IJAYUSH
International Journal of AYUSH
AYURVEDA • YOGA • UNANI • SIDDHA • HOMEOPATHY
https://internationaljournal.org.in/journal/index.php/ijayush/
ISSN: 2349-7025
OPEN ACCESS
★ PEER REVIEWED ★
MONTHLY

Original Research Article	Volume 15 Issue 07	July 2026

A COMPARATIVE PHARMACEUTICO-ANALYTICAL STUDY OF KRISHNADI CHURNA PREPARED WITH PIPPALI COLLECTED FROM DIFFERENT GEOGRAPHICAL REGIONS OF INDIA

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ABSTRACT

Background: *Krishnadi Churna* is a classical polyherbal formulation containing *Pippali*, *Ativisha*, *Musta* and *Karkatashringi*. It is traditionally indicated in childhood conditions such as *Jwara*, *Atisara*, *Vamana*, *Shwasa* and *Kasa*. The geographical origin of medicinal plants may influence their pharmaceutical behaviour and phytochemical composition; therefore, comparative standardization is necessary. **AIM** To conduct a comparative pharmaceutico-analytical evaluation of *Krishnadi Churna* prepared with *Pippali* collected from different geographical regions of India. **OBJECTIVES** To prepare *Krishnadi Churna* containing *Pippali* obtained from Karnataka, Assam, Uttar Pradesh and Gujarat. To document the pharmaceutical

observations and final yield of three preparation batches. To evaluate the organoleptic and physicochemical characteristics of the four samples. To estimate and compare piperine content by ultraviolet spectrophotometry. To evaluate the microbiological quality of the prepared samples. To assess the possible influence of geographical source on the quality characteristics of the finished formulation. **Materials and Methods:** Four samples were coded as P-1, P-2, P-3 and P-4. Three pharmaceutical batches were prepared by cleaning, drying, separate powdering, sieving and homogeneous mixing of the ingredients. Analytical evaluation included organoleptic properties, pH, water-soluble extractive, alcohol-soluble extractive, acid-insoluble ash, loss on drying, piperine by ultraviolet spectrophotometry and microbiological examination. Pharmaceutical yield was summarized using descriptive statistics. **Results:** Final yield varied from 38 g to 58 g from a recorded initial quantity of 100 g. Batch 1 was most uniform. The pH ranged from 5.87 to 6.83. Uttar Pradesh showed the highest water-soluble extractive value (24.14%), Karnataka the highest alcohol-soluble extractive value (23.00%), Gujarat the lowest acid-insoluble ash (0.84%), and Karnataka the lowest loss on drying (9.01%). Piperine was highest in Assam (0.89%). Specified pathogens were absent, but the reported total microbial plate counts were above the limit stated in the presentation. **Conclusion:** Geographical source was associated with variation in pharmaceutical yield and analytical characteristics. No single sample was superior in every parameter. Assam was richest in the measured piperine marker, while other regions performed better for yield, extractive value, ash or loss on drying. Verification of the original microbiological reports and analytical-method details is required before journal submission.

Keywords: *Krishnadi Churna*; *Pippali*; geographical variation; piperine; pharmaceutical standardization; physicochemical analysis

INTRODUCTION

Ayurveda emphasizes preservation of health and management of disease through appropriate diet, lifestyle and medicinal intervention. *Bhaishajya Kalpana* describes the pharmaceutical procedures through which raw medicinal materials are transformed into stable, acceptable and therapeutically suitable dosage forms.[1]

Churna Kalpana is a commonly employed solid dosage form prepared by drying, powdering and sieving single or multiple medicinal substances. Its fine particle size increases the exposed surface area and permits administration with a suitable *Anupana* such as *Madhu*, *Ghrita*, milk or warm water. The classical definition and method of *Churna* are described in the *Ashtanga Samgraha* and *Sharangadhara Samhita*.[2]

Krishnadi Churna is described in the *Sharangadhara Samhita*, *Madhyama Khanda* 6/16. The formulation comprises *Pippali*, *Ativisha*, *Musta* and *Karkatashringi* and is traditionally indicated in paediatric conditions such as *Jwara*, *Atisara*, *Vamana*, *Shwasa* and *Kasa*.[3]

Pippali is an important medicinal drug and piperine is one of its principal chemical markers. Variations in climate, soil, rainfall, harvesting, maturity and post-harvest processing may influence the pharmaceutical and analytical characteristics of plant material. Therefore, the geographical origin of *Pippali* may contribute to differences in the quality attributes of the finished formulation.

