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Research Article

ANANDABHAIRAVA RASA: A COMPARATIVE ANALYSIS OF TWO *VATSANABHA SODHANA* APPROACHES

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ABSTRACT

Anandabhairava Rasa (ABR) is an Ayurvedic herbo-mineral formulation traditionally used to treat fever and diarrhea. It contains *Vatsanabha* (*Aconitum ferox* Wall.), a highly toxic herb. In Ayurveda, *Śodhana* (purification) is a process that renders toxic substances safe and therapeutically effective. This study investigates the effects of two distinct *Śodhana* media-cow urine *(Gomutra)* and cow milk *(Godugdha)* on the physicochemical properties of *Vatsanabha* and the final ABR formulations. **Materials and Methods**: *Vatsanabha* tubers was separately purified using classical (*Śodhana*) methods using cow urine and cow milk. Subsequently, the two distinct batches of ABR were prepared by triturating the ingredients and compressing them into tablets. The raw materials were and final products were subjected to analytical study, including organoleptic characterization, physicochemical parameter assessment and chromatographic profiling. The Thin Layer Chromatography (TLC) of unpurified *Vatsanabha* and purified *Vatsanabha* and High-Performance Thin Layer Chromatography (HPTLC) of *Anandabhairava Rasa* (ABR) prepared from both types of purified *Vatsanabha*. **Results and Discussion**: The purification of *Vatsanabha* using cow urine and cow milk resulted in notable physicochemical alterations, including a significant shift in pH, differences in water-soluble extracts, and distinct TLC patterns. Although the resulting *Anandabhairava Rasa* (ABR) formulations exhibited similar size and texture, slight differences were observed in hardness and disintegration time. **Conclusion:** The study demonstrates that the use of cow urine and cow milk as *Śodhana* media for *Vatsanabha* results in two chemically distinct *Anandabhairava Rasa* formulations. Further quantitative and clinical studies are necessary to evaluate the precise therapeutic implications of these variations.

INTRODUCTION

In ancient India, Ayurveda, has a long-standing tradition of using herbo-mineral formulations for holistic healing. *Rasaśastra*, a specialized branch of Ayurveda, focuses on the preparation and standardization of *Rasaoushadhis* (herbo-mineral formulations) that exhibit potent therapeutic effects. *Rasoushadhis* are classified as *Kharaliya*, *Parpati, Pottali* and *Kupipakwa rasayan* as per the method of preparation.

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Anandabhairava Rasa[1] is well-known *Kharaliya rasayan (*herbo-mineral formulation) documented in many classical texts like Rasa Yog Sagar and Bhaishajya Ratnavali, with slight variation in composition, thus the indications. It is recognized for its effectiveness in treating of *Jvarastisar* (febrile diarrhoea), *Jvara* (fever) and *Atisara* (diarrhea), *Kasa* (cough), *Shwasa* (respiratory disorders), *Mandagni* (weak digestive fire) etc. It contains *Vatsanabha* (Aconitum ferox), *Hingula, Ṭankana,* as a key-ingredients, which requires proper purification (*Śodhana*) before its use to mitigate their inherent toxicity and enhance therapeutic potency.

Vatsanabha botanically identified as *Aconitum ferox* Wall. of *Ranunculaceae* family is a deciduous perennial herb with racemes of blue zygomorphic

flowers and numerous stamens. *Vatsanabha* is potent poisonous plant explained in almost all the classical ayurveda text as tuber root poison (*Kanda Visha*) and is categorized as *Sthavara Visha* (poison derived from plant sources), and *Mahavisha* (highly poisonous substance) due to the presence of alkaloids, which can cause severe cardiovascular ill-effects if consumed unprocessed. In Ayurveda, the concept of *"Śodhana"* is crucial for the detoxification and purification of poisonous substances like *Vatsanabha*. To ensure its safety, *Śodhana*[2] (purification) techniques using specific media is not merely a detoxification process but it also enhances the drug's potency and efficacy. *Śodhana* is essential to mitigate *Vatsanabha's* toxicity and enhance its therapeutic efficacy by converting harmful diterpene alkaloids like aconitine and pseudoaconitine, aconine etc. into less toxic forms.[3] Various media are used in the *"Śodhana* process to neutralize the toxic components of *Vatsanabha.* The two widely practiced *Śodhana* methods of *Vatsanabha* involve using *Gomutra* (cow urine) and *Godugdha* (cow milk)[4]. Several studies have explored the *Śodhana* process of *Vatsanabha* using different media and their impact on its physico-chemical properties[2–10]. *Anandabhairava Rasa* (ABR) is been proven for its anti-pyretic activity[11], anti-microbial[12], anti-amoebiasis[13]. However, a focused comparative study that specifically investigates the pharmaceutical and analytical differences in *Anandabhairava Rasa* formulations prepared using *Vatsanabha* processed with two distinct *Śodhana* approaches is lacking.

This study aims to compare two different purification methods, using cow urine and cow milk as media of purification- on the physicochemical properties of *Vatsanabha* and the resultant *Anandabhairava Rasa* (ABR) which is prepared as per *Bhaishajyaratnavali Jwaratisaar- rogadhikar*[14]. The comparative analysis contributes to the understand how purification methods influence various physicochemical parameters, and organoleptic characteristics of formulation.

