

Pharmaceutical Development, Quality Standardization, and Clinical Evaluation of *Trunamooladi* Granules for the Management of *Stanyakshaya*: A Randomized Controlled Trial

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Abstract:

Background

Ayurvedic decoctions (*Kwatha*) are of medicinal relevance but suffer from constraints including limited shelf-life, poor palatability and difficulties in routine administration.

The current work was performed to generate a stable granule dosage form of *Trunamooladi Kwatha* via an altered pharmaceutical method.

Methods

Trunamooladi Kwatha was made by classical principles of *Kwatha Kalpana* and turned into granules with modified *Khanda Kalpana* approach by *Khadi Sharkara*.

Organoleptic characters, physicochemical parameters, microbiological quality and heavy metal concentration of the produced granules were assessed as per established pharmacopoeial methods.

Results:

The discovered *Trunamooladi* Granules exhibited good organoleptic qualities and uniform batch wise yield. Physicochemical investigation found acceptable quality characteristics such as low loss on drying (0.59% w/w), near neutral pH (7.76), low ash values, high water soluble extractive value (82.99% w/w) and total sugar content 82%. Microbial examination found that bacteria counts were within allowed ranges while heavy metal analysis showed that Lead, Arsenic, Cadmium and Mercury were within specified safety limits.

Conclusion:

The present study has effectively developed a reproducible pharmaceutical methodology for conversion of classical *Trunamooladi Kwatha* into standardised granule dosage form. The proposed formulation exhibited good quality qualities and

can be used as a reference for future pharmaceutical manufacture, quality control and further clinical evaluation.

Keywords: Ayurvedic formulation; Modified *Khanda Kalpana*; Pharmaceutical standardization; Physicochemical evaluation; Quality control; *Trunamooladi* Granules; *Trunamooladi Kwatha*

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1. Introduction

Breastfeeding is the preferred method of nutrition for newborns, offering vital nutrients and immune defence. The World Health Organization recommends exclusive breastfeeding for the first six months of life and continuous breastfeeding with adequate supplemental nutrition up to two years of age or beyond.(1) Insufficient breast milk supply is a key reason for early supplementing and termination of exclusive breastfeeding. Insufficiency of lactation is multifaceted and could be connected with maternal poor nutrition, ineffective suckling, delayed breastfeeding beginning, maternal stress, hormonal changes and other maternal or infant-related variables.(2,3)

Breast milk (*Stanya*) is an *Upadhatu* of *Rasa Dhatu* in Ayurveda and is thought to be necessary for sustenance and development of the newborn. *Stanyakshaya* is a condition of insufficient secretion of breast milk and has been reported in the light of abnormalities affecting the maternal nutrition, digestion, *Rasa Dhatu* and *Stanyavaha Strotasa*. Ayurvedic care is based on correction of causative factors, proper nutrition and use of *Stanyajanana* formulations for the support of lactation.(4,5) Among the classical formulations advised for *Stanyakshaya*, *Trunamooladi Yoga* is mentioned in *Kashyapa Samhita*. The formulation is prepared from *Darbha* (*Imperata cylindrica*), *Kusha* (*Desmostachya bipinnata*), *Gundra* (*Typha*

angustifolia), *Shar* (*Saccharum munja*), *Sariva* (*Hemidesmus indicus*), *Ushira* (*Vetiveria zizanioides*), *Ikshu* (*Saccharum officinarum*) and *Kash* (*Saccharum spontaneum*). Traditionally, the formulation is prescribed as *shastika shali* rice processed with *Kwatha* (decoction) of this medicine along with milk. It comprises mostly medications belonging to the *Stanyajanana Mahakashaya* given by *Aacharya Charaka*, with constituents traditionally defined for nourishment and *Stanyajanana* characteristics.(5,6)

Among the *Panchavidha Kashaya Kalpana*, *Kwatha* is an important dosage form and is created by water extraction of coarse powder of the medications. However, its routine usage is accompanied with practical restrictions such as the requirement for fresh preparation, limited storage stability, cumbersome handling and poor palatability, which may adversely affect patient convenience and compliance, particularly when protracted administration is necessary.(7,8) In pharmaceutical development, the evolution of classical decoctions into more practical dosage forms could lead to better handling, storage, dose administration and acceptance, while maintaining their classical medicinal essence.

Granules are a useful form of medicinal dosage for such progress because they offer simplicity of handling, better storage and transport facilities, convenience of dosing and suitability for standardised manufacture. However, the conversion of a classical decoction into granules demands systematic supervision of the production process and the assessment of the finished product in an effort to guarantee reproducibility and quality. Organoleptic, physicochemical, microbial load and heavy-metal test are significant aspects of the pharmaceutical quality assessment of herbal preparations.(8-11)

Although the individual ingredients of *Trunamooladi Yoga* and Ayurvedic *Stanyajanana* drugs have been studied in different pharmacological, pharmacognostical and phytochemical studies, scanty published evidence is available regarding pharmaceutical development and standardisation of *Trunamooladi* Granules. Also, an integrated assessment of pharmaceutical development, quality assessment and clinical evaluation of this pharmaceutically advanced dosage form has not been properly recorded.(6)

Hence the present study was undertaken to prepare *Trunamooladi* granules from classical *Trunamooladi kwatha* by modified *Khanda Kalpana*, to set the pharmaceutical and analytical quality parameters and to evaluate the clinical efficacy in lactating mothers with *Stanyakshaya* by randomised controlled clinical trial. The present investigation was designed to give systematic evidence from pharmaceutical development and quality standardisation to clinical evaluation of the generated formulation.