The present study compared *Krishnadi Churna* prepared with *Pippali* collected from four geographical regions of India. Pharmaceutical yield, physicochemical characteristics, piperine content and microbiological quality were evaluated to identify regional variation and generate preliminary standardization data.[4]

AIM

To conduct a comparative pharmaceutico-analytical evaluation of *Krishnadi Churna* prepared with *Pippali* collected from different geographical regions of India.

OBJECTIVES

1. To prepare *Krishnadi Churna* containing *Pippali* obtained from Karnataka, Assam, Uttar Pradesh and Gujarat.
2. To document the pharmaceutical observations and final yield of three preparation batches.
3. To evaluate the organoleptic and physicochemical characteristics of the four samples.
4. To estimate and compare piperine content by ultraviolet spectrophotometry.
5. To evaluate the microbiological quality of the prepared samples.
6. To assess the possible influence of geographical source on the quality characteristics of the finished formulation.

MATERIALS AND METHODS

Study Design

The work was designed as a comparative pharmaceutical and analytical study of four *Krishnadi Churna* samples prepared using *Pippali* collected from four geographical regions of India.

Place of Study

The pharmaceutical work was reported to have been carried out in the Department of *Rasa Shastra* and *Bhaishajya Kalpana*, Government P.G. Ayurvedic College and Hospital, Varanasi. Analytical testing was reported according to laboratory guidelines followed by Vasu Pharmaceuticals, Vadodara. The complete laboratory guideline and test-method documents were not included in the presentation.

Table No. 1: Composition of Krishnadi Churna

S. No.	Ayurvedic name	Botanical name	Family
1	*Musta*	*Cyperus rotundus*	Cyperaceae
2	*Ativisha*	*Aconitum heterophyllum*	Ranunculaceae
3	*Pippali*	*Piper longum*	Piperaceae
4	*Karkatashringi*	*Pistacia integerrima*	Anacardiaceae

Table No. 2: Coding of Geographical Samples

Sample code	Geographical origin of ***Pippali***
P-1	Karnataka
P-2	Assam
P-3	Uttar Pradesh
P-4	Gujarat

Pharmaceutical Equipment

The equipment documented in the pharmaceutical study included a mortar and pestle, mixer-grinder, weighing machine, stainless-steel plates, beakers, spoons and sieve No. 80.

Pharmaceutical Procedure

The raw drugs were cleaned and dried properly. *Ativisha*, *Musta* and *Karkatashringi* were initially converted into *Yavakutta* or coarse powder. *Pippali* and the other ingredients were finely powdered separately. The powders were passed through sieve No. 80, weighed, mixed in equal proportions and blended until a homogeneous *Churna* was obtained. The finished formulation was packed in an airtight container and stored in a dry place.[5]

Three batches were prepared on 22 July 2025, 4 August 2025 and 11 August 2025. The total processing duration reported in the comparative tables was approximately 2 hours and 30 minutes. The reported temperature ranged from 35°C to 40°C.

Analytical Parameters

Organoleptic evaluation: *Rupa* (colour and appearance), *Gandha* (odour), *Rasa* (taste) and *Sparsha* (consistency).

Physicochemical evaluation: pH, water-soluble extractive, alcohol-soluble extractive, acid-insoluble ash and loss on drying. Total ash was mentioned in the proposed methodology, but no result was supplied.[6]

Instrumental analysis: piperine was quantified by ultraviolet spectrophotometry. Wavelength, extraction procedure, reference-standard preparation, calibration curve and validation data were not supplied.[7]

Microbiological analysis: total microbial plate count, total yeast and mould count, *Staphylococcus aureus*, *Salmonella* species, *Pseudomonas aeruginosa* and *Escherichia coli*.[8]

Statistical Analysis

The pharmaceutical findings were analysed descriptively. Mean, sample standard deviation and coefficient of variation were calculated from the final-yield values reported for the three batches. Inferential statistical testing was not applied because replicate-level analytical observations were unavailable.