MATERIAL AND METHODS

Raw Material Collection and Authentication

On January 30, 2020, *Vatsanabha* tubers, black pepper, long pepper, lemon, *Hingula*, and *Ṭankana* were sourced from a verified seller in Nagpur, Maharashtra, India. All raw drugs were authenticated by the HOD (Head of Department) of the *Dravyaguna* and HOD *Rasaśastra* and *Bhaiṣajyakalpana* at an Ayurvedic college. The specimens were then deposited in the departmental herbarium with the following voucher numbers: BMAM-DG-2020-005 for *Aconitum ferox* Wall., 006 for *Piper nigrum* Linn., 007 for *Piper longum* Linn., and 008 for *Citrus limon* (L.) Burm. f. Additionally, fresh cow urine and cow milk were procured from a local cowshed.

Anandabhairava Rasa Preparation[14]

Anandabhairava Rasa is a *Kharliya* Ayurvedic formulation, meaning its preparation involves the meticulous trituration of ingredients in a mortar and pestle. The final product was prepared through the Bhavana (wet trituration) process using a reference formulation [14]. The ingredients, *Śodhita Vatsanabha (Aconitum ferox* Wall.), *Śodhita Hingula* (Cinnabar), *Śodhita Ṭankana* (Borax), Marich *(Piper nigrum* Linn.), *and Pippali (Piper longum* Linn.), were each used in equal proportions of 100g. Lemon juice (*Citrus limon* (L.) Burm. f) was added in a sufficient quantity (Q.S.) as the trituration medium. The proportions are detailed in the table below.

Table 1: Ingredients and proportions

Drug Name	Latin / English Name	Part used	Proportion
Śodhita Vatsanabha	*Aconitum ferox* Wall. (Ranunculaceae)	Tubers	100gm
Śodhita Hingula	Cinnabar	-	100gm
Śodhita Ṭankana	Borax	-	100gm
Marich	Black pepper / *Piper nigrum* Linn (Piperaceae)	Fruit	100gm
Pippali	Long pepper / *Piper longum* Linn (Piperaceae)	Fruit	100gm
Lemon Juice		Fruit	Q.S

Pharmaceutical study

Pharmaceutical study of ABR was carried out at Teaching Pharmacy of Ayurvedic College. Preparation of *Anandabhairava Rasa* was done in 6 phases namely - 1) *Śodhana* of *Vatsanabha* in cow urine and 2) *Śodhana* of *Vatsanabha* in cow milk. 3) *Śodhana* of *Hingula* 4) *Śodhana* of *Ṭankana*. 5) Preparation of *Anandabhairava Rasa* using cow urine *Śodhita Vatsanabha*. 6) Preparation of *Anandabhairava Rasa* using cow milk *Śodhita Vatsanabha.*

FIGURE NO. 1 PHARMACEUTICAL STUDY OF COMPOSITION OF *ANANDBHAIRAV RASA*

***Śodhana* of *Vatsanabha* in Cow urine**[15] **(figure 1) (CUSV)–** 400gm of crude *Vatsanabha* was cut into small pea-size pieces and immersed in a stainless-steel vessel containing 2000ml of fresh cow urine under intense sunlight. Each day cow urine was replaced with fresh cow urine for 3 consecutive days[15]. On 4th day, it was washed with normal water, and the outer layer was peeled off with the help of knife, and the pieces were sun-dried for two days. The dried pieces were grinded to get the powder form and stored in air tight container with label cow urine *Śodhita Vatsanabha (CUSV)*[15].

***Śodhana* of *Vatsanabha* in cow milk**[16] **(figure 1) (CMSV) -** 400gm of crude *Vatsanabha* was cut into small pea- sized pieces, tied in a cloth to form a *Pottali* (bag), tied in *Ḍolayantra* (An apparatus used to detoxify the materials by suspending the bundle in specified liquids for a specified time period), with 3000ml of fresh cow milk under moderate heat for 4 hours at 100°C -110°C. Afterwards the pieces was washed with lukewarm water. Same process was carried out for three days. Further, the outer layer of Aconite was peeled off with help of knife, and the pieces were sun-dried for two days. The dried pieces were grinded into the powder form and stored in air tight container with label cow milk *Śodhita Vatsanabha (CMSV)*[16].

***Śodhana* of *Hingula* (figure 1)-** 250gm of *Ashuddha Hingula* was placed in a stone mortar and pestle, and finely powdered. *Śodhana* of *Hingula* was done by *Bhavana* (wet trituration) method. For the first *Bhavana*, 100ml of lemon juice was added, and the mixture was triturated until completely dry. This process was repeated seven times using fresh lemon juice each time. After the final *Bhavana*, the mixture was washed thoroughly, allowed to settle for six hours in a stainless-steel vessel, and the water was decanted carefully so as to avoid loss of *Hingula*. The purified *Hingula* was then dried and stored in an airtight glass container[17].

Śodhana* of *Ṭankana[18] **(figure 1)-** 400gm of *Ashuddha tankana* was pounded into a fine powder using a mortar and pestle and heated in an iron vessel on low flame, continuous stirring was ensured for the complete evaporation of water content, yielding white, puffy *Śodhita Ṭankana*, which was further allowed to cool and then powdered in mortar and pestle to obtain fine *Śodhita Ṭankana* powder[18].

Preparation of Powders of Piper nigrum and Piper longum (figure 1) - Physical impurities from crude Piper nigrum and Piper longum were removed, and was pounded in a clean, dry mortar and pestle, then finely powdered in grinder and filtered through a muslin cloth.