2. Materials and Methods

2.1 Study Design

The present study was designed as a prospective pharmaceutico-analytical investigation coupled with a randomized controlled clinical trial to evaluate *Trunamooladi Granules* in the management of *Stanyakshaya*.

The investigation comprised three sequential phases:

- (i) Pharmaceutical development of *Trunamooladi* Granules from the classical *Trunamooladi Kwatha*
- (ii) Analytical evaluation of the developed formulation through organoleptic, physicochemical, microbial load and heavy-metal test
- (iii) Randomized controlled clinical evaluation in lactating mothers diagnosed with *Stanyakshaya*.

The clinical study protocol was approved by the Institutional Ethics Committee and was prospectively registered with the Clinical Trials Registry–India (CTRI Registration No.: CTRI/2024/12/078723).(12)

2.2 Pharmaceutical Development of *Trunamooladi* Granules Procurement and Authentication of Raw Materials

All herbal raw materials required for the preparation of *Trunamooladi Yoga* were procured from an authenticated supplier of Ayurvedic raw drugs. Prior to pharmaceutical processing, each ingredient was authenticated and quality evaluated according to the standards prescribed in the Ayurvedic Pharmacopoeia of India. Only pharmacopoeially compliant raw materials were utilized for the preparation of the formulation. Standardised *Khadi Sharkara* (rock sugar) was used as the granulating base during the modified *Khanda Kalpana* process.(11,12)

Table 1. Composition of *Trunamooladi Yoga*

Sr. No.	Drug	Botanical Name	Part Used	Quantity
1	<i>Darbha</i>	<i>Imperata cylindrica</i>	Root	500 g
2	<i>Kusha</i>	<i>Desmostachya bipinnata</i>	Root	1000 g
3	<i>Gundrha</i>	<i>Typha angustifolia</i>	Root	500 g
4	<i>Shar</i>	<i>Saccharum munja</i>	Root	500 g
5	<i>Sariva</i>	<i>Hemidesmus indicus</i>	Root	500 g
6	<i>Ushir</i>	<i>Vetiveria zizanioides</i>	Root	500 g

7	<i>Ikshu</i>	<i>Saccharum officinarum</i>	Root	500 g
8	<i>Kash</i>	<i>Saccharum spontaneum</i>	Root	500 g

Composition of Trunamooladi Yoga

The formulation was composed of the mentioned dravya in table 1 in a given quantity. The ingredients were added in classical proportion as indicated in *Kashyapa Samhita* with adaptation for pharmaceutical formulation. *Kusha dravya* was taken 2 times as it was referenced twice in reference shloka.

Preparation of Yavakuta Churna

All the validated crude drugs were cleansed individually to remove the foreign matter and roughly pulverised by the classical procedure of *Yavakuta Churna* described in *Sharangadhara Samhita*. The particle size was kept coarse to provide successful extraction of water soluble phytoconstituents and to facilitate filtration following preparation of decoction.(7)

Preparation of Trunamooladi Kwatha

4.5 kg of *Yavakuta Churna* was moved to a food grade stainless steel vessel and mixed with 36 L potable water keeping the classical drug to water ratio 1:8 in view of its Moderate hardness quality. The mixture was thoroughly combined and allowed to soak for twelve hours at room temperature (*Nimajjana*). The mixture was soaked overnight and heated over *Mandagni* utilising LPG heat source. The heating temperature was maintained at 90°C to 100°C with constant intermittent stirring to achieve equal distribution of heat and to prevent sedimentation or charring of the herbal material. The heat was sustained till the volume had reduced to one eighth of the starting quantity (approx. 4.5 L) and classical *Kwatha Siddhi Lakshanas* were obtained.(7) The prepared decoction was cooled to around 41°C and filtered through a double layered muslin cloth into a clean stainless steel vessel sterilised. The marc was gently pressed to recover the filtrate without allowing the passage of coarse particles. The volume of the prepared *Kwatha* was measured and recorded. The critical pharmaceutical parameters including soaking time, heating temperature, reduction time, organoleptic properties and ultimate yield were recorded in the Batch Manufacturing Record for reproducibility.

Table 2. Chronological Pharmaceutical Observations During Preparation of Trunamooladi Kwatha

Time	Temperature (°C)	Observation
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6:00 PM 01/12/2025	27.6	Water was at 27.6°C before addition of the drugs.
6:00 PM	25.8	After immersion of the drugs, the <i>Yavakuta Churna</i> was mixed with water in a 1:8 ratio and soaking (<i>Nimajjana</i>) was commenced.
6:00 AM 02/12/2025	26.8	Soaking was completed after 12 hours. A slight brown hue and typical herbal fragrance were noticed.
6:05 AM	26.8	Heating (<i>Kwatha Paka</i>) was commenced over <i>Mandagni</i> .
6:30 AM	48.7	Gentle boiling commenced. Characteristic herbal aroma developed.
7:15 AM	62.3	Uniform boiling was maintained with intermittent stirring.
8:30 AM	82.3	The colour changed to dark brown and the volume started reducing noticeably. Central boiling was observed, and the temperature was subsequently reduced.
10:00 AM	98.8	The decoction continued to concentrate with progressive

		reduction in volume.
11:30 AM	85.2	The decoction became more concentrated and the aroma intensified.
1:00 PM	87.1	Further reduction in volume was observed; continuous stirring was carried out to prevent sedimentation.
2:30 PM	91.2	The <i>Kwatha</i> approached <i>Siddhi Lakshana</i> , with characteristic colour, odour and consistency observed.
3:30 PM	88.3	The final volume was reduced from 36 L to approximately 4.5 L. Heating was stopped.
3:50 PM	41.1	The <i>Kwatha</i> was allowed to cool naturally and strained through muslin fabric to obtain the clear decoction.
4:00 PM	30.8	Filtration through muslin cloth was completed, yielding a clear, dark-brown decoction free from coarse particles.

