RESEARCH PAPER

DEVELOPING A MULTI-HERB CHOORNA AS AN ADJUNCTIVE THERAPY FOR CHRONIC KIDNEY DISEASE: A FOCUS ON PHARMACOGNOSTIC, PHYSICOCHEMICAL, NUTRITIONAL PROPERTIES AND PRE-CLINICAL VALIDATION

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ABSTRACT

Introduction

Chronic Kidney Disease (CKD) is a progressive disorder characterized by inflammation, oxidative stress, metabolic imbalance, and protein–energy wasting. Conventional therapies inadequately address nutritional deficits, long-term safety, and patient adherence, creating a need for adjunctive therapies that integrate nephroprotection with nutritional support.

Methods

A renal-specific multi-herb choorna formulated using ten botanicals was evaluated through pharmacological studies. The formulation underwent comprehensive evaluation, including pharmacognostic, physicochemical characterization, qualitative phytochemical screening, HPTLC fingerprinting, GC–MS profiling, antimicrobial testing, nutritional and safety assessment, sensory evaluation, and preclinical validation in an adenine-induced CKD Wistar rat model.

Results

Pharmacognostic and physicochemical analyses confirmed botanical authenticity, stability and quality compliance. Phytochemical and chromatographic profiling identified flavonoids, phenolics, alkaloids, and bioactive fatty acids with antioxidant and anti-inflammatory activity. The formulation demonstrated broad-spectrum antimicrobial activity, renal-safe electrolyte composition, acceptable protein levels, and negligible heavy metal contamination and favourable acceptability. In-vivo studies showed significant improvement in renal biomarkers, electrolyte balance, and histopathological architecture in the treated groups compared to the disease controls.

Conclusion

This study establishes a scientifically validated, nutritionally compatible multi-herb choorna as a promising adjunctive therapy for CKD, bridging traditional medicine with modern renal nutrition science and supporting its further clinical translation.

Keywords: Chronic Kidney Disease; Polyherbal formulation; Choorna; Nephroprotection; Pharmacognostic standardization; Renal nutrition.

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1. INTRODUCTION

Chronic Kidney Disease (CKD), categorized by a progressive renal function decline, accumulation of metabolic waste, electrolyte imbalance, and systemic complications, including chronic inflammation, oxidative stress, anemia and protein-energy wasting[1]. Closely linked to diabetes, hypertension and cardiovascular disease, its prevalence continues to rise, in low- and middle-income countries, where access to renal replacement therapy remains limited[2]. Conventional management with renin–angiotensin system inhibitors is insufficient to address nutritional deficiencies, chronic inflammation, and oxidative damage central to CKD, often limited by cost, adverse effects, and poor patient adherence and nutritional risk[3].

These limitations have generated interest in adjunctive approaches. Ayurvedic, polyherbal formulations documented to have diuretic, antioxidant, anti-inflammatory, antimicrobial, and nephroprotective properties mediated by flavonoids, phenolic acids, alkaloids, and glycosides that act synergically across multiple CKD pathways[4,5]. Despite their therapeutic potential, they have been hindered by insufficient standardization, safety validation and lacks in accounting for sodium, potassium, phosphorus, and protein restrictions critical for renal safety[6]. Choorna, a traditional dry powdered dosage form, offers prominent gaps such as shelf-stable, precise dosing and incorporation into renal-friendly diet. Realizing this potential, however, requires rigorous pharmacognostic, physicochemical, phytochemical, nutritional, and safety assessments, which are essential[7,8].

The study addresses this critical gap and develops and validates a multi-herb choorna for non-dialysis CKD, through pharmacognostic, chromatographic, physicochemical, antimicrobial, nutritional, sensory evaluation, and toxicological assessment, together with preclinical validation in an adenine-induced CKD rat model, to establish its therapeutic relevance, safety, and dietary compatibility of the formulation as a standardized, evidence-based adjunct to conventional CKD management[9,10,11].

2. METHODOLOGY

The present study was designed to develop and scientifically validate a renal-specific multi-herb choorna intended for use as adjunctive therapy in non-dialysis Chronic Kidney Disease (CKD). All animal procedures were approved by the Institutional Animal Ethics Committee (IAEC) in accordance with CPCSEA guidelines (Registration No. MB/IAEC/25/01/11).

