DOI: 10.7860/JCDR/2026/82196.23957

Original Article

Pharmacology Section

Evaluation and Standardisation of *Tripurabhairava Rasa*: A Laboratory-based Pharmaceutical Analytical Experimental Study

SUKESHINI JULME[1], MUJAHID KHAN[2], RASHMI ZADE[3]

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ABSTRACT

Introduction: *Tripurabhairava Rasa* (TBR) is a classical herbo-mineral formulation described in the *Bharat Bhaishajya Ratnakara*, traditionally used in managing various types of *Jwara* (fever). It contains *Shuddha Vatsanabha* (*Aconitum ferox*), *Shunthi* (*Zingiber officinale*), *Maricha* (*Piper nigrum*), and *Shuddha Tankana* (Borax). The presence of toxic substances like *Vatsanabha* necessitates thorough pharmaceutical and toxicity evaluation. The formulation is indicated in conditions such as *Sannipatik Jwara* (severe condition of any disease), *Vataroga* (disorders caused by *vata*), *Shleshmaroga* (disorders caused by *kapha*), *Maha Jwara* (long duration of pyrexia), *mastakasya roga* (disorders of meninges), and *pida of Udara* (pain in the lower abdomen).

Aim: To evaluate and standardise *TBR* using validated pharmaceutical and analytical parameters.

Materials and Methods: The present study is a laboratory-based pharmaceutical-analytical experimental study conducted at the Department of Rasashastra evam Bhaishajya Kalpana, Mahatma Gandhi Ayurved College and Research Centre (MGACH & RC), Salod (H), Wardha, Maharashtra, India, from January 2024 to February 2024. The formulation was prepared using classical Ayurvedic methods, including *Shodhana* (purification), *Churna Nirmana* (powdering), *Bhavana* (levigation), and *Vati* (tablet preparation). Physicochemical analyses, including loss on drying, total ash, water and alcohol-soluble extractives, acid-insoluble ash, extractive values, and pH, were carried out at Cotex Laxmi Health Care Pvt., Ltd., Wardha, and MGACH & RC, Salod (H), Maharashtra, India. Chemical profiling of the formulation was performed using High-Performance Thin Layer Chromatography (HPTLC). As this was a pharmaceutical-analytical study, demographic parameters were not applicable. Data were analysed descriptively and the results were expressed as mean values, with no inferential statistical analysis performed.

Results: The formulation showed the following analytical values: Loss on drying- 1.59%, Total ash- 32.5%, Water-soluble ash- 0.32%, Acid-insoluble ash-0.01%, Alcohol-soluble extractive- 9.88%, Water-soluble extractive- 52.94%, and pH-10.3. HPTLC analysis revealed five distinct spots with Rf values of 0.32, 0.72, 0.77, 0.82, and 0.90.

Conclusion: The present study presents the first pharmaceutical and analytical evaluation of *TBR* prepared strictly according to classical references. The findings suggest the formulation maintains acceptable pharmaceutical standards. Further research on pharmacological activity and toxicity is warranted to support its safe therapeutic application.

Keywords: Chromatography, Drug standardisation, Herbal medicine, Pharmaceutical preparations

INTRODUCTION

Rasashastra and *Bhaishajya Kalpana* are branches of Ayurveda focused on preparing herbo-mineral and metal substances. These materials are processed in a way that makes them safe for consumption, ensuring they are effective at the correct dosage without causing harm to the body, and are easily absorbed and assimilated [1]. *Ayurveda* describes particular physiochemical techniques, including sublimation and heating, to purify metals and mitigate their toxic effects [2].

TBR is a type of *Rasoushadha* that contains *Rasa Dravya* (metal and minerals) as well as *Kastadravya,* or plants. *TBR* is a complex concoction of many different medications that treat the same symptoms at the same time. The 13th century Acharya Sharangadhara provided a detailed explanation of the many *Kalpanas*. One of the five basic Ayurvedic treatments is *Vati Kalpana*, which includes tablets and capsules. These are important in Ayurvedic pharmaceutics due to their simplicity of use, palatability, and portability. *Vati Kalpana* is the process of combining raw herbal or herbomineral powders with *Kashayam*, juice, or honey to produce pills or tablet [3].

