RESEARCH PAPER

CHROMATOGRAPHIC EVALUATION OF A TRADITIONAL POLYHERBAL MEDICATED OIL (TAILA) USING PHYSICOCHEMICAL, PHYTOCHEMICAL, AND HPTLC-BASED ANALYTICAL METHODS

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Abstract

Ayurveda employs medicated oils (Taila) as a widely used dosage form for the topical management of skin disorders, inflammation, wounds, and musculoskeletal conditions. Because such polyherbal formulations frequently lack rigorous analytical standardization, the present study aimed to prepare and evaluate a traditional polyherbal medicated oil formulated from Daruharidra (Berberisaristata stem), Lodhra (Symplocosracemosa stem bark), and Mañjiṣṭhā (Rubiacordifolia stem) through organoleptic, physicochemical, phytochemical, and chromatographic (TLC/HPTLC) analyses. The formulation exhibited a reddish-brown colour, characteristic herbal odour, clear appearance, and smooth oily consistency. Physicochemical parameters — acid value (3.5 mg KOH/g), peroxide value (5.8 meq/kg), pH (5.2), specific gravity (0.90) — fell within acceptable ranges. Preliminary phytochemical screening confirmed the presence of alkaloids, anthraquinones, phenolics, and flavonoids. TLC screening detected a faint spot corresponding to the berberine standard but did not detect purpurin or gallic acid, indicating limited sensitivity for the complex oil matrix. Subsequent HPTLC analysis, performed on silica gel 60 F254 aluminium plates with chloroform: ethyl acetate: methanol: formic acid (4:2:1:0.2, v/v/v) as the mobile phase and densitometric scanning at 254/366 nm, confirmed the presence of berberine with a well-resolved peak. Quantification using a linear calibration curve (R^2 = 0.999202; CV = 1.52%) established a berberine content of 3.074 µg per 300 mg/mL of oil, corresponding to 0.00102% w/w. Purpurin and gallic acid remained undetected under the optimized HPTLC conditions, possibly due to low concentration, limited methanolic extractability, or poor partitioning from the oil matrix. These findings support HPTLC as a suitable, sensitive, and reproducible technique for the chromatographic evaluation and quality control of traditional herbal oil formulations, while underscoring the need for further method validation, stability studies, and pharmacological evaluation.

Keywords: Ayurveda; Taila; medicated oil; HPTLC; berberine; phytochemical screening; standardization

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.1. Introduction [1-4]

Ayurveda is a holistic health care system that prescribes the use of various medicated oils, applied to the body with or without massage, for therapeutic benefit against specific indications. Detailed processing methods for such oils are described in classical Ayurvedic texts recognized under the Drugs and Cosmetics Act and in the Ayurvedic Formulary of India (AFI).

Among the various forms of herbal preparations, medicated oils are widely used topically in the treatment of skin disorders, inflammation, wounds, and infections. These oils are typically prepared by extracting the active constituents of medicinal plants into an oil base through controlled heating, yielding a formulation rich in phytochemicals that contribute to therapeutic activity. Medicinal plants used in such preparations may involve whole parts, roots, seeds, leaves, flowers, or bark, and may be administered orally, applied to the skin, or inhaled. Many medicinal plants contain phytochemicals such as alkaloids, phenolics, flavonoids, and anthraquinones, several of which exhibit antimicrobial, antioxidant, anti-inflammatory, and wound-healing properties.

Skin is continuously exposed to environmental stressors such as pollution, UV radiation, and microbial challenge, which can lead to inflammation, oxidative damage, and uneven pigmentation. This has driven growing interest in natural, plant-based remedies that soothe, protect, and restore the skin without causing irritation or adverse reactions. Herbal constituents such as berberine, purpurin, and gallic

acid are well known for their anti-inflammatory and antioxidant properties, making them valuable in topical applications by reducing oxidative stress, calming inflammation, and supporting overall skin health. Consequently, the scientific evaluation of such herbal preparations has gained considerable importance in recent research.

