Comparative Qualitative Assessment Of Nishaamalaki Formulations Prepared From Seasonal Variations Of Amalaki Using Different Bhavana Methods

Short Title: *Qualitative profile of Nishaamalaki samples*

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Abstract:
Nishaamalaki, a traditional Ayurvedic formulation comprising of Haridra(*Curcuma longa*) and Amalaki *(Phyllanthus emblica*) is described in classical texts such as Bruhatrayi as an effective formulation in the management of *Prameha* and related disorders. However, the traditional way of administration as mixture of fresh Amalaki juice with Haridra powder poses practical challenges, reducing compliance of patient. Additionally, seasonal variations in Amalaki (winter and summer) may influence its pharmacological profile and therapeutic potential. The present study is aimed to develop and evaluate the qualitative fingerprinting of four Nishaamalaki (NA) formulations using two seasonal varieties of Amalaki and two processing methods (direct mixing and wet trituration).

Four formulations were prepared by incorporating sixteen parts of seasonal Amalaki juice into fine Haridra powder using two distinct Bhavana methods Evaluation was conducted with organoleptic, physicochemical , phytochemical parameters, along with LC-MS profiling.

Organoleptic variations were observed in color and odour, while all formulations retained sour taste and smooth texture. Physicochemical analysis showed higher moisture and extractive values in winter formulations, with uniform ash values and acidic pH across the samples. Qualitative phytochemical screening confirmed the presence of major bioactive groups in all formulations. Quantitative analysis revealed higher β-sitosterol and ferulic acid in summer formulations, whereas winter formulations were enriched in curcumin, gallic acid, and piperine. Quercetin remained stable across all formulations.

Study established that seasonal variation and processing methods significantly alter the phytochemical profile of Nishaamalaki. Necessity of standardized preparation methods to maintain reproducible phytochemical fingerprint is evidenced.

Keywords: Nishaamalaki, wet trituration, direct mixing method, LC-MS

Key messages

- The seasonal variation of Amalaki significantly dictates the variation in concentration of key phytoconstituents in the formulation.
- The specific methods of processing functions as a critical variable that affects the quatification of phytoconstituents present in Nishaamalaki.
- The study establishes a scientific rationale for selecting specific seasons and processing techniques to ensure the consistency and therapeutic potency of Nishaamalaki.

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Inroduction:
Nishaamalaki [NA], a classical Ayurvedic formulation comprising Nisha (Turmeric, *Curcuma longa*) and Amalaki (Indian Gooseberry, *Phyllanthus emblica*), represents a synergistic therapeutic approach in the management of *Prameha* (~ Diabetes mellitus) and associated complications. Both constituents are extensively

described in Ayurvedic literature for their wide-ranging pharmacological properties. Individually, Nisha and Amalaki exhibit actions such as Vayasthapana (anti-aging), Rasayana (rejuvenative), Pramehaghna (anti-diabetic), Tridoshahara (balancing all three doshas), Shleshmasanshamana (kapha-pacifying), Chakshushya (beneficial for vision), and Deepana (enhancing metabolic activity), indicating their potential in metabolic and degenerative disorders. [1,2] Extensive preclinical and clinical research have been conducted to validate the therapeutic potential of Nishaamalaki, particularly in the management of Madhumeha (~Type 2 Diabetes Mellitus) . It is revealed in few clinical studies that diverse dosage forms are used, ranging from traditional powder to modernized capsules and tablets.[3, 4] In previous research work Nishaamalaki yoga was prepared by sixteen times levigation of Amalaki juice to mixture of fine powders of turmeric and Amalaki and investigated its antihyperglycemic activity in animal models. [5] Recent pharmacognostical evaluations have highlighted the importance of Bhavana processes wherein seven cycles of Amalaki juice levigation significantly alter the phytochemical profile of Nisha. [6.] In one case study, the effectiveness of Amalaki swaras bhavit Haridra powder in Diabedtic Dyslipidemia was reported.[7] Another study provided comparative phytochemical profile and HPTLC analysis of Nisha (turmeric), Amalaki (Indian gooseberry), and their combined formulation, prepared with mixing of Nisha-Amalaki Churna in equal proportion, was confirmed the presence of key secondary metabolites, such as steroids, alkaloids, and amino acids, establishing essential physicochemical standards for the authentication and quality control of this traditional polyherbal preparations.[8]