In Rasa Shastra, the techniques of *Shodhana* (purification), *Bhavana* (levigation or impregnation), and *Marana* (calcination or incineration) are employed to convert toxic raw materials into safe and therapeutically potent Ayurvedic formulations known as *Rasaushadhis*. However, concerns about their safety and efficacy have emerged due to lack of proper standardisation and defined physical or chemical parameters [4]. Pharmaceutical research is the science of drug discovery and application, focusing on how and why drugs are used. In the context of Ayurvedic medicine, it is crucial as it involves all herbo-mineral preparations that must undergo processes such as *Shodhana, Jarana,* and *Marana* [5].

The current study is important because *TBR*, a traditional Ayurvedic treatment for fever, has not been consistently prepared, standardised, or scientifically assessed. The absence of proper pharmaceutical and analytical standards creates concerns about its safety, effectiveness, and consistency. The growing use of traditional medicine in modern healthcare calls for thorough scientific validation [6].

Shuddha Vatsanabha (*Aconitum ferox*), *Shunthi* (ginger), *Maricha* (black pepper), and *Shuddha Tankana* (borax) are the ingredients of *TBR,* a herbo-mineral preparation. The typical dosage is one *Masha* with *Ardraka swarasa* (ginger juice) and *Madhu* (honey), and it is used to treat various types of fever. Mentioned in the *Bharat Bhaishajya Ratnakar,* it is effective for conditions such as

Sannipatik Jwara (vitiation of all doshas/severe condition of any disease), *Vataroga* (disorders caused by vata), *Shleshmaroga* (disorders caused by kapha), *Maha Jwara* (long duration of pyrexia), *mastakasya roga* (disorders of meninges), and *pida of Udara* (pain in the lower abdomen) [7,8].

Standardising herbal and herbomineral formulations present significant challenges. However, the primary goal of drug or formulation standardisation is to ensure the final product's quality, efficacy, and consistency. This can be achieved through physical-chemical and instrumental analysis. Therefore, the aim of the present study was to establish pharmaceutical and analytical standards for *TBR.*

The aim of the study is to assess and standardise *TBR* through validated pharmaceutical and analytical methods, ensuring its consistency, safety, effectiveness, and overall quality.

Study objectives

- To formulate *TBR* following traditional Ayurvedic procedures.
- To evaluate the organoleptic and physicochemical characteristics of the ingredients and the final formulation.
- To conduct standard quality control assessments, including pH, loss on drying, ash content.
- To apply instrumental techniques such as HPTLC for analytical profiling.

MATERIALS AND METHODS

The present study was designed as a laboratory-based pharmaceutical-analytical experimental investigation and was conducted at the Department of Rasashastra evam Bhaishajya Kalpana, Mahatma Gandhi Ayurved College and Research Centre (MGACH & RC), Salod (H), Wardha, Maharashtra, India, from January 2024 to February 2024. As the study focused on pharmaceutical and analytical evaluations, informed consent was not applicable.

The formulation was prepared in three independent batches to ensure reproducibility and to assess batch-to-batch variability. The selection of three batches was based on standard pharmaceutical-analytical practice to evaluate consistency of the preparation process. Batch-to-batch variability was assessed by comparing organoleptic characters, tablet parameters, physicochemical values, microbiological findings, and HPTLC profiles across all batches.

Collection of Raw Materials

Vatsanabha and *Tankana* were procured from Cotex Laxmi Health Care Pvt., Ltd., Wardha, while *Shunthi, Maricha, Nagavalli, Ardraka*, and *Nimbu* were sourced from the local market. The materials were then examined macroscopically for key botanical features, and any physical impurities were removed.

Authentication of Raw Materials

Mineral drugs were authenticated based on their mineralogical properties by the Department of Rasashastra evam Bhaishajya Kalpana, and herbal drugs were identified through morphological traits at the Department of Dravyaguna, Mahatma Gandhi Ayurved College and Research Center, Salod (H), Wardha, Maharashtra, India.

