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RESEARCH ARTICLE

Integrated Assessment of Antioxidant Activity and HPTLC Fingerprinting of *Nishaamalaki Churna*: A Multifaceted Approach

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Abstract

Background: *Nishaamalaki churna* is a classical ayurvedic formulation comprising *Haridra* (*Curcuma longa* L.) and *Aamalaki* (*Phyllanthus emblica* L.). It is traditionally prescribed in the management of *Madhumeha* (diabetes mellitus) in the *Charaka Samhita.* **Aim:** This study aimed to scientifically validate *Nishaamalaki Churna* (NC) through analytical standardisation and antioxidant profiling, focusing on quantifying curcuminoids and gallic acid. **Methods:** Organoleptic characteristics and physicochemical parameters were evaluated as per Ayurvedic Pharmacopoeia standards. Quantification of curcuminoids and gallic acid was performed using HPTLC. Antioxidant potential was assessed through multiple in vitro assays, including DPPH, FRAP, ABTS, and Lipid Peroxidation (LPO) inhibition, along with Total Phenolic Content (TPC) estimation. **Results:** NC exhibited a yellowish colour, characteristic odour, and sour taste. Physicochemical parameters were within pharmacopeial limits (pH-3.91, LOD-4.6%, total ash-8.23%, water-soluble extractive-47.62%). HPTLC quantified curcuminoids (10.44 mg/g) and gallic acid (5.295 mg/g). The formulation exhibited strong antioxidant activity, DPPH (IC_{50} 84 µg/mL), FRAP (100.00 mg GAE/g (IC_{50} 41.7 µg/mL), LPO (IC_{50} 290 µg/mL) and TPC (7.63 mg GAE/g). Confirming its potent redox-modulating and therapeutic potential. **Conclusion:** NC contains substantial amounts of bioactive curcuminoids and gallic acid, contributing to its antioxidant, anti-inflammatory, and antidiabetic properties. These findings scientifically substantiate its traditional therapeutic application in managing *Madhumeha* and oxidative stress–mediated disorders.

Major Findings: The study established the analytical standardisation and antioxidant profile of NC, confirming the presence of key bioactive markers, curcuminoids (10.44 mg/g) and gallic acid (5.9 mg/g). The formulation demonstrated potent antioxidant activity across DPPH, FRAP, ABTS, and LPO assays.

Keywords: Anti-oxidant, Curcuminoids, Gallic Acid, HPTLC, Total Polyphenol, *Nishaamalaki churna*

1. Introduction

Diabetes Mellitus (DM) is a major global health concern characterised by chronic hyperglycaemia that leads to progressive microvascular and macrovascular complications affecting multiple organ systems. As of 2024, more than 800 million adults worldwide are living with diabetes, representing a fourfold increase since 1990. Projections suggest that by 2050, this number may reach approximately 853 million, underscoring the urgent need for effective strategies for prevention and management[1,2]. India contributes substantially to this global burden, accounting for an estimated 101 million cases, the second-highest prevalence worldwide. This epidemiological trend highlights the critical necessity for population-specific therapeutic interventions that are effective, safe, and affordable[3]. Among the various formulations prescribed for diabetes in *Ayurveda*, *Nishaamalaki Churna* (NC), a combination of *Haridra* (*C. longa* L.) and *Aamalaki* (*P. emblica* L.) is a widely

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used formulation in the management of *Prameha* (Diabetes mellitus).