Existing research works documents the use of Nishaamalaki in varied forms, such as powder or tablet form. Moreover, many contemporary studies on Nishaamalaki prepared with equal ratio of ingredients have been studied and reported. However, the guideline for specific proportion of Amalaki Juice and Haridra powder as well as its qualitative profile is not yet explored.

Thus, the present study addresses this gap by adhering to the specific classical guidelines outlined in the Sushrut Samhita wherein 1 part Haridra and 16 parts Amalaki swaras is suggested while administration of Nishaamalaki formulation.[9] Also, application of pharmaceutical principle of Ayurvedic concept of Bhavana process was focused and its subsequent impact on qualitative profile of the formulation.

Considering the seasonal availability of fresh Amalaki fruits and the potential influence of seasonal variation on drug potency, the focus is given to collect fruits in winter and summer seasons and were utilized in preparation of NA formulations. As well, considering the ayurvedic pharmaceutical guidelines, two methods such as direct mixing and wet trituration were employed. Accordingly, four distinct formulations of Nishaamalaki were prepared using two variations of Amalaki fruits and two different preparation methods, with the aim of comparative evaluation through organoleptic, physicochemical parameters and phytochemical, LC–MS analysis.

Materials and Methodology

Preparation of Nishaamalaki [NA] formulations: Four formulations of NA were prepared using two seasonal varieties of Amalaki fruits. Fresh Amalaki fruits harvested during the winter (November–December) and summer (March–April) seasons were procured from an authentic source, BNK Foods and Herbals, Pune. Haridra rhizomes were procured from the renowned Mahagaonkar Agro Industry, Maharashtra. Individually, drugs were identified and authenticated by taxonomists and Ayurveda experts.

Step-wise preparation of formulations

Preparation of Amalaki Swaras (Juice): Freshly procured Amalaki fruits were washed, shade-dried, and deseeded. 2kg of fruit was mechanically pulverized and pulp was squeezed through a clean cotton cloth. The resulting Swaras (juice) was collected in a sterile stainless-steel vessel for further use.

Preparation of Haridra Choorna [Powder]

Haridra rhizomes were powdered using grinder and sieved to obtain fine particle sized Haridra choorna [mesh size 80] which was then standardized and used for preparation of Nishaamalaki formulations.

In-house testing of Haridra choorna and Amalaki swaras

Testing of the raw materials (Haridra choorna and Amalaki Swaras) was carried out in the in house laboratory. Haridra choorna was evaluated through organoleptic tests and physicochemical parameters such as pH, alcohol-soluble extractive, water-soluble extractive, moisture content and ash value. Amalaki Swaras was evaluated using organoleptic parameters along with physicochemical parameters including pH, specific gravity, total solid content, and viscosity.

Preparation of four formulations of Niashaamalaki (NA1A, NA1B, NA2A, NA2B)

Four variants of Nishaamalaki (NA1A, NA1B, NA2A, NA2B) were prepared using a 1:16 ratio of Haridra powder to Amalaki juice, as mandated by the Sushrut Samhita (Su. Chi 11/8). Thus, in present study sixteen times of Amalaki juice was added to Haridra powder depicted in table 1. Two distinct processing methods were employed: Direct Mixing, where the liquid was absorbed without mechanical intervention (NA1A, NA2A), and Wet Trituration, involving continuous grinding in a mortar and pestle

until complete liquid absorption takes place. (NA1B, NA2B). [10, 11]