Analytical Study

Physicochemical analyses, including total ash, acid-insoluble ash, water and alcohol-soluble extractives, loss on drying, pH, and microbial tests for pathogens such as *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, were conducted at Cotex Laxmi Health Care Pvt., Ltd., and MGACH & RC, Salod (H), Wardha, Maharashtra, India Additionally, HPTLC was performed using the CAMAG win CATS Linomat 5 system.

Pharmaceutical Study

Methods of *Shodhana* of ingredients:

***Vatsanabha Shodhana* [9]:** The roots of *Vatsanabha* (*Aconitum ferox*) were cut into small, pea-sized fragments and placed in an earthen pot filled with cow urine. The cow urine was replaced daily for three consecutive days. On the 4th day, the urine was not changed, and the pot was kept in direct sunlight. Subsequently, the root pieces were washed with cold water, and the upper layer was separated and rinsed again with warm water. These pieces were then immediately dried under sunlight and ground into a fine powder as shown in [Table/Fig-1].

***Tankana Shodhana* [10]:** The raw *Tankana* (Borax) was put into a clean, dry mortar and carefully ground into a fine powder. The powder was transferred to a clean, wide-mouthed iron vessel and heated over a flame with continuous stirring. Heating was stopped once the substance lost all moisture and became light and brittle, resembling *Lajja*. The final product was then stored in an airtight container. as shown in [Table/Fig-2].

Preparation of *Shuddha Vatsanabha Churna* [11]: *Shuddha Vatsanabha* (*Aconitum ferox*) pieces were finely ground using a *Khalva Yantra*, then the powder was sifted through a cloth to produce a smooth, fine powder, as depicted in [Table/Fig-1].

Preparation of *Shunthi churna* [12]: *Shunthi* (dry ginger) was procured and examined to remove any external contaminants. After thorough cleaning, it was ground in a *Khalva Yantra* and passed through a cloth sieve to produce a fine powder, as shown in [Table/Fig-3].

Initial weight of *Vatsanabha*	Weight after purification	Weight loss	Percentage yield
100 g	70 g	30 g	70%

[Table/Fig-1]: Results of *Vatsanabha shodhana.*

Initial weight of *Tankana*	Weight After purification	Weight loss	Percentage yield
500 g	425 g	75 g	85%

[Table/Fig-2]: Results of *Tankana* (Borax) Purification *(Shodhana).*

Net weight of raw *shunthi* before *churna*	Final weight of *shunthi* after *churna*	Loss (g)	Yield (%)
300 g	270 g	30 g	90%

[Table/Fig-3]: Results of *Shunthi churna.*

Preparation of *Maricha Churna* [13]: *Maricha* (black pepper) was gathered and inspected for impurities. After cleaning, it was ground using a *Khalva Yantra* and then sieved through a cloth to achieve a fine powder, as illustrated in [Table/Fig-4].

Net weight of raw *Maricha* before *churna*	Final weight of *Maricha* after *churna*	Loss (g)	Yield (%)
300 g	270 g	30 g	90%

[Table/Fig-4]: Results of *Maricha churna.*

Preparation of *Nagavalli Patra Swarasa:* *Nagavalli* leaves were sourced from the local market, thoroughly washed with tap water, and chopped into small pieces. These leaves were then ground into a smooth paste and filtered through a clean cloth into a stainless-steel container. The extracted juice *(swarasa)* was subsequently utilised in the *bhavana* process.

Preparation of *Ardraka Swarasa:* Fresh *Ardraka* (ginger) was gathered, cleaned, and peeled. It was then chopped into small pieces and ground into a paste using a *Khalva Yantra*. The juice *(swarasa)* was obtained by pressing the paste through a clean cloth and collected in a beaker.

Preparation of *Nimbu Swarasa:* Fresh *Nimbu* (lemon) was collected and washed. It was then cut into small pieces, and the juice was extracted using a lemon squeezer. The *swarasa* was filtered through a clean cloth, and the filtered *swarasa* was then collected in a beaker.

The preparation *of Nagavelli Patra Swarasa, Ardraka Swarasa,* and *Nimbu Swarasa* was done according to the *Sharngadhara Samhita*, using the traditional method of juice extraction through pounding as shown in [Table/Fig-5] [14].