Maintaining the quality of herbal products remains challenging, as it requires a strict set of processes to ensure consistency within specified limits; traditional medicine formulations often lack adequate scientific evaluation and analytical standardization. Phytochemical and chromatographic analysis is therefore essential to ensure the quality, safety, and effectiveness of herbal products. Modern analytical techniques such as High-Performance Thin Layer Chromatography (HPTLC) are widely used to identify marker compounds and establish chromatographic fingerprints of herbal formulations.

The choice of dosage form is critical to therapeutic efficacy, stability, patient compliance, and overall formulation performance. In Ayurveda, Taila (medicated oil) is among the most extensively used dosage forms for topical administration, owing to its ability to deliver herbal phytoconstituents into the skin while simultaneously providing nourishment and protection. The preparation of medicated oils through the process of SnehaKalpana is described in classical Ayurvedic texts and continues to be widely practiced in the management of skin disorders, wounds, inflammatory conditions, and musculoskeletal diseases. Accordingly, the present study was undertaken to evaluate a traditional herbal oil formulation through physical, phytochemical, and chromatographic analysis.

1.1 High-Performance Thin Layer Chromatography (HPTLC) [5-13]

HPTLC is a well-established separation technique that has evolved considerably from conventional thin-layer chromatography through automation, higher resolution, and more accurate quantitative capability. The HPTLC layer is uniform and thin, which reduces analysis time, improves resolution, and supports in situ quantification. The stationary phase is a finely divided solid coated with a thin layer of inert support material — most commonly silica gel for adsorptive TLC, or modified silica such as octadecylsilyl (ODS) phases for reversed-phase chromatography — while the mobile phase is selected according to the nature of the solute and stationary phase.

Although HPLC offers superior separation efficiency, HPTLC provides rapid qualitative results and high-resolution separations comparable in accuracy and precision to HPLC and GC, and is recognized as a reliable method for quantitative analysis at the micro-, nano-, and even picogram level, even in complex formulations. As an offline technique performed on an open, disposable bed, HPTLC offers higher throughput and lower cost per analysis than online column methods such as HPLC, while allowing simultaneous analysis of standards and samples on a single plate under identical conditions — enabling true in-system calibration with low uncertainty. Key advantages include the ability for multiple analysts to work simultaneously, reduced need for internal standards, low maintenance and mobile-phase cost, minimal sample pre-treatment, an open system permitting visual recognition, and fresh stationary/mobile phases for every run, eliminating carryover contamination.

Method development in HPTLC begins with defining the analytical goal (qualitative categorization, quantitative measurement, mixture separation, or analysis-time optimization) and requires basic knowledge of analyte structure, stability, volatility, polarity, and solubility. Silica gel is typically used as the starting stationary phase. Mobile phase optimization generally follows a three-level approach: a single solvent is first screened for average separating ability; solvent strength is then adjusted using water or hexane; and finally, mixtures of the selected solvents are optimized with modifiers such as acids or bases. Detection is performed in absorption or fluorescence mode, with pre- or post-chromatographic derivatization employed if analytes are not adequately visualized. Chromatographic conditions primarily influence two parameters — peak purity and retention factor (Rf). Peak purity is assessed by comparing the first peak-slope spectrum to the peak-maximum spectrum, and the peak-maximum spectrum to the down-slope/peak-end spectrum, with a 1% error probability rejected only when the test value is 2.576 or greater. The retention factor, Rf, is calculated as the ratio of the migration distance of the substance to the migration distance of the solvent front from the origin.

2. Rationale of the Study

The present study was undertaken to bridge the gap between traditional Ayurvedic knowledge and modern analytical science by evaluating a polyherbal medicated oil prepared from Mañjiṣṭhā (Rubiacordifolia), Lodhra (Symplocosracemosa), and Daruharidra (Berberisaristata). These medicinal plants have long been used in Ayurveda for maintaining skin health and managing inflammatory skin conditions. However, despite their therapeutic significance, limited chromatographic data are available on their combined oil formulation, and establishing a chromatographic profile is essential for ensuring the formulation's quality, identity, and consistency.

