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Research Article

Pharmaceutico Analytical and Experimental Study of Amalakyadi Churna in Jwara with Special Reference to Antipyretic Activity

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ABSTRACT

Background: Rasashastra and Bhaishajya Kalpana provide the pharmaceutical basis for preparing Ayurvedic medicines. Churna Kalpana is a dosage form in which dried medicinal ingredients are finely powdered and mixed. Amalakyadi Churna is one such formulation, described in Bharata Bhaishajya Ratnakara for Jwara and possessing Bhedana, Ruchikara, Dipana, Pachana and Kaphahara properties. This study evaluated its pharmaceutical preparation, analytical characteristics and experimental antipyretic activity. Materials and Methods: The study comprised pharmaceutical, analytical and experimental phases. Amalakyadi Churna was prepared following the general Churna preparation procedure described in Sharangadhara Samhita. Organoleptic, physicochemical and HPTLC evaluations were performed. Eighteen Wistar albino rats were randomly divided into Control, Standard and Test groups of six animals each. Following Brewer's yeast-induced pyrexia, the groups received distilled water, Paracetamol at 100 mg/kg body weight and Amalakyadi Churna at the calculated rat dose, respectively. Treatments were administered orally 18 hours after yeast injection, and rectal temperatures were assessed up to 24 hours. Experimental data were analyzed using ANOVA. Results: The formulation exhibited characteristic organoleptic and physicochemical properties, and HPTLC demonstrated a characteristic chromatographic profile. Amalakyadi Churna showed temperature reductions at some observation intervals, but its antipyretic effect was statistically non-significant compared with Control. Conclusion: Amalakyadi Churna was prepared and analytically characterized,

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but statistically significant antipyretic activity was not demonstrated under the experimental conditions.

INTRODUCTION

Ayurvedic pharmaceuticals involves processing medicinal substances into dosage forms suitable for administration. Churna Kalpana is an Upakalpana in which dried raw drugs are reduced to a fine powder and mixed according to the formulation. The preparation method described in Sharangadhara Samhita provides the pharmaceutical basis for processing and combining the ingredients.¹

Jwara occupies an important position in Ayurvedic literature and is discussed both as a disease and as a manifestation associated with other conditions. Classical descriptions emphasize the involvement of Dosha, Agni and Ama in its development.² In contemporary physiology, fever or pyrexia involves an increase in body temperature associated with elevation of the hypothalamic thermoregulatory set point.³ Experimental evaluation of an Ayurvedic Jwarahara formulation can therefore assess its effect on induced pyrexia while retaining the distinction between the classical disease concept and the experimental temperature endpoint.

Amalakyadi Churna, described in Bharata Bhaishajya Ratnakara, contains Amalaki, Chitraka, Pathya or Haritaki, Pippali and Saindhava Lavana in equal proportions. The formulation is traditionally indicated for Jwara and is described as possessing Bhedana, Ruchikara, Dipana, Pachana and Kaphahara properties.⁴ Its ingredients are readily available, and the powder preparation is comparatively simple to perform.

The need for scientific evaluation of the classical indication of Amalakyadi Churna in Jwara prompted the

present study. Hence, the study was conducted to evaluate its pharmaceutical preparation, analytical characteristics and experimental antipyretic activity.

MATERIALS AND METHODS

The study comprised three components: pharmaceutical preparation, analytical evaluation and experimental assessment of the antipyretic activity of Amalakyadi Churna.

1. Pharmaceutical Study

1.1 Preparation of Churnodaka and Chitraka Shodhana

Churnodaka was prepared using 12.5 g of Sudha Churna and 3 L of water. The mixture was left undisturbed for nine hours, after which the clear liquid was collected.⁵ Chitraka roots were cut into small pieces and immersed in sufficient Churnodaka overnight. The material was collected on the following day and dried. The Shodhana procedure followed Rasa Tarangini, chapter 24, verse 575.⁶

During Churnodaka preparation, the freshly mixed liquid appeared milky white and subsequently became clear, with a white surface layer. During Chitraka Shodhana, the liquid changed from light brownish pink to dark brownish pink after approximately nine hours.

1.2 Preparation of Amalakyadi Churna

Amalakyadi Churna was prepared according to Bharata Bhaishajya Ratnakara, following the general Churna preparation procedure described in Sharangadhara Samhita. Raw materials were checked for authenticity before use.¹

Table 1: Ingredients and Quantities of Amalakyadi Churna

Ingredient	Botanical or mineral identity	Part used	Quantity
Amalaki	Emblica officinalis Gaertn. / Phyllanthus emblica L.	Fruit	200 g
Chitraka	Plumbago zeylanica L.	Root	200 g
Haritaki or Pathya	Terminalia chebula Retz.	Fruit	200 g
Pippali	Piper longum L.	Fruit	200 g
Saindhava Lavana	Rock salt	Mineral ingredient	200 g



Cleaning and drying

The herbal ingredients were cleaned, washed and dried under sunlight in a clean place. Materials were monitored for fungal infestation and contamination. Chitraka was dried after Shodhana. Each ingredient was weighed according to the prescribed proportion. Saindhava Lavana was handled separately.