Free radicals, also known as Reactive Oxygen Species (ROS), are highly reactive and unstable molecules generated through both endogenous metabolic processes and exogenous environmental exposures. Under physiological conditions, a balance exists between ROS production and antioxidant defences; however, when this equilibrium is disrupted, it leads to oxidative stress that causes oxidative modification of vital biomolecules such as lipids, proteins, and nucleic acids, impairing cellular structure and function. Persistent oxidative damage has been implicated in the pathogenesis of numerous chronic and degenerative diseases, including diabetes mellitus, cancer, cardiovascular disorders and inflammatory diseases, *etc.*[4]. Antioxidants play a crucial protective role by scavenging free radicals, inhibiting their formation, and preventing oxidative chain reactions[5]. In chronic diseases like diabetes, endogenous defences are often insufficient. Therefore, phytochemical antioxidants from natural sources have emerged as a promising strategy for mitigating oxidative stress and preventing disease progression due to their potential to reinforce endogenous defences. Both *Haridra* and *Aamalaki,* the primary components of NC, are rich in natural antioxidants[6,7] that contribute to the reduction of oxidative stress, enhance insulin sensitivity, reduce LPO, and modulate inflammatory pathways leading to improved glycaemic parameters. The combination of these botanicals not only aligns with Ayurvedic principles of synergism (*Yogavahi*) but also represents a scientifically plausible formulation relevant to diabetes management. Despite the long-standing traditional use of NC in managing diabetes mellitus, systematic scientific validation of its antioxidant activity and marker identification of this formulation remain limited. The present study, therefore, aims to evaluate the antioxidant potential of NC and the presence of marker compounds through integrated analytical approaches. This work seeks to bridge the concepts of *Ayurveda* and contemporary scientific validation, thereby reinforcing the evidence base for its use in the management of diabetes mellitus.

2. Materials and Methods

2.1 Raw Drug Procurement and Identification

The raw drugs *Haridra* and *Aamalaki* were procured from Khari Bawli Market, New Delhi. Both ingredients were identified and authenticated at the Regional Raw Drug Repository (RRDR), All India Institute of Ayurveda (AIIA), New Delhi. The authentication numbers assigned were RRDR/AIIA/Phg.132-133.

2.2 Preparation of NC

Nishaamalaki Churna (NC) was prepared in accordance with the standard procedure described in the Ayurvedic Formulary of India[8]. Each raw ingredient was thoroughly washed, shade-dried, and pulverised separately to obtain a fine powder. The powders were then passed through sieve number 80[9], to ensure uniform particle size and subsequently blended in equal proportions to achieve a homogeneous mixture. The botanical sources and plant parts used in the preparation of NC are presented in Table 1.

2.3 Analytical Evaluation

2.3.1 Chemicals and Reagents

All chemicals used in this study were of analytical HPLC grade. Hydrochloric acid, ethanol, methanol, toluene, ethyl acetate, and formic acid were procured from Sigma Aldrich (India). Standard reference compounds, Curcuminoids and Gallic acid were also obtained from the same source. Analytical reagents, including 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, sodium bicarbonate, potassium ferricyanide ($K_3[Fe(CN)_6]$), Trichloroacetic Acid (TCA), ferric chloride ($FeCl_3$), and ABTS [2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] diammonium salt, were procured from SRL India. Potassium phosphate monobasic and dibasic salts were used to prepare a 0.2 M phosphate buffer (pH 6.6). All chemicals and reagents used were handled under controlled laboratory conditions to ensure accuracy and reproducibility.

Table 1. Ingredients of NC with botanical name, part used and amount

S. No.	Contents	Botanical Name	Family	Part used	Quantity
1	*Haridra*	*Curcuma Longa L.*	*Zingiberaceae*	Rhizome	1 part
2	*Aamalaki*	*Phyllanthus emblica L.*	*Phyllanthaceae*	Pericarp	1 part

2.3.2 Physicochemical Analysis

Physicochemical parameters, including pH[10], loss on drying (moisture content)[11], water-soluble extractives, alcohol-soluble extractives, total ash[12] and acid-insoluble ash[13] were determined in accordance with the Ayurvedic Pharmacopoeia of India (API) guidelines. These analyses were conducted to ensure compliance with pharmacopeial quality standards and to characterise the physicochemical profile of *Aamalaki, Haridra* and NC.

2.3.3 Sample Preparation

A 500 mg portion of NC was mixed with 10 mL of methanol (99.8%) in a conical flask and subjected to continuous agitation on a rotary shaker at 250 rpm for 6 h. The mixture was then allowed to stand undisturbed for 18 h, followed by centrifugation at 4000 rpm. The clear supernatant was filtered and subsequently used for HPTLC fingerprinting, free radical scavenging assays, and TPC estimation.