2.1. Formulation Design and Ingredient Selection: Ten botanicals (Table 1) were incorporated to target multiple pathological mechanisms involved in CKD progression, including antioxidant, anti-inflammation, nephroprotective, diuretic activity, and nutritional deficiencies[12].

Table 1. Composition of Multi-Herb Choorna and Rationale for Selection

Common Name	Botanical Name	Part Used	Key Bioactive Constituents	Therapeutic Rationale in CKD
Punarnava	*Boerhavia diffusa*	Root	Punarnavine, flavonoids	Diuretic, anti-fibrotic, reduces inflammation and edema
Woolly Aerva	*Aerva lanata*	Whole plant	Alkaloids, phenolics	Nephroprotective, reduces crystal deposition
Kantakari	*Solanum xanthocarpum*	Whole plant	Steroidal alkaloids	Anti-inflammatory, supports renal clearance
Indian Gooseberry	*Phyllanthus emblica*	Fruit	Vitamin C, tannins	Potent antioxidant, reduces oxidative stress
Turmeric	*Curcuma longa*	Rhizome	Curcumin	Anti-inflammatory, anti-fibrotic
Black Pepper	*Piper nigrum*	Fruit	Piperine	Enhances bioavailability, antioxidant
Long Pepper	*Piper longum*	Fruit	Piperlongumine	Immunomodulatory, improves absorption
Haritaki	*Terminalia chebula*	Fruit	Tannins, chebulinic acid	Antioxidant, detoxifying
Cinnamon	*Cinnamomum verum*	Bark	Cinnamaldehyde	Anti-inflammatory, metabolic support
Bermuda grass	*Cynodon*	Whole	Flavonoids,	Anti-inflammat

	dactylo n	plan t	chloroph yll	ory, renal support

2.2. Processing and Standardization of Raw Materials: All herbal raw materials were manually cleaned, washed with distilled water, blotted dry, then dried in a hot-air oven at 60°C, individually pulverized using a stainless-steel grinder and passed through a 100-mesh sieve. Accurately weighed quantities of each powder ingredient were blended using a sterile manual paddle mixer until a homogeneous mixture.

2.3. Quality and Phytochemical Characterization: Pharmacognostic evaluation: Macroscopic assessment examines the colour, odour, texture, particle size, and overall appearance. Microscopic evaluation was performed using compound light microscopy, which examined the powder for trichomes, fibres, xylem vessels, starch grains, calcium oxalate crystals, and parenchyma cells.

Physicochemical analysis: The evaluated parameters included moisture content, loss on drying, total ash value, acid-insoluble ash, water-soluble ash, pH, and water-extractive values were determined per standard pharmacopeial methods[13].

Phytochemical screening: Standard qualitative tests detected alkaloids, flavonoids, phenolic compounds, glycosides, tannins, saponins, and steroids.

High-Performance Thin-Layer Chromatography (HPTLC) Fingerprint: Methanolic extracts were applied to pre-coated silica gel plates using an automated applicator developed in an optimized solvent system and visualized under UV light at 254 and 366 nm; Rf values, band intensities, and fluorescence patterns were recorded.

Gas chromatography-mass spectrometry (GC-MS): Methanolic extracts obtained by Soxhlet extraction were analyzed under electron-impact ionization conditions. The detected compounds were identified by comparing the mass spectra with established spectral libraries.

2.4. Biological, Nutritional and Sensory Evaluation: Antimicrobial activity: Inhibitory potential against *Escherichia coli, Salmonella* spp., *Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans*, and *Aspergillus niger* was evaluated by agar well diffusion using standardized microbial suspensions and graded extract concentrations, with standard antimicrobial agents and solvent as positive and negative controls; zones of inhibition were measured after incubation.

Nutritional and Safety Profiling: Macronutrients (energy, carbohydrates, protein, fat, and fiber) were quantified using standard AOAC methods. Micronutrients (iron, calcium, zinc, and vitamin B12) were quantified using ICP-OES and immunoassays and electrolytes (sodium, potassium, and phosphorus) by flame photometry and spectrophotometry[14]. Heavy metals (lead, arsenic, mercury, cadmium, and chromium) were measured by atomic absorption spectroscopy. Anti-nutritional factors, such as oxalates and phytates, were quantified.

