

RESEARCH ARTICLE



Evaluation of Natriuretic, Kaliuretic, and Chloruretic Responses of Hydroalcoholic Leaf Extract of *Bacopa monnieri* (L.) Wettst. in Wistar Albino Rats

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Abstract: Renal fluid and electrolyte management is vital for mitigating volume overload in systemic hypertension, congestive heart failure, and nephrotic conditions, yet conventional synthetic therapeutics frequently precipitate severe metabolic derangements and potassium wasting. Botanical secondary metabolites represent chemically diverse templates capable of modulating tubular transport with favorable safety profiles. The present investigation quantitatively establishes the acute saluretic, natriuretic, and diuretic profile of a standardized hydroalcoholic leaf extract of *Bacopa monnieri* (L.) Wettst. in normotensive male and female Wistar albino rats employing the classic Lipschitz bioassay. Qualitative phytochemical screening revealed abundant specialized secondary metabolites, including saponins, flavonoids, phenolic fractions, tannins, alkaloids, and phytosterols, with confirmed presence of terpenoid and glycosidic nuclei corresponding to dammarane saponin architecture. Oral administration of the hydroalcoholic extract across graded dose cohorts (10, 50, and 100 mg/kg body weight) following standardized physiological saline pre-loading elicited statistically significant, dose-dependent increments in cumulative five-hour urinary excretion relative to vehicle controls. Test cohorts receiving the maximal botanical dose demonstrated an elevated urine output index accompanied by marked renal clearances of sodium, potassium, and chloride, approaching 92% of the ceiling natriuretic efficacy induced by loop diuretic standard furosemide (10 mg/kg). Notably, urinary sodium-to-potassium ratios across extract cohorts remained stable near physiological parity, avoiding excessive kaliuretic divergence. Histomorphological evaluation of renal cortical sections demonstrated intact tubular and glomerular integrity without cytopathic signs of parenchymal necrosis. These results confirm that hydroalcoholic constituents of *Bacopa monnieri* exert potent saluretic action through tubular transport modulation.

Keywords: *Bacopa monnieri*; Diuretic index; Lipschitz bioassay; Natriuresis; Saluretic activity.

1. Introduction

Regulation of extracellular fluid volume and solute homeostasis by the mammalian kidney is an essential physiological process governing mean arterial blood pressure, cardiac preload, and systemic tissue perfusion [1]. Pathophysiological expansion of plasma and interstitial fluid compartments is a primary clinical feature across cardiovascular and renal diseases, notably essential hypertension, congestive heart failure, hepatic cirrhosis with ascites, and nephrotic syndrome [2]. Diuretic pharmacotherapy serves as a first-line clinical strategy designed to reduce extracellular fluid hypervolemia through the inhibition of renal tubular solute transport, principally promoting the simultaneous urinary excretion of sodium ions and water [3]. Synthetic diuretic classes such as high-ceiling loop agents (e.g., furosemide, bumetanide) and thiazide-type sulfonamides specifically disrupt luminal ion co-transporters along the thick ascending limb of Henle's loop and the distal convoluted tubule, respectively [3, 4].

Despite their high pharmacodynamic efficacy, chronic administration of modern synthetic diuretics is frequently limited by clinically significant adverse effects [4]. Foremost among these liabilities is excessive kaliuresis, wherein augmented distal tubular delivery of sodium and elevated luminal fluid flow stimulate potassium and hydrogen ion secretion, resulting in symptomatic hypokalemia, cardiac arrhythmias, and metabolic alkalosis [3, 5]. Loop and thiazide agents frequently induce hyperuricemia through competitive transport mechanisms along the organic anion transporter 1 and 3 (OAT1/OAT3) pathways in the proximal tubule, exacerbating gouty arthritis [4]. These agents can also induce neurohormonal counter-activation of the renin-angiotensin-aldosterone axis, ototoxicity, and prerenal azotemia [5]. Secondary to systemic hemodynamic and fluid-volume considerations, disorders of the urinary tract, particularly nephrolithiasis and urolithiasis, constitute widespread pathological burdens worldwide [6]. The promotion of sustained, high-volume urinary flow through pharmacological diuresis reduces supersaturation of sparingly soluble salts like calcium oxalate and uric acid, providing critical prophylaxis against crystal nucleation, aggregation, and retention within renal papillae

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Due to the adverse event profile, tolerance liabilities, and metabolic disturbances associated with conventional synthetic sulfonamide derivatives, extensive pharmacological exploration has targeted natural plant extracts as safe, multi-mechanistic alternatives possessing balanced saluretic and natriuretic properties [4, 7].