2.3.4 Preparation of Standards (Curcuminoids and Gallic acid)

Standard stock solutions (1 mg/mL) were prepared by dissolving 1 mg each of Curcuminoids and Gallic acid in 1 mL of methanol. The solutions were vortex-mixed and sonicated for 10 and 20 minutes, respectively, followed by filtration. Working standard solutions (100 µg/mL) were prepared by dilution for use in HPTLC analysis. Similarly, ascorbic acid (1 mg/mL) was used as the standard for Radical Scavenging Assays (RSA), and gallic acid (500 µg/mL) served as the standard for TPC, FRAP, and LPO assays.

2.3.5 HPTLC (High Performance Thin Layer Chromatography) Fingerprinting

HPTLC profiling of NC was performed using a CAMAG Linomat V sample applicator. The methanolic extract was applied in 2-10 µL volumes, while Curcuminoids and Gallic acid standards were spotted in the ranges of 1-4 µL and 1-5 µL, respectively. Mobile phase compositions used were Toluene: Glacial acetic acid (8 : 2, v/v) for Curcuminoids and Toluene: Ethyl acetate : Formic acid (5 : 5 : 1, v/v/v) for Gallic acid. Densitometric scanning was carried out using a CAMAG TLC Scanner (230698) at 420 nm for Curcuminoids and 280 nm for Gallic acid. Calibration curves were constructed for both standards, and the quantities present in NC were expressed as mg of marker compound per g of sample (mg/g).

2.3.6 TPC Estimation

The TPC of the NC aqueous extract was estimated using the modified Folin–Ciocalteu colorimetric method[14]. Aliquots of 1.0 mL containing 10, 15, 20, 25, 50, and 100 µg/mL of gallic acid were prepared and mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and 2.0 mL of 7.5% sodium carbonate solution. The mixtures were vortexed for 5 min and incubated at 25°C for 45 min. After that, absorbance was measured at 765 nm using a UV spectrophotometer (Analytik Jena Specord 200 Plus, Germany). For the NC samples, 1.0 mL aliquots of the aqueous extract were prepared and processed in the same manner as the gallic acid standards. Analyses were performed in triplicate and result was expressed as mg gallic acid equivalents per g of dry extract (mg GAE/g) using the equation: $C = C_1 \times V/m$, where C = TPC (mg GAE/g), C_1 = concentration determined from the standard curve (mg/mL), V = volume (mL), and m = mass of dry extract (g).

2.3.7 DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Assay

The free-radical scavenging potential of NC extract and ascorbic acid (standard) was evaluated using the DPPH assay[15] with slight modifications. A 0.5 mM DPPH solution was prepared, and aliquots containing 5-70 µg/mL of ascorbic acid and 2-6 mg/mL of NC extract were mixed with 2 mL DPPH reagent. The mixtures were vortexed for 5 min and incubated at 25°C for 30 min in the dark. Absorbance was measured at 517 nm using a UV spectrophotometer (Analytikajena, Specord 200 plus, Germany) against a blank (methanol + DPPH). The experiment was executed in triplicate. The decolourisation of DPPH from purple to yellow indicated the scavenging potential of the extract. The percentage inhibition of DPPH was computed using the equation: % Inhibition = $A_{DPPH} - A_{Sample} / A_{DPPH} \times 100$, where A_{DPPH} = Absorbance of DPPH, A_{Sample} = Absorbance of sample. Furthermore, the inhibition percentage was plotted against the sample concentration and the IC_{50} (half-maximal inhibitory concentration) value was measured.

2.3.8 Ferric Reducing Power (FRAP-Potassium Ferricyanide Method) Assay

The reducing power of NC was determined using the Potassium Ferricyanide method. Samples or standards (1.0 mL) were mixed with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide, followed by incubation at 50°C for 30 min in triplicate. The reaction was terminated by adding 2.5 mL of 10% TCA, and the mixture was centrifuged. The supernatant (2.5 mL) was then reacted with 0.5 mL of 0.1% ferric chloride, and absorbance was measured at 700 nm. Results were expressed as mg gallic acid equivalents per g of dry extract (mg GAE/g).