Preparation of Cow urine *Śodhita Vatsanabha-Anandabhairava Rasa* (CUSV-ABR) figure 3– Cow urine *Śodhita Vatsanabha, Śodhita Hingula, Śodhita Ṭankana,* black pepper, long pepper powders each 100g were triturated in *Khalva Yantra* (mortar and pestle) and mixed thoroughly. Lemon juice was added and triturated until dry. This was repeated for three times. On drying the dried reddish-brown powder was then compressed into 250mg tablets using a tableting machine.

Preparation of Cow milk *Śodhita Vatsanabha-Anandabhairava Rasa* (CMSV-ABR) figure 2– Cow milk *Śodhita Vatsanabha, Śodhita Hingula, Śodhita Ṭankana,* black pepper, long pepper 100g each were mixed and triturated in a *Khalva Yantra (*mortar and pestle*).* Lemon juice was added and triturated until dry, this was repeated for three times. The dried mixture formed a smooth reddish-brown powder, which was then compressed into 250 mg tablets using a tableting machine.

FIGURE NO. 2 PHARMACEUTICAL STUDY OF *ANANDBHAIRAV RASA - COW MILK SHODHITA VATSANABHA*

FIGURE NO. 3- PHARMACEUTICAL STUDY OF *ANANDBHAIRAV RASA - COW URINE SHODHITA VATSANABHA*

Analytical study

Physicochemical Analysis[19]**:** Organoleptic characteristics, such as color, odour, touch, taste and appearance and physicochemical parameters such as ash values, pH, extractive values, etc. were tested for Raw *Vatsanabha,* CMSV, CUSV[6], *Ashuddha Hingula*, *Śodhita Hingula, Ashuddha Ṭankana, Śodhita Ṭankana,* black pepper, long pepper were recorded.

pH: This procedure was carried out to find out the acidity and alkalinity of the samples. 1gm of sample was taken in 10 ml of distilled water, stirred for ½ hour, filtered and pH of filtrate was noted with pH meter[6,19,20].

Total Ash value: Sample was taken in a weighted dish and was strongly heated in muffle furnace at 450°C for 3 hours. Continued heating was done until constant weight was obtained. The dish was cooled in desiccator and weighed[20]. Percentage of total ash with reference to air dried sample was calculated as[6,19,20]-

Total ash % = $\frac{\text{Weight of ash}}{\text{Weight of sample taken}} \times 100$

Water soluble Ash: Total ash obtained by incineration of 1 gm of sample up to constant weight, it was boiled for few minutes with 5 ml of distilled water. The insoluble matter was collected on Whatman's ashless filter paper and kept in muffle furnace at about 450^0 C and weighed. The water soluble ash is obtained by subtracting this ash from total ash[6,19,20].

Percentage of Water-soluble ash was calculated with formula

Water soluble ash% = $\frac{\text{Water soluble ash}}{\text{3w Weight. sample taken}} \times 100$

Acid insoluble ash: The ash obtained in total ash above was boiled with 25ml of 2 N hydrochloric acid

(HCl) for 5 minutes. The insoluble matter was collected on Whatman's ashless filter paper. It was washed with hot water and the filter paper residue was incinerated for about 10-15 minutes, cooled and weighed. Heating was continued until constant weight was obtained. Percentage of acid insoluble ash was calculated[6,19,20]-

$$\text{Acid insoluble ash\%} = \frac{\text{Acid insoluble ash}}{\text{Wt. sample taken}} \times 100$$

Moisture content: This procedure determines the percentage of water in a sample. The sample is weighed wet, then dried at temperature not exceeding 239°F (115°C) until it reaches a constant weight. After cooling the dried sample is weighed again. Then the moisture content is calculated as the percentage of water relative to the final dry weight[6,19,20].

Uniformity of Weight: 10 tablets of *Anandabhairava Rasa* were taken randomly and weighed individually. The average weight was calculated. The weight of the individual tablet was compared with the average weight[6,19,20].

Hardness Test: Ten random tablets were tested for hardness using a Monsanto tester. Each tablet was held vertically between the tester's jaws, and the force applied was gradually increased by hand until the tablet broke. The breaking force was recorded, and the mean breaking force of the ten tablets was calculated[20].

Friability Test: Randomly selected tablets were weighed and placed in a friability test apparatus. The friability test apparatus revolves 100 times at 25 revolution per minute, causing tablets to fall 6" per revolution. After 100 revolutions, tablets were reweighed. The weight loss was noted, and the percentage of friability was calculated[20].

Rekhapurnatva[21]**-** The test involves the rubbing of powder in thumb and fore finger. If the powder is fine enough, it will completely fill the tiny lines and crevices on the fingertips[21]. This indicates fineness and quality of powder ensuring its therapeutic efficacy and safety. It was tested for *Śodhita Hingula* and *Śodhita Ṭankana*

Varitara[21]**:** When the powder in small quantity is sprinkled in a small quantity on the surface of still water. If the powder is light enough and has been properly processed, it will float on the water without sinking. It was tested for *Śodhita Hingula and Śodhita Ṭankana.*

AAS **(Atomic Absorption spectroscopy):** AAS is a sensitive technique that can precisely quantify the concentration of specific minerals in a sample by detecting the absorption of light at specific wavelength by the atoms of that element in a flame[22]. The sample to be analyzed is ashed and dissolved in an aqueous solution. A beam of radiation is passes through atomized sample, and the absorption of radiation is measured at specific wavelength corresponding to the mineral of interest[22,23]. The percentage of mercury and sulfur present in *Anandabhairava Rasa* was calculated.