TLC was employed as a preliminary qualitative tool to evaluate the marker compounds and optimize chromatographic conditions, while HPTLC was subsequently used to establish a chromatographic fingerprint and quantify the selected marker compound, berberine. The findings of this study are expected to contribute to the preliminary standardization of the formulation and provide a foundation for further analytical work.

3. Literature Survey

JatyadiTaila, a classical Ayurvedic formulation, has been standardized using physico-chemical parameters together with an HPTLC fingerprint, with chromatographic analysis performed on silica gel 60 F254 precoated aluminium plates using toluene:ethylacetate:formic acid (6:2:1, v/v/v) as the mobile phase and densitometric scanning at 254 and 366 nm; the resulting fingerprint, showing multiple distinct bands at different Rf values, serves as a reference for identification, standardization, and quality control of the formulation.[14]

A stability-indicating HPTLC method has also been developed and validated for the simultaneous estimation of curcumin, gallic acid, and ursolic acid in JatyadiTaila — a well-known Ayurvedic wound-healing product comprising sixteen medicinally important plants, including Curcuma longa, Terminaliachebula, and Jasminumofficinale. The marker constituents were resolved using the same toluene–ethyl acetate–formic acid (6:2:1, v/v/v) mobile phase, with ursolic acid estimated following derivatization; the developed plates were further subjected to HPTLC-MS analysis, forced degradation studies to confirm stability-indicating capability, and greenness evaluation using the AGREE methodology.[15]

Similarly, AnuTaila, another classical Ayurvedic medicated oil, has been analytically evaluated using both HPTLC and HPLC for the identification and quantification of bioactive constituents. HPTLC, using a toluene:ethylacetate:formic acid mobile phase, provided well-resolved fingerprint profiles of phytoconstituents, while HPLC enabled precise estimation of marker compounds such as berberine — collectively demonstrating that chromatographic techniques are effective tools for establishing the quality, consistency, and standardization of Ayurvedictaila formulations.[16]

4. Aim and Objectives

Aim: To formulate and evaluate a traditional polyherbal medicated oil containing Mañjiṣṭhā (Rubiacordifolia), Lodhra (Symplocosracemosa), and Daruharidra (Berberisaristata) through physicochemical, phytochemical, and chromatographic analysis using Thin Layer Chromatography (TLC) and High-Performance Thin Layer Chromatography (HPTLC).

Objectives:

- To prepare a traditional polyherbal medicated oil using Mañjiṣṭhā, Lodhra, Daruharidra, and sesame oil according to Ayurvedic principles.
- To evaluate the formulation for its organoleptic characteristics.
- To determine the physicochemical parameters, including acid value and peroxide value.
- To perform preliminary phytochemical screening of the formulation.
- To develop suitable chromatographic conditions for Thin Layer Chromatography (TLC).
- To compare the herbal extracts with their respective marker compounds using TLC.
- To establish a chromatographic fingerprint of the formulation using HPTLC.
- To identify and quantify the selected marker compound (berberine) by HPTLC.
- To evaluate the quality and preliminary standardization profile of the formulation.
- To provide scientific documentation supporting the analytical evaluation of the traditional herbal oil.

5. Materials and Methods

5.1 Plant Materials

Three medicinally important plant drugs were selected for formulation of the polyherbal medicated oil, each authenticated and monographed according to the Ayurvedic Pharmacopoeia of India. The crude drugs (Mañjiṣṭhā, Lodhra, and Daruharidra) and the base oil (sesame oil) were procured from Yucca Enterprises, Antop Hill Warehousing Co., Barkat Ali Naka, VIT Marg, Wadala (E), Mumbai 400037, India. The marker standards — purpurin, gallic acid, and berberine — were obtained from the same supplier.