RESULTS

Pharmaceutical Study

Table No. 3: Pharmaceutical Yield in Batch 1

Sample	Region	Initial quantity	After *Yavakutta*	Final yield	Calculated loss	Loss (%)
P-1	Karnataka	100 g	95 g	50 g	50 g	50%
P-2	Assam	100 g	93 g	48 g	52 g	52%
P-3	Uttar Pradesh	100 g	93 g	50 g	50 g	50%
P-4	Gujarat	100 g	96 g	50 g	50 g	50%

Table No. 4: Pharmaceutical Yield in Batch 2

Sample	Region	Initial quantity	After *Yavakutta*	Final yield	Calculated loss	Loss (%)
P-1	Karnataka	100 g	95 g	45 g	55 g	55%
P-2	Assam	100 g	96 g	50 g	50 g	50%
P-3	Uttar Pradesh	100 g	90 g	48 g	52 g	52%
P-4	Gujarat	100 g	94 g	42 g	58 g	58%

Table No. 5: Pharmaceutical Yield in Batch 3

Sample	Region	Initial quantity	After *Yavakutta*	Final yield	Calculated loss	Loss (%)
P-1	Karnataka	100 g	94 g	43 g	57 g	57%
P-2	Assam	100 g	90 g	38 g	62 g	62%
P-3	Uttar Pradesh	100 g	95 g	53 g	47 g	47%
P-4	Gujarat	100 g	93 g	58 g	42 g	42%

Table No. 6: Batch-Wise Final Yield

Batch	Mean yield
Batch 1	49.50 g
Batch 2	46.25 g
Batch 3	48.00 g

Table No. 7: Region-Wise Mean Yield Across Three Batches

Region	Batch 1	Batch 2	Batch 3
Karnataka	95 g	95 g	94 g
Assam	93 g	96 g	90 g
Uttar Pradesh	93 g	90 g	95 g
Gujarat	96 g	94 g	93 g

Organoleptic Analysis

Table No. 8: Organoleptic Characteristics

Parameter	P-1 Karnataka	P-2 Assam	P-3 Uttar Pradesh	P-4 Gujarat
Description/colour	Brown powder	Brown powder	Brown powder	Brown powder
Odour	Strong pungent	Strong pungent	Strong pungent	Strong pungent
Taste	Characteristic	Characteristic	Characteristic	Characteristic

Physicochemical and Instrumental Analysis

Table No. 9: Comparative Analytical Results

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 by UV	0.56%	0.89%	0.18%	0.30%

Interpretation of Analytical Parameters

The pH ranged from 5.87 to 6.83. Karnataka was the most acidic sample, whereas Uttar Pradesh was closest to neutral. The observed difference may reflect variation in the chemical constituents contributed by geographically different *Pippali* samples.

The highest water-soluble extractive was recorded in Uttar Pradesh at 24.14%, followed by Gujarat at 19.10%, Karnataka at 18.74% and Assam at 16.94%. This indicated a greater proportion of constituent's extractable in water in the Uttar Pradesh sample under the stated test conditions.

Karnataka showed the highest alcohol-soluble extractive value of 23.00%, followed by Assam at 20.49%. Uttar Pradesh and Gujarat showed values of 15.52% and 14.05%, respectively. These findings demonstrated variation in the concentration of alcohol-soluble constituents.

Acid-insoluble ash was lowest in Gujarat at 0.84% and highest in Karnataka at 6.22%. Uttar Pradesh and Assam showed values of 6.00% and 4.58%, respectively. The low value in Gujarat suggested a comparatively smaller amount of acid-insoluble inorganic material; however, raw-drug cleaning and environmental contamination should also be considered.

Loss on drying ranged from 9.01% to 11.22%. Karnataka showed the lowest value, while Gujarat showed the highest value, indicating a comparatively greater amount of moisture and other volatile matter in the Gujarat sample under the test conditions.

The Assam sample contained the highest piperine concentration at 0.89%, followed by Karnataka at 0.56%, Gujarat at 0.30% and Uttar Pradesh at 0.18%. Assam was therefore superior specifically for the measured piperine marker. In the absence of analytical replicates and clinical outcomes, this finding cannot independently establish superior therapeutic efficacy.