Preparation of Trunamooladi Granules

The prepared *Trunamooladi Kwatha* was split into three equal halves of 1.5 L each. Each batch was moved into a stainless-steel jar and 6 kg of standardised *Khadi Sharkara* was added slowly with continuous stirring. The combination was cooked over *Mandagni* at a temperature of between 90 °C

and 110 °C until all the sugar was dissolved and the preparation slowly concentrated.

The heating was continued till the appearance of the characteristic *Khanda Siddhi Lakshanas* such as *Darvipralepa*, *Tantumatra*, *Apsumajjana*, visible base of the vessel during stirring, characteristic aroma of caramel and cake cutting appearance indicating the attainment of the desired concentration suitable for granulation.(2,7)

Table 3. Comparative In-process Pharmaceutical Observations Recorded During Three Batches of Trunamooladi Granule Preparation

Parameter	Measurement	Batch A	Batch B	Batch C	Average
Kwatha volume	L	1.5	1.5	1.5	1.5
Sugar added	kg	6.0	6.0	6.0	6.0
Temperature at start	°C	37.0	32.0	33.0	34.0
Frothing started	Time (min)	10	9	9	9.33
	Temperature (°C)	45.9	52.0	46.8	48.23
Large bubbling started	Time (min)	22	19	21	20.67
	Temperature (°C)	61.6	63.7	62.4	62.56
1-thread stage	Time (min)	35	33	29	32.33
	Temperature (°C)	89.6	88.3	83.9	87.26
Sticking to periphery	Time (min)	39	36	38	37.67

	Temperature (°C)	88.3	83.6	87.0	86.3
Caramel smell	Time (min)	44	39	42	41.67
	Temperature (°C)	89.6	87.9	88.7	88.73
Darvipral eptva	Time (min)	56	59	57	57.33
	Temperature (°C)	94.3	87.7	86.9	89.63
2-thread stage	Time (min)	63	60	60	61
	Temperature (°C)	99.7	103.2	97.6	100.16
Cake-cutting-like appearance	Time (min)	69	60	64	64.33
	Temperature (°C)	103.4	101.7	98.8	101.3
Apsu Nimajjana	Time (min)	69	63	64	65.33
	Temperature (°C)	98.7	94.6	106.4	99.9
Heating stopped and sieve granulation performed	Temperature (°C)	51	53	47	50.33
Final yield	kg	5.90	5.86	5.88	5.88

Granulation and Drying

At the end of concentration the pharmaceutical mass was allowed to cool naturally to a temperature of around 45–55°C. At this stage, the semi solid material was instantly passed through stainless steel sieve to give uniformly sized wet granules. The granules were dried under controlled conditions to constant weight and then sieved again to acquire uniform particle size. The oversized granules were gently ground and re-sieved to uniform particle size. The prepared granules were kept in air-tight, moisture-proof containers until they were evaluated analytically and administered clinically.

Standardization of the Pharmaceutical Process

For reproducibility of manufacture, predetermined critical process parameters such as drug-to-water ratio, soaking duration, heating temperature, reduction end point, *Kwatha-to-Khadi Sharkara* ratio, sugar concentration, achievement of *Khanda Siddhi Lakshanas*, granulation temperature, drying conditions and particle-size uniformity were standardised and documented in each manufacturing batch.

Table 4. Batch-wise Yield of Trunamooladi Granules

Batch	Decoction Used (L)	<i>Khadi Sharkara</i> (kg)	Final Yield (kg)
Batch I	1.5	6.0	5.90
Batch II	1.5	6.0	5.86
Batch III	1.5	6.0	5.88
Average	1.5	6.0	5.88

2.3 Analytical Standardization

The prepared *Trunamooladi* Granules were evaluated by detailed analytical studies as per established pharmacopeial methods.

Organoleptic Evaluation

The completed composition was subjected to colour (*Varna*), aroma (*Gandha*), taste (*Rasa*), appearance, consistency and touch (*Sparsha*) via standard sensory evaluation protocols.(9,10)

Table 5. Organoleptic Evaluation of Trunamooladi Kwatha & Granules

Organoleptic Parameter	<i>Trunamooladi Kwatha</i>	<i>Trunamooladi Granules</i>
Colour (Varna)	Dark brown	Brownish white

Odour (Gandha)	Characteristic aromatic herbal odour	Characteristic aromatic odour
Taste (Rasa)	Predominantly <i>Kashaya</i> with slight <i>Madhura</i>	Predominantly <i>Madhura</i> with slight <i>Tikta-Kashaya</i> aftertaste
Appearance	Clear, homogeneous liquid free from suspended particles	Free-flowing, uniform crystalline granules
Consistency	Thin liquid decoction	Dry, coarse granular consistency
Touch (Sparsha)	Smooth and free from coarse particles	Smooth, non-sticky and free-flowing

Physicochemical Evaluation

Physicochemical analysis was done for loss on drying, pH, total ash, acid-insoluble ash, water-soluble extractive value, alcohol-soluble extractive value and total sugar content by standard analytical procedures suggested for Ayurvedic herbal formulations.(9,11)

