

Formulation, Evaluation and Antiurolithiatic Activity of Polyherbal Granules Containing *Boerhavia diffusa*, *Celosia argentea* and *Plumeria rubra*

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Abstract: The study was undertaken to develop and evaluate polyherbal granules containing *Boerhavia diffusa*, *Celosia argentea* and *Plumeria rubra* and to investigate their antiurolithiatic activity in an ethylene glycol-induced Wistar rat model. The optimized herbal ratio was 4:1:1 (*B. diffusa*:*C. argentea*:*P. rubra*). Granules were prepared by wet granulation using a decoction of *B. diffusa* and powdered *C. argentea* seeds and *P. rubra* seed pods. The formulation showed acceptable organoleptic and physicochemical characteristics, including pH 7.8, loss on drying 3.2%, bulk density 0.41 g/mL, tapped density 0.44 g/mL and angle of repose 33.37°. HPTLC fingerprinting demonstrated the presence of rutin, gallic acid, quercetin and luteolin in the formulation. In vivo, ethylene glycol produced changes in urinary calcium, oxalate, phosphorus and magnesium and increased serum creatinine and uric acid. Treatment with the polyherbal preparation at 200 and 400 mg/kg was associated with improvement in these parameters. Histopathological observations also documented calcium oxalate-like material in renal tubules, with less crystal material reported in the 400 mg/kg group. The findings support further controlled investigation of the standardized polyherbal preparation.

Keywords: Polyherbal granules; antiurolithiatic activity; ethylene glycol; Wistar rats; HPTLC; *Boerhavia diffusa*; *Celosia argentea*; *Plumeria rubra*.

I. INTRODUCTION

The thesis work was designed to develop a polyherbal preparation for kidney-stone management and to evaluate its physicochemical characteristics, phytochemical markers and antiurolithiatic activity. Initial in-vitro optimization identified the 4:1:1 combination of *B. diffusa*, *C. argentea* and *P. rubra* as the combination carried forward for in-vivo study.

A wet-granulated dosage form was selected to provide a standardized solid herbal preparation. Evaluation included organoleptic characteristics, pH, moisture content, ash values, extractive values, particle size, density and angle of repose. HPTLC fingerprinting was used to assess selected bioactive markers, while an ethylene glycol model was used to evaluate antiurolithiatic activity.

II. MATERIALS AND METHODS

2.1 Formulation development

The formulation contained *B. diffusa* whole-plant powder (16 g), *C. argentea* seed powder (4 g) and *P. rubra* seed-pod powder (4 g). A decoction of *B. diffusa* was prepared by boiling the powdered material in 100 mL water and reducing the volume to one-third, followed by sieving and evaporation. Fine powders of *C. argentea* and *P. rubra* were passed



through sieve no. 22 and incorporated into the cooled semisolid decoction with excipients. Polyherbal granules were prepared by wet granulation.

2.2 Physicochemical evaluation

Parameter	Observed value
Appearance	Granular
Colour	Dark greenish brown
Odour	Slightly bitter
Taste	Mild bitter/characteristic
pH	7.8
Loss on drying	3.2%
Bulk density	0.41 g/mL
Tap density	0.44 g/mL
Hausner ratio	1.07
Total ash	6.254%
Water-soluble extractive	12.26%
Alcohol-soluble extractive	3.36%
Angle of repose	33.37°

2.3 HPTLC fingerprinting

HPTLC was carried out using the optimized solvent system toluene:ethyl acetate:methanol:formic acid (6:4:0.5:0.5). The thesis reports application of gallic acid, rutin, quercetin, ursolic acid and luteolin standards with methanolic extract and formulation samples. Marker compounds identified in the polyherbal granule formulation were rutin, gallic acid, quercetin and luteolin.

Marker	Rf value	Amount present (as reported)
Rutin	0.04	31.92
Gallic acid	0.33	7.41
Quercetin	0.48	10.70
Luteolin	0.48	10.70

2.4 In-vivo antiurolithiatic study

The animal study used adult male Wistar albino rats weighing 150–250 g. The thesis reports CPCSEA/IAEC approval no. 1401/PO/RE/S/11/IAEC/2020-21/07/02. Following acclimatization, animals were divided into five groups of six. Urolithiasis was induced using ethylene glycol (0.75% v/v) in drinking water for 28 days. The standard group received Cystone 750 mg/kg from days 15–28. Test groups received the effective polyherbal extract at 200 or 400 mg/kg from days 1–28.

Group	Treatment	n
I	Regular diet and water	6
II	Ethylene glycol 0.75% v/v for 28 days	6
III	Ethylene glycol + Cystone 750 mg/kg (days 15–28)	6
IV	Ethylene glycol + effective extract 200 mg/kg (days 1–28)	6
V	Ethylene glycol + effective extract 400 mg/kg (days 1–28)	6



On day 28, urine was collected in metabolic cages and evaluated for volume, pH, calcium, oxalate, phosphorus and magnesium. Serum parameters included creatinine and uric acid. Histopathology was performed on formalin-fixed kidney tissue with hematoxylin and eosin staining. Statistical analysis in the thesis used one-way ANOVA followed by Student unpaired t-test with Dunnett correction, with results expressed as mean $\pm$ SEM and n=6.

