Research Article

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INVESTIGATING AUTHENTICITY, QUALITY AND PHYTOCHEMICAL COMPOSITION OF TRIMADA CHURNA: AN HPTLC-BASED APPROACH

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ABSTRACT

Trimada churna is one of the widely prescribed churna stated Charaka Samhita and Bhaishajya Ratnavali, used in various kinds of gut dilemmas which are achieved by the powerful digestive and stimulant properties of Chitraka, whereas the other ingredients concurrently exhibit their particular medicinal effects. This study aimed to establish analytical parameters and perform HPTLC fingerprinting of Trimada churna. This involved comparing the analytical data with another sample of Trimada churna prepared from commercially available ingredients, specifically Vidanga, Mustaka, and Chitraka. Additionally, the study focused on quantifying Embelin from Vidanga and Plumbagin from Chitraka. Qualitative tests showed the presence of alkaloids, glycosides, flavonoids and phenols. All two samples showed significantly fewer variations in physicochemical parameters, but the acid-insoluble ash content of market-sampled Chitraka was found to be slightly higher (1.9% w/w) compared to both the original sample (0.3% w/w) and the values recorded in standard books. The HPTLC analysis revealed the absence of the chief secondary metabolite, plumbagin, in Chitraka's market sample.

Keywords: Trimada churna, HPTLC, Physico-chemical parameters, Standardization, Embelin, Plumbagin.

INTRODUCTION

Ayurveda, the ancient Indian system of medicine, offers a holistic approach to health and wellness that has been practised for over 5,000 years. Translating to "the science of life," it emphasises achieving balance and harmony within the body, mind, and spirit, providing a unique perspective on wellness that remains relevant today.[1-3] Churnas represent a significant category of formulations widely used in treating and managing various diseases, making them an important subject for research and exploration in traditional medicine.[4] Trimada is a traditional Ayurvedic polyherbal formulation comprising the tubers of Mustaka (*Cyperus rotundus*), fruits of Vidanga (*Embelia ribes*), and roots of Chitraka (*Plumbago zeylanica*). This formulation has garnered attention for its multifaceted benefits, including acting as an appetiser, expelling intestinal worms, and aiding in weight management by preventing weight gain. Furthermore, Trimada is noted for its potential to eliminate plaque deposits in the body, enhance blood circulation, inhibit fat accumulation, and avoid water retention, commonly associated with obesity.[5] We conducted thin-layer chromatography (TLC) on Trimada churna to analyse its components and verify the presence of active phytochemicals. In this study, we aimed to conduct a comparative pharmaceutical-analytical examination of Trimada churna, utilising fresh, authentic raw ingredients and those sourced from the market. This approach allows us to evaluate the differences in quality and overall formulation characteristics.

Aims and Objectives

- To formulate standards for analysing Trimada churna to ensure consistent processing and end product quality.
- To develop a comprehensive HPTLC fingerprint of Trimada churna.
- To quantify the principal chemical constituents, embelin was derived from Vidanga, and plumbagin was obtained from Chitraka.
- To compare analytical data with market samples.

MATERIALS AND METHODS

The current work was planned for the following stages: Viz.

- Procurement of raw drugs and Preparation of Trimada churna.
- Finished product standardisation with comparative study to market sample.
- Quantifying principal bioactive markers, embelin from the Vidanga and plumbagin from the Chitraka.

Procurement of raw drugs

As discussed earlier, we planned to prepare Trimada churna using fresh, authentic raw ingredients and those available from the market. Accordingly, we procured Vidanga from the Bhore region in Pune, Mustaka from the manufacturing unit of the ITRA Pharmacy, and Chitraka from the botanical garden of the ITRA Pharmacy College, constituting one set of raw materials. Another set of raw materials was obtained from the local market. Trimada

churna, which is made from the second set of raw materials, is referred to as the market sample (MS). All raw materials were stored correctly.

Preparation of Trimada churna

Each raw ingredient was carefully cleaned to eliminate any impurities and then dried in the shade to maintain their medicinal qualities while avoiding exposure to direct sunlight. After drying, each raw ingredient was pounded individually using the Udukhalva yantra (Iron mortar-pestle) to break them into smaller particles. The resulting powders were sifted through a fine mesh sieve (#85) to ensure uniform particle size and minimise coarseness. Equal portions of the finely sifted powders of Vidanga, Mustaka, and Chitraka were measured out, combined in a container, and mixed thoroughly to create a uniform blend, guaranteeing an even distribution of all components within the formulation. The composition of Trimada churna, as per Bhaishajya Ratnavali, has been presented in Table 1.[6]