Table 6. Physicochemical Parameters of Trunamooladi Granules

Parameter	Result	Interpretation
Loss on Drying	0.59% w/w	Low moisture content
pH (10% aqueous solution)	7.76	Near-neutral pH
Total Ash	0.79% w/w	Low total inorganic residue
Acid-Insoluble Ash	0.03% w/w	Minimal acid-insoluble matter

Water-Soluble Extractive	82.99% w/w	High proportion of water-soluble extractable matter
Alcohol-Soluble Extractive	2.82% w/w	Low proportion of alcohol-soluble extractable matter
Total Sugar	82.00%	High sugar content, consistent with the use of Khadi Sharkara in granule preparation

Microbial Count Evaluation

The microbial load assessment was performed by enumeration of the total aerobic microbial count, total yeast and mould count and detection of specified pathogenic microorganisms like *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa* and members of *Enterobacteriaceae* to ensure compliance with permissible pharmacopeial limits.(9,10)

Table 7. Microbial Load of Trunamooladi Granules

Parameter	Result	AYUS H Limit	Interpretation
Total Microbial Count	110 CFU/g	NMT 100,000 CFU/g	Pass
Total Yeast and Mould Count	<10 CFU/g	NMT 1,000 CFU/g	Pass
<i>Enterobacteriaceae</i>	Not detected	Absent	Pass
<i>Escherichia coli</i>	Not detected	Absent	Pass
<i>Salmonella</i> spp.	Not detected	Absent	Pass
<i>Staphylococcus aureus</i>	Not detected	Absent	Pass

<i>Pseudomonas aeruginosa</i>	Not detected	Absent	Pass
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Heavy Metal Test

The estimate of heavy metals lead (Pb), arsenic (As), cadmium (Cd) and mercury (Hg) was done by validated analytical techniques as per acknowledged pharmacopeial criteria to assess the safety profile of the formulated product.(9,10)

Table 8. Heavy Metal test of Trunamooladi Granules

Heavy Metal	Result	Permissible Limit	Status
Lead (Pb)	2.102 mg/kg	NMT 10 mg/kg	Complied with the specified permissible limits
Arsenic (As)	BLQ (<0.1 mg/kg)	NMT 3 mg/kg	
Cadmium (Cd)	BLQ (<0.1 mg/kg)	NMT 0.3 mg/kg	
Mercury (Hg)	0.159 mg/kg	NMT 1 mg/kg	

2.4 Clinical Evaluation

Study Setting

The randomised controlled clinical experiment was performed at the Department of *Prasuti Tantra and Stree Roga* in association with the Department of *Rasashastra and Bhaishajya Kalpana*, Shri Ayurved Mahavidyalaya, Nagpur, India.

Study Population

The study population consisted of the lactating mothers diagnosed with *Stanyakshaya* and who fulfilled the pre-specified inclusion criteria. Written informed consent was obtained and the participants were recruited from the outpatient and inpatient departments of the study center during the study period. In total, sixty mothers were enrolled.

Sample Size

Sample size was calculated on the basis of anticipated change in the score of breast laxity/engorgement from pre-intervention to post-intervention in the *Shatavari* Granules group, considering the impact magnitudes from a previously completed study in a comparable setup. It was calculated using a pre-test mean of 1.47, post-test mean of 1.10, pre-test standard deviation of 0.507 and post-test standard deviation of 0.346, estimated effect size 0.37. Assuming a two-sided alpha level of 1% and 90% statistical power, a required sample size of 23 participants per group was calculated. Sample size was inflated to 30

participants per group to account for an expected 20% attrition. This meant that 60 people were to be enrolled.

Randomization

Eligible participants were randomised to the Trial Group (Group A) or Control Group (Group B) by simple randomisation. Allocation was by lottery technique as described in the study protocol. The study was an open label clinical experiment.

The study was designed as a randomized, parallel-group, open-label controlled clinical trial, with participants and investigators aware of the assigned treatment.

Study Groups

Sr No	PARAMETER	TRAIL GROUP	CONTROL GROUP
1	No. of patients	30	30
2	Treated with	<i>Trunmooladi</i> granules	<i>Shatavari</i> granules
3	Route of drug administration	Oral	Oral
4	Time	BD after meal	BD after meal
5	Anupana	<i>Shali shashitik</i> with cow milk	Cow milk
6	Intervention	45 Days	45 Days
7	Observation time	55 Days	55 Days
8	Follow up	on 1 st , 2nd, 4th, 6 th , 8 th Weeks	on 1 st , 2nd, 4th, 6 th , 8 th Weeks

Trial Group (Group A) : The participants were given *Trunamooladi* Granules in a dose of 10 g twice daily after meals for 45 days continuously by oral route with lukewarm cow's milk and *Shashitik Shali* rice as Anupana.

Control Group (Group B): This group was given *Shatavari* Granules at the amount of 10 g twice daily after meals for 45 consecutive days orally with lukewarm cow's milk as *Anupana*.

The treatment period was 45 days. Participants were observed for 10 further days after completion of therapy, with Day 55 indicating the observation-period assessment rather than the end of treatment.

Outcome Measures

The efficacy of treatment was evaluated clinically with the use of pre-defined subjective and objective measures. Clinical factors studied were breast *Stanmlanata*/laxity, milk ejection, rate of infant's weight increase, infant sleep, infant crying and infant stool characteristics. Breast laxity, milk ejection, newborn sleep, infant crying and infant stool characteristics were measured by applying pre-defined grading criteria and infant weight increase was measured as an objective continuous measure based on recorded infant weight. The major evaluation of treatment was completed on day 45, at the end of the 45-day intervention period. Results of Day 55 were assessed independently within the 10-day observation period.