Microbiological Analysis

Table No. 10: Microbiological Findings

Parameter	**P-1 Karnataka**	**P-2 Assam**	**P-3 Uttar Pradesh**	**P-4 Gujarat**	**Limit stated in PPT**
Total microbial plate count	3169 cfu/g	3132 cfu/g	3404 cfu/g	3096 cfu/g	NMT 10^3 cfu/g
Total yeast and mould count	206 cfu/g	241 cfu/g	254 cfu/g	207 cfu/g	NMT 10^3 cfu/g
Staphylococcus aureus	Absent	Absent	Absent	Absent	Absent/g
Salmonella species	Absent	Absent	Absent	Absent	Absent/g
Pseudomonas aeruginosa	Absent	Absent	Absent	Absent	Absent/g
Escherichia coli	Absent	Absent	Absent	Absent	Absent/g

The total yeast and mould counts were within the limit of NMT 10^3 cfu/g stated in the source presentation, and all specified pathogenic organisms were absent. However, the total microbial plate counts ranged from 3096 to 3404 cfu/g and were above the same stated limit. Therefore, the samples cannot be described as fully compliant with the presented microbiological specification unless the original laboratory reports confirm a different permissible limit or corrected count.

DISCUSSION

The pharmaceutical study was conducted to evaluate the processing behaviour, reproducibility, and yield of *Krishnadi Churna* prepared using *Pippali* collected from Karnataka, Assam, Uttar Pradesh, and Gujarat. Although the initial quantity of each sample was maintained at 100 g, variations were observed in the quantity obtained after *Yavakutta* and in the final yield. These variations indicated that the geographical source of *Pippali*, physical characteristics of the raw drugs, moisture content, fibrous matter, particle size, and losses during pulverization and sieving may have influenced the pharmaceutical yield.

As shown in Table No. 3, Batch 1 demonstrated comparatively uniform pharmaceutical processing. The quantity after *Yavakutta* ranged from 93 to 96 g, while the final yield ranged from 48 to 50 g. Karnataka, Uttar Pradesh, and Gujarat samples each produced a final yield of 50 g, whereas the Assam sample produced 48 g. The total loss ranged from 50% to 52%, indicating minimal regional variation in this batch. Therefore, Batch 1 showed the greatest uniformity and reproducibility among the three batches.

In Batch 2, presented in Table No. 4, the quantity obtained after *Yavakutta* ranged from 90 to 96 g, whereas the final yield ranged from 42 to 50 g. The Assam sample produced the highest final yield of 50 g, followed by Uttar Pradesh with 48 g, Karnataka with 45 g, and Gujarat with 42 g. The percentage loss varied from 50% to 58%. The lower yield of the Gujarat sample may have resulted from a greater proportion of coarse, fibrous, or non-sieveable material being removed during the final preparation process.

Table No. 5 showed greater variation in Batch 3. The final yield ranged from 38 to 58 g, with Gujarat producing the highest yield of 58 g and Assam producing the lowest yield of 38 g. Uttar Pradesh and Karnataka yielded 53 g and 43 g, respectively. Correspondingly, the pharmaceutical loss ranged from 42% in Gujarat to 62% in Assam. This wide variation suggested that Batch 3 was less uniform than Batches 1 and 2. Differences in the hardness,

dryness, fibre content, maturity, or storage condition of the regional samples may have affected their behaviour during pulverization and sieving.

The batch-wise mean yield shown in Table No. 6 was highest in Batch 1 at 49.50 g, followed by Batch 3 at 48.00 g and Batch 2 at 46.25 g. The difference between the highest and lowest batch means was only 3.25 g. Thus, despite individual regional variations, the overall batch-level yield remained relatively comparable. However, Batch 1 was more consistent, while Batch 3 showed the greatest inter-sample variation.

Across the three batches, the calculated mean final yield was approximately 50.33 g for Uttar Pradesh, 50.00 g for Gujarat, 46.00 g for Karnataka, and 45.33 g for Assam. Uttar Pradesh therefore demonstrated the highest and most consistent average final yield. Gujarat also showed a high mean yield but exhibited greater batch-to-batch variation, ranging from 42 to 58 g. The Assam sample showed the lowest regional mean yield, mainly because of the comparatively low yield of 38 g obtained in Batch 3.

The values presented in Table No. 7 represent the quantities obtained after *Yavakutta*. These ranged from 90 to 96 g, indicating that the initial loss during coarse powdering was comparatively low and generally limited to 4–10%. The average post-*Yavakutta* quantities were approximately 94.67 g for Karnataka, 94.33 g for Gujarat, 93.00 g for Assam, and 92.67 g for Uttar Pradesh. Therefore, most of the pharmaceutical loss occurred during the subsequent fine pulverization and sieving stages rather than during the initial *Yavakutta* process.