III. RESULTS AND DISCUSSION

3.1 Physicochemical characteristics

The granules exhibited a granular appearance and dark greenish-brown colour, with slightly bitter odour and mild bitter characteristic taste. The measured pH was 7.8 and loss on drying was 3.2%. Bulk and tapped densities were 0.41 and 0.44 g/mL, respectively. The angle of repose was 33.37°, which the thesis interpreted as indicating good flow. Total ash, water-soluble extractive and alcohol-soluble extractive values were 6.254%, 12.26% and 3.36%, respectively.

3.2 HPTLC standardization

The HPTLC fingerprint supported the presence of selected marker compounds in the formulation. Rutin, gallic acid, quercetin and luteolin were reported at R_f values of 0.04, 0.33, 0.48 and 0.48, respectively. The combination extract additionally showed ursolic acid at R_f 0.65. These chromatographic fingerprints can serve as a basis for identity and batch-to-batch standardization, although additional validation would be required for quantitative quality control.

3.3 Urinary parameters

Parameter	Normal (I)	Disease control (II)	Standard (III)	PHF 200 mg/kg (IV)	PHF 400 mg/kg (V)
Urine volume	2.57 $\pm$ 0.11	1.60 $\pm$ 0.23	5.40 $\pm$ 0.14	3.35 $\pm$ 0.54	5.19 $\pm$ 0.32
pH	7.25 $\pm$ 0.04	5.70 $\pm$ 0.13	7.29 $\pm$ 0.45	6.30 $\pm$ 0.24	7.24 $\pm$ 0.04
Calcium	9.54 $\pm$ 0.10	13.56 $\pm$ 0.08	10.24 $\pm$ 0.11	12.81 $\pm$ 0.15	10.36 $\pm$ 0.16
Oxalate	1.86 $\pm$ 0.07	4.39 $\pm$ 0.17	2.11 $\pm$ 0.07	3.80 $\pm$ 0.12	2.55 $\pm$ 0.10
Phosphorus	5.15 $\pm$ 0.20	7.13 $\pm$ 0.17	5.18 $\pm$ 0.14	6.33 $\pm$ 0.14	5.28 $\pm$ 0.11
Magnesium	4.16 $\pm$ 0.08	1.73 $\pm$ 0.12	3.33 $\pm$ 0.11	2.11 $\pm$ 0.09	3.18 $\pm$ 0.07

Values are mean $\pm$ SEM, n=6. The thesis reports that disease-control animals showed decreased urine volume and pH and increased urinary calcium, oxalate and phosphorus with reduced magnesium compared with normal animals. Treatment groups showed movement of these parameters toward the normal pattern. The 400 mg/kg group showed urinary calcium, oxalate and phosphorus values closer to the normal group than the 200 mg/kg group, while magnesium was also higher than in the 200 mg/kg group.

3.4 Serum parameters

Parameter	Normal (I)	Disease control (II)	Standard (III)	PHF 200 mg/kg (IV)	PHF 400 mg/kg (V)
Creatinine	2.65 $\pm$ 0.12	3.33 $\pm$ 0.08	2.43 $\pm$ 0.11	2.91 $\pm$ 0.07	2.63 $\pm$ 0.11
Uric acid	2.70 $\pm$ 0.05	4.43 $\pm$ 0.09	2.95 $\pm$ 0.07	3.98 $\pm$ 0.09	3.21 $\pm$ 0.08

The disease-control group showed higher serum creatinine and uric acid than the normal group. The thesis reports significant reductions in these parameters following treatment, with the 400 mg/kg group approaching the normal values more closely than the 200 mg/kg group for the reported measures.

3.5 Histopathology

Histological examination described renal cortex and medulla with dilated tubules containing polyhedral/rhomboid eosinophilic material suggestive of calcium oxalate crystals. In the 400 mg/kg treatment group, the thesis described



very little such material and fewer congested glomeruli, while some chronic inflammatory infiltrate and focal hemorrhage were still reported. These observations are consistent with the biochemical findings but should be interpreted as descriptive histopathological observations.

IV. CONCLUSION

The optimized polyherbal preparation containing *B. diffusa*, *C. argentea* and *P. rubra* was successfully prepared as granules and exhibited measurable physicochemical and chromatographic characteristics. In the ethylene glycol-induced Wistar rat model, the preparation at 200 and 400 mg/kg was associated with improvement in urinary stone-forming/inhibitory parameters and serum creatinine and uric acid. The 400 mg/kg group showed values generally closer to the normal group in the reported dataset. The formulation therefore merits further standardized pharmacological, toxicological and mechanistic evaluation before any clinical application is considered.

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