Assessment Parameters

Sr No	Parameter	Details	Score
1	Laxity Of Breast	Prakrut	0
		Alpa	1
		Madhyam	2
		Adhik	3
2	Baby weight Gain rate	Prakrut (>200 gm/week)	0
		Hras (100-200 gm/week)	1
		Adhik Hras (<100 gm/week)	2
		Prakrut	0
3	Sleep	Alpa Khandit	1
		Anidra	2
		Occasionally crying and stops after feeding	1
		Frequent crying and stops after feeding	2
4	Cry For Demand feeding	Frequent crying that doesn't stop after feeding	2
		Prakrut (more than 7 feeds/day)	0
		Alpa (Less than 7 feeds/Day)	1
		Apravartana	2
5	Milk Ejection	Prakrut/Normal	0
		Dravamatprasvuti/watery stool	1
		Maitasidhata/Hard Stool	2
		Prakrut/Normal	0
6	Stool Study	Prakrut/Normal	0
		Dravamatprasvuti/watery stool	1
		Maitasidhata/Hard Stool	2
		Prakrut/Normal	0

Statistical Analysis

Within-group changes in subjective ordinal parameters were analyzed using the Wilcoxon signed-rank test, while infant weight was analyzed using the paired t-test. Between-group comparisons of subjective ordinal parameters were performed using the Mann-Whitney U test, while infant weight gain was compared using the unpaired t-test. A two-sided p-value <0.05 was considered statistically significant.

3. Results

3.1 Pharmaceutical Development of Trunamooladi Granules

Trunamooladi Kwatha was successfully prepared according to the principles of *Kwatha Kalpana* and subsequently converted into *Trunamooladi* Granules using a modified *Khanda Kalpana* technique. Critical pharmaceutical parameters, including drug-to-water ratio, soaking duration, heating conditions, endpoint of decoction reduction, quantity of *Khadi Sharkara*, granulation temperature and drying conditions were recorded and standardized.

Three independent batches were prepared to assess process reproducibility. Each batch was prepared using 1.5 L of *Trunamooladi Kwatha* and 6.0 kg of

Khadi Sharkara. The final yields were 5.90 kg, 5.86 kg and 5.88 kg for Batches I, II and III, respectively, with an average yield of 5.88 kg.

Characteristic sequential changes were observed during granule preparation, including frothing, progressive bubbling and concentration, development of caramel odour, *Darvipralepatva*, thread formation, cake-cutting-like appearance and *Apsu Nimajjana*. Sieve granulation was performed at temperatures ranging from 47–53°C across the three batches.

3.2 Organoleptic Evaluation

The prepared *Trunamooladi* Granules exhibited brownish-white colour, characteristic aromatic odour and predominantly *Madhura* taste with a slight *Tikta-Kashaya* aftertaste. The finished product showed a dry, coarse, non-sticky and free-flowing granular consistency.

The original *Trunamooladi Kwatha* was dark brown, with characteristic herbal odour and predominantly *Kashaya* taste with slight *Madhura* taste. It was obtained as a clear, homogeneous liquid free from coarse particles.

3.3 Physicochemical Evaluation

The prepared *Trunamooladi* Granules showed a loss on drying of 0.59% w/w and a pH of 7.76 in a 10% aqueous solution. Total ash and acid-insoluble ash values were 0.79% w/w and 0.03% w/w, respectively.

The water-soluble extractive value was 82.99% w/w, while the alcohol-soluble extractive value was 2.82% w/w. Total sugar content was 82.00%.

3.4 Microbial Evaluation

The total microbial count was 110 CFU/g against the specified limit of NMT 100,000 CFU/g. The total yeast and mould count was <10 CFU/g against the specified limit of NMT 1,000 CFU/g.

Specified pathogens, including *Enterobacteriaceae*, *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus* and *Pseudomonas aeruginosa*, were not detected in the finished formulation.

3.5 Heavy Metal Test Analysis

Heavy metal test analysis showed a lead concentration of 2.102 mg/kg and mercury concentration of 0.159 mg/kg. Arsenic and cadmium were below the limit of quantification (<0.1 mg/kg). All tested heavy metals were within their respective permissible limits.

3.6 Clinical Evaluation

A total of 60 lactating mothers fulfilling the eligibility criteria were enrolled and randomly allocated into two groups of 30 participants each: *Trunamooladi* Granules (n = 30) and *Shatavari* Granules (n = 30). All participants completed the treatment period and scheduled assessments.

The treatment duration was 45 days, with a subsequent 10-day observation period. Clinical assessment included breast laxity, milk ejection, infant weight gain, infant crying, infant sleep and

infant stool characteristics. Infant weight was recorded serially as an objective parameter.

3.6.1 Baseline Characteristics

Baseline demographic characteristics and clinical variables were comparable between the two groups, indicating adequate homogeneity before initiation of treatment.

3.6.2 Within-Group Clinical Outcomes

Both treatment groups demonstrated improvement from baseline in the assessed maternal and infant-related clinical parameters during the 45-day treatment period.

Within-group analysis demonstrated statistically significant improvement from baseline in all assessed clinical parameters in both treatment groups at the end of the 45-day treatment period ($p < 0.05$).