Sensory evaluation: An expert panel of 10 professional evaluators comprising registered dietitians, nutrition and pharmacy academicians, independently scored appearance, aroma, taste, texture, and overall acceptability on a 7-point rating scale (1 = extremely unacceptable; 7= excellent). Mean scores and standard deviations were calculated.

2.5. Preclinical Validation: Preclinical efficacy was evaluated using an adenine-induced CKD model in male Wistar rats, randomized into six groups (n=4/group): normal control, disease control, standard treatment (enalapril 10mg/kg), synergistic (enalapril+ choorna 100mg/kg), high dose choorna (300 mg/kg), and prophylactic/low dose choorna (100mg/kg). CKD was induced with oral and intraperitoneal adenine over Days 1-30, followed by the treatment phase (Days 31-60). Renal function was assessed via serum creatinine, urea, electrolytes, protein and albumin; urine analysis for proteinuria and creatinine clearance; body and organ weight; and histopathological examination of kidney, liver, heart and spleen for tubular integrity, inflammation and fibrosis.

3. RESULTS AND DISCUSSION

The developed multi-herb choorna meets stringent quality, safety, nutritional, and biological efficacy criteria, supporting its potential as an adjunctive intervention for non-dialysis CKD, as demonstrated across pharmacognostic and physicochemical validation, phytochemical and chromatographic profiling, antimicrobial efficacy, nutritional and safety assessment, sensory acceptability, and preclinical in-vivo evaluation.

3.1. Pharmacognostic Authentication and Physicochemical Stability: Macroscopic and microscopic evaluations confirmed the botanical authenticity and purity of all the constituent herbs: the finished choorna exhibited a uniform fine-powder consistency, characteristic herbal aroma, and homogeneous coloration, with anatomical features (trichomes, lignified xylem vessels, parenchymatous cells, fibres, and calcium-oxalate crystals) consistently present across samples, verified raw material integrity and compliance with pharmacognostic standards. Physicochemical characterization demonstrated that the moisture content 5.96%, total ash 7.0%, acid-insoluble ash 2.0%, pH 6.9, loss on drying 20%, and extractive valve 1.5%, all within pharmacopeial limits, indicating low microbial growth risk, minimal inorganic and siliceous contamination. The gastrointestinal compatibility relevant to CKD patients with gastric discomfort, and adequate solubility of bioactive constituents. Collectively,

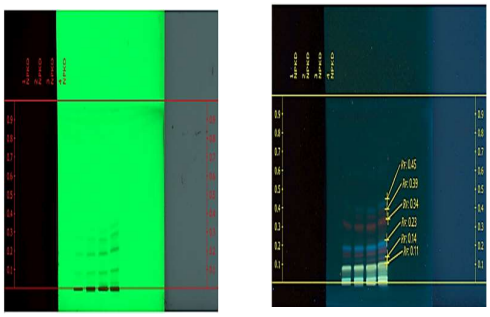
these results confirm that choorna is physicochemically stable, reproducible, and suitable for long-term storage and dietary use.

3.2. Phytochemical Composition and Chromatographic Profiling: Qualitative Screening: Phytochemical screening revealed that flavonoids and phenolic in high abundance, whereas alkaloids and glycosides are at moderate levels, tannins and saponins are at low levels, whereas steroids were absent. This distribution is particularly relevant to CKD: flavonoids and phenolics are strongly associated with antioxidant, anti-inflammatory, and vasoprotective effects, which counter oxidative stress and chronic inflammation, both central drivers of CKD progression[8], while alkaloids and glycosides contribute to nephroprotection and metabolic regulation. The balanced presence of multiple phytochemical classes also suggests synergistic interactions characteristic of polyherbal formulation.

