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PHARMACEUTICAL STANDARDISATION OF *KRISHNADI CHURNA* PREPARED WITH *PIPPALI* COLLECTED FROM DIFFERENT GEOGRAPHICAL ZONES

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ABSTRACT

Background: *Krishnadi Churna* is a classical polyherbal powder containing *Pippali*, *Ativisha*, *Musta* and *Karkatashringi*. As plant-derived raw materials may vary with geographical origin, a source-wise pharmaceutico-analytical assessment is required to support batch consistency and rational quality standards **Aim** To prepare *Krishnadi Churna* using *Pippali* collected from different geographical zones in the Department of *Rasa Shastra and Bhaishajya Kalpana*, RAC Varanasi **Objectives** To compile the literature regarding *Krishna (Pippali)*, *Atis (Ativisha)*, *Mustak (Motha)* and *Shringikadama (Karkatshringi)*. To analyse variation in activity, concentration of phytochemicals, consistency, stability and shelf life of the ingredients with reference to particle size. **Materials and Methods:** Four coded formulations were prepared in

three independent batches by maintaining the same ingredients and method while changing the geographical source of *Pippali*. Organoleptic characters, pH, water-soluble extractive, alcohol-soluble extractive, acid-insoluble ash, loss on drying, piperine by UV spectrophotometry and microbiological parameters were evaluated. Final yields were descriptively analysed as mean, standard deviation and coefficient of variation. **Results:** All samples were brown, strongly pungent powders with characteristic taste. The final powder weights were In Batch 1, final powder weights were 95 g for Karnataka, 93g for Assam, 93g for Uttar Pradesh and 96g for Gujarat. In Batch 2, the respective values were 95 g, 96 g, 90g and 94g. In Batch 3, they were 94g, 90 g, 95 g and 93 g. pH ranged from 5.87 to 6.83; water-soluble extractive from 16.94% to 24.14%; alcohol-soluble extractive from 14.05% to 23.00%; acid-insoluble ash from 0.84% to 6.22%; and loss on drying from 9.01% to 11.22%. Piperine was highest in the Assam sample (0.89%) and lowest in the Uttar Pradesh sample (0.18%). Specified pathogens were absent in all samples. **Conclusion:** Geographical source of *Pippali* produced measurable variation in pharmaceutical behaviour and chemical-marker content of *Krishnadi Churna*. Assam yielded the highest piperine value, Uttar Pradesh the highest water-soluble extractive, Karnataka the highest alcohol-soluble extractive and lowest moisture, and Gujarat the lowest acid-insoluble ash. These results support multi-parameter, source-sensitive standardisation rather than selection based on a single marker alone.

Keywords: *Krishnadi Churna*; *Pippali*; *Piper longum*; piperine; geographical variation; *Churna Kalpana*; pharmaceutical standardisation.

INTRODUCTION

In *Bhaishajya Kalpana*, conversion of authenticated raw drugs into stable and administrable dosage forms is central to reproducible therapeutic use. *Churna Kalpana* is a basic solid dosage form prepared by drying, powdering, sieving and homogeneous blending of one or more medicinal substances. Its simplicity, low processing requirement and adaptable dosing have made it one of the most frequently used classical preparations.[1]

Krishnadi Churna is described in the classical literature as a paediatric formulation comprising *Krishna* or *Pippali*, *Ativisha*, *Musta* and *Karkatashringi* in equal proportion. It is traditionally indicated in childhood conditions such as *Jwara*, *Atisara*, *Vamana*, *Shwasa* and *Kasa*. The Ayurvedic formulary approach requires identity, purity and correct proportion of every constituent because variability in any one ingredient may influence the final formulation.[2]

Quality evaluation of herbal powders must integrate sensory identity with objective parameters such as moisture, ash, extractive values, marker-compound estimation and microbial quality. International guidance recognises that representative sampling, powder fineness and validated analytical procedures are essential for defining the identity, purity and content of medicinal plant materials.[3]