Table 2. Organoleptic evaluation of NC

Sample	Parameters			
	Colour	Odour	Texture	Taste
Haridra	Yellow	Characteristic	Powder	Bitter
Aamalaki	Brown	Characteristic	Powder	Sour, Sweet
Nisha Aamalaki	Pale Yellow	Characteristic	Powder	Sour, Bitter

2.3.9 ABTS Radical Scavenging Assay

The ABTS radical cation ($ABTS^{\bullet+}$) was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate (1:1) and allowing the mixture to stand in the dark for 12-16 h. The $ABTS^{\bullet+}$ solution was then diluted with methanol to achieve an absorbance of 0.700 ± 0.02 at 734 nm. To 3.995 mL of this solution, 5 µL of NC extract was added, mixed thoroughly, and incubated for 30 minutes at room temperature. Absorbance was measured at 734 nm, and Trolox was used as the reference standard. All measurements were performed in triplicate, and results were expressed as per cent inhibition of absorbance at 734 nm[16].

2.3.10 LPO Inhibition Activity

The Thiobarbituric Acid (TBA) method was employed to assess the LPO inhibitory activity of NC. Different concentrations of NC extract were mixed with 30% TCA and centrifuged at 8000 rpm for 10 min. The supernatant was reacted with 1.5 mL TBA, and the mixture was heated at 100°C for 10 min. After cooling,

Table 3. Physicochemical evaluation of *Aamalaki, Haridra* and NC

S. No.	Parameters	*Aamalaki*	*Haridra*	NC
1	pH (5% Aqueous solution)	5.16 ± 0.012	5.89 ± 0.015	3.91±0.008
2	Loss on Drying (110°C)	6.84 ± 0.046	7.06 ± 0.053	4.60±0.057
3	Total Ash Value (w/w) %	3.70 ± 0.021	7.36 ± 0.034	8.23±0.014
4	Acid insoluble ash value (w/w) %	1.10 ± 0.008	0.85 ± 0.006	1.93±0.005
5	Water soluble extractive (w/w) %	42.80 ± 0.065	11.08 ± 0.052	47.62±0.061
6	Alcohol soluble extractive (w/w) %	20.00 ± 0.042	24.10 ± 0.038	20.64±0.030

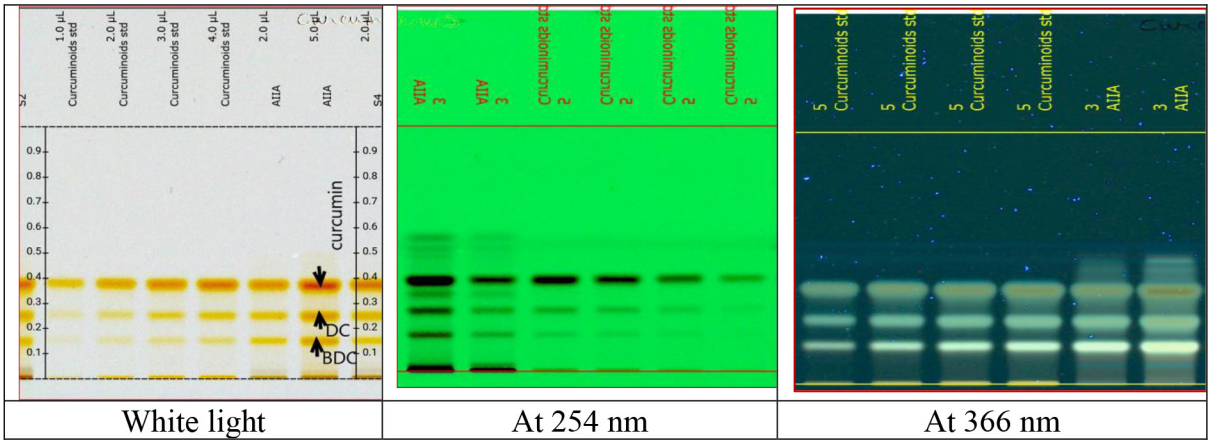


Figure 1. Chromatogram of NC and Curcuminoids under different wavelengths.