5.1.1 Daruharidra (stem): [17] Consists of the dried stem of Berberisaristata DC. (Fam. Berberidaceae), an erect, spinous, deciduous shrub 1.8–3.6 m in height, found in the Himalayan ranges (1000–3000 m elevation) and the Nilgiri hills of South India. Macroscopically the drug occurs in pieces of variable length and thickness with a pale yellowish-brown, furrowed, brittle bark (0.4–0.8 cm thick) and a hard yellow xylem; taste is bitter. Microscopically, the stem shows rhytidoma with cork of thin-walled, radially arranged cells; phloem fibres in tangential rows; secondary phloem traversed by multiseriate phloem rays containing prismatic calcium oxalate crystals and stone cells; and a broad secondary xylem with numerous lignified, spirally-thickened vessels

and thick-walled fibres. The powder is yellow, showing cork fragments, phloem fibres, stone cells, prismatic crystals, and xylem vessels with spiral thickening. Its major constituents are alkaloids (Rasa: Tikta; Guna: Ruksa; Vīrya: Usna), and it is used in formulations such as BhṛṅgarājaTaila, Aśvagandhādyāriṣṭa, KhadirādiGuṭika, Khadirāriṣṭa, JātyādiTaila, and TriphalāGhṛta, with therapeutic application in Kaṇḍu, Medoroga, Mukharoga, Vraṇa, Āmātisāra, Urustambha, Kapharoga, Karṇaroga, Netraroga, and Meha. Dose: 5–10 mL of the drug in Kvatha (decoction) form.

5.1.2 Lodhrā (stem bark): [18] Consists of the dried stem bark of SymplocosracemosaRoxb. (Fam. Symplocaceae), an evergreen tree 6–8.5 m tall found abundantly in the plains and lower hills of India. Mature bark occurs in channelled or curved pieces up to 1 cm thick, grayish-brown to grey externally and pale to whitish-brown internally, with a short, granular fracture and an astringent, feebly bitter taste. Microscopically it shows a wide cork of thin-walled rectangular cells, a secondary cortex with stone cells and prismatic/cluster calcium oxalate crystals, and a wide secondary phloem containing sieve elements, phloem parenchyma, phloem fibres, and stone cells with uni- to multiseriate medullary rays. The powder is greyish-brown. Identity parameters include foreign matter (not more than nil%), total ash (not more than 12%), acid-insoluble ash (not more than 1%), alcohol-soluble extractive (not less than 9%), and water-soluble extractive (not less than 15%). Major constituents are unreported in the assay/TLC sections of the monograph consulted; properties include Rasa: Kaṣāya, Guṇa: Laghu, Vīrya: Śīta, Vipāka: Kaṭu. It is used in formulations such as Rodhrāsava (Lodhrāsava), PuṣyānugaCūrṇa, and BṛhatGaṅgādharaCūrṇa, with therapeutic use in Raktapitta, Atisāra, Śotha, Pradara, and Netraroga. Dose: 3–5 g of the drug in powder form, or 20–30 g in decoction.

5.1.3 Mañjiṣṭhā (stem): [19] Consists of the dried roots of Rubiacordifolia Linn. (Fam. Rubiaceae), a perennial herbaceous prickly creeper or climber up to 10 m long, found throughout the country up to 3750 m elevation. The stem is slender, cylindrical, wiry (about 0.5 cm thick), brown to purple, with a scabrous, grooved surface and prickles in the immature stem. Microscopically it shows exfoliating cork, a secondary cortex containing acicular calcium oxalate crystals, a wide reddish secondary phloem, and a continuous reddish secondary xylem cylinder; the powder is pink, showing cork fragments, lignified xylem vessels, and acicular/sandy crystals. Identity parameters include foreign matter (not more than 1%), total ash (not more than 12%), acid-insoluble ash (not more than 0.5%), alcohol-soluble extractive (not less than 3%), and water-soluble extractive (not less than 17%). TLC of the alcoholic extract on silica gel 'G' using n-butanol:aceticacid:water (4:1:5) shows two spots at Rf 0.92 (grey) and 0.98 (green) in visible light; under UV (366 nm), fluorescent zones appear at Rf 0.92 (green) and 0.98 (pink); on exposure to iodine vapour, six yellow spots appear at Rf 0.28, 0.37, 0.53, 0.72, 0.92, and 0.98; and on spraying with 5% methanolic sulphuric acid reagent followed by heating at 110 °C for ten minutes, six spots appear at Rf 0.28 and 0.37 (grey), 0.53 (bluish-grey), 0.72 and 0.92 (grey), and 0.98 (violet). Major constituents are glycosides (Rasa: Kashāya, Tikta, Madhura; Guna: Guru; Vīrya: Uṣṇa; Vipāka: Kaṭu). It is used in formulations such as Aravindāsava, Aśvagandhāriṣṭa, Uśīrāsava, Candanasava, BrahmanjiṣṭhādiKvātha, ManjiṣṭhādiTaila, and KhadirādiGuṭikā (Mukha), with therapeutic use in VraṇaRopa, AkṣiRoga, ŚleṣmaŚotha, KarṇaRoga, MañjiṣṭhāMeha, Raktadoṣa, Kuṣṭha, Visarpa, Prameha, Śvāsa, Raktapitta, Bhagandara, Arśa, and Vātarakta. Dose: 2–4 g of the drug.