Bacopa monnieri (L.) Wettst. (commonly referred to in Indian traditional medicine as *Brahmi*, family Plantaginaceae, historically categorized under Scrophulariaceae) is a small, creeping, perennial succulent herb indigenous to wet, marshy, and damp wetland ecosystems throughout tropical and subtropical regions across the globe [8]. The species possesses an extensive history of documented medicinal utilization within ancient Ayurvedic medical literature, being classified as a premier *Medhya Rasayana*—an herbal rejuvenator primarily administered to alleviate mental fatigue, enhance intellectual acuity, and manage nervous system disorders [1, 8].



Figure 1. Leaves and Flowers of *Bacopa monnieri*

Phytochemical investigations over several decades have established that the secondary metabolome of *Bacopa monnieri* is characterized by complex dammarane-type triterpenoid saponins, designated collectively as bacosides [8, 9]. Structurally, these bioactive principles consist of aglycone pseudojубogenin or jубogenin triterpene skeletons covalently bonded through acetal linkages to carbohydrate moieties such as D-glucopyranose, L-arabinopyranose, and L-arabinofuranose [9]. Major individual congeners include bacoside A3, bacoside I, bacoside II, and bacosaponin C [8, 10]. In addition to these triterpenoid saponin glycosides, the crude aerial leaves synthesize diverse auxiliary phytochemical classes, including monomeric flavonoids (e.g., apigenin, luteolin), phenylethanoid glycosides, polyhydroxy phenolic acids, condensed tannins, free phytosterols (stigmasterol, β -sitosterol), and minor alkaloidal fractions including brahmine and herpestine [8, 11].

Pharmacological studies on crude extract preparations and isolated saponin fractions of *Bacopa monnieri* have primarily investigated central nervous system models, confirming prominent nootropic, neuroprotective, anxiolytic, and antidepressant activities [9, 10]. These activities are mediated largely through the modulation of central cholinergic transmission, upregulation of monoamine neurotransmitters, and transcriptional attenuation of oxidative stress cascades [10, 11]. Peripheral bioactivities have demonstrated substantial systemic free-radical scavenging capacity, membrane-stabilizing efficacy, hepatoprotective activity against xenobiotic insults, and anti-hyperlipidemic properties [8, 12, 13]. Within the renal physiological axis, *Bacopa monnieri* extract suppresses oxidative, nitrosative, and peroxidative damage in the renal cortices of diabetic rodent models, upregulating key endogenous enzymatic defenses including superoxide dismutase, catalase, and glutathione peroxidase [12].

Despite detailed neuropharmacological and cytoprotective characterizations of *Bacopa monnieri*, rigorous experimental investigations into its acute renal hemodynamics, tubular transport alterations, and physiological diuretic actions remain under reported in the scientific literature. Herbal remedies displaying high saponin, flavonoid, and phenolic titers frequently exert profound actions on renal microvasculature and tubular epithelial transport [4, 14]. Certain bioflavonoids inhibit vascular adenosine deaminase, augment renal prostacyclin synthesis, and antagonize adenosine A_1 receptors in the proximal nephron, simultaneously augmenting cortical blood flow and promoting solute clearance [14, 15]. Dammarane and oleanane saponin glycosides selectively alter the permeability of tubular epithelial membranes, modifying passive and active ion fluxes in the renal medullary architecture [4].

Given the therapeutic need for saluretic interventions that mitigate potassium wasting, evaluating the diuretic properties of *Bacopa monnieri* holds significant pharmacological promise. This study quantitatively characterizes the preliminary phytochemical composition and acute dose-dependent diuretic, natriuretic, kaliuretic, and chloruretic responses to an orally administered hydroalcoholic leaf extract of *Bacopa monnieri* (HAEBM) in normotensive Wistar albino rats, using the validated Lipschitz bioassay framework with furosemide as an active reference standard.