Table 1: Trimada churna – Ingredients

Ingredient	Botanical name	Part used	Quantity
Vidanga	*Embelia ribes* Burm F.	Fruit	1 Part
Mustaka	*Cyperus rotundus* Linn.	Tuber	1 Part
Chitraka	*Plumbago zeylenica* Linn.	Root	1 Part

Finished product standardisation with comparative study to market sample

The main goal of this study is to create guidelines for standardising Trimada churna by setting clear standards. A detailed analysis of an in-house sample was conducted, and its results were compared with those from market samples. The data obtained from the in-house sample served as the baseline for formulating the standardisation protocol. Each sample was analysed using a range of pharmacognostic and physicochemical parameters alongside chromatographic and other instrumental methods.

Chromatographic method (Thin Layer Chromatography)

A popular analytical technique for separating and identifying components in a mixture based on their varying affinities toward a stationary phase and a mobile phase is thin layer chromatography (TLC).[7-9]

Sample preparation of Trimada churna for TLC

Five grams of Trimada churna were weighed into a conical flask, and 100 ml of methanol was added. The mixture was vigorously stirred using a magnetic stirrer for 3-4 hours, then allowed to settle for 5-6 hours for sedimentation. The supernatant was filtered through filter paper #1, and this filtrate was used for TLC analysis.[10]

Sample preparation of individual ingredients (Vidanga, Mustaka and Chitraka) for TLC

For the sample preparation of the individual ingredients Vidanga, Mustaka and Chitraka, the same procedure is followed which was used in the sample preparation for Trimada churna.

Mobile phase: Toluene: Ethyl acetate: Formic acid: Methanol (6:3:0.1:1 v/v/v/v)

Table 2: Details of TLC plate

Track	Description
T1	Trimada OG
T2	Trimada MS
V	Vidanga
M	Mustaka
C	Chitraka

Instrumental method (High-Performance Thin Layer Chromatography)

HPTLC studies were carried out using pre-coated silica gel G F254 plates (Merck). The samples and reference standards were applied with the CAMAG ATS 4 applicator. The plates were developed in twin trough chambers, and spots were detected in short UV (254 nm), Long UV (366 nm) and after derivatisation.[11]

Sample preparation of Trimada churna for HPTLC

Five grams of Trimada churna were weighed into a conical flask, and 100 ml of methanol was added. The mixture was vigorously stirred using a magnetic stirrer for 3-4 hours, then allowed to settle for 5-6 hours for sedimentation. The supernatant was filtered through filter paper #1, and this filtrate was used for TLC analysis.[12]

Application setting

- Applicator: CAMAG ATS 4
- Chromatographic development setting:
- Tank: Twin trough chamber 20×10
- Mobile phase: Toluene: Ethyl acetate: Formic acid: Methanol (6:3:0.1:1 v/v/v/v)
- Saturation time: 20 minutes
- Use saturation pad: True
- Volume front: 10 ml.
- Volume rear: 10 ml.
- Drying time: 5 minutes
- Drying temperature: Room temperature
- Scanner: CAMAG TLC Scanner 4
- Detectors: Deuterium and Tungsten lamp
- Scanning wavelengths: 254 nm (Short UV), 366 nm (Long UV)
- Derivatisation (CAMAG Derivatizer): Anisaldehyde sulphuric acid
- Documentation/Data system: VisionCats software

Table 3: Details of HPTLC plate 1 (Fingerprinting))

Track	Vial ID	Description	Volume	Position	Type
1	SA2425019	Trimada OG 50mg/ml	5.0 µL	A1	Sample
2	SA2425019	Trimada OG 50mg/ml	10.0 µL	A1	Sample
3	SB2425019	Trimada MM 50mg/ml	5.0 µL	A2	Sample
4	SC2425019	Trimada BK 50mg/ml	5.0 µL	A3	Sample
5	RA2425019	Embelin STD 1mg/ml	2.0 µL	A4	Reference
6	RA2425019	Embelin STD 1mg/ml	6.0 µL	A4	Reference
7	SD2425019	Vidanga OG 50mg/ml	5.0 µL	A5	Sample
8	SD2425019	Vidanga OG 50mg/ml	10.0 µL	A5	Sample
9	SE2425019	Vidanga Pharmacy 50mg/ml	5.0 µL	A6	Sample
10	SF2425019	Mustaka 50mg/ml	5.0 µL	A7	Sample
11	RB2425019	Plumbagin 1mg/ml	2.0 µL	A8	Reference
12	RB2425019	Plumbagin 1mg/ml	6.0 µL	A8	Reference
13	SH2425019	Chitraka OG 50mg/ml	5.0 µL	A9	Sample
14	SH2425019	Chitraka OG 50mg/ml	10.0 µL	A9	Sample
15	SG2425019	Chitraka AYU	5.0 µL	A10	Sample

Quantification of principal chemical constituents, embelin from the Vidanga and plumbagin from the Chitraka

All the conditions for every step of HPTLC for the 2nd plate are the same as per the 1st plate except the preparation of standards Mobile phase.