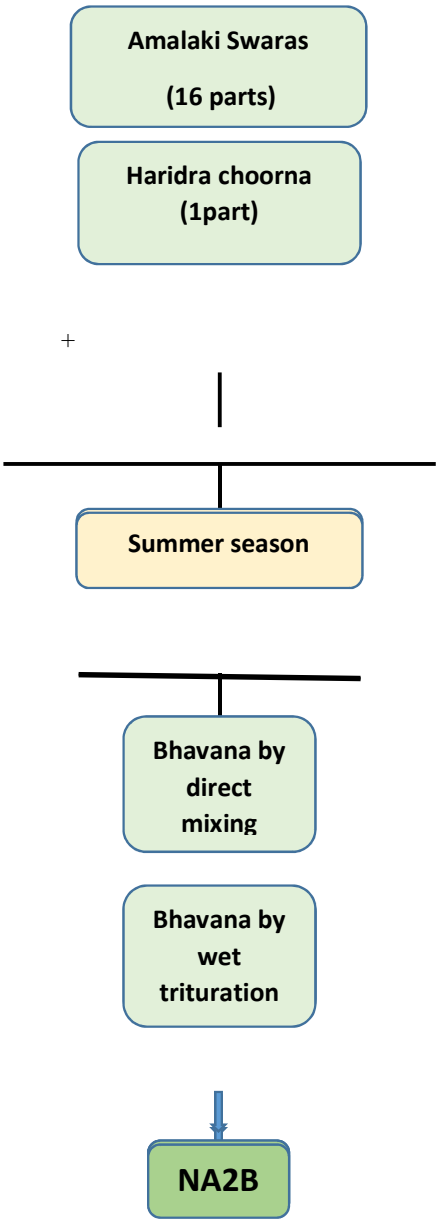
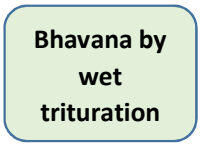


Table 1: Quantity of ingredients in preparation of NA1A, NA2A, NA1B and NA2B

Sample	Haridra powder	Amalaki juice
NA1A (winter-direct mixing)	30 g	480ml
NA2A (Summer-direct mixing)	30g	480 ml



NA1B (winter-wet trituration)	30 g	480ml
NA2B (summer-wet trituration)	30g	480 ml

Method of preparation for NA1A and NA2A

Thirty grams of Haridra powder was placed in a clean, empty stainless steel plate. Sixteen parts (480 ml) of Amalaki juice was gradually added and thoroughly mixed to ensure uniform incorporation. The plate was covered with a clean cotton cloth and exposed to sunlight for drying, with daily observations recorded to monitor physicochemical changes during the process. Upon complete drying, the material was aseptically scraped using a sterile scraper and triturated in dry form to obtain a fine particle-sized Nishaamalaki formulation. The final product was then labelled and stored in an airtight container.

Method of preparation for NA1B and NA2B

Thirty grams of Haridra powder was placed in a clean mortar and required quantity of Amalaki juice was gradually added. Wet trituration was performed until the powder was fully saturated. The resulting mixture was spread on a plate and dried under sunlight, with the plate covered using a clean cloth to prevent contamination. The dried material was then powdered, and the process of wet trituration was repeated until a total of 480 ml of Amalaki juice had been completely incorporated. After final drying, the material was collected and pounded to obtain a uniform, smooth formulation, which was subsequently stored in an airtight container.

Qualitative assessment of four formulations of Nishaamalaki: Four formulations were prepared in a single batch, analyzed with organoleptic, for physicochemical, phytochemical evaluation and LC-MS characterization. Colour, odor, touch, taste evaluation and physic-chemical analysis (pH, alcohol soluble extractive, water soluble extractive, moisture content, ash value) of four formulations of Nishaamalakai was done using API parameters. Further, formulations were tested at GMP approved laboratory for phytochemical analysis to estimate functional groups and studied using LC-MS technique for quantification of significant biomolecules- Curcumin and Quercetin.