Previous standardisation studies on classical *Churna* formulations have demonstrated that organoleptic, physicochemical and chromatographic parameters together provide a more reliable quality profile than visual examination alone. Such multi-parameter evaluation is particularly important for polyherbal medicines in which natural source variation can be compounded during grinding, sieving, mixing and storage.[4]

Pippali (*Piper longum* L.) contains piperine as an important chemical marker. Climate, soil, plant chemotype, harvest stage, drying and storage may alter the abundance of piperine and other extractable constituents. Therefore, comparative evaluation of *Krishnadi Churna* prepared using *Pippali* from different geographical zones can help identify source-associated variability and generate preliminary standards for raw-material selection and finished-product control.[5]

AIM AND OBJECTIVES

Aim

To prepare *Krishnadi Churna* using *Pippali* collected from different geographical zones in the Department of *Rasa Shastra and Bhaishajya Kalpana*, RAC Varanasi.

Objectives

1. To compile the literature regarding *Krishna (Pippali)*, *Atis (Ativisha)*, *Mustak (Motha)* and *Shringikadama (Karkatshringi)*.
2. To analyse variation in activity, concentration of phytochemicals, consistency, stability and shelf life of the ingredients with reference to particle size.

MATERIALS AND METHODS

Study design and setting

A comparative pharmaceutico-analytical study was conducted in the Department of Rasa Shastra and Bhaishajya Kalpana, Government P.G. Ayurvedic College and Hospital, Varanasi. Three pharmaceutical batches were prepared on 22 July 2025, 4 August 2025 and 11 August

2025. Analytical evaluation was performed according to the laboratory protocol used for the submitted samples.

Raw materials and sample coding

Authenticated raw materials of *Ativisha*, *Musta* and *Karkatashringi* were maintained as common ingredients. The geographical source of *Pippali* constituted the study variable. The finished samples were coded P-1 for Karnataka, P-2 for Assam, P-3 for Uttar Pradesh and P-4 for Gujarat. The botanical identities used were *Aconitum heterophyllum* Wall. ex Royle, *Cyperus rotundus* L., *Piper longum* L. and *Pistacia integerrima* J.L. Stewart ex Brandis.

Table No. 1. Composition and sample coding of Krishnadi Churna

Ayurvedic name	**Botanical identity / source**	**Proportion**
Ativisha	Aconitum heterophyllum Wall. ex-Royle	1 part
Musta	Cyperus rotundus L.	1 part
Pippali	Piper longum	1 part
Karkatashringi	Pistacia integerrima J.L. Stewart ex Brandis	1 part

Pharmaceutical procedure

The raw drugs were cleaned, dried and examined for visible foreign matter. Each ingredient was separately reduced to coarse powder and then finely powdered. The powders were passed through sieve No. 80, weighed and mixed in equal proportion until a homogeneous blend was obtained. The finished *Churna* was packed in clean, dry, airtight containers. Each geographical sample started with 100 g of raw material for batch-wise yield observation. The working temperature ranged from 35 degrees C to 40 degrees C, and the reported processing duration was approximately 2 hours 30 minutes per batch.

Pharmaceutical observations and calculations

Weights after *Yavakutta* and final powdering were recorded for each geographical sample in all three batches. The quantities, reported total yield, total weight loss and percentage loss were reproduced batch-wise .

Analytical evaluation

Organoleptic evaluation included description or colour, odour and taste. Physicochemical evaluation included pH, water-soluble extractive, alcohol-soluble extractive, acid-insoluble ash and loss on drying. These parameters were selected to provide complementary information on acidity, extractable constituents, siliceous matter and residual moisture in the powdered formulation.

Piperine was quantified by UV spectrophotometry and reported as percentage of the finished formulation. Marker-based estimation is a useful quality-control approach for Ayurvedic powders containing species of the Piperaceae family, although full validation data and a chromatographic fingerprint are desirable for definitive routine standardisation.[6]

Microbiological testing included total microbial plate count, total yeast and mould count, and absence testing for *Staphylococcus aureus*, *Salmonella* species, *Pseudomonas aeruginosa* and *Escherichia coli*.