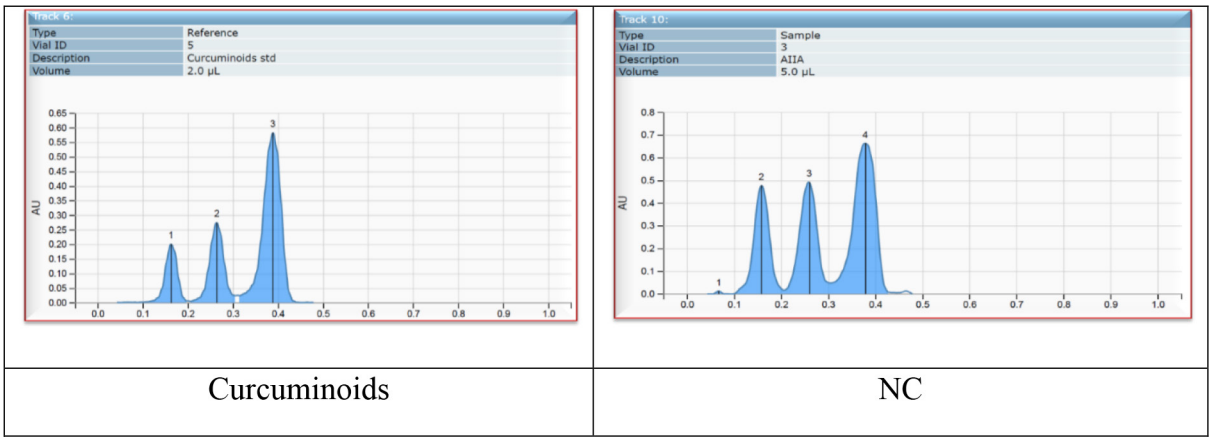


Figure 2. Peak densitogram of Curcuminoids in NC.

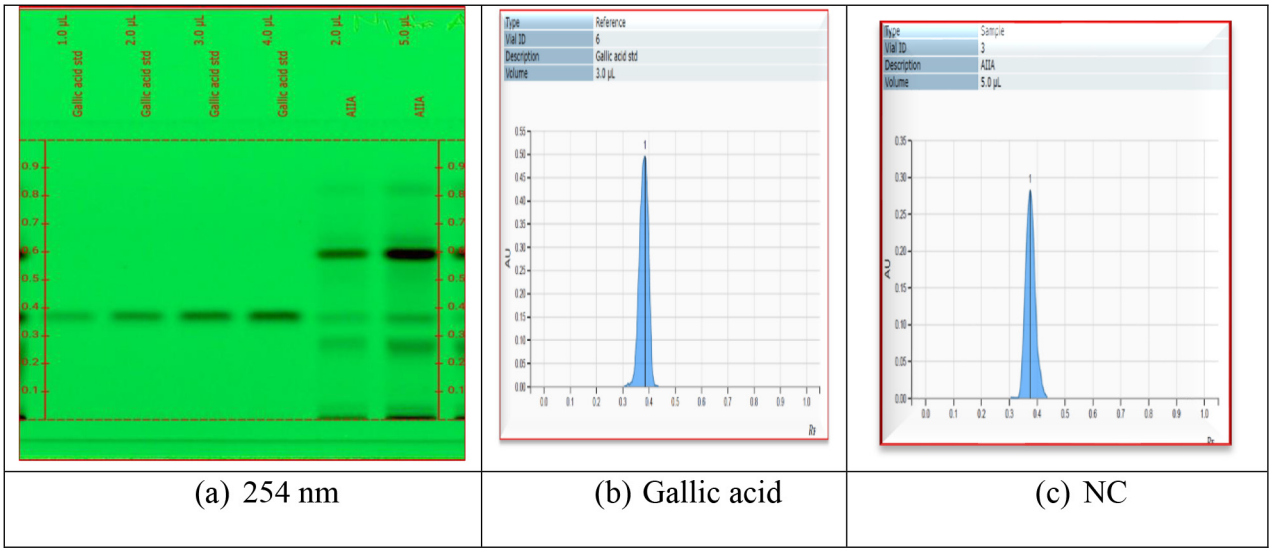


Figure 3. HPTLC chromatogram of (a) NC and gallic acid, (b) peak for standard gallic acid and (c) NC sample.

absorbance was recorded at 532 nm. The results were calculated as per cent inhibition and expressed in terms of IC_{50} value, with ascorbic acid serving as the positive control[17].