2. Materials and Methods

2.1. Collection of Plant Material

Fresh, disease-free whole leaves of mature *Bacopa monnieri* (L.) Wettst. were harvested during early morning hours from natural wetland habitats across the Tirupati geographic zone of Andhra Pradesh, India. Taxonomic identification, botanical authentication, and nomenclatural verification were formally performed by Dr. K. Madhava Chetty, Assistant Professor in the Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh. A voucher herbarium specimen was assigned accession cataloging and deposited in the departmental herbarium repository at the Department of Pharmacology, Malla Reddy College of Pharmacy, Secunderabad, Telangana, India, for retrospective taxonomy and archival verification.

Harvested botanical material was systematically sorted to eliminate extraneous biomass, washed thoroughly under continuous running tap water to remove superficial debris, soil particulate matter, and epiphytic contaminants, and subsequently rinsed with sterile deionized water. The cleaned foliage was uniformly spread over sterile absorbent surfaces and subjected to continuous air-drying in a darkened room under active dehumidification and controlled room temperature (22–25 °C) for a duration of three weeks to avoid solar UV-mediated degradation of thermolabile constituents. Following complete drying, the leaf material was pulverized into a coarse, uniform powder using an industrial stainless steel mechanical mill and stored in air-tight, light-resistant glass containers inside a vacuum desiccator.

2.2. Extraction and Determination of Yield

The coarsely pulverized leaf matrix (500 g) was packed into an analytical-grade cellulose extraction thimble and mounted inside a classic continuous hot Soxhlet solvent extraction assembly. Continuous solid-liquid exhaustive extraction was conducted using an analytical hydroalcoholic solvent system consisting of ethanol and deionized water in a 70:30 volume-to-volume ratio (70% v/v). Hot solvent percolation was sustained across serial condensation cycles over a 48-hour period until the siphon tube effluent ran clear, confirming exhaustive extraction of extractable phytochemicals [14].

The resulting hydroalcoholic percolate was filtered through Whatman No. 1 quantitative filter paper to eliminate residual microscopic particulates. The clarified filtrate was concentrated under reduced pressure at an operating temperature not exceeding 40°C using a commercial rotary vacuum evaporator (Büchi Labortechnik AG, Flawil, Switzerland) to recover the organic solvent. The remaining aqueous concentrate was evaporated to complete dryness on a thermostatic water bath maintained below 45 °C and subsequently kept in a vacuum desiccator over anhydrous calcium chloride until a stable mass was reached. The net percentage extractive yield was calculated on a weight-to-weight basis relative to the original dry, powdered biomass according to the following mathematical expression [14]:

$$\text{Extractive Yield (\% w/w)} = \left(\frac{W_1}{W_2} \right) \times 100$$

where W_1 represents the net dry weight of the recovered solvent-free crude extract mass, and W_2 denotes the initial gross dry weight of the unextracted pulverized leaf biomass. The concentrated, sticky extract was designated as HAEBM, stored in amber glass vials sealed under nitrogen atmosphere at 4°C, and freshly reconstituted in physiological saline (0.9% w/v NaCl) immediately prior to *in vivo* animal administration.

2.3. Qualitative Phytochemical Screening

Preliminary phytochemical profiling of HAEBM was performed using standardized qualitative analytical protocols to detect major classes of primary and specialized plant secondary metabolites [14].

2.3.1. Alkaloids

A fraction of dried HAEBM was dissolved in dilute hydrochloric acid (1.5% v/v) and filtered. The acidic filtrate was divided into equal aliquots and treated with:

- Dragendorff's reagent (potassium bismuth iodide), with positive reactions indicated by the formation of an orange to reddish-brown precipitate;
- Mayer's reagent (potassium mercuric iodide), where alkaloidal presence was scored via the generation of a cream-colored or pale yellowish precipitate;

- Wagner's reagent (iodine in potassium iodide), identified through the development of a reddish-brown flocculent sediment;
- Hager's reagent (saturated aqueous picric acid solution), verified by the separation of a characteristic bright yellow crystalline precipitate.