High-Performance Thin-Layer Chromatography (HPTLC) Fingerprint: HPTLC analysis produced a distinct, reproducible chromatographic fingerprint: under UV at 254 nm, multiple well-resolved bands with consistent Rf values indicated the presence of alkaloids and other UV-absorbing compounds. At 366 nm, prominent fluorescent bands corresponding to flavonoids and phenolic compounds were evident, further confirming the phytochemical richness identified qualitatively (Figure 1). The consistency of the band patterns, Rf values, and intensities across replicate analyses demonstrated excellent batch-to-batch reproducibility. This chromatographic fingerprint serves as a robust quality control marker, ensuring formulation authenticity and standardization, which are essential for regulatory approval and future clinical translation.

Figure 1: HPTLC profile of choorna under UV light at 254 nm and 366 nm

Remission 254nm Remission 366nm



GC–MS Analysis: GC–MS profiling identified fifteen volatile and semi-volatile compounds (Figure 2), dominated by long-chain saturated fatty acids, such as palmitic, stearic, myristic, arachidic acids, heptadecanoic, and nonadecanoic acids, along with antioxidant-associated esters, such as ascorbic acid 2,6-dihexadecanoate, with palmitic acid being the dominant. whereas several minor constituents contributed to the overall bioactivity profile. The presence of these compounds is reported to have anti-inflammatory, antioxidant, lipid-modulating and membrane-protective, supporting a role for the formulation in reducing oxidative damage and maintaining cellular integrity in renal tissues. The GC–MS data complemented the HPTLC findings, providing molecular-level confirmation of phytochemical diversity and reinforcing the standardization of the formulation. The GC–MS chromatogram illustrating the peak distribution and compound abundance is presented in Figure 2.

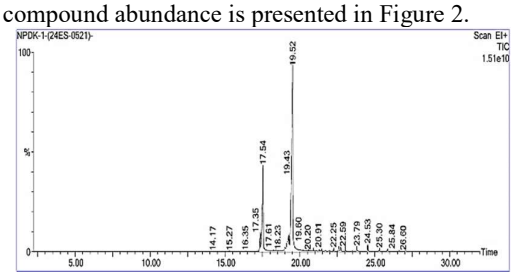


Figure 2. GC-MS chromatogram showing peak distribution and compound abundance

3.3. Antimicrobial Activity: The multi-herb choorna exhibited broad-spectrum, concentration-dependent antimicrobial activity by agar well diffusion, with a significant dose-response relationship ($p<0.05$), and zones of inhibition highest against *Pseudomonas aeruginosa* (21±1.5mm) and *Escherichia coli* (19±1.2mm), followed by *Salmonella* spp. (19±1.1mm), and *Candida albicans* (20±1.3mm), a spanning organism relevant to urinary tract, nosocomial, systemic, and opportunistic infections. At higher extract concentrations, inhibition zones approached those of standard antimicrobial agents. The antimicrobial activity is attributable to phenolic, flavonoids, alkaloids, and fatty acids constituents identified above, is particularly relevant for CKD patients, who face elevated infection risk due to immune dysregulation, adding a valuable secondary benefit to the formulation.

3.4. Nutritional Profile and Safety Evaluation: Nutritional analysis per 10 g serving of choorna (table 2) provided 27.33 Kcal, 2.01 g of carbohydrate, 4.60 g of protein, 0.10 g of fat, and 0.38 g of fibre. This macronutrient profile is consistent with the protein-restriction guidelines for non-dialysis CKD, which will minimize the nitrogenous waste burden. Electrolytes, which are key for renal safety, are within limits: sodium, potassium, and phosphorus each below 2% of the Recommended Dietary Allowance (RDA) per serving, indicating that the formulation does not worsen hyperkalemia, hyperphosphatemia, or sodium overload. Particularly, iron and zinc reached 169% and 133% of RDA per serving, respectively,

with calcium and vitamin B12 also nutritionally meaningful, which addresses the common deficiency in CKD that contributes to anemia, bone disorder, and immune dysfunction. Heavy metals were below the detectable or within WHO permissible limits, and anti-nutrient factors were present at minimal risk of mineral chelation or nephrolithiasis. Collectively, these findings confirm that choorna is both nutritionally supportive and safe for the CKD dietary pattern.