TLC (Thin layer Chromatography): TLC analysis was conducted for both purified (*Śodhita*) and unpurified (*Aśodhita*) *Vatsanabha.* A 4gm sample was soaked in 40 ml of chloroform, left overnight, boiled for 10 minutes, and then filtered. The filtrate was concentrated and adjusted to a final volume of 10 ml in a standard flask. A 20µl aliquot of this solution was applied to a Merck aluminum plate 60F 250 precoated with silica gel of 0.02mm thickness. The plate was developed using a solvent system of Toluene: Ethyl acetate in a 7:1.5 ratio.

After drying, the plate was examined under UV light at 254 nm and 366nm, and photographs were taken. The plate was then dipped in a methanolic-sulfuric acid reagent and heated in an oven at 105°C until spot colors appeared, followed by photographic documentation. The Rf values were recorded. The same procedure was repeated using iodine vapors and Dragendorff reagent for further analysis.

HPTLC analysis: The High Performance Thin layer Chromatography (HPTLC)[24,25] analysis of sample of *Anandabhairava Rasa* tablets, was conducted to compare the chemical profiles of ABR prepared separately using CMSV and CUSV. The study was performed using CAMAG HPTLC. A 2gm of CMSV-ABR and CUSV- ABR was dissolved in 20 ml methanol and allowed to extract for 24 hours to prepare the methanolic extract. The optimal solvent system for HPTLC analysis was determined through multiple trials as Toluene: Ethyl acetate: Formic acid in a 5:4:1 ratio.

The prepared sample was applied onto precoated silica gel 60F254 plates (5.0×10cm) using a CAMAG Linomat 5 applicator under controlled conditions. The application was performed using inert gas spray, ensuring uniform sample distribution. The dosage speed was set to 150nL/s, and a pre-dosage volume of 0.2 µl was maintained for precision. Two tracks were created, corresponding to CUSV-ABR and CMSV-ABR of 10 mm band length.

A rectangular twin-trough glass chamber (20×10 cm) using mobile phase was optimized for separation of active constituents. The solvent front position was set at 85.0mm and a total 10 mL of mobile phase was used. Further plates were dried in an oven at 60°C for 5 minutes. CAMAG TLC Scanner 3 was used for detection, scanning of plates was done at multiple wavelengths (254nm, 366nm, and White R). The resulting Chromatogram provided RF values, Peak heights, and areas. The data was processed using winCATS Planar Chromatography Manager.

Table 2: Results of physico-chemical analysis of cow milk *Śodhita Vatsanabha* (CMSV) and cow urine *Śodhita Vatsanabha* CUSV, Raw *Hingula,* Purified *Hingula,* Raw *Ṭankana, Śodhita Ṭankana,* Black pepper, Long pepper

Tests	**Crude *Vatsanabha***	**CMSV**	**CUSV**	***Aśodhita Hingula***	***Śodhita Hingula***	***Aśodhita Ṭankana***	***Śodhita Ṭankana***	**Black pepper**	**Long pepper**
Description	Blackish Brown colored longitudinal root with pungent smell & taste	Light yellowish brown colored powder with Aromatic smell & taste	Light yellowish brown colored powder with characteristic smell and taste	Bright red, crystalline, scarlet streak, translucent to opaque	Brick red colored powder	White crystalline lumps with alkaline taste	white colored powder	Greyish black hard wrinkled fruit	Blackish dark green colored longitudinal fruit
Color	Blackish brown	Light yellowish brown	Light yellowish brown	Bright red	Saffron red	White shiny	White	Greyish black	Blackish
Taste	-	-	-	Tasteless	Acrid	*Katu* (pungent)- Alkaline	*Katu* (pungent) - Alkaline	Pungent	Pungent
Odor	Pungent	Aromatic	Characteristic	Odorless	Citrus aroma	Odorless	Odorless	Aromatic	Pungent
Texture	hard	smooth	smooth	Hard, rough	Fine, smooth	Rough. Hard	Smooth, fine	soft	soft
Foreign matter	0.88%	Nil	Nil	-	-	-	-	-	-
Water soluble extractive value	26.231%	36.31%	32.931%	-	-	-	-	10.94%	12.91%
Alcohol soluble extractive	10.712%	6.992%	8.112%	-	-	-	-	8.51%	9.14%
pH(5% solution)	6.445	5.15	7.85	5.82	6.9	-	-	5.21	4.81
Total ash	4.37%	6.55%	7.97%	4.09%	5.26%	-	-	3.42%	4.77%
Mercury Content	-	-	-	78.885%	68.195%	-	-	-	-
Sulphur Content	-	-	-	12.849%	8.339%	-	-	-	-
Solubility in water	-	-	-	Insoluble	Insolubl e	complies	complies	-	-

Acid Insoluble Ash	1.12%	1.11%	3.992%	2.555% w/w	3.915% w/w	-	-	0.44%	0.35%
Solubility in HCL	-	-	-	Soluble	Soluble	complies	complies	-	-
Solubility in H2SO4	-	-	-	Soluble	Soluble	complies	complies	-	-
Niścandra	-	-	-	NO	Yes	-	-	-	-
Rekhapurnatva	-	-	-	NO	Yes	-	-	-	-
Varitara	-	-	-	NO	Yes	Sinks to the bottom	Yes	-	-
Boron Trioxide (B2O3)	-	-	-	-	-	42.445%	38.58%	-	-
Sodium	-	-	-	-	-	18.75%	14.95%	-	-
Moisture content	-	-	-	-	-			3.445%	4.85%