S. No.	Ingredients name	Chemical formula/ Latin name	Quantity used
1.	*Shuddha Vatsanabha*	*Aconitum ferox* Wall.	1 Part
2.	*Shunthi*	*Zingiber officinale* Roscoe	4 Parts
3.	*Maricha*	*Piper nigrum Linn*.	4 Parts
4.	*Shuddha Tankana*	$Na_2B_4O_7.10\ H_2O$ (Borax)	6 Parts
5.	*Nagavalli Swarasa*	Juice of *Piper betle* leaves	QS
6.	*Ardraka Swarasa*	Juice of *Zingiber officinale*	QS
7.	*Nimbu Swarasa*	Juice of fresh lemon	QS

[Table/Fig-5]: Showing ingredients with their quantity used in *Tripurabhairava Rasa* (TBR) preparation [14].

The preparation of *Vati of Tripurabhairava Rasa* (TBR) [7]: *Suddha Vatsanabha* (*Aconitum ferox*), *Shunthi churna* (*Zingiber officinale* roscoe), *Maricha churna* (*Piper nigrum Linn*), and *Suddha Tankana* (Borax) were placed in a clean, dry mortar and pestle *(khalva yantra)* for grinding and triturated into a very fine powder. This mixture underwent 3-3 *bhavana* with *Nagavalli swarasa* (*Piper betle*), *Ardraka swarasa* (ginger juice), and *Nimbu swarasa* (juice of *Citrus Medica*) until the *bhavita lakshana* was achieved. A *vatika* of 250mg was prepared. The *vatika* (pills) were dried in the shade, and after complete drying, they were collected and preserved in an airtight container.

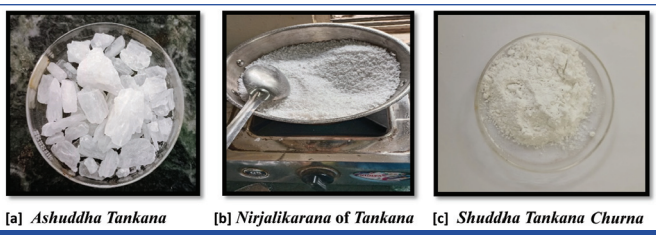
[Table/Fig-6-10] demonstrate the entire process for preparation.

[Table/Fig-6]: Images of *Vatsanabha Shodhana*

[Table/Fig-7]: *Shunthi* and *maricha churna.*

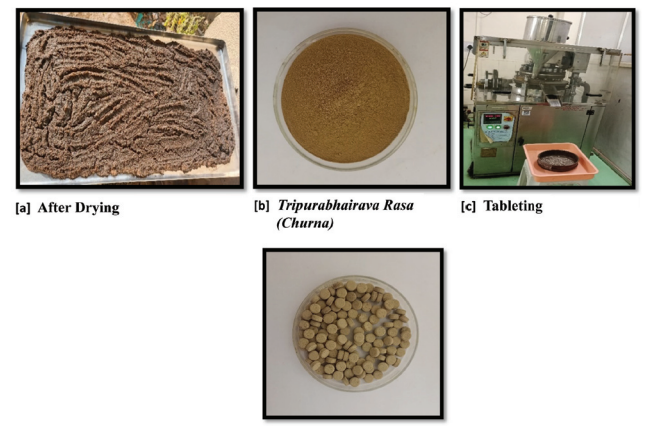
STATISTICAL ANALYSIS

Data were analysed descriptively and the results were expressed as mean values with no inferential statistical analysis performed.



[Table/Fig-8]: *Tankana Shodhana.*

[Table/Fig-9]: *Bhavana* process.



[Table/Fig-10]: Preparation of *Tripurabhairava Rasa (TBR) (Vati).*

RESULTS

Preparation of *Bhavita Tripurabhairava Rasa* (TBR) (*B-TBR churna*)

In the preparation of *Bhavita TBR,* the *Bhavana* process was performed three times using *Nagavalli Swarasa, Nimbu Swarasa,* and *Ardraka Swarasa*. The average quantity of *Swarasa* used per *Bhavana* cycle was 150 ml, with each *Bhavana* having a duration of approximately 180 minutes (3 hours). Following the third *Bhavana*, the average weight gain recorded was 5.33 grams, corresponding to a 3.34% increase in mass. This indicates successful absorption of the liquid media and effective trituration. The detailed observations are summarised in [Table/Fig-11].