RESULTS

Pharmaceutical yield

The following tables present the complete batch-wise pharmaceutical data extracted. Values are retained exactly as reported in the source tables.

Table No. 2. Batch No. 1: Pharmaceutical observations of Krishnadi Churna

Parameter	**P-1 Karnataka**	**P-2 Assam**	**P-3 Uttar Pradesh**	**P-4 Gujarat**
Date of preparation	22/07/2025	22/07/2025	22/07/2025	22/07/2025
Quantity taken (g)	100	100	100	100
Weight after Yavakutta (g)	95 g	93g	93 g	96g
Weight after powdering (g)	50	48	50	50
Total weight loss (g)	50	52	50	50
Percentage loss (%)	50	52	50	50

Parameter	P-1 Karnataka	P-2 Assam	P-3 Uttar Pradesh	P-4 Gujarat
Total duration required	2:30 h	2:30 h	2:30 h	2:30 h
Temperature observed (degree C)	35	40	35	40

Table No. 3. Batch No. 2: Pharmaceutical observations of Krishnadi Churna

Parameter	P-1 Karnataka	P-2 Assam	P-3 Uttar Pradesh	P-4 Gujarat
Date of preparation	04/08/2025	04/08/2025	04/08/2025	04/08/2025
Quantity taken (g)	100	100	100	100
Weight after Yavakutta (g)	95 g	96 g	90g	94 g
Weight after powdering (g)	45	50	48	42
Total yield (g)	45	50	48	42
Total weight loss (g)	55	50	52	48
Percentage loss (%)	55	50	52	48
Total duration required	2:30 h	2:30 h	2:30 h	2:30 h
Temperature observed (degree C)	35	40	35	40

Table No. 4. Batch No. 3: Pharmaceutical observations of Krishnadi Churna

Parameter	P-1 Karnataka	P-2 Assam	P-3 Uttar Pradesh	P-4 Gujarat
Date of preparation	11/08/2025	11/08/2025	11/08/2025	11/08/2025
Quantity taken (g)	100	100	100	100
Weight after Yavakutta (g)	94g	90 g	95g	93 g
Weight after powdering (g)	43	38	53	58
Total yield (g)	43	38	53	58
Total weight loss (g)	47	62	47	42
Percentage loss (%)	47	62	47	42
Total duration required	2:30 h	2:30 h	2:30 h	2:30 h
Temperature observed (degree C)	35	40	35	40

Organoleptic findings

All four samples were brown-coloured powders with strong pungent odour and characteristic taste. No gross organoleptic difference was reported among the geographical groups.

Table No. 5. Organoleptic characteristics

Parameter	P-1 Karnataka	P-2 Assam	P-3 Uttar Pradesh	P-4 Gujarat
Colour / description	Brown powder	Brown powder	Brown powder	Brown powder
Odour	Strong pungent	Strong pungent	Strong pungent	Strong pungent

Parameter	P-1 Karnataka	P-2 Assam	P-3 Uttar Pradesh	P-4 Gujarat
Taste	Characteristic	Characteristic	Characteristic	Characteristic

Physicochemical findings

The pH values indicated a mildly acidic to near-neutral profile. Uttar Pradesh showed the highest water-soluble extractive, Karnataka the highest alcohol-soluble extractive and lowest loss on drying, and Gujarat the lowest acid-insoluble ash. The four samples therefore showed different quality advantages rather than a single uniformly superior profile.