OBSERVATIONS AND RESULTS

Pharmaceutical study

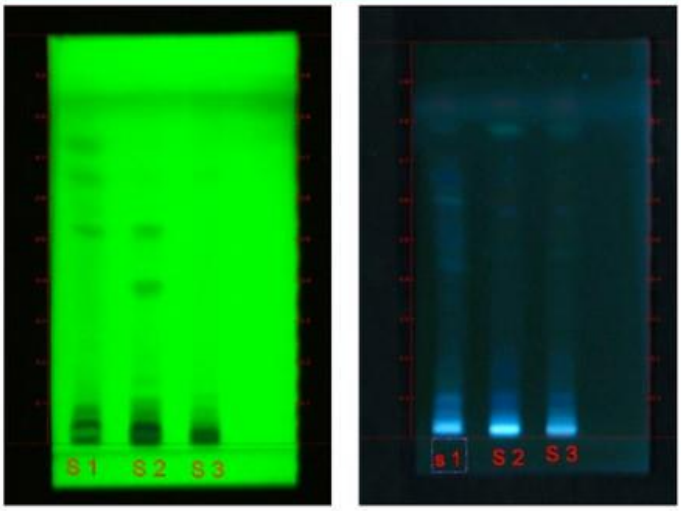
Aśodhita Vatsanabha is externally dark blackish brown and resemble like cow's udder. After *Śodhana* there was significant weight reduction in Cow urine *Śodhita Vatsanabha* from 400g to 175g, while cow milk *Śodhita Vatsanabha* also leads to reduction but final weight was higher at 190 g. Unlike *Vatsanabha, Hingula* shows an increase in weight after *Śodhana*, going from 250g to 270g. *Śodhana* of *Hingula* required 9 days, total hours taken were 17 hrs and 48 mins. *Aśodhita Hingula* were hard solid, bright red shinning blocks, without any specific odor while *Śodhita Hingula* is soft, fine, saffron red lustureless powder with citrus aroma. *Aśodhita Tankana* is crystalline, translucent, irregular solid. Weight of *Tankana* also gets reduced from 400g to 245g after *Śodhana* which is about 38.75% loss in weight. *Total* duration of *Tankana Śodhana* was 2 hours and 15 minutes. Black pepper was blackish brown in colour when crude and converted to greyish black after powdered with soft touch and pungent taste. Powder of long pepper was dark green in colour, soft touch and pungent in taste. Black pepper and long pepper showed marginal decrease in weight after powdering, from 250g to 240g for black pepper, 250g to 235g for long pepper.

Analytical study

Vatsanabha: Crude *Vatsanabha* is characterized by its blackish-brown color and pungent odor. Both CMSV and CUSV exhibit a light yellowish-brown color, and smooth, however, they differ in odor, with CMSV having an aromatic smell and CUSV a characteristic cow urine odor. Crude *Vatsanabha* contains 0.88% foreign matter, whereas both CMSV and CUSV show its complete absence. CMSV has a higher water-soluble extract value (36.31%) compared to CUSV (32.931%) and raw *Vatsanabha* (26.231%). However, CUSV has a higher alcohol-soluble extract value (8.112%) than CMSV (6.992%), with raw *Vatsanabha* at 10.712%. Total ash content is higher in both CMSV (6.55%) and CUSV (7.97%) than in raw *Vatsanabha* (4.37%), indicating an increase in mineral content post-purification. CUSV also has a considerably higher acid-insoluble ash content (3.992%) compared to CMSV (1.11%) and raw *Vatsanabha* (1.12%). Crude *Vatsanabha* has a pH of 6.445, CMSV is more acidic at 5.15, and CUSV is slightly alkaline at 7.85.

Thin layer Chromatography (TLC)-of *Vatsanabha* (refer figure 4)

FIGURE NO. 4 - TLC IMAGES OF VATSANĀBHA



TLC at 254nm TLC at 366nm

S1 Cow urine Śodhita Vatsanābha (CUSV)
S2- Aśodhita Vatsanābha
S3- Cow milk Śodhita Vatsanābha (CMSV)

TLC of *Aśodhita Vatsanabha (S2*) Showed following characters

1. Iodine vapours - major spots at Rf – 0.01, 0.05, 0.1, 0.15, 0.4, 0.5, 0.8 (all sky blue).
2. Dragendroft Reagent – major spot Sports Rf -0.01, 0.18, 0.6, 0.8 (all light violet).

TLC of Cow urine *Śodhita Vatsanabha* (S1) Showed following characters

1. Iodine Vapours – Major spots at Rf- 0.05, 0.09, 0.52, 0.66, 0.75 (all sky blue).
2. Dragendrolf Reagent – major spot Rf – 0.1,0.6, 0.8 (all light blue).