Formation of *Tripurabhairava Rasa (TBR) Vati* (Tablet)

Following the *Bhavana samskara*, the resulting wet mass was converted into *Vati* (tablet) form. Each tablet was uniformly

prepared, with an average weight of 250 mg and a size comparable to a thumb in thickness. From Batch 1 (82.5 g), Batch 2 (165.0 g), and Batch 3 (247.5 g) of *Bhavita* TBR *Churna*, approximately 320, 640, and 960 tablets were prepared respectively. The average weight loss during tablet formation was found to be 3.03%, which was consistent across batches. These findings are presented in [Table/Fig-12].

Analytical study

A comprehensive analytical evaluation was carried out on the raw ingredients and the final formulation of *TBR*. Observations were systematically recorded in tabular form. Preliminary assessments focused on organoleptic properties such as colour, odour, taste and touch, as detailed in [Table/Fig-13,14]. Physicochemical parameters-including tablet testing, loss on drying at 105°C, water soluble ash, total ash, acid insoluble ash, alcohol soluble extractive, water soluble extractive, and pH were analysed, with the results summarised in [Table/Fig-15-17]. Microbial testing followed Central Council for Research in Ayurvedic Sciences (CCRAS) guidelines and included assessments for total viable count, total fungal count, Enterobacteriaceae, Salmonella species, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, as detailed in [Table/Fig-18]. Furthermore, chemical profiling was carried out using HPTLC with the CAMAG win CATS system and Linomat 5, with findings presented in [Table/Fig-19,20].

DISCUSSION

Pharmaceutical Insights

The TBR is a classical herbo-mineral formulation described in *Bharat Bhaishajya Ratnakara*, traditionally used for managing various forms of Jwara (fever). It contains *Shuddha Vatsanabha* (1 part), *Shunthi* (4 parts), *Maricha* (4 parts), and *Shuddha Tankana* (6 parts), triturated with *Nagavalli, Ardraka,* and *Nimbu Swarasa* to achieve a uniform pill consistency.

Although *Vatsanabha* contains toxic aconitine, proper *Shodhana* using *Gomutra* converts harmful alkaloids into safer derivatives (aconine and benzylaconine), reducing cardiac and neurotoxicity while retaining therapeutic efficacy [15,16]. This ensures *Vatsanabha* maintains its antipyretic, anti-inflammatory, and *Rasayana* properties in TBR [17].

Tankana (Borax), traditionally an antidote to *Vatsanabha*, has digestive, wound-healing, and anti-inflammatory activities. *Shodhana* via *Bharjana* (dry heating) removes moisture and impurities, converts $Na_2B_4O7{\cdot}10H_2O$ to $Na_2B_4O_7{\cdot}5H_2O$, and increases boron content from 10.08% to 13.48%, improving bioavailability and reducing toxicity [18,19].

Batch	Wt. of TBR *Churna*(g)	No. of *Bhavana*	Bhavana Dravya used (mL)	Durationof *bhavana* (min)	Wt. after *bhavana* (g)	Weight of wet mass after *Bhavana* (g)	% Gain
B-TBR 1	75	1st	75	180	78.0	3.0	4.00
	78.0	2nd	75	180	80.5	2.5	3.21
	80.5	3rd	75	180	82.5	2.0	2.48
B-TBR 2	150	1st	150	180	157.5	7.5	5.00
	157.5	2nd	150	180	162.5	5.0	3.17
	162.5	3rd	150	180	165	2.5	1.54
B-TBR 3	225	1st	225	180	232.5	7.5	3.33
	232.5	2nd	225	180	240.0	7.5	3.22
	240.0	3rd	225	180	247.5	7.5	3.13
Avg	150.0	-	150.0	180	165	5.00	3.23

[Table/Fig-11]: Observation during Trituration of *Tripurabhairava Rasa* (*TBR*) with *Bhavana Dravya* for preparation of *Bhavita Tripurabhairava Rasa* (TBR) (B-TBR *Churna*).

