Gallic acid protects lens epithelial cells by reducing oxidative damage, stabilizing mitochondrial function and preventing apoptosis. (Table 7)

Table 7: Gallic acid

Parameters	Results
Gallic acid	7.72 % w/w

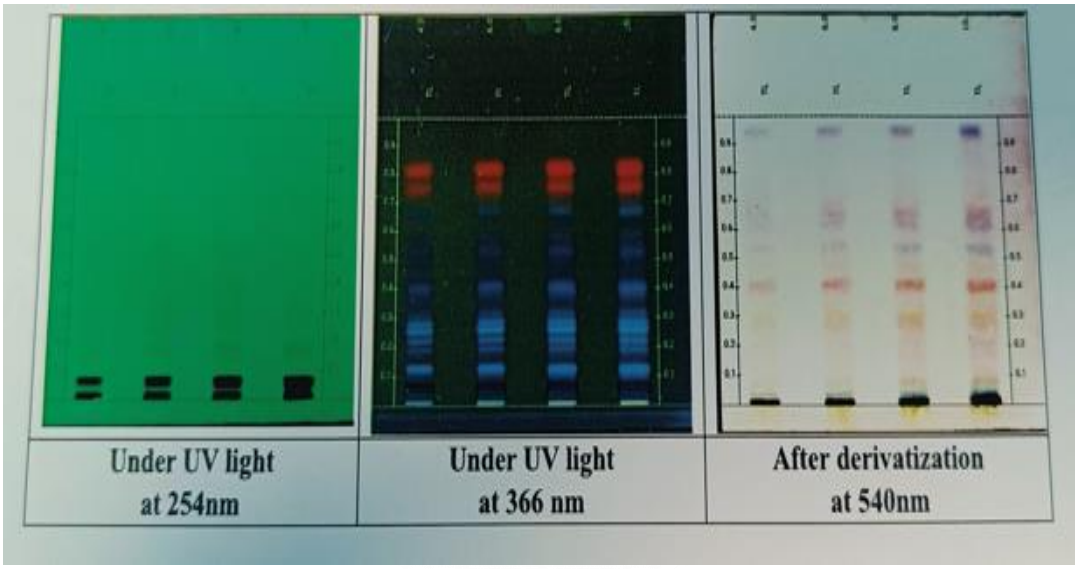
High-Performance Thin-Layer Chromatography (HPTLC)

Thin layer chromatography is the most common chromatographic method to detect the number of compounds in a product. It also helps to determine the purity of the sample[8]. (Table 8) (Insert Figure 1 here)

Table 8: Fingerprint of *Timirahara Lauha*

Sample preparation	
Extract	Methanolic extract
Stationary Phase	TLC precoated plate silica gel 60 F_{254} of 0.2 mm thickness
Volume of Sample	4.0,6.0,8.0 & 10.0 µl
Mobile phase	Toluene:Ethyl acetate: Formic Acid (7:3:0.1) V/V/V/
Observation (wavelength)	254nm, 366nm & 540 nm
Result	
Wavelength	R_f (10 µl)
At 254 nm	0.15, 0.28, 0.41
At 366 nm	0.10, 0.18, 0.21, 0.25, 0.27, 0.41, 0.54, 0.70, 0.79, 0.86
At 540 nm	0.06, 0.20, 0.28, 0.41, 0.55, 0.68, 0.80

Fig 1: HPTLC of *Timirahara Lauha* capsule



DISCUSSION

The physicochemical parameters assessed in the study, including moisture content, ash values (total ash, acid-insoluble ash) and extractive values (alcohol and water soluble), fell within acceptable limits as per the standards prescribed in the Ayurvedic pharmacopoeia of India (API). The high ash content and acid-insoluble ash percentage highlighting the significant inorganic content due to the presence of *Lauha Bhasma*. The low moisture content (3.85%) indicates reduced risk of microbial contamination and good stability. Extractive values suggest the presence of water and alcohol soluble phytoconstituents contributing to therapeutic activity. Safety parameters confirm the absence of heavy metal toxicity, microbial contamination and mycotoxins, ensuring consumer safety. Acidic pH may influence bioavailability. Gallic acid, a known antioxidant was quantified at 7.72% w/w, supporting its role in preventing oxidative stress related ocular damage. These parameters serve as essential quality control tools to ensure batch-to-batch consistency and to detect adulteration or substandard raw materials.

The High-Performance Thin Layer Chromatography (HPTLC) study of *Timirahara Lauha* capsule was conducted to generate a detailed chemical fingerprint, which serves as an essential tool for quality control and standardization of the formulation.

The methanolic extract of the capsule was analyzed using precoated silica gel $GF_{60(254)}$ TLC plates, with Toluene: Ethyl acetate: Formic acid (7:3:0.1 v/v/v) as the mobile phase. The developed chromatograms were visualized under UV light at 254nm and 366nm, and also post-derivatization at 540nm, to detect a broad range of phytoconstituents.

Under short-wave UV light at 254nm, distinct dark bands were observed, indicating the presence of compounds that absorb in the UV region, primarily phenolic and other aromatic compounds. The chromatogram at 366nm revealed bright fluorescent bands of various colors and intensities, suggesting the presence of flavonoids, coumarins, and other UV-fluorescent phytoconstituents.

After derivatization, the plate observed at 540nm displayed multiple well-separated colored bands, confirming the presence of various classes of secondary metabolites, including terpenoids and glycosides that develop color reactions with the derivatizing reagent.

The Rf values obtained ranged from 0.06 to 0.86, with notable reproducibility across all lanes. The presence of multiple bands with varying Rf values reflects the polyherbal nature of *Timirahara Lauha* and the diversity of its phytochemical constituents. This chemical fingerprint can serve as a reference standard for future batch verification, ensuring consistency and authenticity of the formulation during manufacturing and quality control.

The well-resolved bands and distinct chromatographic pattern demonstrate that the capsule contains a broad spectrum of bioactive principles, supporting its traditional therapeutic claims for ocular health. Furthermore, the reproducibility and clarity of the bands in this study reinforce the reliability of HPTLC as a rapid and cost-effective analytical tool for routine monitoring of complex Ayurvedic formulations.

CONCLUSION

The analytical study validates that *Timirahara Lauha* capsules are pharmaceutically acceptable, safe and standardized. The characteristic HPTLC fingerprint profile can be used as a reference marker for routine quality control and to detect any adulteration or deviation during large-scale manufacturing.

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Authors Contribution

Conceptualization, writing, review and editing by SS. Supervision and review by NB and MR. All authors have read and agreed to the published version of the manuscript.

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