TLC of Cow milk *Śodhita Vatsanabha* (S3) Showed following characters

1. Iodine vapours – Major spots at Rf- 0.05, 0.1, 0.68 (all sky blue).

2. Dragendorff Reagent – major spot Rf –0.8 (light blue).

***Hingula*:** Unpurified *Hingula* is bright red, crystalline, scarlet streak, opaque while purified *Hingula* is a brick red colored powder. Raw *Hingula* is odorless, but purified *Hingula* has a citrus smell. The texture of raw *Hingula* changes from hard and rough to fine and smooth. The pH increases from 5.82 to 6.9 and ash content increases from 4.09% to 5.26% after purification of *Hingula.* Raw and purified *Hingula* remains insoluble in water, while soluble in HCl and H_2SO_4 in both raw and purified forms. In purified *Hingula* there was absence of metallic shine *(Niścandra), Rekhapurnatva, and Varitara,* which are indicative of finer particles and proper processing.

Tankana: Unpurified Ṭankana are white crystalline lumps with alkaline taste while purified *Ṭankana* is a white colored powder. The color remains white, and both forms are odorless and the taste is *Katu* (pungent)- Alkaline for both. The texture changes from rough and hard to smooth and fine. The content of boron trioxide and sodium decreases after purification. Purified *Tankana* floats on water *(Varitara)* and is too fine to exhibit *Rekhapurnatva.*

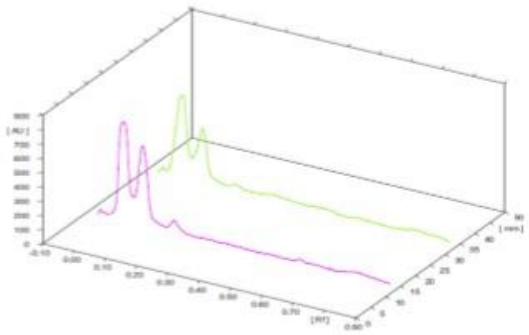
Black Pepper and Long Pepper: Black pepper and long pepper when powdered gets converted to greyish black color with aromatic odor and blackish green color with pungent odor respectively, while both are pungent in taste.

***Anandabhairava Rasa* -** (CMSV-ABR) and (CUSV-ABR), both formulations have similar characteristics - *Snigdha* (unctuous) touch, brownish-black color, and a citrus smell and biconvex tablets were formed. The average weight is slightly different, with CMSV-ABR at 258mg and CUSV-ABR at 252mg. The diameter is the same for both (8.20 mm), but the thickness varies slightly (3.74mm for CMSV-ABR and 3.70mm for CUSV-ABR). CUSV-ABR tablets are harder 2.5kg/cm^2 than CMSV-ABR tablets 2.0 kg/cm^2. Disintegration time is longer for CUSV-ABR 12 minutes compared to CMSV-ABR 10 minutes. Friability is also slightly higher for CUSV-ABR. The pH is similar for both formulations 6.45 for CMSV-ABR and 6.34 for CUSV-ABR. Total ash value and acid-insoluble ash vary slightly 7.044% for CMSV-ABR and 7.25% for CUSV-ABR. Alcohol-soluble and water-soluble extractive values are 8.992% for CMSV-ABR and 7.280% for CUSV-ABR. Concentrations of mercury 8.005 % in CMSV-ABR and7.335% in CUSV-ABR; and sulfur concentration was 3.115% was in CMSV-ABR and 3.45% in CUSV-ABR are close but not identical.

Table 3: Physico-chemical Analysis of *Anandabhairava Rasa*

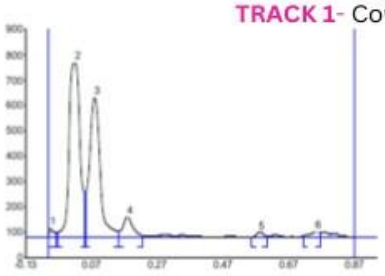
Tests	**CMSV-ABR**	**CUSV-ABR**
Sparsha	*Snigdha* (uncutous)	*Snigdha* (uncutous)
Roopa	*Brownish black*	*Brownish black*
Gandha	*Citrus smell*	*Citrus smell*
Description	Biconvex	Biconvex
Color	Brown	Brown
Average weight	258 mg	252 mg
Diameter	8.20 mm	8.20 mm
Thickness	3.74 mm	3.70 mm
Hardness	2.0 kg/cm^2	2.5kg/cm^2
Disintegration	10 min	12 min
Friability	0.1600 kg/cm^2	0.1873 kg/cm^2
pH	6.45	6.34
Total ash value	7.044%	7.25%
Acid insoluble ash	1.33%	2.40%
Alcohol soluble extractive	8.992%	7.280%
Water soluble extractive	16.711%	14.117%
Moisture content	5.12%	4.32%
Concentration of mercury	8.005%	7.335%
Concentration of sulfur	3.115%	3.45%

FIGURE NO. 5. HPTLC IMAGES OF ANANDBHAIRAV RASA



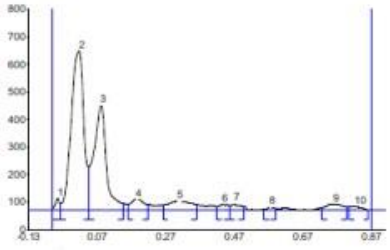
Measurement mode -Absorption

TRACK 1- Cow urine *Shodita Vatsanabha- Anandbhairav Ras*



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.07	3.9	-0.06	39.3	2.78	-0.04	20.5	478.2	1.21
2	-0.04	21.1	0.02	693.2	48.98	0.05	178.6	19941.7	50.32
3	0.05	179.0	0.08	554.3	39.17	0.15	23.3	15945.7	40.24
4	0.15	23.6	0.18	82.3	5.81	0.22	7.5	2217.4	5.60
5	0.55	1.5	0.58	22.0	1.55	0.60	3.6	408.9	1.03
6	0.71	4.6	0.75	24.2	1.71	0.76	20.6	635.0	1.60