Table 2:Tests performed for analysis of Phytochemicals Nishaamalaki formulations

Phyto chemicals	Tests
Alkaloids	Dragendorff test, Hager's test
Phenolic compounds	Ferric chloride test, Lead acetate test
Flavonoids	Ferric chloride test, Shinoda test
Tannins	Ferric chloride test, Vanillin HCL test
Steroids	Salowski's test

Liquid chromatography mass spectroscopy analysis:

0.5 g of Nishaamalaki choorna was accurately weighed and transferred to a clean extraction vessel. Methanol was added, and the extraction process was carried out. Following extraction, the crude methanolic extract was filtered to remove particulate matter. To bring the analyte concentration within the linear dynamic range of the detector, a 40-fold dilution (1:40) was performed using an appropriate solvent system. The diluted formulations were then filtered through a 0.22 µm syringe filter to ensure suitability for injection.

A precise volume of the processed sample was injected into the LC-MS system for chromatographic separation. The instrument used was Agilent 1260 Infinity coupled with a 6460 Triple Quadrupole (QQQ) mass spectrometer. As the compounds eluted from the column, they were introduced into the mass spectrometer via an electrospray ionization (ESI) source. The data were acquired and analyzed**.**

Observations

- Organoleptic and physicochemical analysis of raw materials:

The organoleptic evaluation of Haridra powder revealed a characteristic deep yellow colour with a distinctly aromatic and pungent odor. Upon tasting, the powder exhibited a slightly bitter flavor. The texture was found to be fine and uniform, consistent with the physical standards. Fresh Amalaki juice of both seasons was evaluated for its sensory attributes, exhibiting a pale green appearance and a distinctive, fruity odour. The liquid was found to be slightly turbid in consistency with a sharp sour-astringent flavor.

The physicochemical analysis of Haridra powder revealed an alcohol-soluble extractive of 15.44% and a water-soluble extractive of 14.4%,.With a moisture content of 9.46%, an ash value of 4.45%, and a nearly neutral pH of 6.5, all parameters were found to be in accordance with Ayurvedic Pharmacopoeia of India (API) standards, confirming the purity and quality of the raw material.

Table 3: Physicochemical analysis-for Amalaki swaras

Test	Amalaki swaras : Winter	Amalaki swaras : Summer
Specific gravity	1.035	1.022
Total solid content	4.126	4.032
Viscosity	1.74	1.74
pH	3	3.2

Table 4: Observations during preparation of NA formulations

Observations	NA1A	NA1B	NA2A	NA2B
Season for preparation	Winter	Winter	Summer	Summer
Duration of process	Mixture Kept in sunlight for 6 hours daily	Wet Trituration daily for 2 hours + Sun drying- 6 hours daily	Mixture Kept in sunlight for 6 hours daily	Wet Trituration 1.5 to 2 hours + Sun drying- 6 hours daily
Time required for preparation of dried formulation	5 days	12 days	3 days	8 days
Change in colour	From yellow to dark yellow	From yellow to greenish dark	From yellow to greenish yellow	From yellow to dark olive green
Weight of final product	34g	42g	35 g	40 g

Table 5: Organoleptic evaluation of four formulations of Nishaamalaki

Parameters / Formulations	NA1A	NA1B	NA2A	NA2B
Color	Dark yellow	Greenish dark	Greenish yellow	Dark olive green
Taste	Amla (sour)	Amla++ (sour)	Amla (sour)	Amla Kashay
Odor	Dominant Haridra specific	Strong mixed +++	Mixed +	Strong Mixed +++
Touch	Smooth	smooth	smooth	Smooth

Table 5 illustrates the organoleptic characteristics of the NA formulations. The formulations prepared using the direct mixing method exhibited a yellowish color, whereas those processed by wet trituration showed a comparatively darker green shade. All formulations possessed *Amla* (sour) *rasa* and a smooth texture upon touch. A characteristic odor specific to *Haridra* was observed in NA1A formulation. In contrast, NA1B and NA2B exhibited a strong odour, while NA2A showed a relatively mild odor.