3.6.3 Intergroup Comparison at the End of Treatment

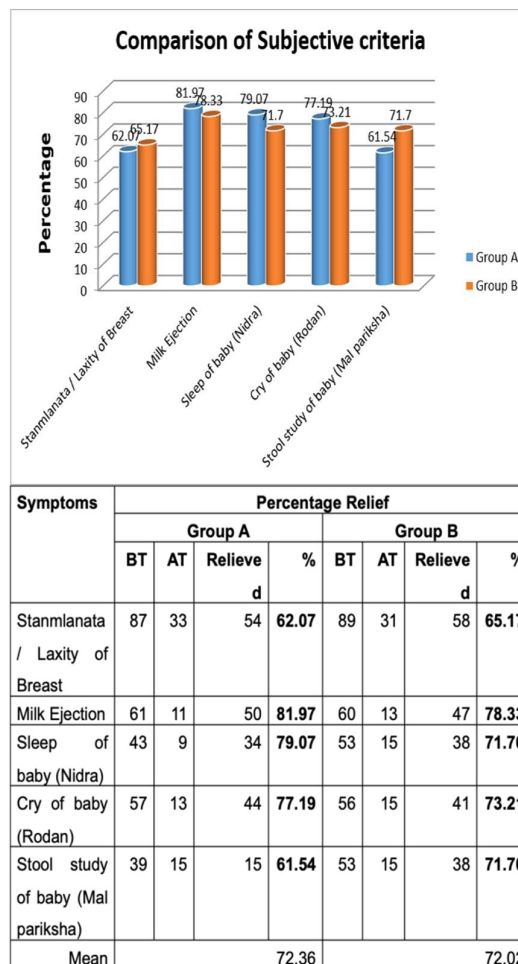
Intergroup comparison was performed using the change in clinical parameters from baseline to Day 45. For subjective ordinal clinical parameters, the Mann–Whitney U test was used, whereas infant weight gain, considered as a continuous objective parameter, was analyzed using the unpaired t-test. For breast laxity, the mean difference score was 1.80 in the *Trunamooladi* group and 1.933 in the *Shatavari* group. The between-group difference was not statistically significant ($p = 0.314$).

For milk ejection, the mean difference score was 1.667 in the *Trunamooladi* group and 1.567 in the *Shatavari* group. The between-group difference was not statistically significant ($p = 0.502$).

For infant sleep, the mean difference score was 1.90 in the *Trunamooladi* group and 0.433 in the *Shatavari* group. A statistically significant between-group difference was observed ($p = 0.001$), favouring the *Trunamooladi* group.

For infant crying, the mean difference score was 1.467 in the *Trunamooladi* group and 1.367 in the *Shatavari* group. The between-group difference was not statistically significant ($p = 0.572$).

For infant stool characteristics, the mean difference score was 1.30 in the *Trunamooladi* group and 0.50 in the *Shatavari* group. A statistically significant between-group difference was observed ($p = 0.001$), favouring the *Trunamooladi* group.



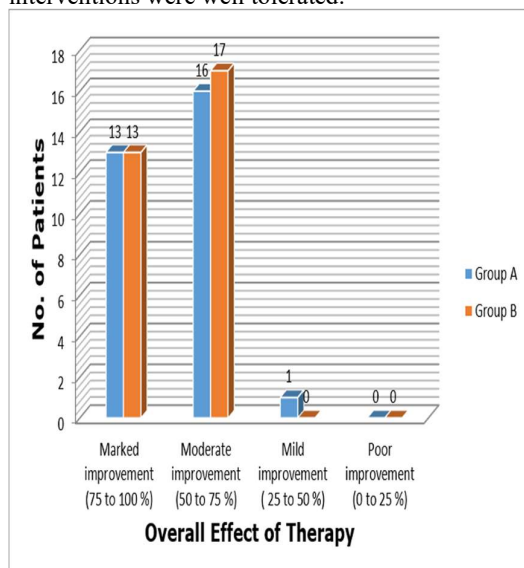
3.6.4 Intergroup Comparison of Infant Weight Gain

Infant weight gain was assessed objectively from serial infant-weight measurements. At the end of the 45-day treatment period, the mean infant weight gain was 814.43 ± 108.16 g in the *Trunamooladi* group and 909.26 ± 97.40 g in the *Shatavari* group. The between-group difference was statistically significant ($t = 3.77$, $p = 0.004$), with greater mean infant weight gain observed in the *Shatavari* group. Thus, the observed difference in infant weight gain favoured the control group rather than the trial group.

3.6.5 Overall Clinical Response

Both treatment groups demonstrated statistically significant improvement from baseline across the assessed clinical parameters during the 45-day treatment period. The comparative response varied according to the individual outcome assessed. Statistically significant between-group differences favouring the *Trunamooladi* group were observed for infant sleep and infant stool characteristics. No statistically significant between-group differences were observed for breast laxity, milk ejection or infant crying. For infant weight gain, a statistically significant between-group difference was observed

($p = 0.004$), with greater mean weight gain in the *Shatavari* group than in the *Trunamooladi* group. No serious adverse drug reactions or treatment-related complications were reported during the treatment or observation period, and both interventions were well tolerated.



Result	Group A		Group B	
	Number of patients	%	Number of patients	%
Marked improvement (75 to 100 %)	13	43.33 %	13	43.33 %
Moderate improvement (50 – 75 %)	16	53.33 %	17	56.67 %
Mild improvement (25 – 50 %)	1	3.33 %	0	0.00 %
Poor improvement (0 to 25 %)	0	0.00 %	0	0.00 %

3.7 Summary of Findings

In the clinical trial, both *Trunamooladi* Granules and *Shatavari* Granules demonstrated statistically significant improvement from baseline across the assessed clinical parameters during the 45-day treatment period. In the between-group analysis, statistically significant differences favouring *Trunamooladi* Granules were observed for infant sleep and infant stool characteristics, whereas no statistically significant differences were observed for breast laxity, milk ejection or infant crying. A statistically significant between-group difference was also observed for infant weight gain ($p = 0.004$), with greater mean weight gain in the *Shatavari* group. The Day-55 findings were considered

separately as part of the 10-day observation period and were not treated as the primary treatment endpoint.