TRACK 2 -Cow milk *Shodita Vatsanabha- Anandbhairav Ras*



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.06	5.7	-0.05	44.4	3.75	-0.04	24.6	461.7	1.23
2	-0.04	25.0	0.01	579.7	49.03	0.04	156.6	18592.3	49.69
3	0.04	157.3	0.08	382.7	32.37	0.14	25.3	12145.0	32.46
4	0.16	22.3	0.18	41.5	3.51	0.22	19.6	1423.9	3.81
5	0.26	19.3	0.30	34.7	2.93	0.36	20.7	2072.6	5.54
6	0.42	12.8	0.43	23.3	1.97	0.45	15.8	520.7	1.39
7	0.45	16.0	0.47	24.9	2.11	0.49	13.5	569.1	1.52
8	0.55	2.4	0.57	12.0	1.01	0.59	6.5	215.0	0.57
9	0.72	9.0	0.76	23.8	2.01	0.79	12.0	992.7	2.65
10	0.80	11.8	0.82	15.3	1.29	0.86	0.1	423.2	1.13

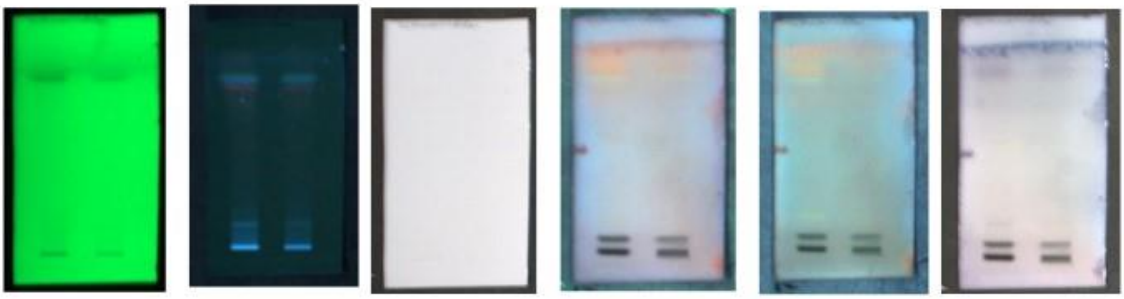


Image at 254 nm | Image at 366 nm | White Image | Image at 254 nm | Image at 366 nm | White Image

TRACK 1 - Methanolic extract of Cow urine Shodita Vatsanabha- Anandbhairav Ras
TRACK 2 - Methanolic extract of Cow milk Shodita Vatsanabha- Anandbhairav Ras

HPTLC of CUSV and CMSV (refer fig 5)

At CUSV total 6 peaks were observed with 2 prominent peaks at maximum Rf- 0.02 and 0.08 has occupied 50.32% and 40.24% area. Total 10 peaks were observed in CMSV-ABR, 1st peak at 0.01Rf occupying 49.69% of area and other peak at 0.08 occupying 32.46% area. At 254nm and 366 nm similar bands were observed for both ABR. White image does not show any bands for both ABR. CUSV-ABR showed 2 orange and 1 yellow band at 254 and 366nm, while such bands were absent in CMSV- ABR. 2 prominent black bands at similar position were present in both ABR at 254nm and 366nm.

DISCUSSION

Studies have shown that raw *Vatsanabha* contains 0.4-0.8% diterpene alkaloids, with the concentration of aconite ranging from 0.3-2.0%[26]. The primary alkaloids present include aconitine, pseudoaconitine, diacetylpseudoaconitine, and aconine.[26] The Ayurvedic detoxification process known as *Shodhana* leads to a reduction in the total alkaloid content [5]. However, this process concurrently

increases the levels of less toxic compounds such as aconine, hypoaconine, and benzylhypoaconine[3,27]. This increase is potentially due to the conversion of toxic aconitine into aconine or the hydrolysis of alkaloids into their corresponding amino alcohols during *Shodhana* [28,29]. Interestingly, treatment with *Gomutra* (cow's urine) transforms aconite, shifting its pharmacological action from a cardiac depressant to a cardiac stimulant [7,27,30–32]. The darkening of *Gomutra* after *Shodhana* suggests the dissolution of toxic substances from *Vatsanabha* into it[4,10].

This study compared several attributes of *Anandabhairava Rasa*, based on the *Śodhita Vatsanabha* (purified Aconitum ferox) treated with either cow urine (*Gomutra*) or cow milk (*Godugdha*) both preparations, from organoleptic characteristics, physical properties to HPTLC. The *Śodhana* process[4,10], involves purification of raw materials, the results showed that the purification of *Vatsanabha* using cow urine resulted in a slightly lesser yield of 3.75% lesser *Vatsanabha* (175g) compared to cow milk (190g), but both methods revealed significant reduction in the raw material's weight, likely due to removal of impurities and unwanted substances. There was gain in weight of *Śodhita Hingula* due to addition of substances from lemon juice during *Bhavana (trituration*) process*.* There was 38.75% of weight loss during *Śodhana* of *Ṭankana,* as it loses all its moisture and impurities, and becomes light and puffed[33]. Changes in the weight of the raw materials, reflects transformative nature of the purification processes in Ayurveda[10]. Organoleptic characteristics play an important role in quality assurance of Ayurvedic formulation. As the color of *Vatsanabha* changes after *Śodhana,* indicates that the purification changes the visual appearance of the herb. CUSV exhibits characteristic smell after *Śodhana* which persisted after drying also and CMSV having aromatic odor, suggesting purification medium changes the odor profile. Difference in the water-soluble and alcohol-soluble extractive value suggest that the purification process and the medium used affects the solubility and extractability of compounds in *Vatsanabha*. In terms of chemical analysis, water soluble extractive value being more in CUSV indicates better bioavailability than CMSV refer (table 2), thus body can easily absorb the active compounds into blood stream, it will be readily processed may result in faster therapeutic action.