3. Result

The Organoleptic and Physicochemical findings are depicted in Tables 2 and 3, respectively. Figure 1 represents the HPTLC fingerprints of NC and curcuminoids, while Figure 2 shows the peak densitogram of curcuminoids in the NC sample. 10.44 mg/g of curcuminoids was detected in the NC sample. HPTLC chromatogram of NC and Gallic Acid peak for standard Gallic acid and NC sample has been depicted in Figure 3. The quantity of gallic acid detected in NC was found to be 5.9 mg/g. In the study, NC has shown a TPC and FRAP value of 7.63 mg GAE/g and 100.00 mg GAE/g respectively. NC exhibited the IC_{50} values of 84 µg/ml, 41.7 µg/mL and 290.00 µg/mL in DPPH, ABTS and LPO assay, respectively.

4. Discussion

Diabetes mellitus is a chronic metabolic disorder that may lead to progressive dysfunction of multiple organ systems. Persistent hyperglycaemia induces micro- and macrovascular complications that substantially increase morbidity and mortality worldwide. India accounts for more than 100 million of these cases,

marking it as one of the epicentres of the global diabetes epidemic[18]. Despite the availability of several modern antidiabetic drugs, long-term glycaemic control remains a challenge, primarily due to drug-related side effects, oxidative stress, and progressive β-cell dysfunction. This scenario underscores the need for safe, cost-effective, and multi-targeted interventions that address both hyperglycaemia and its associated oxidative complications. Free radicals are highly reactive molecules generated as byproducts of normal metabolic processes or through external exposures such as pollution and diet. In diabetes, chronic hyperglycaemia accelerates ROS production via glucose autoxidation, protein glycation, and activation of the polyol and hexosamine pathways. This oxidative overload leads to LPO, DNA damage, and protein oxidation, culminating in β-cell dysfunction, insulin resistance, and vascular complications. The resultant oxidative stress is thus recognised as a pivotal contributor to the onset and progression of diabetes and its secondary pathologies, including neuropathy, nephropathy, and retinopathy[19]. To counter these deleterious effects, the body employs endogenous antioxidant defence systems such as superoxide dismutase, catalase, and glutathione peroxidase[20,] but they become depleted and often found to be insufficient in conditions like DM. Hence, exogenous antioxidants, particularly from plant-derived sources rich in polyphenols and flavonoids, *viz*. NC can emerge as a promising therapeutic strategy. Antioxidants mitigate oxidative damage by neutralising free radicals, thereby interrupting oxidative chain reactions and protecting biomolecules from oxidative damage. In the context of diabetes, they have been shown to improve insulin sensitivity by protecting β-cells from oxidative destruction, enhance endothelial function, thus reducing microvascular and macrovascular complications, lower oxidative inflammation and reduce inflammatory cytokines (TNF-α, IL-6), via inhibition of NF-κB signalling and cytokine release[21]. In the present study, multiple antioxidant assays, *viz*. DPPH, FRAP, ABTS, and LPO comprehensively examine the NC ability to scavenge free radicals, reduce oxidants, and inhibit LPO, thereby elucidating its multifaceted antioxidant mechanism. The first component of NC, *i.e.*, *Haridra,* is rich in curcuminoids, notably curcumin, demethoxycurcumin, and bisdemethoxycurcumin and known for potent anti-inflammatory, antioxidant, and hypoglycaemic properties. Curcumin has been shown to modulate multiple molecular pathways, including AMPK activation, inhibition of TNF-α and IL-6, and downregulation of oxidative enzymes, thereby improving glucose tolerance and reducing insulin resistance[22]. *Aamalaki*, on the other hand, is a rich source of vitamin C, gallic acid, ellagic acid, and tannins, compounds with well-documented free-radical scavenging, β-cell protective, and lipid-lowering effects. Studies have demonstrated *Aamalaki* extracts to enhance hepatic glycogen storage, regulate gluconeogenic enzymes, and improve antioxidant enzyme activity in diabetic models[23]. Thus, NC represents a synergistic polyherbal formulation, wherein the lipophilic curcuminoids and hydrophilic phenolics may confer complementary antioxidant and antidiabetic effects. Previous investigations on NC have reported its organoleptic, qualitative and phytochemical profiles. The present study expands upon these findings by incorporating quantitative estimations of curcuminoids, gallic acid, TPC and a comprehensive assessment of antioxidant activity. The observed congruence in physicochemical parameters, phytochemical constituents, and HPTLC *Rf* values reaffirms the reproducibility and analytical reliability of the formulation, thereby strengthening its standardisation framework. Physicochemical parameters serve as essential benchmarks for quality assurance, reflecting the formulation's purity, stability, and therapeutic consistency. The present study integrates physicochemical, chromatographic, and antioxidant assays to establish a multidimensional analytical profile of NC. Physicochemical parameters such as pH (3.91 ± 0.008), loss on drying (4.60 ± 0.057%), total ash (8.23 ± 0.014%), and acid-insoluble ash (1.93 ± 0.005%) conform to pharmacopeial standards, ensuring purity, stability, and reproducibility. NC exhibited a slightly acidic pH, possibly due to the presence of gallic and phenolic compounds inherent to *Aamalaki* and *Haridra*. The total ash value, a measure of total inorganic content found to be within pharmacopeial limits, indicating minimal extraneous contamination, while acid-insoluble ash confirmed negligible siliceous