4. Discussion

4.1 Pharmaceutical Advancement of *Trunamooladi Kwatha* into Granules

The present study successfully transformed the classical *Trunamooladi Kwatha* into granule dosage form by employing modified *Khanda Kalpana* technique. The procedure involved controlled concentration of *Kwatha*, inclusion of *Khadi Sharkara*, monitoring of typical processing phases, granulation and controlled drying. This modification has overcome the practical drawbacks of conventional *Kwatha* administration, such as preparation and handling, while keeping the classical formulation intact.(2,7,8)

The pharmaceutical method was reproducible in three independent batches. The same quantity of *Kwatha* (1.5 L) and *Khadi Sharkara* (6 kg) were used to prepare each batch. The final yields were 5.90, 5.86 and 5.88 kg accordingly. The average yield was 5.88 kg. The close spread of yields suggests uniformity in the standardised production process.

4.2 Standardization of the Pharmaceutical Process

The formulation was developed based on standardisation of crucial processing parameters. The typical 1:8 ratio of medication to water, 12 hour maceration, regulated heating and reduction to around one-eighth volume were preserved in the *Kwatha* stage. In-process observations indicated progressive changes in temperature, colour, fragrance and concentration till completion of decoction.(7)

Characteristic stages of granule formation including foaming, one thread stage, *Darvipralepatva*, two thread stage and *Apsu Nimajjana* were observed and documented batch wise. Similar final yields were obtained with small changes in processing time and temperature. These results suggest that the selected process parameters were sufficiently repeatable under the conditions of the present study and provide a basis for further scale-up and process validation.(2)

4.3 Analytical Standardization

The resulting granules were having uniform organoleptic properties such as brownish white colour, characteristic fragrant odour, primarily sweet taste and free flowing granular texture. These observations give descriptive quality parameters which can be used for routine identification and comparison of subsequent batches.(9,10)

The physicochemical profile indicated loss on drying 0.59%w/w, pH 7.76, total ash 0.79%w/w, acid insoluble ash 0.03%w/w, water soluble extractive 82.99%w/w, alcohol soluble extractive 2.82%w/w and total sugar content 82.00%. The high water-soluble extractive value is in agreement with

the formulation being an aqueous decoction, while the high total sugar content is due to the incorporation of *Khadi Sharkara* in the manufacture of granules. Collectively, these findings offer a preliminary physicochemical quality profile that may be used as reference data for future batch-wise evaluation and further standardisation of *Trunamooladi* Granules.(9,10)

4.4 Microbial and Heavy Metal Evaluation

The overall microbial count and yeast and mould count of the final formulation were 110 CFU/g and <10 CFU/g respectively within the acceptability limits prescribed. No specified pathogens tested were found. The results obtained show that the analysed batch has good microbial quality, under the conditions of fabrication and analysis.

In the heavy metal examination, lead (2.102 mg/kg) and mercury (0.159 mg/kg) were detected while arsenic and cadmium were below the limit of detection (<0.1 mg/kg). All four parameters were within their designated allowed limits. These results are consistent with conformity of the tested formulation with the relevant heavy-metal standards, but should not be used as proof of overall safety in the clinic independent of the clinical and toxicological evaluation.(9,10)

4.5 Clinical Evaluation of *Trunamooladi* Granules

The randomized controlled clinical evaluation demonstrated statistically significant improvement from baseline across the assessed clinical parameters in both treatment groups during the 45-day intervention period. In the between-group analysis, *Trunamooladi* Granules demonstrated statistically significant greater improvement in infant sleep and infant stool characteristics. No statistically significant differences were observed between the groups for breast laxity, milk ejection or infant crying.

For objective infant weight gain, a statistically significant between-group difference was observed ($p = 0.004$), with greater mean weight gain in the *Shatavari* group than in the *Trunamooladi* group. This finding indicates that although both interventions were associated with improvement from baseline, the magnitude of improvement in infant weight gain was greater in the control group. Thus, the comparative findings were outcome-specific and did not demonstrate uniform superiority of *Trunamooladi* Granules across all assessed parameters. The significant differences favouring *Trunamooladi* in infant sleep and stool characteristics nevertheless indicate potential benefits of the developed formulation in selected infant-related outcomes.

4.6 Possible Mechanism of Action

The therapeutic rationale for *Trunamooladi Yoga* can be interpreted from both Ayurvedic and contemporary perspectives. In Ayurveda, *Stanyakshaya* is associated with disturbances

involving *Rasa Dhatu*, *Stanyavaha Srotasa* and *Dosha* balance, and management emphasizes nourishment and *Stanyajanana* measures. The constituent drugs of *Trunamooladi Yoga* are traditionally described with properties including *Balya*, *Brimhana*, *Rasayana*, *Pittashamaka* and *Stanyajanana*, providing a classical rationale for its use in lactation insufficiency.(4-6)

From a contemporary perspective, individual constituent drugs have been reported to contain diverse phytochemical constituents with nutritional, antioxidant and other biological activities.(6) However, the present study did not investigate specific marker compounds, hormonal responses or molecular mechanisms. Therefore, these mechanisms remain hypothetical and require confirmation through targeted phytochemical, pharmacological and hormonal studies.