Organoleptic Analysis

The organoleptic findings presented in Table No. 8 were uniform among all four regional samples. Each preparation was observed as a brown powder with a strong pungent odour and characteristic taste. The similarity in colour, odour, taste, and general appearance indicated that the identity and basic sensory characteristics of *Krishnadi Churna* were maintained irrespective of the geographical source of *Pippali*. Thus, geographical variation did not produce any prominent alteration in the organoleptic profile of the finished formulation.

The strong pungent odour observed in all samples may be associated with the presence of volatile and pungent constituents of *Pippali* and other ingredients of the formulation. The uniform brown colour also suggested adequate mixing and comparable processing of the ingredients. However, organoleptic examination alone could not differentiate the samples according to their chemical quality, as considerable variation was subsequently observed in the physicochemical parameters and piperine content.

Physicochemical Analysis

The pH values of the four samples ranged from 5.87 to 6.83, indicating that all preparations were mildly acidic to nearly neutral. The Uttar Pradesh sample had the highest pH of 6.83, followed by Gujarat at 6.32, Assam at 6.20, and Karnataka at 5.87. Although some regional variation was present, the values remained within a relatively narrow range. This suggested that the overall acid–base characteristics of the formulation were broadly comparable, while minor differences may have resulted from variations in the chemical composition of the regional *Pippali* samples.

The water-soluble extractive value was highest in the Uttar Pradesh sample at 24.14%, followed by Gujarat at 19.10%, Karnataka at 18.74%, and Assam at 16.94%. A higher water-soluble extractive value indicates the presence of a comparatively greater quantity of water-soluble constituents. Therefore, the Uttar Pradesh sample appeared to contain the highest proportion of polar and water-soluble components, whereas the Assam sample contained the lowest proportion.

In contrast, the alcohol-soluble extractive value was highest in the Karnataka sample at 23.00%, followed by Assam at 20.49%, Uttar Pradesh at 15.52%, and Gujarat at 14.05%. This indicated that the Karnataka and Assam samples contained comparatively higher quantities of alcohol-soluble phytoconstituents. The difference between the water-soluble and alcohol-soluble extractive values demonstrated that the chemical constituents of *Pippali* varied according to geographical source and possessed different solvent affinities.

The acid-insoluble ash value showed marked variation among the four samples. Karnataka recorded the highest value at 6.22%, followed by Uttar Pradesh at 6.00%, Assam at 4.58%, and Gujarat at only 0.84%. Acid-insoluble ash mainly represents siliceous matter, such as sand, soil, and other acid-insoluble inorganic materials. Therefore, the comparatively higher values in the Karnataka and Uttar Pradesh samples may be related to intrinsic mineral content or the presence of adhering soil and siliceous matter. These findings emphasize the importance of proper cleaning, handling, and quality control of the raw materials before formulation.

Loss on drying ranged from 9.01% to 11.22%. The Gujarat sample showed the highest value at 11.22%, followed by Uttar Pradesh at 9.69%, Assam at 9.67%, and Karnataka at 9.01%. The higher loss on drying in the Gujarat sample indicated comparatively greater moisture and volatile matter. Excess moisture may affect powder flow, microbial stability, shelf life, and

preservation of the formulation. Therefore, adequate drying and moisture-protective storage would be particularly important for samples showing higher loss on drying.

Piperine Estimation by UV Spectrophotometry

Piperine content showed considerable geographical variation and served as the most important differentiating analytical parameter. The Assam sample contained the highest piperine content at 0.89%, followed by Karnataka at 0.56%, Gujarat at 0.30%, and Uttar Pradesh at 0.18%. Thus, the piperine content of the Assam sample was nearly five times higher than that of the Uttar Pradesh sample. This variation may be associated with differences in climate, soil composition, altitude, rainfall, maturity at harvesting, drying conditions, storage, and genetic characteristics of the plant material.