Preparation of standard: 1mg/ml with methanol

Mobile phase: n propanol: n butanol: Ammonia (7:1:2 v/v/v)

Table 4: Details of HPTLC plate 2 (Quantification of Embelin)

Track	Vial ID	Description	Volume	Position	Type
1	RA2425019	Embelin STD 1mg/ml	0.5 µL	A4	Reference
2	SA2425019	Trimada OG 50mg/ml	5.0 µL	A1	Sample
3	RA2425019	Embelin STD 1mg/ml	1.0 µL	A4	Reference
4	SA2425019	Trimada OG 50mg/ml	5.0 µL	A1	Sample
5	RA2425019	Embelin STD 1mg/ml	1.5 µL	A4	Reference
6	SA2425019	Trimada OG 50mg/ml	5.0 µL	A1	Sample
7	RA2425019	Embelin STD 1mg/ml	2.0 µL	A4	Reference
8	RA2425019	Embelin STD 1mg/ml	2.5 µL	A4	Reference
9	RA2425019	Embelin STD 1mg/ml	3.0 µL	A4	Reference
10	SC2425019	Trimada BK 50mg/ml	5.0 µL	A3	Sample
11	SC2425019	Trimada BK 50mg/ml	5.0 µL	A3	Sample
12	SD2425019	Vidanga OG 50mg/ml	5.0 µL	A5	Sample
13	SD2425019	Vidanga OG 50mg/ml	5.0 µL	A5	Sample
14	SE2425019	Vidanga Pharmacy 50mg/ml	5.0 µL	A6	Sample
15	SE2425019	Vidanga Pharmacy 50mg/ml	5.0 µL	A6	Sample

All the conditions for every step of HPTLC for the 3rd plate are the same as those for the 1st plate except for the Mobile phase.

Preparation of standard: 1mg/ml with methanol

Mobile phase: Toluene: Formic acid (9.5: 0.5 v/v)

Table 5: Details of HPTLC plate 3 (Quantification of Plumbagin)

Track	Vial ID	Description	Volume	Position	Type
1					
2	RB2425019	Plumbagin 1mg/ml	0.5 µL	A8	Reference
3	SA2425019	Trimada OG 50mg/ml	5.0 µL	A1	Sample
4	RB2425019	Plumbagin 1mg/ml	1.0 µL	A8	Reference
5	SA2425019	Trimada OG 50mg/ml	5.0 µL	A1	Sample
6	RB2425019	Plumbagin 1mg/ml	1.5 µL	A8	Reference
7	SA2425019	Trimada OG 50mg/ml	5.0 µL	A1	Sample
8	RB2425019	Plumbagin 1mg/ml	2.0 µL	A8	Reference
9	RB2425019	Plumbagin 1mg/ml	2.5 µL	A8	Reference
10	RB2425019	Plumbagin 1mg/ml	3.0 µL	A8	Reference
11	RB2425019	Plumbagin 1mg/ml	3.5 µL	A8	Reference
12					
13	SH2425019	Chitraka OG 50mg/ml	5.0 µL	A9	Sample
14	SH2425019	Chitraka OG 50mg/ml	5.0 µL	A9	Sample
15	SH2425019	Chitraka OG 50mg/ml	5.0 µL	A9	Sample

RESULTS AND DISCUSSION

Table 6: Organoleptic characters for Trimada churna

	Samples	
	In house	**MS**
Colour	Reddish brown	Brown
Odour	Characteristic bitter	Characteristic bitter
Taste	Astringent followed by slightly bitter	Astringent followed by slightly bitter
Texture	Fine coarse	Fine coarse