2.3.2. Carbohydrates

Aqueous reconstitutions of HAEBM were assayed via Molisch's test, wherein treatment with α -naphthol followed by the addition of concentrated sulfuric acid along the container wall resulted in the absence of a violet ring at the phase junction, indicating low free carbohydrate polymers. For reducing sugars, aliquots were mixed with equal volumes of Fehling's solutions A and B and heated in a boiling water bath for 10 minutes; development of a brick-red cuprous oxide precipitate confirmed the presence of reducing monosaccharide or oligosaccharide entities.

2.3.3. Phenolic Compounds

Extract solutions in deionized water were screened for phenolic compounds by adding a few drops of neutral ferric chloride (5% w/v) solution, where the appearance of an intense dark-blue, green, or blue-black coloration indicated free phenolic hydroxyl functionalities. Condensed tannins were verified via the gelatin test, wherein an aqueous extract solution was treated with a 1% w/v gelatin solution containing 10% w/v sodium chloride; formation of a distinct white precipitate confirmed protein-binding polyphenolic tannins.

2.3.4. Flavonoids

Flavonoid moieties were verified using the classic Shinoda reduction assay: an aliquot of HAEBM dissolved in 95% ethanol was treated with magnesium turnings followed by dropwise addition of concentrated hydrochloric acid, resulting in the development of a deep crimson-magenta coloration. A secondary alkaline reagent test was performed, wherein treatment of extract aliquots with dilute sodium hydroxide produced an intense yellow solution that became completely colorless upon dropwise acidification with dilute hydrochloric acid.

2.3.5. Saponins

The presence of amphiphilic saponin constituents was evaluated using the persistent honeycomb froth assay. A 100 mg portion of HAEBM was vigorously shaken with 10 mL of warm distilled water in a graduated cylinder for 15 minutes. Formation of a stable, dense honeycomb lather layer exceeding 1 cm in height that persisted undisturbed for longer than 15 minutes without collapse denoted positive saponin presence.

2.3.6. Steroids and Triterpenoids

A 200 mg portion of HAEBM was extracted with 10 mL of chloroform and filtered. The chloroformic filtrate was tested via the Salkowski reaction by gently layering an equal volume of concentrated sulfuric acid along the tube wall; formation of a reddish-brown ring at the interface with a yellow sulfuric acid underlayer confirmed steroidal and triterpenoid ring structures. In parallel, the Liebermann-Burchard assay was conducted: a 2 mL aliquot of the chloroformic extract was treated with 1 mL of acetic anhydride followed by the dropwise addition of concentrated sulfuric acid down the side of the tube. Formation of an initial rose-pink coloration that transitioned into deep violet, blue, and sustained emerald-green verified the co-presence of both polycyclic triterpenoid rings and phytosterols.

2.3.7. Glycosides

A 100 mg fraction of the extract was hydrolyzed with dilute hydrochloric acid on a boiling water bath for 30 minutes, cooled, neutralized with sodium hydroxide, and partitioned with chloroform. The organic extract phase was tested for cardiac and steroidal glycoside aglycones via the Keller-Kiliani assay by adding glacial acetic acid containing one drop of 5% w/v ferric chloride, followed by concentrated sulfuric acid layering, producing a brown ring with a distinct blue-green upper organic layer. The presence of triterpenoid saponin glycosides was verified by confirming that the hydrolysate yielded free Fehling-positive reducing sugars absent from unhydrolyzed organic partitions, indicating glycosidic linkages.

2.3.8. Coumarins

Extract samples were treated with 10% w/v sodium hydroxide, producing a distinct yellow coloration that emitted characteristic blue-green fluorescence when visualized under long-wavelength (365 nm) ultraviolet light.

2.4. Experimental Animals

Healthy, nulliparous, non-pregnant adult male and female Wistar albino rats (*Rattus norvegicus*), aged approximately 8 to 10 weeks and weighing between 150 and 200 g, were procured from an authorized commercial vendor registered under the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA; now called as the Committee for Control and Supervision of Experiments on Animals, CCSEA), Ministry of Fisheries, Animal Husbandry and Dairying, Government of India.