impurities, ensuring raw-material purity. The water-soluble extractive value (47.62 ± 0.061%) was substantially higher than the alcohol-soluble extractive (20.64 ± 0.030%), reflecting a dominance of polar bioactive constituents such as glycosides and phenolics. HPTLC fingerprinting effectively identified and quantified curcuminoids and gallic acid, which serve as key phytochemical markers of *Haridra* and *Aamalaki*[24], respectively. In HPTLC analysis, *Rf* values of 0.16, 0.26, and 0.38, corresponding to curcumin, demethoxycurcumin, and bisdemethoxycurcumin, were detected. The total curcuminoid content was quantified at 10.44 mg/g of NC, confirming substantial enrichment with these bioactive polyphenols. Curcuminoids are extensively recognised for their anti-inflammatory, antioxidant, and glucose-modulatory effects, aligning with the therapeutic rationale of NC in diabetes mellitus. In gallic acid quantification, the NC sample exhibited a distinct *Rf* value of 0.38, consistent with the gallic acid standard and revealed a gallic acid content of 5.9 mg/g. Gallic acid exhibits potent antioxidant and antihyperglycemic activities, supporting insulin sensitivity and countering oxidative stress-mediated diabetic complications. The presence of both curcuminoids and gallic acid, in significant and reproducible quantities, reinforces the phytochemical consistency, analytical validity, and therapeutic relevance of NC. These compounds thus serve as robust standardisation markers for quality control and pharmacological validation. Phenolic compounds play a pivotal role in antioxidant defence and metabolic regulation. The TPC analysis demonstrated strong linearity for the gallic acid calibration curve ($R^2 = 0.9987$), confirming methodological precision. The extract exhibited a TPC of 7.63 mg GAE/g, indicating a significant concentration of phenolic metabolites. These phenolics, primarily derived from *Aamalaki* and *Haridra*, contribute to antioxidant, anti-inflammatory, and antidiabetic activities through Reactive Oxygen Species (ROS) neutralisation and oxidative stress attenuation, the key mechanisms in diabetic pathophysiology[25]. The DPPH assay revealed a concentration-dependent increase in scavenging activity ($R^2 = 0.9807$). At concentrations between 2-6 mg/mL, the percentage inhibition detected an IC_{50} of 84 µg/mL, indicating potent antioxidant potential. This activity is possibly attributed to the high content of polyphenolic compounds, particularly curcuminoids and gallic acid, which possess proven radical-neutralising properties. The results substantiate the role of NC in reducing oxidative damage, potentially mitigating diabetic complications through improved redox homeostasis. Oxidative stress is a major etiological factor in the development of diabetic complications. Therefore, the antioxidant efficacy of NC was evaluated through DPPH, FRAP, ABTS, and LPO assays, which collectively assess radical-scavenging ability, reducing power, and LPO inhibition. The FRAP assay demonstrated a highly linear standard curve for gallic acid ($R^2 = 0.9996$), validating methodological robustness. NC exhibited a strong reducing capacity of 100.00 ± 0.01 mg GAE/g, signifying abundant reducing equivalents capable of electron donation to Fe^3 ions, thereby halting free-radical chain propagation. This reducing potential reflects the presence of phenolic antioxidants that stabilise transition-metal-induced oxidative reactions. The ABTS radical scavenging assay confirmed significant radical-trapping capacity, with a dose-dependent increase in inhibition and an IC_{50} value of 41.7 µg/mL. The low IC_{50} value signifies strong antioxidant potency, suggesting that NC contains compounds with strong hydrogen-donating and electron-transfer abilities. These properties are particularly relevant in systemic oxidative stress management. The TBA-based LPO assay demonstrated dose-dependent inhibition of LPO, with an IC_{50} value of 290.0 µg/mL, indicating moderate protection against membrane lipid oxidation, highlighting NC's role in preserving cellular integrity and preventing oxidative damage to lipids, proteins, and DNA. The synergistic combination of *Haridra* and *Aamalaki* contributes to a multifaceted therapeutic profile encompassing antioxidant, anti-inflammatory, and antidiabetic mechanisms. The use of multiple antioxidant assays, each probing distinct reaction mechanisms, provides a comprehensive evaluation of NC's redox-modulating potential. The strong correlation between high phenolic content and robust antioxidant activity supports its antidiabetic potential. Thus, the study substantiates the scientific validation of NC and provides a foundation for future pharmacological and clinical studies aimed at