Batch	Quantity of B-*TBR Churna* (g)	Total weight of preparedtablets (g)	Weight of each tablet(mg)	Weight loss(g)	% loss	No. of tablets
TBR-1	82.5	80.0	250	2.5	3.03	320
TBR-2	165.0	160.0	250	5.0	3.03	640
TBR-3	247.5	240.0	250	7.5	3.03	960
Avg	165.0	160.0	250	5.0	3.03	640

[Table/Fig-12]: Observation during the Preparation of TBR *(Vati).*

S. No.	Organoleptic characters	*Vatsanabha*	*Shunthi*	*Marich*	*Tankana*
1	Colour	Brown to blackish	Light brown	Blackish brown	White, crystalline
2	Odour	Slight, unpleasant	Aromatic, spicy	Spicy, penetrating	Odourless
3	Taste	Bitter, acrid	Pungent	Pungent, slightly bitter	Alkaline, salty
4	Touch	Hard, coarse	Rough, fibrous	Hard, wrinkled	Smooth, powdery, cool

[Table/Fig-13]: Organoleptic characters of raw materials.

S. No.	Organoleptic Characters	Batch 1	Batch 2	Batch 3
1	Colour	Buff colour	Buff colour	Buff colour
2	Odour	Pungent, *Ati-tikshna*	Pungent, *Tikshna*	Pungent, *Tikshna*
3	Taste	Characteristic, *ati katu*	Characteristic, Pungent	Characteristic, Pungent
4	Touch/consistency	*Churna* batch- Fine Powder with a soft touch		
		Vati batch- semisolid after the *bhavana* process and the final product is solid and smooth.		

[Table/Fig-14]: Organoleptic characters of *Tripurabhairava Rasa* (*TBR*).

S. No.	Parameters	Batch 1	Batch 2	Batch 3	Average Value
1	Hardness	3.6 kg/cm²	3.8 kg/cm²	2.6 kg/cm²	3.33 kg/cm²
2	Friability	0.6%	0.7%	0.6%	0.63%
3	Disintegration time	5 min	8 min	6 min	6.33 min
4	Uniformity of weight	0.266 g	0.267 g	0.256 g	0.263 g

[Table/Fig-15]: Analysis of *Tripurabhairava Rasa* (TBR) tablet samples.

S. No.	Physicochemical parameters	*Vatsanabha*	*Shunthi*	*Marich*	*Tankana*	Average %
1	Loss on drying at 105°C (%)	0.98%	1.4%	1.9%	0.44%	1.18%
2	Total ash (%)	5.57%	6.83%	3.65%	0.19%	4.06%
3	Water soluble ash (%)	3.03%	2.35%	2.27%	0.44%	2.02%
4	Acid insoluble ash (%)	1.02%	0.5%	0.5%	0.02%	0.51%
5	Water soluble extractive (%)	21.6%	15.7%	11.7%	17.8%	16.7%
6	Alcohol soluble extractive (%)	5.92%	15.9%	1.99%	1.96%	6.44%
7	pH	6.2	4.9	7.3	12.4	7.7

[Table/Fig-16]: Physicochemical analysis of raw material.

S. No.	Physicochemical parameter	Batch 1	Batch 2	Batch 3	Average (%)
1	Loss on drying at 105°C (%)	1.59%	1.65%	1.56%	1.60%
2	Total ash (%)	32.5%	32.7%	32.8%	32.67%
3	Water soluble ash (%)	0.32%	0.38%	0.34%	0.35%
4	Acid insoluble ash (%)	0.01 %	0.02 %	0.01 %	0.01 %
5	Water soluble extractive (%)	52.94 %	52.92 %	52.93 %	52.93 %
6	Alcohol soluble extractive (%)	9.88%	9.85%	9.87%	9.87%
7	pH	10.3	10.5	10.2	10.33

[Table/Fig-17]: Physicochemical parameters of *Tripurabhairava Rasa* (*TBR*).

S. No.	Parameters	TBR
1	Total viable count	Absent
2	Enterobacteriaceae	Absent
3	Total fungus count	Absent
4	*Escherichia coli*	Absent
5	Salmonella	Absent
6	*Staphylococcus aureus*	Absent
7	*Pseudomonas aeruginosa*	Absent

[Table/Fig-18]: Microbiological parameter of *Tripurabhairava Rasa* (*TBR*).