Figure 1: Epicarp cells

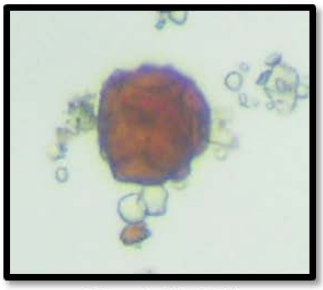

Figure 2: Oil globule

Figure 3: Stone cell

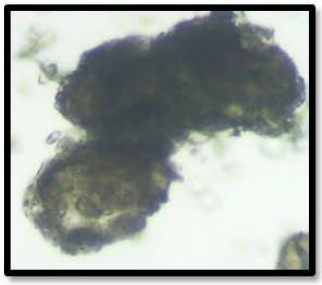

Figure 4: Group of Stone cells

Figure 5: Pitted Stone cells

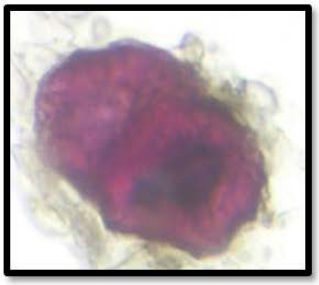

Figure 6: Lignified Stone cells

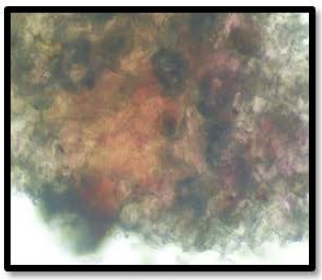

Figure 7: Endosperm cell

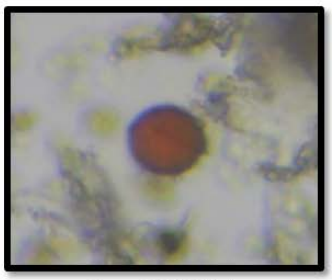

Figure 8: Oil globules

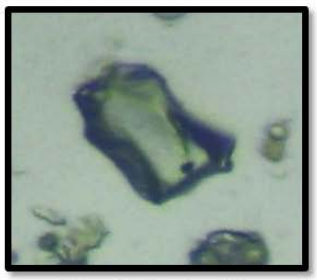

Figure 9: Silica deposition

Figure 10: Simple starch grain

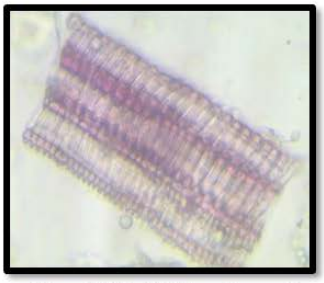

Figure 11: Lignified Annular vessel

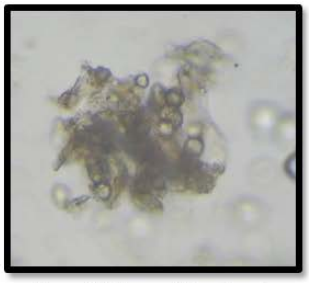

Figure 12: Group of starch grains

Table 7: Analytical values of Physicochemical parameters for Vidanga, Mustaka and Chitraka

Parameters	**Samples**					
	Vidanga		**Mustaka**		**Chitraka**	
	OG	**MS**	**OG**	**MS**	**OG**	**MS**
Loss on Drying (LOD)	0.23% w/w	0.30% w/w	8.55% w/w	7.89% w/w	23% w/w	5.9% w/w
Total ash	2.71% w/w	1.33% w/w	5.89% w/w	6.23% w/w	1.9% w/w	2.3% w/w
Acid insoluble ash	0.89% w/w	1% w/w	2.13% w/w	2.42% w/w	0.3% w/w	1.9% w/w
Water Soluble Extractive Value	15.35% w/v	16.71% w/v	32% w/v	34.90% w/v	25.2% w/v	20.8% w/v
Alcohol Soluble Extractive Value	21% w/v	19.81% w/v	27% w/v	25.92% w/v	21.9% w/v	17.25% w/v

Table 8: Analytical values of Physicochemical parameters for Trimada churna

Parameters	Samples	
	In house	MS
Loss on Drying (LOD)	9.8% w/w	9.43% w/w
Total ash	7.23% w/w	8.09% w/w
Acid insoluble ash	1.27% w/w	2.1% w/w
Water Soluble Extractive Value	18.34% w/v	13.97% w/v
Alcohol Soluble Extractive Value	24.25% w/v	19.41% w/v

Table 9: Qualitative tests for Trimada churna

Test for	Samples	
	In house	MS
Alkaloids	+	-
Carbohydrates	+	-
Tannins	+	+
Glycosides	+	+
Phenols	+	+
Saponins	+	+
Phytosterols	-	-
Flavonoids	-	-
Amino acids	-	-
Diterpenes	-	-

Table 10: Comparative Rf values of all samples (TLC)