5.2 Solvents, Chemicals, and Marker Standards

Solvents and chemicals: methanol (extra pure), chloroform (99%), ethyl acetate (AR grade), and formic acid (85%) — all obtained from Research-Lab Fine Chem Industries, Mumbai 400002, India — together with hydrochloric acid, ferric chloride, and Dragendorff's reagent used for phytochemical screening, and distilled water.

Marker compounds: berberine, purpurin, and gallic acid, procured from Yucca Enterprises, Mumbai.

Instruments and equipment: analytical balance, hot plate/heating mantle, magnetic stirrer, pH meter, mechanical grinder, muslin cloth for filtration, stainless-steel vessel, TLC developing chamber, silica gel TLC/HPTLC plates, capillary tubes/sample applicator, CAMAG Linomat 5 applicator, CAMAG twin-trough chamber, UV chamber/UV cabinet, and CAMAG TLC scanner.

5.3 Preparation of the Herbal Oil Formulation

The polyherbal medicated oil (Taila) was prepared from Mañjiṣṭhā, Lodhra, and Daruharidra using sesame oil as the base, following the classical Ayurvedic method of SnehaKalpana (Kalka: oil: Kwatha 1:4:16). The crude drugs were first cleaned manually to remove extraneous matter, then separately pulverized using a mechanical grinder.

- Coarse powder: The dried, cleaned crude drugs were pulverized into a coarse powder (12.5 g of each drug) for preparation of the herbal decoction (Kwatha).
- Fine powder: A separate portion of the cleaned crude drugs was further pulverized into a fine powder (12.5 g of each drug) for preparation of the herbal paste (Kalka).

- Kwatha (decoction): The coarse powder (37.5 g total, i.e. 12.5 g of each drug) was mixed with 2400 mL of purified water and heated under controlled conditions until the volume was reduced to 600 mL, following the classical Ayurvedic procedure. The decoction was filtered through muslin cloth to remove the herbal residue, and the filtrate was collected as Kwatha.
- Kalka (herbal paste): The fine powder (37.5 g total, i.e. 12.5 g of each drug) was mixed with a sufficient quantity of purified water and triturated to obtain a smooth, homogeneous paste (Kalka).
- Taila (medicated oil): Sesame oil (150mL), the prepared Kwatha, and the prepared Kalka were combined in a stainless-steel vessel and heated under controlled conditions with continuous stirring until the aqueous portion was removed. Completion of the process was assessed by the classical Taila Siddhi Lakshana (endpoint signs of proper oil preparation).
- Filtration and storage: The prepared oil (150 mL) was filtered while still warm through clean muslin cloth, allowed to cool, transferred into clean, dry, amber-coloured containers, and stored appropriately until further evaluation.

5.4 Organoleptic and Physical Evaluation

The prepared oil formulation was evaluated for colour, odour, appearance, and texture/consistency, providing preliminary information on the quality and stability of the formulation.

5.5 Physicochemical Evaluation

Physicochemical evaluation comprised pH determination, specific gravity, acid value, and peroxide value, to assess chemical stability and overall quality of the oil formulation.

5.6 Preliminary Phytochemical Screening

Preliminary phytochemical tests were performed to detect major classes of phytoconstituents, including tests for alkaloids, phenolic compounds, anthraquinones, and flavonoids.