Solvent system - Toluene: Ethyl Acetate 7:3				
S. No.	Samples	Conditions	No. of Spots	Rf values
1.	*Vatsanabha*	Short UV-254	3	0.38, 0.60, 0.77
2.	*Shunthi*	Short UV-254	2	0.79, 0.94
3.	*Maricha*	Short UV-254	4	0.14, 0.72, 0.84, 0.90
4.	*Tankan*	Short UV-254	3	0.05, 0.20, 0.80
5.	*Tripurabhairava Rasa (TBR)*	Short UV-254	5	0.32, 0.72, 0.77, 0.82, 0.90

[Table/Fig-19]: HPTLC profile of *Tripurabhairava Rasa* (*TBR*).

The *Bhavana* process with *Nagavalli, Ardraka,* and *Nimbu Swarasa* facilitates trituration, imparts digestive, anti-inflammatory, and antioxidant properties, supports *Samprapti Vighatana,* and forms a uniform herbomineral complex, enhancing stability, potency, and bioavailability [20,21].

The consistency and therapeutic reliability of *TBR* can be influenced by variations in its raw materials, which may arise from differences in geographic origin, seasonal factors, harvesting time, storage conditions, and processing techniques [22]. Such variations can alter the chemical composition, physicochemical characteristics, and concentration of bioactive constituents in *Shunthi, Maricha,* and *Vatsanabha,* as well as affect the purity of *Tankana,* potentially impacting the potency, reproducibility, and safety of the final formulation [23]. To minimise these batch-to-batch differences, it is essential to perform careful authentication and standardisation of raw materials and to strictly adhere to classical processing methods, including *Shodhana, Bhavana,* and *Vati* preparation, thereby ensuring uniformity, stability, and therapeutic efficacy [24].

Analytical Findings

Evaluation of three batches demonstrated consistent organoleptic, physicochemical, and microbiological properties. Tablets (Vati) exhibited uniform weight (250 mg), low processing loss (3.03%), hardness (2.6-3.8 kg/cm²), low friability (0.600.7%), and rapid disintegration (5-8 minutes). Physicochemical parameters showed low moisture (1.60%), total ash (32.67%), higher water-soluble extractive (52.93%) than alcohol-soluble extractive (9.87%), and alkaline pH (10.33), indicating good stability, extractability, and potential gastrointestinal absorption.

Microbiological evaluation confirmed the absence of *Escherichia coli*, Salmonella spp., *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, ensuring microbial safety. HPTLC profiling (toluene: ethyl acetate 7:3, 254 nm) revealed five spots with Rf values 0.32, 0.72, 0.77, 0.82, and 0.90, where 0.72 and 0.77 corresponded to *Maricha* and *Vatsanabha,* confirming chemical integrity and providing a reproducible chromatographic fingerprint. These results support the standardisation, quality control, and clinical safety of TBR.

Implications for Standardisation

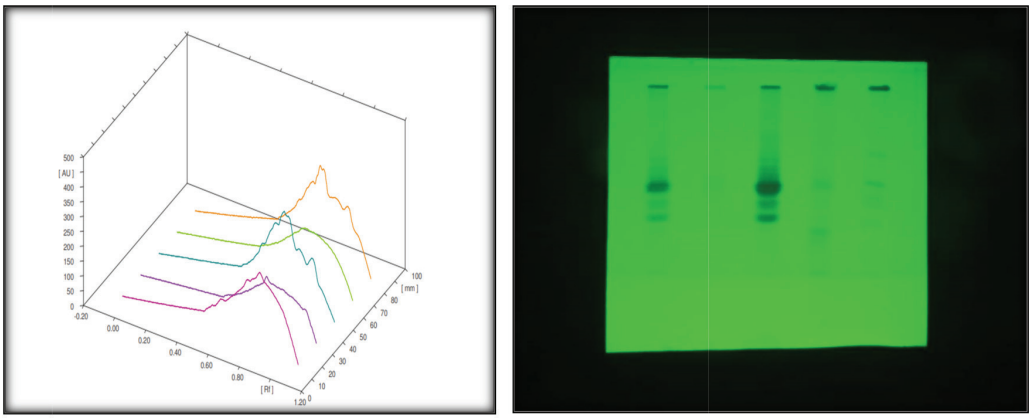
The findings demonstrate batch-to-batch consistency, chemical integrity, and safety, supporting the standardisation of *TBR.* Combining classical Ayurvedic pharmaceutical techniques with modern analytical assessment ensures the formulation is both therapeutically effective and pharmaceutically reliable. Reproducible physicochemical, mechanical, and chromatographic parameters provide a strong foundation for quality control and consistent clinical outcomes. To further strengthen translational relevance, future studies should incorporate advanced chemical profiling techniques, such as Liquid Chromatography-Mass Spectrometry (LC-MS), to comprehensively characterise bioactive constituents, alongside pharmacological and clinical validation to confirm long-term safety, efficacy, and optimal dosing in humans.