The pharmaceutical conversion may offer practical advantages in terms of handling, administration and acceptability compared with the conventional decoction dosage form. However, the present study did not independently evaluate the contribution of pharmaceutical convenience to treatment adherence or clinical outcome. Therefore, any effect of dosage-form modification on adherence remains a hypothesis requiring dedicated investigation.

4.7 Comparison with Previous Studies

Published evidence specifically addressing the pharmaceutical development and clinical evaluation of *Trunamooladi* Granules remains limited. Previous Ayurvedic research has predominantly focused on classical descriptions, individual constituent drugs, pharmacognostical or phytochemical investigations and clinical evaluation of other galactagogue formulations.(6,15,16)

The present investigation extends the available evidence by integrating pharmaceutical process development, batch-wise reproducibility, analytical quality assessment and randomized clinical evaluation of the same formulation. The pharmaceutical findings demonstrate the feasibility of converting the classical *Kwatha* into a granule dosage form under controlled laboratory conditions, while the clinical findings provide preliminary evidence of improvement in *Stanyakshaya*-related clinical parameters.

The comparative clinical findings were outcome-specific, with significant between-group differences favouring *Trunamooladi* Granules for infant sleep and stool characteristics, while no significant differences were observed for breast laxity, milk ejection and infant crying. These findings should be interpreted in the context of the limited sample size, single-centre design and other methodological limitations of the study.

Further comparative studies with larger samples, standardized co-interventions and longer follow-up are required to determine the reproducibility and broader clinical relevance of these findings.

4.8 Strengths of the Study

The major strength of the present study was its integrated design combining pharmaceutical development, process standardization, analytical quality assessment and randomized clinical evaluation of *Trunamooladi* Granules. Reproducibility of the pharmaceutical process was assessed through three batches, while organoleptic, physicochemical, microbial and heavy-metal test provided a comprehensive preliminary quality profile. The randomized controlled design enabled comparative clinical evaluation of the developed formulation under defined study conditions.

4.9 Limitations of the Study

The study was conducted at a single centre with a relatively small sample size of 60 participants, which may limit the generalizability of the findings. The 45-day treatment period and subsequent 10-day observation period were adequate for assessing short-term clinical changes but insufficient to determine long-term maintenance of lactation, recurrence of *Stanyakshaya* or long-term safety.

Objective hormonal and biochemical parameters, quantitative phytochemical profiling and marker-compound analysis were not performed. Long-term stability, shelf-life, packaging validation and industrial-scale GMP process validation were also beyond the scope of the present investigation.

Lactation outcomes may additionally have been influenced by maternal nutritional and psychosocial factors, breastfeeding technique and infant feeding behaviour. Importantly, the *Anupana* differed between groups, as *Trunamooladi* Granules were administered with warm milk and *Shashtik Shali* rice whereas *Shatavari* Granules were administered with warm milk alone. This difference may represent a potential confounding factor and limits attribution of the observed comparative effects solely to the formulations.

Future multicentric randomized trials with larger sample sizes, longer follow-up, common *Anupana*, objective lactation-related biomarkers, phytochemical characterization and pharmaceutical stability studies are warranted.

The observed between-group differences across individual clinical outcomes, including the greater infant weight gain observed in the *Shatavari* group, indicate that the comparative effects may vary according to the outcome assessed and should not be generalized to overall therapeutic superiority.

5. Conclusion

The present study successfully developed *Trunamooladi* Granules from the classical *Trunamooladi Kwatha* using a modified *Khanda Kalpana* approach. The pharmaceutical process demonstrated reproducible processing characteristics across three batches and produced a granule dosage form with defined organoleptic and physicochemical characteristics. Analytical evaluation provided a preliminary quality profile

based on physicochemical, microbial count and heavy-metal test parameters.

In the randomized controlled clinical evaluation, both *Trunamooladi* Granules and *Shatavari* Granules demonstrated statistically significant improvement from baseline across the assessed clinical parameters during the 45-day treatment period. Between-group analysis demonstrated statistically significant differences favouring *Trunamooladi* Granules for infant sleep and infant stool characteristics, while no statistically significant differences were observed for breast laxity, milk ejection and infant crying. A statistically significant between-group difference was observed in infant weight gain, with greater mean weight gain in the *Shatavari* group ($t = 3.77$, $p = 0.004$).

Thus, *Trunamooladi* Granules demonstrated beneficial effects in selected clinical outcomes but did not demonstrate uniform superiority over *Shatavari* Granules across all assessed parameters. The findings provide preliminary clinical evidence supporting the potential usefulness of *Trunamooladi* Granules in the management of *Stanyakshaya* and support the feasibility of pharmaceutical advancement of the classical *Kwatha* into a convenient granule dosage form. Larger multicentric randomized studies with longer follow-up, standardized co-interventions, objective lactation-related outcomes, phytochemical characterization and stability evaluation are warranted to confirm these findings.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Ethics Approval

The study protocol was reviewed and approved by the Institutional Ethics Committee of Shri Ayurved Mahavidyalaya, Nagpur. The clinical trial was

prospectively registered with the Clinical Trials Registry–India (CTRI No.- CTRI/2024/12/078723).

Informed Consent

Written informed consent was obtained from all study participants before enrolment in the clinical trial. Confidentiality of participant information was maintained throughout the study in accordance with institutional ethical guidelines and the principles of the Declaration of Helsinki.

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