Sr.No.	Sample	UV 254 (Short UV)		UV 366 (Long UV)		After derivatisation	
		No. of spots	Rf Value	No. of spots	Rf value	No. of spots	Rf value
T1	Trimada OG	4	0.16,0.21,0.34, 0.43	2	0.91,0.96	2	0.82,0.83
T2	Trimada MS	3	0.36,0.39,0.46	3	0.44,0.90,0.95	3	0.44,0.82,0.83
V	Vidanga	1	0.42	4	0.42,0.5,0.90,0.95	2	0.44,0.83
M	Mustaka	5	0.13,0.17,0.23,0.35,0.89	4	0.18,0.32,0.36,0.90	4	0.42,0.43, 0.83,0.95
C	Chitraka	5	0.18,0.21,0.26,0.31,0.37	1	0.27	-	-

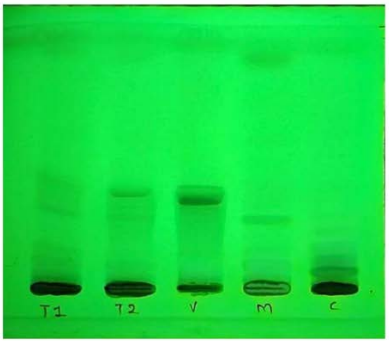

Figure 13: Short UV-254nm

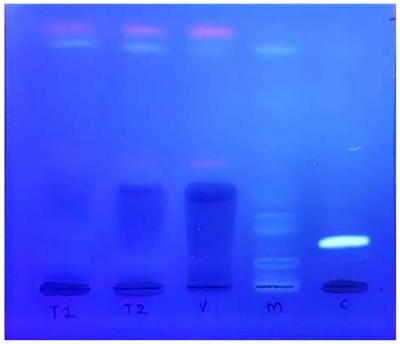

Figure 14: Long UV-366nm

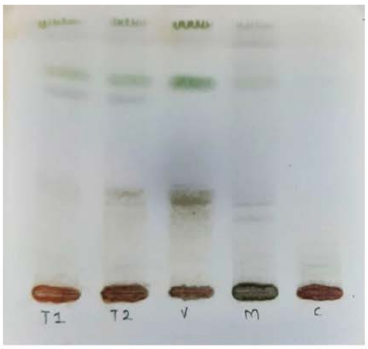

Figure 15: After derivatization

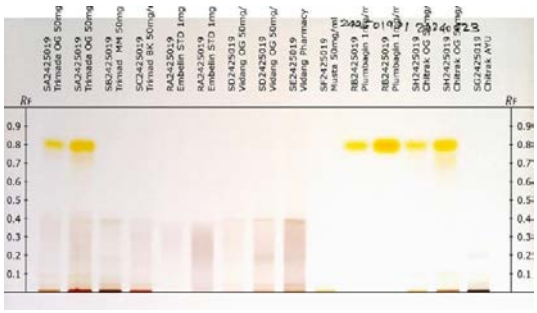

Figure 16: Developed plate image under white light (fingerprinting)

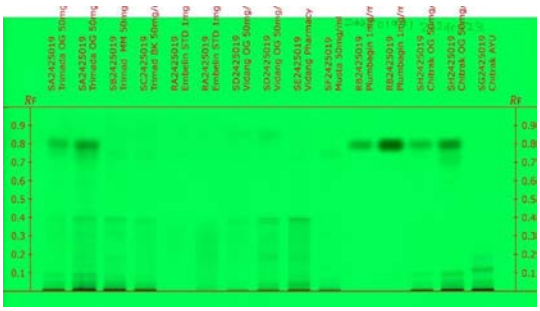

Figure 17: Developed plate image under short uv-254nm (fingerprinting)

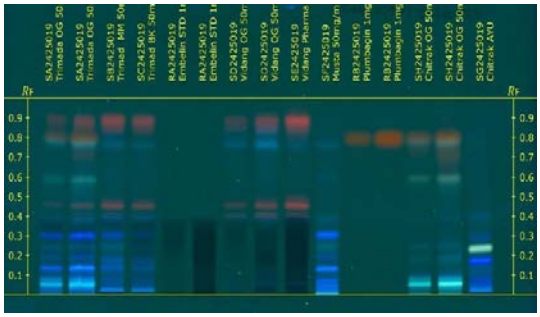

Figure 18: Developed plate image under long uv-366nm (fingerprinting)

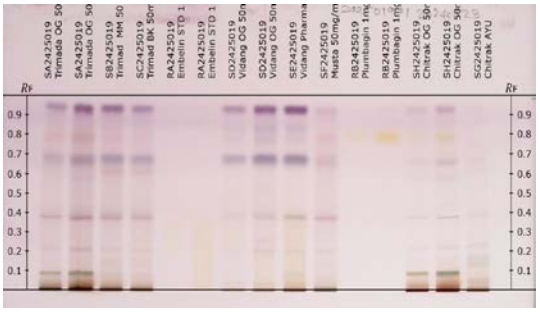

Figure 19: Derivatised plate image under white light (fingerprinting)