5.7 Chromatographic Evaluation

Preparation of standard solution: Approximately 10 mg each of berberine, purpurin, and gallic acid were accurately weighed separately, transferred into individual 10 mL volumetric flasks, and made up to volume with methanol to obtain a concentration of 1 mg/mL. The solutions were sonicated until complete dissolution and filtered prior to application.

Preparation of sample solution: The medicated oil was extracted with methanol prior to chromatographic analysis. Approximately 3 g of medicated oil was accurately weighed and transferred into a centrifuge tube; 10 mL of methanol was added and the mixture was vortexed thoroughly and sonicated for about 15–20 minutes to facilitate extraction of phytoconstituents. The sample was centrifuged to separate the oil layer, and the methanolic supernatant was carefully collected and filtered through Whatman filter paper before HPTLC analysis. The resulting clear methanolic extract served as the sample solution.

Chromatographic conditions employed for HPTLC analysis are summarized in Table 1.

Parameter	**Condition**
Stationary phase	Silica Gel 60 F_{254} aluminium plate
Plate size	10 × 10 cm
Sample applicator	CAMAG Linomat 5
Chamber	CAMAG Twin Trough Chamber
Mobile phase	Chloroform : Ethyl acetate : Methanol : Formic acid
Chamber saturation	Approximately 20 minutes
Development distance	Approximately 80 mm
Drying	Air dried after development
Detection	UV at 254 nm and 366 nm
Scanner	CAMAG TLC Scanner

Standard solutions and the methanolic extract of the medicated oil were applied as bands on the silica gel plate using the CAMAG Linomat 5 applicator. The mobile phase (chloroform:ethylacetate:methanol:formic acid 4:2:1:0.2 v/v/v/v) was freshly prepared and transferred into a CAMAG twin-trough chamber, which was saturated with the mobile phase for approximately 20 minutes. The plate was developed to a migration distance of approximately 80 mm, removed, and air-dried at room temperature. The developed plate was scanned using a CAMAG TLC Scanner under UV detection, and chromatograms were recorded using VisionCATS software; peak areas, peak heights, and Rf values were obtained for the marker compounds.

For the calibration curve, different concentrations of berberine standard were applied to the HPTLC plate, and a calibration curve was constructed by plotting peak area (or peak height) against berberine concentration. Linear regression analysis was performed, and the regression equation and correlation coefficient (R^2) were calculated.

Identification of berberine in the sample was carried out by comparing its Rf value, peak profile, peak height, and peak area with those of the authentic berberine standard; a matching Rf value and peak profile confirmed the presence of berberine in the formulation. For quantitative estimation, 3 g of the prepared polyherbal medicated oil was extracted with

10 mL methanol to obtain the sample extract for HPTLC analysis. The amount of berberine present was determined from the calibration curve, with the concentration in the methanolic extract calculated using the regression equation, and the result expressed as the amount of berberine present in the analyzed oil sample.

6. Results and Discussion

6.1 Organoleptic Evaluation

Parameter	Observation
Colour	Reddish brown
Odour	Characteristic herbal odour
Appearance	Clear, free from suspended particles
Consistency	Smooth, oily
Clarity	Clear

The formulation showed acceptable organoleptic properties, indicating proper preparation and the absence of contamination.

6.2 Physicochemical Evaluation

Parameter	Result	Standard range
Acid Value	3.5	< 5 mg KOH/g
Peroxide Value	5.8	< 10 meq/kg
pH	5.2	4.7–5.75
Specific Gravity	0.90	0.90–0.93

All observed physicochemical values fell within acceptable limits, indicating satisfactory stability and suitability of the formulation for topical use.

6.3 Phytochemical Screening

Phytoconstituent	Test performed	Observation	Result
Alkaloids	Dragendorff's test; Mayer's test; Hager's test	Orange–red ppt; yellowish-white ppt; yellow ppt	+
Anthraquinones	Borntrager's test	Pink/red colour observed	+
Phenolics	Shinoda's test	Deep pink colour observed	+
Flavonoids	Ferric chloride test	Blue/black/green coloured precipitate	+

The presence of multiple phytoconstituents supports the therapeutic potential of the formulation, particularly for anti-inflammatory and skin-protective activity.