Table No. 6. Comparative physicochemical parameters

Parameter	P-1 Karnataka	P-2 Assam	P-3 Uttar Pradesh	P-4 Gujarat
pH	5.87	6.20	6.83	6.32
Water-soluble extractive (%)	18.74	16.94	24.14	19.10
Alcohol-soluble extractive (%)	23.00	20.49	15.52	14.05
Acid-insoluble ash (%)	6.22	4.58	6.00	0.84
Loss on drying (%)	9.01	9.67	9.69	11.22

Piperine content

Piperine content varied almost five-fold among the samples. Assam showed the highest value at 0.89%, followed by Karnataka at 0.56%, Gujarat at 0.30% and Uttar Pradesh at 0.18%.

Table No. 7. Piperine content by UV spectrophotometry

Parameter	P-1 Karnataka	P-2 Assam	P-3 Uttar Pradesh	P-4 Gujarat
Piperine (%)	0.56	0.89	0.18	0.30

Microbiological findings

Total microbial plate counts ranged from 3096 to 3404 CFU/g, while total yeast and mould counts ranged from 206 to 254 CFU/g. *Staphylococcus aureus*, *Salmonella* species, *Pseudomonas aeruginosa* and *Escherichia coli* were absent in all samples. The limit of NMT 10^3 CFU/g for both count parameters; therefore, the reported total microbial plate counts should be verified against the applicable standard.

Table No. 8. Microbiological quality of Krishnadi Churna samples

Parameter	**P-1**	**P-2**	**P-3**	**P-4**	**Limit reported in API**
Total aerobic microbial count (CFU/g)	3169	3132	3404	3096	NMT 10^3 cfu/g
Total yeast and mould count (CFU/g)	206	241	254	207	NMT 10^3 cfu/g
Staphylococcus aureus	Absent	Absent	Absent	Absent	Absent/g
Salmonella species	Absent	Absent	Absent	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent	Absent	Absent	Absent/g
Escherichia coli	Absent	Absent	Absent	Absent	Absent/g

Table No. 9. Source-wise standardization profile

Sample	**Principal favorable observation**	**Parameter requiring attention**
P-1 Karnataka	Highest alcohol-soluble extractive (23.00%) and lowest LOD (9.01%)	Highest acid-insoluble ash (6.22%)
P-2 Assam	Highest piperine content (0.89%)	Lowest water-soluble extractive (16.94%); reported final yield ranged from 38 g to 50 g

Sample	Principal favorable observation	Parameter requiring attention
P-3 Uttar Pradesh	Highest water-soluble extractive (24.14%); reported final yields 50 g, 48 g and 53 g	Lowest piperine content (0.18%)
P-4 Gujarat	Lowest acid-insoluble ash (0.84%)	Highest LOD (11.22%); reported final yield ranged from 42 g to 58 g

DISCUSSION

The present study established a comparative pharmaceutical and analytical profile for four *Krishnadi Churna* samples in which the geographical source of *Pippali* was varied. The results demonstrate why a classical name and organoleptic identity alone are insufficient for finished-product control. Although all samples looked and tasted similar, their yields, extractive values, ash, moisture and piperine content differed materially. Similar standardization work on other polyherbal *Churna* formulations has shown that a combined battery of tests is required to support reproducibility and quality assurance.[8]

Pharmaceutical yield varied among both geographical sources and batches. In Batch 1, final powder weights were 95 g for Karnataka, 93g for Assam, 93g for Uttar Pradesh and 96g for Gujarat. In Batch 2, the respective values were 95 g, 96 g, 90g and 94g. In Batch 3, they were 94g, 90 g, 95 g and 93 g. Batch 1 showed the narrowest range, while Batch 3 showed the widest variation. These observations indicate that raw-drug characteristics and processing behaviour influenced the pharmaceutical yield of *Krishnadi Churna*.

The physicochemical profile was multidimensional. A higher water-soluble extractive in the Uttar Pradesh sample indicates a larger fraction of constituent's extractable in water under the test conditions, whereas the higher alcohol-soluble extractive in Karnataka indicates greater recovery of comparatively less polar constituents. Acid-insoluble ash was lowest in Gujarat, suggesting the least siliceous or earthy residue among the tested samples. Karnataka had the lowest LOD, while Gujarat had the highest, which may affect flow, microbial stability and storage behaviour. These interpretations are quality-control inferences and should not be equated directly with clinical superiority.