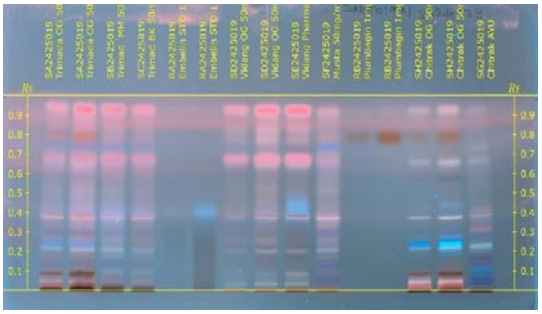

Figure 20: Derivatised plate image under 366 fluorescent light (fingerprinting)

Table 11: Quantitative estimation of Embelin in various samples at 343nm

Sample and Quantity of Embelin	Average concentration	Concentration standard deviation	Assignment count
Sample SA2425019 (Trimada OG) 50 mg.	260 µg	15.93%	3
Sample SC2425019 (Trimada MS) 50 mg.	306.1 µg	12.32%	2
Sample SD2425019 (Vidanga OG) 50 mg.	327.1 µg	15.89%	2
Sample SE2425019 (Vidanga MS) 50 mg.	419.6 µg	6.78%	2

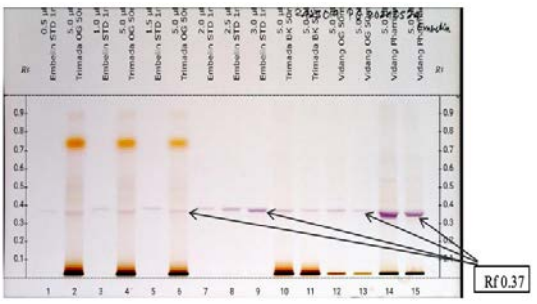

Figure 21: Developed plate image under white light (quantification of embelin from Vidanga)

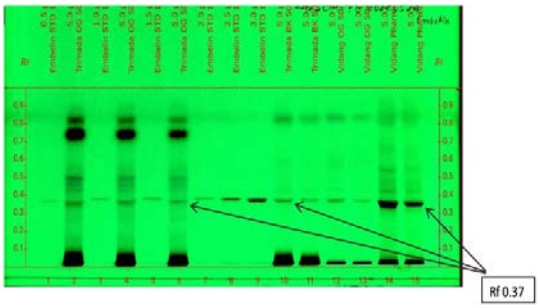

Figure 22: Developed plate image under short uv-254nm (quantification of embelin from Vidanga)

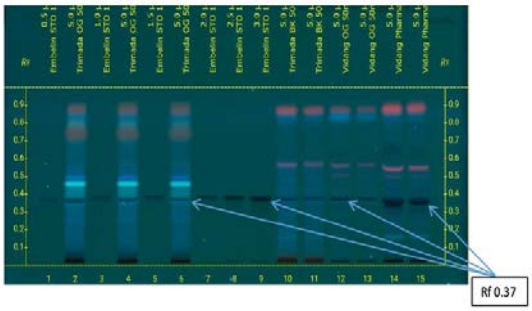

Figure 23: Developed plate image under short uv-366nm (quantification of embelin from Vidanga)