6.4 TLC Analysis

Marker compound	Standard observation	Oil extract observation	Result
Purpurin	Clear spot	Spot not observed	Not detected
Gallic acid	Clear spot	Spot not observed	Not detected
Berberine	Clear spot	Faint spot observed	Preliminary detection
		corresponding to standard	

These findings indicate that TLC was effective for preliminary screening of the individual crude drug extracts but had limited sensitivity for the complex oil formulation, necessitating further analysis by HPTLC.

6.5 HPTLC Analysis

Parameter	Observation
Marker used for quantification	Berberine
Sample	Polyherbal oil
Detection	HPTLC
Mobile phase	Chloroform : Ethyl acetate : Methanol : Formic acid (4:2:1:0.2 v/v/v)
Berberine identified	Yes
Berberine quantified	3.074 µg per 300 mg/mL of oil
Purpurin detected	No
Gallic acid detected	No

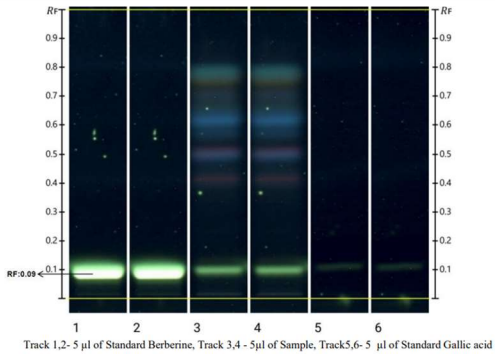


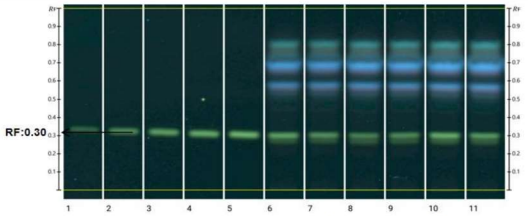
Fig. 1 Identification of Berberine and Gallic acid in sample

The HPTLC chromatogram showed a well-resolved peak corresponding to berberine, confirming its presence in the formulation. Purpurin and gallic acid, however, were not detected under the optimized chromatographic conditions. The absence of these markers may be attributed to their lower concentration in the final oil, limited extraction into methanol, poor partitioning from the oil matrix, or concentrations below the detection limit of the method.

6.6 Quantification of Berberine

Parameter	Observation
Regression mode	Linear-2
Calibration type	Peak Height
Calibration function	$y = 2.447 \times 10^{-6}x + 1.74 \times 10^{-2}$
Correlation coefficient (R^2)	0.999202
Coefficient of Variation (CV)	1.52%

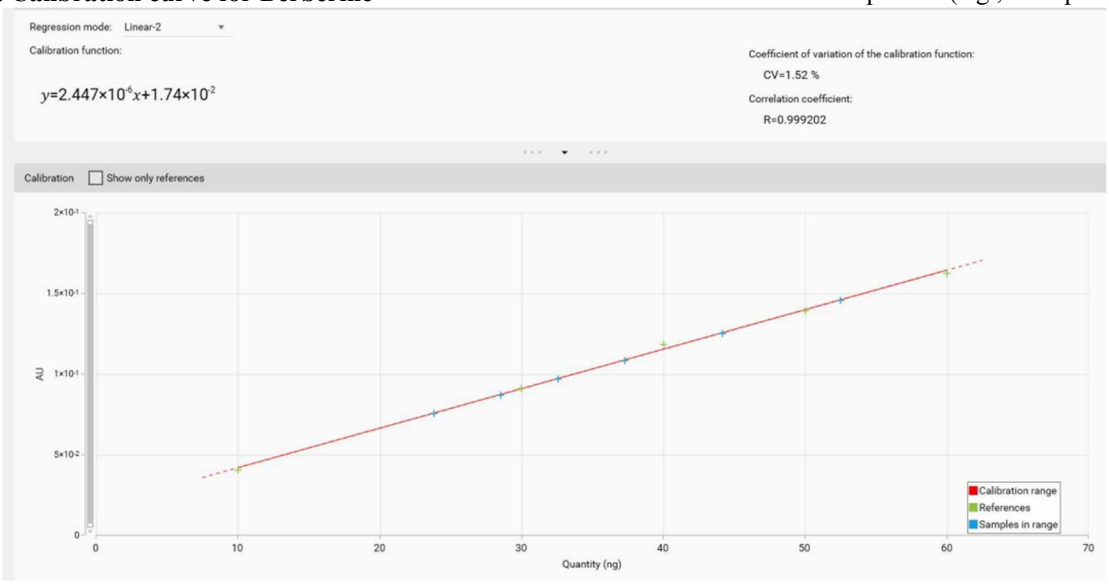
Amount of berberine detected	3.074 µg in 300 mg/mL of oil
Berberine content	0.00102% (w/w)