Table 6 : Physicochemical evaluation of four formulations of Nishaamalaki

Parameters	**NA1A**	**NA1B**	**NA2A**	**NA2B**
Alcohol soluble extractive	33.4%	32.64%	16.8%	20.18%
Water soluble extractive	55.4%	63.74%	37.76%	36.64%
Moisture content	16.87%	10.5%	9.96%	7.87%
Ash value	4.3%	5.92%	4.6%	5.4%
pH	3.09	3.05	5.68	3.30

Table 6 presents the physicochemical parameters of the formulations. The moisture content was highest in NA1A among all four formulations. Overall, moisture content was slightly higher in formulations prepared during the winter season and those processed by direct mixing; however, all values remained within permissible limits. The ash values of all formulations were found to be comparable, indicating uniformity. The pH of NA2A was higher, whereas the remaining three formulations exhibited similar pH values, suggesting an overall acidic nature. Furthermore, formulations prepared in the winter season demonstrated higher extractive values compared to those prepared in the summer season.

Phytochemical analysis of formulations:

Qualitative phytochemical analysis revealed that all formulations of Nishaamalaki contained important bioactive constituents, including alkaloids, phenolic compounds, flavonoids, tannins, and steroids, indicating a consistent phytochemical profile across all variants.

LC-MS Analysis

The quantitative estimation of phytochemicals across the four NA formulations (NA1A, NA1B, NA2A, and NA2B) demonstrated considerable variation in the concentration of bioactive constituents. The values were given in mg/kg.

Table 7: Quantification of Phytochemicals in Nishaamalaki formulations through LCMS

Phytochemicals	**NA1A** (mg/kg)	**NA1B** (mg/kg)	**NA2A** (mg/kg)	**NA2B** (mg/kg)
Beta sitosterol	11.683	20.210	1085.226	1144.224
Piperine	5.094	3.967	1.683	1.666
Curcumin	14805.768	17659.270	877.136	757.395
Gallic acid	895.013	BLQ	57.226	28.631
Caffeic acid	0.825	1.203	0.999	0.667
Rutin	0.746	0.548	0.41	0.342
P-Cumaric acid	46.675	BLQ	40.481	23.685
Quercetin	0.356	0.429	0.0691	0.127
Ferulic acid	163.808	BLQ	235.641	134.105

Table 7 indicates that β-sitosterol content was markedly higher in NA2A (1085.226) and NA2B (1144.224) compared to NA1A (11.683) and NA1B (20.210), indicating a substantial increase in formulations associated with the summer season. Similarly, ferulic acid was found in higher concentrations in NA2A (235.641) and NA2B (134.105), whereas it was lower in NA1A (163.808) and below the limit of quantification (BLQ) in NA1B. These findings suggest that summer formulations favour the accumulation of certain sterols and phenolic acids.

In contrast, curcumin content was significantly higher in NA1 formulations, particularly in NA1B (17659.270), followed by NA1A (14805.768), while markedly lower levels were observed in NA2A (877.136) and NA2B (757.395). This indicates that winter season formulations are richer in curcumin content, with NA1B showing the highest concentration among all.

Gallic acid was detected at a high level in NA1A (895.013), while it was below the limit of quantification in NA1B and present in much lower concentrations in NA2A (57.226) and NA2B (28.631). This highlights NA1A as the formulation with the highest gallic acid content.

Piperine content was comparatively higher in NA1A (5.094) and NA1B (3.967) than in NA2A (1.683) and NA2B (1.666), further supporting the observation that winter formulations retain higher levels of certain alkaloids.

Other phenolic compounds such as caffeic acid, rutin, and p-coumaric acid were present in relatively low concentrations across all formulations, with minor variations. Notably, p-coumaric acid was below the limit of quantification in NA1B but present in the remaining formulations.