Powdering sieving and mixing

Amalaki, Pippali, Chitraka and Haritaki were pounded separately using Khalwa Yantra and then powdered individually using a mixer grinder. The fibrous nature of Chitraka required thorough pounding. Saindhava Lavana was finely powdered separately. The herbal powders were individually sieved, combined and mixed thoroughly to obtain a homogeneous preparation. Saindhava Lavana was added at the end. The finished Churna was stored in an airtight container.

Precautions included thorough drying of the raw drugs, use of Shodhita Chitraka, separate processing of the ingredients and avoidance of moisture during storage. Weight changes during grinding and sieving were recorded.

2. Analytical Evaluation

The prepared Amalakyadi Churna was subjected to organoleptic and physicochemical evaluation for characterization of the formulation. Powder microscopy and HPTLC fingerprinting were also performed.

Physical appearance, colour, odour, taste and consistency were examined. The physicochemical assessment included pH, loss on drying, total ash, acid-insoluble ash, water-soluble ash, alcohol-soluble extractive value, water-soluble extractive value, bulk density, tapped density, Carr's index and Hausner's ratio.⁷

For loss on drying, 10 g of the sample was dried at 105 °C for five hours and further dried to constant weight. Total ash was determined by incinerating 2 g of sample at a temperature not exceeding 450 °C until carbon-free ash was obtained. Acid-insoluble ash was determined from the ash residue using dilute hydrochloric acid, filtration and ignition to constant weight. Water-soluble

ash was determined by boiling the ash with water and subtracting the insoluble residue from total ash.⁷

For extractive values, 4 g of the sample was treated with 100 mL of the respective solvent, shaken intermittently for six hours and left to stand for 18 hours. A measured portion of the filtrate was evaporated, dried at 105 °C and weighed. The aqueous sample preparation for pH measurement was 1% w/v, and the instrument was calibrated using standard buffers. Bulk and tapped densities were recorded, with the latter measured after 50 tappings. Powder microscopy was performed after mounting the sample in glycerine, using a Zeiss Axio trinocular microscope with an attached camera.

One gram of Amalakyadi Churna was extracted with 10 mL of ethanol. Aliquots of 3, 6 and 9 µL were applied as 7 mm bands to precoated silica gel F₂₅₄ aluminium plates using a Linomat 5 applicator. The mobile phase was Toluene:Ethyl acetate:Formic acid (5.0:4.0:1.0). Developed plates were visualized under short and long ultraviolet light and after derivatization with vanillin-sulphuric acid. Densitometric scans were recorded at 254, 366 and 620 nm, the latter following derivatization. R_f values and band colours were documented.

3. Experimental Study

The antipyretic activity of Amalakyadi Churna was evaluated using Brewer's yeast-induced pyrexia in healthy Wistar albino rats of either sex, weighing 180–250 g. Animals were housed in standard plastic cages with stainless-steel coverings and wheat-straw bedding under controlled environmental conditions, with a balanced pelleted diet and water. Pregnant animals, diseased animals and animals involved in other experiments were excluded.

The experimental protocol was approved by the Institutional Animal Ethics Committee under approval number SDMCRA/IAEC/SDM-KAMC-MR-14.

Eighteen rats were randomly divided into three groups of six animals each. The Control group received distilled water, the Standard group received Paracetamol and the Test group received Amalakyadi Churna.



Table 2: Experimental Grouping

Group	Number of rats	Treatment
Control	6	Distilled water
Standard	6	Paracetamol, 100 mg/kg body weight
Test	6	Amalakyadi Churna, calculated rat dose

3.1 Induction of Pyrexia

Animals were fasted overnight before the experiment. Initial normal rectal temperatures were recorded using a digital thermometer. Pyrexia was induced by subcutaneous injection of Brewer's yeast suspension at 1 mL/100 g body weight. Rectal temperature was recorded 18 hours after injection to confirm pyrexia.

3.2 Dose Fixation and Treatment

Following confirmation of pyrexia, the corresponding treatments were administered orally using a feeding tube. The Control group received distilled water, the Standard group received Paracetamol at 100 mg/kg body weight and the Test group received Amalakyadi Churna at the calculated rat dose.

The rat dose was calculated using the formula: human dose $\times 0.018 \times 5$ per kilogram body weight. The human dose was one Karsha, equivalent to 12 g, corresponding to a calculated rat dose of 1.08 g/kg body weight.