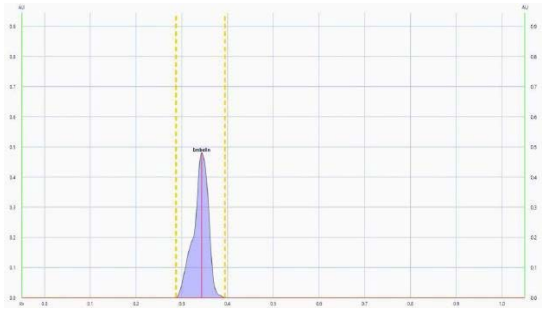

Figure 24: Integrated peak of embelin standard

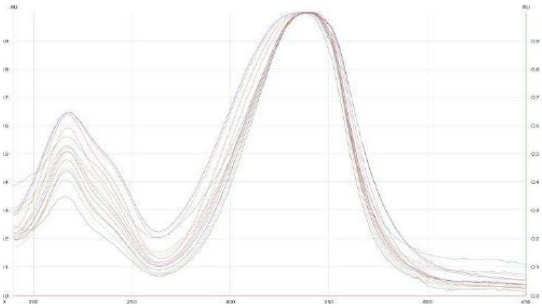

Figure 25: Spectrum of embelin band at Rf 0.37

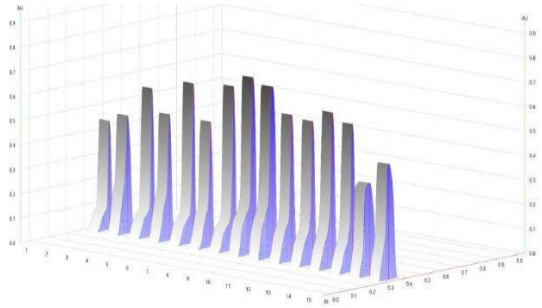

Figure 26: 3D profile of embelin peak from formulation & herb tracks at 343nm

Table 12: Quantitative estimation of Plumbagin in various samples at 271nm

Sample and Quantity of Plumbagin	Average concentration	Concentration standard deviation	Assignment count
Sample SA2425019 (Trimada OG) 50 mg.	302.8 µg	20.91%	3
Sample SH2425019 (Chitraka OG) 50 mg.	349.8 µg	18.47%	3

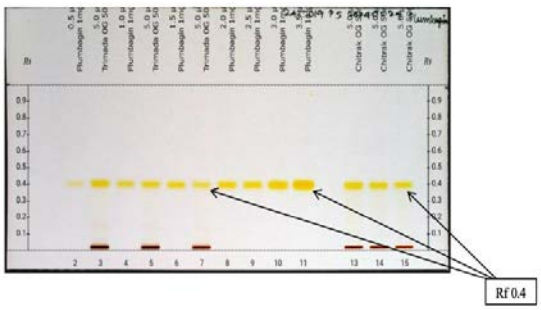

Figure 27: Developed plate image under white light (quantification of plumbagin from Chitraka)

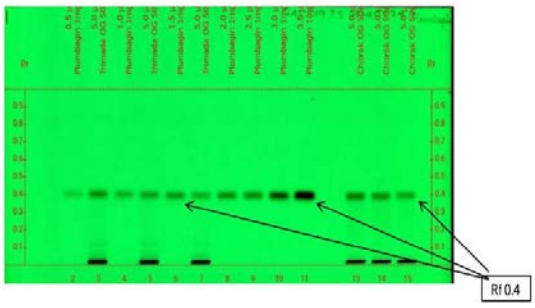

Figure 28: Developed plate image under short uv-254nm (quantification of plumbagin from Chitraka)

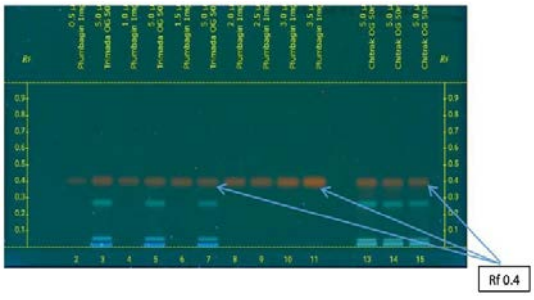

Figure 29: Developed plate image under long uv-366nm (quantification of plumbagin from Chitraka)