Piperine showed the most pronounced chemical variation, ranging from 0.18% to 0.89%. The Assam formulation contained approximately 4.9 times the piperine measured in the Uttar

Pradesh formulation. Independent research has likewise demonstrated substantial intra-specific and geographical variation in piperine among *Piper longum* chemotypes, supporting the use of source-specific chemical evaluation when selecting raw *Pippali* for pharmaceutical manufacture.[9]

The high piperine result in Assam identifies it as the strongest sample for this particular marker, but piperine should not be treated as the sole determinant of overall quality. *Piper longum* contains multiple alkaloids, lignans, volatile constituents and other metabolites, and the finished formulation also contains three additional drugs. A complete standard should therefore integrate identity, purity, extractives, moisture, marker assay and chromatographic fingerprint rather than assign therapeutic potency solely from one UV value.[10]

Piperine is widely used as a chemical marker in Ayurvedic formulations containing Piperaceae ingredients, and validated chromatographic methods have been developed for quantitative control. The present UV results provide a useful preliminary comparison, but publication-grade method reporting should include wavelength, standard purity, calibration equation, linearity range, precision, accuracy, recovery, specificity, limit of detection and limit of quantification. HPTLC or HPLC fingerprinting would also distinguish marker variation from matrix interference.[6]

Microbiological analysis showed total microbial plate counts of 3096-3404 CFU/g and total yeast and mould counts of 206-254 CFU/g. The specified pathogenic organisms were absent in all four samples. As the API listed NMT 10^3 CFU/g for both count parameters, the total microbial plate-count values require verification before final compliance is stated.

The collective findings do not identify one sample as superior in every respect. Assam was strongest for piperine, Uttar Pradesh for water-soluble extractives and yield consistency, Karnataka for alcohol-soluble extractives and lower moisture, and Gujarat for low acid-insoluble ash. This pattern favours development of a multi-attribute raw-material specification. For example, manufacturers may define minimum piperine, acceptable moisture and ash limits, extractive-value ranges and batch-yield tolerances together, rather than procuring *Pippali* only by region or appearance.

Geographical origin can influence piperine through genetic, climatic, edaphic and post-harvest factors. However, the present data cannot separate these effects because cultivar, soil, altitude, harvest season, maturity, drying method, storage duration and supplier practices were

not documented. Controlled sourcing studies that record these variables would help distinguish true regional effects from supply-chain and processing effects.[10]

CONCLUSION

The pharmaceutical standardization of *Krishnadi Churna* prepared with *Pippali* from Karnataka, Assam, Uttar Pradesh and Gujarat demonstrated source-associated variation despite uniform organoleptic characters. In Batch 1, final powder weights were 95 g for Karnataka, 93g for Assam, 93g for Uttar Pradesh and 96g for Gujarat. In Batch 2, the respective values were 95 g, 96 g, 90g and 94g. In Batch 3, they were 94g, 90 g, 95 g and 93 g. The physicochemical values also varied among samples, and piperine was highest in Assam at 0.89%. Specified pathogenic microorganisms were absent in all samples. No single geographical sample was optimal for every parameter; therefore, quality control should integrate pharmaceutical yield, moisture, ash, extractive values, piperine content and microbiological quality.

LIMITATIONS

The study had several limitations. Voucher-specimen numbers and detailed authentication reports were not available; analytical measurements were reported as single values without replicate variance; total ash, particle-size distribution, bulk density, tapped density and flow properties were not reported; the UV piperine method lacked validation details; chromatographic fingerprinting was not performed; stability and shelf-life were not evaluated; and only four geographical sources were included. Consequently, the findings should be regarded as preliminary standardization data rather than final pharmacopoeial specifications.

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