Track 1 - 1 µl of Standard Berberine, Track 2 - 3 µl of Standard Berberine, Track 3- 4 µl of Standard Berberine, Track 4 - 5 µl of Standard Berberine, Track 5 - 6 µl of Standard Berberine, Track 6,7- 10 µl of sample, Track 8,9- 12 µl of sample, Track 10,11 - 14 µl of sample

Fig. 2. Quantification of Berberine

Fig. 3. Calibration curve for Berberine



The calibration curve for berberine, constructed by plotting peak height against concentration, followed the linear regression equation $y = 2.447 \times 10^{-6}x + 1.74 \times 10^{-2}$, where y represents the peak height and x represents the concentration of berberine (in the instrument's response units). The excellent linearity ($R^2 = 0.999202$) and low coefficient of variation (1.52%) demonstrate that the developed HPTLC method is reliable and reproducible for the quantification of berberine in the polyherbal oil formulation.

7. Limitations and Future Scope

The present study has certain limitations that should be considered when interpreting the findings. Only berberine could be identified and quantified by HPTLC; purpurin and gallic acid were not detected in the methanolic extract of the oil, which may reflect their low native concentration in the formulation, incomplete partitioning from the lipophilic oil matrix into methanol, or levels below the detection limit of the method as optimized. Quantification was performed for a single marker compound without an accompanying method validation study (specificity, accuracy, precision, LOD/LOQ, and robustness as per ICH Q2(R1) guidelines), and no forced-degradation or stability data were generated to establish a stability-indicating profile. The study was also limited to a single batch of the formulation, without inter-batch or accelerated stability assessment, and no pharmacological or microbiological efficacy data were generated to correlate the chemical findings with therapeutic activity.

Future work should therefore focus on full validation of the HPTLC method for all three marker compounds, exploration of alternative extraction solvents or techniques (e.g., liquid–liquid partitioning, solid-phase extraction) to improve recovery of purpurin and gallic acid from the oil matrix, multi-batch and accelerated stability studies, and pharmacological evaluation (e.g., anti-inflammatory, antioxidant, and wound-healing assays) to substantiate the therapeutic claims associated with the formulation.

8. Conclusion

The present study focused on the chromatographic and preliminary evaluation of a traditional polyherbal medicated oil using organoleptic, physicochemical, phytochemical, and chromatographic analyses. The prepared formulation exhibited acceptable organoleptic and physicochemical characteristics, indicating satisfactory quality under the conditions of the study. Phytochemical screening confirmed the presence of bioactive phytoconstituents in the formulation.

TLC analysis served as a preliminary screening technique for the identification of marker compounds in the individual plant extracts, whereas HPTLC analysis confirmed the presence and quantified berberine as the selected marker compound. The

HPTLC method demonstrated good linearity (R^2 = 0.999202), with 3.074 µg of berberine detected in the extract corresponding to 300 mg of the oil sample (0.00102% w/w).

Overall, the findings support the application of HPTLC as a suitable analytical technique for the chromatographic evaluation and quality assessment of the prepared herbal oil formulation. Further studies on method validation, stability, and pharmacological evaluation are recommended to establish its long-term quality and therapeutic potential

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