3.3 Assessment of Antipyretic Activity

Antipyretic activity was assessed by recording rectal temperature before and after yeast administration and

at subsequent observation intervals following treatment. The stated protocol called for hourly readings for five hours and a further reading at 24 hours. Temperature responses and percentage comparisons were recorded for the three groups.

PGE₂ results were evaluated for the Control, Standard and Test groups as a biochemical outcome alongside rectal temperature.

3.4 Statistical Analysis

Experimental data were statistically analyzed using analysis of variance (ANOVA).

RESULTS

Pharmaceutical findings

Amalakyadi Churna was prepared as a fine brown powder with the characteristic odour of its ingredients. No fungal infestation was observed during preparation. Processing losses occurred during grinding, sieving and handling, with some fibrous material remaining after grinding. The weights recovered from the individual ingredients are shown in Table 3.

Table 3: Weight Changes During Grinding and Sieving

Ingredient	Before processing (g)	After processing (g)	Loss (g)	Loss (%)
Amalaki	200	188	12	6
Pippali	200	190	10	5
Chitraka	200	184	16	8
Haritaki	200	188	12	6
Saindhava Lavana	200	198	2	1

Table 4: Organoleptic Characteristics

Characteristic	Observation
Physical appearance	Powder
Colour	Brown
Odour	Characteristic
Taste	Astringent, sweet, slightly pungent and salty



Characteristic	Observation
Consistency	Fine

Analytical findings**Table 5: Physicochemical Parameters of Amalakyadi Churna**

Parameter	Reported result
pH	7.05
Loss on drying (%)	7.55 ± 0.01
Total ash (%)	22.1 ± 0.07
Acid-insoluble ash (%)	3.86 ± 0.01
Water-soluble ash (%)	49.05 ± 0.07
Alcohol-soluble extractive value (%)	30.33 ± 0.00
Water-soluble extractive value (%)	78.16 ± 0.01
Bulk density	0.4695
Tapped density	0.5313
Carr's index	11.63
Hausner's ratio	1.13

Parameter	Reported result
Particle size (µm)	100

HPTLC demonstrated multiple bands under different visualization conditions. Under short UV, bands were reported at Rf 0.26, 0.48, 0.56 and 0.78. Under long UV, bands were observed at Rf 0.56, 0.65 and 0.73. Following derivatization, bands were recorded at Rf 0.15, 0.28, 0.47, 0.80 and 0.87.

Experimental findings

Brewer's yeast administration increased rectal temperatures in all three groups. This increase was reported as statistically significant relative to the initial normal temperature. The reported means after yeast injection were 39.63 ± 0.1961 °C in Control, 40.30 ± 0.1183 °C in Standard and 39.72 ± 0.2272 °C in Test. The rectal temperatures are presented in Table 6.

Table 6: Reported Rectal Temperature Summary

Observation interval	Control (°C)	Standard (°C)	Test (°C)
After yeast induction	39.63 ± 0.1961	40.30 ± 0.1183	39.72 ± 0.2272
1st hour	39.60 ± 0.1592	39.06 ± 0.3614	39.22 ± 0.2227
2nd hour	39.27 ± 0.3694	38.74 ± 0.4226	39.23 ± 0.2667
3rd hour	39.40 ± 0.3502	39.04 ± 0.4718	39.22 ± 0.1600
4th hour	39.58 ± 0.4505	39.20 ± 0.4012	39.10 ± 0.1461
5th hour	39.44 ± 0.1631	39.92 ± 0.2417	39.27 ± 0.2305

Mean ± SEM.

Amalakyadi Churna did not produce statistically significant antipyretic activity compared with Control. At the first, third and fourth hourly observations, the Test

group had lower reported mean temperatures than Control. The reported percentage comparisons are shown in Table 7. Temperature reductions were reported for the Standard group at several observations.

Table 7: Reported Percentage Comparisons in Rectal Temperature

Observation interval	Standard versus Control	Test versus Control
1st hour	1.36% decrease	0.95% decrease
2nd hour	1.34% decrease	0.10% increase
3rd hour	0.91% decrease	0.45% decrease
4th hour	0.96% decrease	1.21% decrease
24th hour	1.21% increase	0.43% decrease

At the 24-hour observation, the Test mean was 39.27 ± 0.2305 °C and Control was 39.44 ± 0.1631 °C. The Standard mean was 39.92 ± 0.2417 °C. The temperature-based antipyretic response of the Test group

remained statistically non-significant compared with Control.



Reported PGE₂ findings

Reported PGE₂ values were 250.34 ± 2.34 in Control, 55.18 ± 1.22 in Standard and 108.56 ± 3.55 in Test. Reported reductions were 77.95% and 56.63% for

Standard and Test, respectively, with the results marked as $p < 0.01$ and $p < 0.05$ (Table 8). This biochemical finding is presented separately from the temperature-based antipyretic conclusion.