Table 2. Nutritional and Safety Profile (Per 10 g Serving)

Macronutrients	**Per 10g (1 Serving)**	**RDA (Per Day)**	**% RDA per serving**
Energy (Kcal)	27.33	2000 Kcal (adult avg.)	1.37%
Carbohydrates (g)	2.01	130 g	1.55 %
Protein (g)	4.60	46 g (F), 56 g (M)	10.0% (F), 8.21% (M)
Fat (g)	0.10	70 g	0.14%
Fiber (g)	0.38	25 g (F), 38 g (M)	1.52% (F), 1.00% (M)
Vitamins	**Per 10g (1 Serving)**	**RDA (Per Day)**	**% RDA per serving**
Vitamin A (IU)	8.43	3000 IU	0.28%
Vitamin D	Traces	600 IU	Negligible
Vitamin E (IU)	0.41	15 IU	2.73%
Vitamin K	Nil	120 mcg	0%
Vitamin C (mg)	8.95	90 mg	9.94%
Vitamin B1 (mg)	0.93	1.2 mg	77.5%
Vitamin B2 (mg)	0.088	1.3 mg	6.77%
Vitamin B3 (mg)	0.047	16 mg	0.29%
Vitamin B6 (mg)	0.176	1.3 mg	13.54%
Vitamin B12 (mcg)	1.53	2.4 mcg	63.75%
Minerals	**Per 10g (1 Serving)**	**RDA (Per Day)**	**% RDA per serving**
Sodium (mg)	42.77	2300 mg	1.86 %
Potassium (mg)	14.57	4700 mg	0.31 %
Phosphorus (mg)	5.43	700 mg	0.77 %
Calcium (mg)	122.16	1000 mg	12.2 %
Phosphate (mg)	10.36	~700 mg	1.48 %
Iodine (mg)	0.24	0.15 mg	162.6%
Iron (mg)	30.43	18 mg	169 %
Zinc (mg)	14.64	11 mg	133.1 %
Heavy Metals	**Per 10g (1 Serving)**	**Permissible Limit**	**Comparison**
Mercury,	Below detectable level	<0.5 ppm	Within permissible limits.
Cadmium		<0.2 ppm	
Lead	<0.2 ppm	<10 ppm	
Arsenic	<0.1 ppm	<1 ppm	
Chromium	Below detectable level	<2 ppm	
Antinutrients	**Per 10g (1 Serving)**	**Permissible Limit**	**Comparison**
Oxalates / Phytates (mg)	0.535 / 0.216	<250 / 100 ppm	Low levels; safe for consumption.

3.5. Sensory Evaluation and Acceptability: An expert panel of 10 trained pharmacy and nutritional professionals using a 7-point rating scale yielded mean scores of 4.7 for taste, 5.1 for aroma, 5.3 for texture, 5.3 for colour, 5.2 for overall acceptability – all at or above the mid-scale threshold with low inter-evaluator variability (SD<0.6). This favourable profile supports the feasibility of long-term dietary incorporation – critical in CKD, where palatability directly determines adherence.

3.6. Preclinical In Vivo Evaluation in CKD Model: Biochemical Parameters: Adenine-induced CKD Wistar rat model, disease control showed significant elevations in serum creatinine, blood urea nitrogen (BUN), and potassium levels

compared to normal controls, confirming successful CKD induction. Choorna, treatment groups demonstrated statistically significant reductions in serum creatinine and urea ($p < 0.05$), corroborated by reduced proteinuria and improved creatinine clearance, collectively indicating attenuation of glomerular and tubular dysfunction.

Body weight, organ weight and gross pathology: Final body weight was highest in normal controls (249.4±77.6 g), and lowest in disease control (175.0±51.2 g), with treated groups (178.6–203.3 g) showing partial recovery; one mortality occurred in the synergic group. Kidney weights, elevated in the disease controls (2.82±0.63 g vs. 2.07±0.14 g in normal controls) due to hypertrophy, were reduced in standard (2.34±0.44 g), high-dose (2.54±0.31 g), and prophylactic (2.49±0.12 g) groups. Gross pathology corroborated biochemical findings, with disease controls displaying chromodacryorrhea, conjunctival redness, and swollen kidneys with salty deposits, while treated groups exhibited progressively milder renal lesions and unremarkable liver, heart, and spleen.