establishing its role as a safe, standardised, and efficacious formulation for diabetes management.

5. Future Scope and Limitations

Although the study provides robust in vitro and analytical evidence for NC's antioxidant potential but further research is warranted to delineate its molecular mechanisms and in vivo pharmacodynamics. Future studies can be planned to evaluate the action mechanism through gene-expression profiling of antioxidant enzymes (SOD, CAT, GPx) and clinical trials for assessing glycaemic control, oxidative biomarkers, and inflammatory mediators in diabetic subjects. Limitations of the present study include the in vitro design, which does not account for biotransformation or bioavailability of the active constituents in physiological systems. Additionally, regional differences in raw-drug phytochemistry necessitate multi-site validation for establishing universal standards.

6. Conclusion

The findings substantiate that NC possesses significant antioxidant activity, primarily mediated by curcuminoids and gallic acid, validating its use in *Madhumeha* or diabetes mellitus. By mitigating oxidative stress, enhancing redox balance, and supporting β-cell protection, NC emerges as a promising Ayurveda formulation in the management of diabetes mellitus. These combined activities firmly establish the formulation as a powerful natural antioxidant source, justifying its traditional therapeutic uses. The study reinforces the relevance of *Ayurvedic Rasayana* formulations as scientifically validated interventions capable of addressing complex metabolic disorders. Further, study findings highlight its potential integration into contemporary diabetes management frameworks, meriting further translational and clinical exploration.

7. Author Contribution

Anjali Upadhyay: Contributed to the concept, design, literature search, experimental studies, data acquisition, data analysis, and manuscript preparation. Rohit Singh: Contributed to the concept, design, literature search, experimental studies, data acquisition, data analysis, statistical analysis, and manuscript preparation. Swapnil Chaudhari: Contributed to the concept, experimental studies, data acquisition, statistical analysis, manuscript editing, and manuscript review. Pramod Yadav: Contributed to the concept, experimental studies, statistical analysis, manuscript editing, and manuscript review. Galib Rukunuddin**:** Contributed to the definition of intellectual content, experimental studies, data analysis, manuscript editing, and manuscript review. All authors have read and agreed to the published version of the manuscript.

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