The piperine content did not show a direct relationship with pharmaceutical yield. Uttar Pradesh had the highest mean final yield and the highest water-soluble extractive value but contained the lowest piperine concentration. Conversely, Assam had the lowest mean final yield but the highest piperine content. Similarly, Gujarat produced a high mean yield but contained only 0.30% piperine. These findings indicated that a higher pharmaceutical yield did not necessarily represent a higher concentration of the principal marker compound.

A partial association was observed between the alcohol-soluble extractive value and piperine content. Assam and Karnataka showed comparatively higher alcohol-soluble extractive values and also contained the two highest piperine concentrations. Since piperine is more readily extracted in organic solvents than in water, the higher alcohol-soluble extractive values in these samples may partly reflect the presence of greater quantities of piperine and other alcohol-soluble constituents. However, the extractive value represents the total soluble matter and cannot be considered a specific measure of piperine alone.

Overall Discussion

The study demonstrated that all four regional samples produced *Krishnadi Churna* with similar organoleptic characteristics but differed considerably in pharmaceutical yield, extractive values, ash content, moisture level, and piperine concentration. Uttar Pradesh and Gujarat samples provided comparatively higher mean pharmaceutical yields, while the Assam sample showed the highest piperine content. Karnataka contained the highest alcohol-soluble extractive value, whereas Uttar Pradesh had the highest water-soluble extractive value.

These findings established that geographical origin influenced the quantitative pharmaceutical and analytical characteristics of *Pippali*, even though the finished formulations appeared

organoleptically similar. Therefore, organoleptic evaluation alone was insufficient for assessing the comparative quality of the samples. Standardization of *Krishnadi Churna* should include pharmaceutical yield, extractive values, ash values, loss on drying, and quantitative estimation of piperine. Among the evaluated samples, Assam appeared superior with respect to piperine content, whereas Uttar Pradesh demonstrated a comparatively better and more consistent pharmaceutical yield. Selection of the source should therefore be based on both manufacturing performance and marker-compound concentration rather than on yield alone.

LIMITATIONS

1. The authentication procedure, voucher specimen numbers and complete procurement records of the raw drugs were not supplied.
2. The precise quantity of each ingredient in each finished formulation batch was not clearly documented in the source presentation.
3. Some pharmaceutical tables contained arithmetic and labelling inconsistencies, for apparent processing loss.
4. Batch-temperature information was inconsistent between the practical headings and comparative tables.
5. Total ash was listed in the methodology, but the corresponding results were absent.
6. Particle-size analysis was not reported beyond the use of sieve No. 80.
7. UV spectrophotometric conditions and analytical-validation data were unavailable.
8. Replicate analytical observations were not provided; therefore, statistical significance between regions could not be calculated.
9. The microbiological conclusion in the presentation conflicted with its stated total microbial plate-count limit.
10. Clinical efficacy and bioavailability were not evaluated; therefore, therapeutic superiority cannot be concluded from piperine concentration alone.

CONCLUSION

The study demonstrated geographical variation in the pharmaceutical and analytical characteristics of *Krishnadi Churna* prepared with *Pippali* obtained from Karnataka, Assam, Uttar Pradesh and Gujarat. Final pharmaceutical yield varied from 38% to 58%, with Batch 1 showing the greatest uniformity. Uttar Pradesh showed the highest mean yield and water-

soluble extractive value. Karnataka showed the highest alcohol-soluble extractive value and lowest loss on drying. Gujarat showed the lowest acid-insoluble ash, while Assam showed the highest piperine concentration of 0.89%. The Assam sample may be considered the richest source specifically in terms of the measured piperine marker. It cannot be designated as universally superior because it did not show superiority in pharmaceutical yield, extractive values, ash or loss on drying. The findings underline the importance of *Desha*, raw-drug authentication, controlled drying, uniform powdering, standardized sieving, validated analytical procedures and microbiological quality control. Verification of the original microbial limit and laboratory reports is necessary before the formulation can be described as fully compliant with quality standards.

ACKNOWLEDGEMENT

The author expresses sincere gratitude to Guide Dr. Richa Pathak, M.D. (Ay.), Ph.D., Assistant Professor, and Co-guide Dr. Atul Kumar, M.D. (Ay.), Assistant Professor, Department of Rasa Shastra and Bhaishajya Kalpana, Government P.G. Ayurvedic College and Hospital, Varanasi, Uttar Pradesh, for their guidance and academic support.

CONFLICT OF INTEREST- nil.

FUNDING – nil

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