Quercetin, an important biomarker of *Emblica officinalis* (*Amalaki*), showed relatively consistent levels across all formulations, with slight variations (0.356–0.429 in NA1 and 0.0691–0.127 in NA2), indicating minimal seasonal or processing influence on this constituent.

Discussion

Although the Sushruta Samhita traditionally recommends the fresh administration of Nishaamalaki , this study employed Bhavana (wet trituration) and Nimmajan (addition of liquid with Direct mixing) as a pharmaceutical procedure (Samskara). These processes are selected to enhance the stability, palatability and potency of the formulation.

The organoleptic evaluation of Nishaamalaki formulations revealed a significant difference based on the preparation methods. The dark olive green color observed in the wet trituration formulations may be attributed due to intensive mechanical shear and subsequent oxidation of phenolic components.[12] On the other hand, the yellow coloration retained in the NA formulations of direct mixing method suggests the preservation of curcuminoids, might be due to preventing the oxidative darkening in a more conservative processing setting. The organoleptic profile of NA formulations of the present study, is found alike to the findings reported by Jawanjal et al[2022] .[8] particularly regarding aroma and taste indicating pharmaceutical process is methodically applied and reflected to obtain quality organoleptic parameters. However, the notable variance in colour and physicochemical parameters may be due to the alteration in method of preparation.

In physicochemical testing notable changes in the values obtained might be due to the application of different methods of preparation. Elevation observed in water and alcohol soluble extractives during winter season may be attributed to peak accumulation of secondary metabolites. Furthermore, it can be interpreted that the cooler ambient temperature is likely to prevent thermal degradation of liable bioactive components. [13]

The observed seasonal variation in phytochemical profiles across the NA formulations aligns well with the data reported in earlier studies on medicinal plant chemistry and processing. Previous investigations in phytochemistry have established that environmental factors, particularly seasonal temperature fluctuations, significantly influence the biosynthesis, accumulation, and stability of secondary metabolites such as phenolics, flavonoids, and phytosterols.[14]

Present study results exhibited the elevated levels of β-sitosterol and ferulic acid in summer formulations (NA2) which may be attributed to enhanced solubility and extraction of soluble constituents at higher temperatures .The higher gallic acid and phenolic concentrations observed in winter formulations (NA1), may be qualified due to comparative lower temperature favouring preservation and optimal extraction of thermo-labile compounds. These findings corroborate with the previous studies report on Phyllanthus emblica, indicating the presence of wide range of bioactive compounds including gallic acid, flavonoids, and sterols in fruits, whose quantitative values are sensitive to environmental and harvest conditions. [15]

Similarly, variations in curcumin and piperine content [derived from Haridra rhizome powder] reported in NA formulations have been attributed might be due to difference in pharmaceutical process with varying temperature range, supporting the higher concentrations observed in NA1 (winter) formulations.

The relatively consistent values of quercetin across all NA formulations signifies that it is less environmentally sensitive biomarker and didn't show any variation due to seasonal variation of Amalaki fruit as well as application of differences in processing methods.

Hence, it can be concluded with the comparative qualitative assessment of four formulations of Nishaamalaki-

The seasonal variation of Amalaki and two different methods of bhavana play an important role in modulating phytochemical compositions.

Overall, the findings indicate that winter formulations are enriched in curcumin and certain phenolics, whereas summer formulations support higher levels of sterols and specific phenolic acids.

The outcomes are emphasizing the changes in extraction of bioactive components in four formulations of Nishaamalaki as per seasonal variations of Amakali fruits along with processing methods. Hence, it can be said that the results generated in the study, might be useful to decide rationale selection of Nishaamalaki formulation for attaining specific therapeutic efficacy as per the concentrations of varied biocomponents.

Conflict of Interest

There is no conflict of interest.

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Use of AI in the preparation of the manuscript

The authors declare that no generative AI tools were used in the manuscript.

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