Limitation(s)

The present study is limited to preclinical evaluation, without human clinical trials. Long-term safety, dosage optimisation, and potential metabolic interactions were not assessed. While HPTLC provided a reproducible chemical fingerprint, advanced analytical methods such as LC-MS or Nuclear Magnetic Resonance (NMR) could offer deeper insight into bioactive constituents. Additionally, raw material variability, seasonal differences, and sourcing could affect reproducibility, highlighting the need for multi-batch and multi-centre validation. Addressing these aspects in future studies will strengthen the evidence for clinical use and standardisation of *TBR.*

CONCLUSION(S)

Standardising pharmaceutical preparations is essential to guarantee the safety, effectiveness, and quality of Ayurvedic medicines. In the case of *TBR*, HPTLC analysis confirmed the presence of active constituents, establishing its chemical composition. The *Shodhana* and *Bhavana* processes played a vital role in removing impurities and



[Table/Fig-20]: Depiction of HPTLC analysis at 254 nm wavelength.

enhancing the formulation's potency and bioavailability. The present study demonstrated the pharmaceutical preparation and analytical standardisation of Tripurabhairava Rasa. Further Pharmacological, toxicological, and clinical studies are warranted to establish its therapeutic efficacy and safety. The preparation methods for *TBR* have been thoroughly validated, providing a solid foundation for future research and clinical applications. The analytical results serve as a benchmark for maintaining the consistency and quality of the formulation, ensuring its safe and reliable use in healthcare environments. Further clinical studies are recommended to verify its therapeutic potential and explore its broader medical applications.

Acknowledgement

The authors express their sincere gratitude to the Department of Rasashastra evam Bhaishajya Kalpana, Mahatma Gandhi Ayurved College and Research Centre (MGACH & RC), Salod (H), Wardha, Maharashtra, India, for providing necessary facilities and support to carry out this research work. The authors also acknowledge Cotex Laxmi Health Care Pvt., Ltd., and MGACH & RC for their assistance in conducting the analytical studies.

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PARTICULARS OF CONTRIBUTORS:
1. Postgraduate Scholar, Department of Rasashastra and Bhaishajya Kalpana, Mahatma Gandhi Ayurved College Research Centre, Wardha, Maharashtra, India.
2. Associate Professor, Department of Rasashastra and Bhaishajya Kalpana, Mahatma Gandhi Ayurved College Research Centre, Wardha, Maharashtra, India.
3. Postgraduate Scholar, Department of Rasashastra and Bhaishajya Kalpana, Mahatma Gandhi Ayurved College Research Centre, Wardha, Maharashtra, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Sukeshini Julme,
Mahatma Gandhi Ayurved College Hospital and Research Centre, Salod (H),
Wardha, Maharashtra, India.
E-mail: sukeshinijulme521@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Nov 25, 2025
- Manual Googling: Mar 12, 2026
- iThenticate Software: Mar 14, 2026 (1%)

ETYMOLOGY: Author Origin

EMENDATIONS: 6

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? NA
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jul 22, 2025**
Date of Peer Review: **Dec 14, 2025**
Date of Acceptance: **Mar 17, 2026**
Date of Publishing: **Aug 01, 2026**