Table 8: Reported Effect on Pge₂ Levels

Group	Reported PGE ₂ value	Reduction (%)
Control	250.34 ± 2.34	Not applicable
Standard	$55.18 \pm 1.22^{**}$	77.95
Test	$108.56 \pm 3.55^{*}$	56.63

Mean $\pm$ SEM; $^{*}p < 0.05$, $^{**}p < 0.01$.

DISCUSSION**Classical rationale and pharmaceutical preparation**

The selection of Amalakyadi Churna was based on its classical indication in Jwara and the properties attributed to its constituents. The classical rationale considers Amalaki in relation to its Sheeta Virya and Rasayana properties; Haritaki in relation to digestion and removal of Ama; Pippali in relation to Katu Rasa, Ushna Virya and Rasayana properties; Chitraka in relation to Dipana, Pachana and Vata-Kapha-hara actions; and Saindhava Lavana in relation to Dipana, Pachana and Ruchya properties. This classical rationale informed the experimental investigation; it does not substitute for demonstration of antipyretic efficacy.

The pharmaceutical process involved cleaning, drying, Chitraka Shodhana, separate pounding and powdering, sieving and mixing. Chitraka was processed in Churnodaka before incorporation into the formulation. Separate powdering allowed the fibrous ingredients to be handled individually, while addition of Saindhava Lavana at the final stage followed the preparation procedure.

The individual processing losses ranged from 1% for Saindhava Lavana to 8% for Chitraka. Losses during grinding, sieving and handling, together with retention of fibrous material, accounted for the reduced recovered weights. The resulting product was a fine brown powder with characteristic odour and mixed astringent, sweet, pungent and salty taste.

Interpretation of the analytical profile

Analytical assessment provided a physicochemical and chromatographic profile of the prepared formulation. The pH of 7.05 indicated a value close to neutral under the test conditions. Loss on drying documented the sample's drying-related weight loss, while total ash, acid-insoluble ash and extractive measurements characterized the preparation. Bulk density, tapped density, Carr's index and Hausner's ratio documented powder packing and flow-related characteristics. The Carr's index and Hausner's ratio were interpreted as indicating good flow properties.

HPTLC demonstrated bands at different R_f values under short UV, long UV and after derivatization, indicating the presence of multiple detectable constituents and a characteristic chromatographic profile of Amalakyadi Churna.

The analytical parameters were reported to be within preliminary standard limits. The measured profile provides a basis for characterization of the prepared sample and comparison with subsequent preparations made using the same procedure.

Interpretation of the experimental findings

In the experimental pyrexia study, Amalakyadi Churna showed reductions in rectal temperature at selected observation intervals. At the first hour, the mean temperature in the Test group was 39.22 ± 0.2227 °C, compared with 39.60 ± 0.1592 °C in Control and 39.06 ± 0.3614 °C in the Standard group. At the third hour, the Test mean was 39.22 ± 0.1600 °C, compared with 39.40 ± 0.3502 °C in Control. At the fourth hour, the Test mean was 39.10 ± 0.1461 °C, compared with 39.58 ± 0.4505 °C in Control and 39.20 ± 0.4012 °C in



the Standard group. Although these observations showed lower mean temperatures than Control, the antipyretic effect of Amalakyadi Churna remained statistically non-significant.

The observed temperature reductions may be considered in relation to the combined Dipana, Pachana, Amapachana and Jwarahara properties attributed to Amalaki, Haritaki, Pippali, Chitraka and Saindhava Lavana. In the Ayurvedic account of Jwara, these properties are considered to address Agnimandya and Ama. They provide the classical rationale for evaluating the formulation's antipyretic activity.

At the 24-hour observation, the mean rectal temperature in the Test group was 39.27 ± 0.2305 °C, compared with 39.44 ± 0.1631 °C in Control. The difference was statistically non-significant. The lower mean temperature represents a numerical trend, while the statistical comparison did not establish a significant antipyretic effect.

Overall, the pharmaceutical and analytical findings characterized the prepared Amalakyadi Churna. The experimental findings showed temperature reductions at selected intervals, but did not establish statistically significant antipyretic activity compared with Control under the study conditions.

CONCLUSION

Amalakyadi Churna was prepared using the selected classical formulation and standard operating procedure, including Chitraka Shodhana followed by separate powdering, sieving and mixing of the ingredients. Organoleptic, physicochemical, microscopic and HPTLC assessments documented the characteristics of the prepared sample. The analytical parameters were reported within preliminary standard limits.

In Brewer's yeast-induced pyrexia in Wistar albino rats, Amalakyadi Churna did not demonstrate statistically significant antipyretic activity compared with Control under the experimental conditions.

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