Total ash and acid insoluble ash was found more in CUSV than CMSV is likely due to deposition of minerals from cow urine onto the rhizomes. As the rhizomes are porous in nature absorbs liquid. *Śodhita Vatsanabha* processed with cow urine showed a more neutral pH of 7.85 compared to 5.15 for cow-milk treated *Vatsanabha*, implying to the potential role of the medium in alterations of the chemical composition.

Thin-layer chromatography (TLC) studies on *Vatsanabha* treated with cow's urine have demonstrated the conversion of pseudoaconitine and aconitine into significantly less toxic compounds, veratroyl pseudoaconine and benzoylaconine, respectively, specifically within the context of traditional Ayurvedic *Shodhana*[3]. When images of TLC (Thin Layer Chromatography) (refer figure 4) were compared between raw *Vatsanabha*, CMSV and CUSV, Raw *Vatsanabha* shows presence of blue spot at 254 nm while it was absent in CMSV and CUSV, indicating its removal after *Śodhana* process. CUSV shows added spots at 0.66 and 0.75 Rf value which were added after *Śodhana* with cow urine. After Dragendorff reagent CMSV shows only one spot at 0.8 indicating large effect of cow milk in decreasing alkaloids Both the CUSV and CMSV treated *Vatsanabha* demonstrated distinct patterns in the spots formed under iodine vapors and Dragendorff reagent, which may indicate different phytochemical constituents in the two samples of *Vatsanabha*. These findings suggest that the purification process can influence the bioactive compounds present in the final product, potentially altering its therapeutic properties.

AAS (Atomic absorption spectroscopy) study estimated of 10% decrease in mercury content and 4% decrease in sulfur content of *Śodhita Hingula. Niścandra* (Absence of metallic shine), *Rekhapurnatva, Varitara of Śodhita Hingula* are suggestive of finer particles of *Hingula.* which may result into increased bioavailability and thus increased therapeutic efficacy.

There was 61.25% loss of weight in *Ṭankana* after *Śodhana* refer due to evaporation of water. There was decrease in boron trioxide after *Śodhana* and decreased sodium in *Ṭankana***.** *Varitara* indicates fineness of *Śodhita Ṭankana* powder. Physicochemical analysis of black pepper[34] and long pepper[35] powder were as per Ayurvedic Pharmacopeia of India refer (table 2).

The final product, *Anandabhairava Rasa* CMSV-ABR and CUSV -ABR refer (ref. table 3), both formulations produced tablets of similar size and texture, though minimal differences in hardness and disintegration time were noted, with the CUSV-ABR based preparation exhibiting a longer disintegration time. This could suggest a difference in the bioavailability of the active compounds between the two forms of *Vatsanabha*. Concentration of mercury and sulfur and pH were found to be near to similar in both formulations. HPTLC of CUSV and CMSV (refer figure no.5) with Rf value 0.02 at peak 2 for CUSV-ABR was occupying maximum area of 50.32% and CMSV-ABR at peak 2 with max Rf value 0.01 was occupying maximum area of 49.69%, which are important constituent of ABR formulation. peak 3 and peak 4 for both CUSV-ABR and CMSV-ABR was at Rf value of 0.08

and 0.18 occupying 40.24%, 5.60% and 32.46%, 3.81% area respectively. This indicates same bioactive components in both the formulations. The observation of similar bands for both ABR under both UV wavelengths suggest the presence of some compounds belonging to common classes that absorbs UV light at these wavelengths. These wavelengths are commonly used to detect compounds with conjugated double bonds or aromatic rings. The absence of visible bands under white light for both ABR indicates that the separated compounds are likely colorless and do not absorb visible light significantly (ref fig 5). The presence of colored bands of orange and yellow in CUSV-ABR at both 254nm and 366nm suggest the presence of specific compounds or classes of compounds in CUSV-ABR that are not present in CMSV-ABR (ref fig 5). Overall, the findings emphasize the significance of purification methods in the preparation of Ayurvedic formulations, which directly affects the pharmacological outcomes of the treatment. Further research is needed to explore the clinical implications of these variations in the therapeutic use[12] of *Anandabhairava Rasa*.

CONCLUSION

In conclusion, the study demonstrates that the purification of *Vatsanabha* using either cow urine (*Gomutra)* or cow milk (*Godugdha*) impacts the final formulation of *Anandabhairava Rasa*. Both purification methods resulted in distinct chemical and physical properties, which may influence the bioavailability and therapeutic efficacy of the product. While both preparations yielded similar results in terms of tablet characteristics, further research is necessary to fully understand the clinical implications of these differences in Ayurvedic treatments.

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