Histopathology: Kidneys sections from the disease control showed severe tubular necrosis, dilation, inflammatory infiltration and fibrosis; treated groups demonstrated a marked reduction in tubular degeneration, inflammatory infiltration, and interstitial fibrosis, most pronounced in high-dose and prophylactic groups (Figure 3). Semi-quantitative lesions revealed a statistically significant improvement versus disease control ($p < 0.05$), supporting dose-dependent and prophylactic nephroprotection. The other organs (spleen, heart and liver) showed no significant pathological alterations across groups (Figure 4), indicating organ-selective protection without systematic toxicity.

The findings revealed the choorna as a nephroprotection, nutritionally compatible, and systematically validated formulation. Limited inherent to pre-clinical models, including interspecies variability, controlled disease induction, and absence of pharmacokinetic data- necessitate controlled clinical trials to confirm efficacy, optimize dosing and evaluate interactions with standard CKD pharmacotherapy.

Figure 3. Representative histopathological sections of kidney tissue from adenine-induced CKD rats (H&E staining, 20× magnification). (A) Normal control, showing intact glomeruli and normal tubular architecture. (B) Disease control group showing severe tubular necrosis, tubular dilatation, inflammatory cell infiltration, and interstitial fibrosis. (C) Standard treatment group showing partial restoration of tubular morphology. (D–F) The treated groups showed a marked reduction in tubular degeneration, inflammatory infiltration, and fibrotic changes, with preservation of the renal architecture.

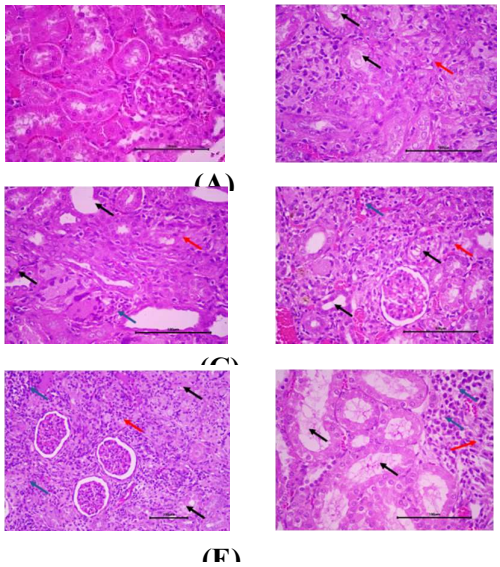
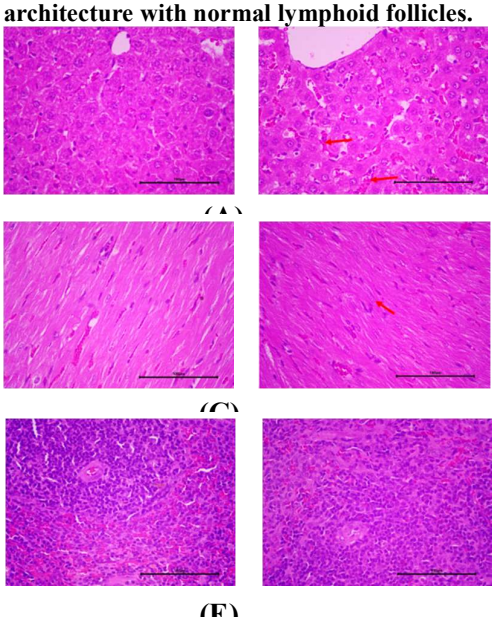


Figure 4. Representative histopathological sections of non-renal organs from adenine-induced CKD rats (H&E staining, 20× magnification). (A–B) Liver sections showing preserved hepatic architecture with normal hepatocyte arrangement in the control and treated groups. (C–D) Heart sections exhibiting normal myocardial architecture without evidence of degeneration or inflammation. (E–F) Spleen sections showing an intact splenic architecture with normal lymphoid follicles.



4. CONCLUSION

The multi-herb choorna demonstrated pharmacognostic authenticity, physicochemical stability, phytochemical richness, renal-safe nutritional compatibility, broad-spectrum antimicrobial activity, and significant nephroprotection in an adenine-induced CKD

model, without systemic toxicity. Its favourable sensory acceptability further supports translational feasibility. Clinical trials are warranted to confirm efficacy and optimize dosing in human CKD populations.

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