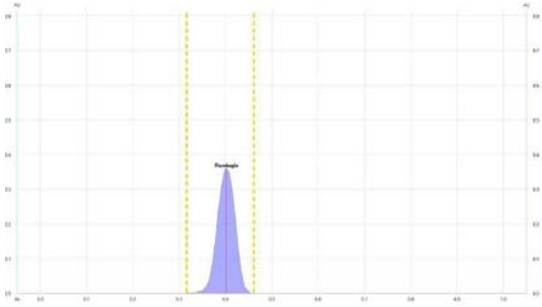

Figure 30: Integrated peak of plumbagin standard

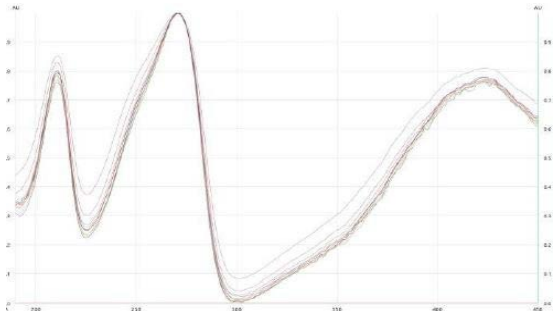

Figure 31: Spectrum of plumbagin band at Rf 0.4

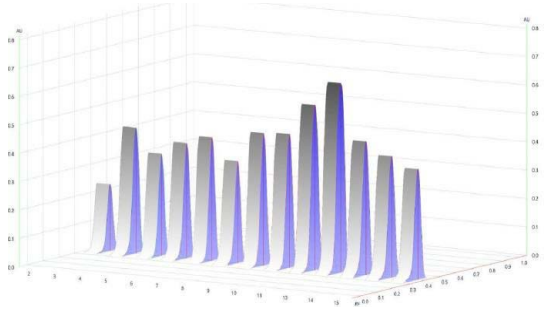

Figure 32: 3D profile of plumbagin peak from formulation & herb tracks at 271nm

The physicochemical analysis of Vidanga, Mustaka, and Chitraka offers vital information regarding the quality and adherence of these medicinal plants to Quality Standards for Indian Medicinal Plants. As shown in Table 7, all assessed parameters complied with the specified limits, affirming the suitability of these herbal materials for medical use. However, the market sample of Chitraka exhibited a slight increase in the acid-insoluble ash content, a measure often used to evaluate the purity and inorganic components of the plant material.[13-15] While this minor deviation does not notably compromise Chitraka's potential, monitoring such factors to maintain consistent quality across various market sources is still crucial.

The physicochemical assessment of Trimada churna, detailed in Table 8, also indicated compliance with the prescribed standards, except for a similar slight exceedance in acid-insoluble ash found in the market sample. This suggests that, although the formulation meets most quality requirements, minor fluctuations in acid-insoluble ash content may occur in commercial offerings, which is important for quality assurance in herbal products.

Regarding chromatographic analysis, the results reveal interesting insights, especially in identifying and measuring active compounds like Embelin and Plumbagin. The chromatographic results (Figures 21- 23) indicated that Trimada OG, Trimada Market sample, Vidanga OG, and Vidanga Pharmacy displayed a shared dark band at Rf 0.37. This band matched the Embelin standard, confirming the presence of Embelin in those samples. Embelin, recognised for its therapeutic attributes and being identified in various samples from different sources, reinforces the reliability and authenticity of these herbal products. Further spectral analysis confirmed that the dark band at Rf 0.37 corresponded to Embelin, validating the chromatographic findings. The quantification of Embelin in Trimada OG analysed at 343 nm (Figure 25) indicated a concentration of 0.52%, affirming the existence of this compound in the formulation and yielding essential data for assessing its potential effectiveness.

Detecting Plumbagin was another crucial finding of this study. Chromatographic analyses under different lighting conditions (Figures 27-29) unveiled a common orange band at Rf 0.4 in Trimada OG and Chitraka OG, which aligned with the Plumbagin standard. Spectral analysis of the orange band confirmed that its pattern matched the Plumbagin standard, reinforcing its identification in these samples. The quantitative evaluation of Plumbagin in Trimada OG, conducted at 271 nm (Figure 31), revealed a concentration of 0.60%, enhancing the support for its therapeutic potential in this formulation. Additionally, Plumbagin was qualitatively detected in Chitraka OG, indicating its presence in the herb.

The outcomes of this research underscore the significance of chromatographic methods in recognising and quantifying active phytochemicals in herbal formulations. Identifying Embelin and Plumbagin in both individual herbs (Vidanga and Chitraka) and the mixture (Trimada OG) confirms the consistency of these active compounds across different products and formulations. Furthermore, quantifying these substances offers critical insights into their concentrations in the final product, ensuring the formulation includes adequate levels of these bioactive components to provide therapeutic benefits.

CONCLUSION

A recent study analysed the raw materials (Vidanga, Mustaka and Chitraka) and formulation (Trimada churna), confirming compliance with the standards. HPTLC fingerprinting quantified Embelin and Plumbagin, crucial marker components, from original and market samples in methanolic fractions. The chromatograms revealed standard peaks, enabling percentage calculations. Notably, market samples showed higher Embelin percentages but lacked Plumbagin peaks.

The study's findings demonstrate the effectiveness of combining organoleptic, pharmacognostic, physicochemical, and chromatographic studies to identify formulation ingredients. The original Trimada churna exhibited superior quality, underscoring its potential anti-obesity effects. This research provides valuable insights for future standardisation efforts in combating global obesity and ensures routine quality control and batch-to-batch variation control.

The HPTLC technique proved effective for identifying and quantifying marker components such as Embelin and Plumbagin in Trimada churna. This method is characterised by its simplicity, precision, specificity, and accuracy, making it highly suitable for routine quality control analysis of Ayurvedic formulations. Its reliable performance can significantly enhance the standardisation and assurance of quality in herbal products.

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