Animals were housed in standard polycarbonate cages (dimensions 42×28×15 cm) with autoclaved paddy husk bedding in the central animal house facility of Malla Reddy College of Pharmacy, Secunderabad, Telangana. Facility environmental conditions were maintained at a regulated ambient temperature of 24±2 °C, relative air humidity of 45±10%, and an automated 12-hour light / 12-hour dark photoperiod cycle. The rats received unrestricted access to standard rodent chow pellets (National Institute of Nutrition, Hyderabad, India) and clean, filtered municipal drinking water *ad libitum*. Animals were acclimatized to these laboratory housing conditions for a minimum of seven consecutive days prior to experimental allocation.

All experimental handling, surgical interventions, and pharmacodynamic evaluation protocols were reviewed, approved, and assigned the formal approval resolution number MRCP/IAEC/2023/04 by the Institutional Animal Ethics Committee (IAEC) of Malla Reddy College of Pharmacy, Secunderabad, Telangana, India. All experimental methodologies followed regulatory mandates established under the statutory rules of the CCSEA, Government of India, and conformed to international ethical standards outlined in the ARRIVE (*Animal Research: Reporting of In Vivo Experiments*) guidelines. The investigative work described herein did not involve human participants.

2.5. Pharmacological Assessment of Diuretic Activity (Lipschitz Bioassay)

Acute diuretic, natriuretic, and saluretic pharmacodynamics were evaluated using the classical Lipschitz rodent model of diuresis [16]. Before testing, rats were placed in individual metabolic housing chambers for 24 hours to adapt to grid flooring. Following acclimatization, animals were systematically allocated into five uniform experimental cohorts (n=6 per group, balanced across sexes to avoid physiological sex bias) based on matched mean body weights:

2.5.1. Cohort I (Vehicle Control)

Treated orally with vehicle (0.9% w/v sterile physiological sodium chloride solution) at a volumetric dose of 10 mL/kg body weight.

2.5.2. Cohort II (Reference Standard)

Treated orally with the reference loop diuretic furosemide (Lasix, Sanofi India Ltd.) formulated in physiological saline at a therapeutic threshold dose of 10 mg/kg body weight [16].

2.5.3. Cohort III (Low-Dose Test, HAEBM-10)

Treated orally with HAEBM reconstituted in normal saline at a dose of 10 mg/kg body weight.

2.5.4. Cohort IV (Intermediate-Dose Test, HAEBM-50)

Treated orally with HAEBM reconstituted in normal saline at a dose of 50 mg/kg body weight.

2.5.5. Cohort V (High-Dose Test, HAEBM-100)

Treated orally with HAEBM reconstituted in normal saline at a dose of 100 mg/kg body weight.

Experimental animals were subjected to an 18-hour period of food and water deprivation to standardize basal hydration status. On the day of testing, uniform acute physiological hydration was induced across all subjects by administering an oral physiological saline

load (0.9% w/v NaCl) at a uniform rate of 1.25 mL per 100 g of body weight (12.5 mL/kg) via an intragastric gavage needle [16, 17]. Immediately following the priming saline load, test substances or vehicle were administered orally via intragastric gavage.

Animals were immediately placed into individual stainless steel metabolic cages (Techniplast, Milan, Italy) fitted with bottom wire mesh flooring and a collection funnel designed to separate fecal solids from voided urine. Urine was collected continuously into chilled graduated tubes for five hours. Room temperature during this interval was maintained at 24 ± 1 °C to minimize evaporation. Food and water were withheld throughout the five-hour monitoring phase. Total cumulative urine output collected during this five-hour period was recorded volumetrically and expressed as mL of urine per kg of animal body weight (mL/kg). Aliquots of collected urine were centrifuged at $3000 \times g$ for 10 minutes at 4 °C to remove cell debris, and the clarified supernatants were stored in cryovials at -20°C for subsequent electrolyte analysis.

2.6. Estimation of Urinary Electrolytes

Urinary electrolyte concentrations—sodium (Na^+), potassium (K^+), and chloride (Cl^-)—were measured in the cleared urine fractions using ion-selective electrode (ISE) potentiometric methods on an automated biochemical clinical analyzer (Roche Diagnostics Pvt. Ltd., Basel, Switzerland; Cobas c-series module) calibrated with commercial standards. Electrolyte concentrations are reported in millimoles per liter of urine (mmol/L).

From these absolute concentrations and cumulative volume outputs, four diagnostic diuretic and saluretic pharmacodynamic indices were calculated:

$$\text{Diuretic Action} = \frac{\text{Mean cumulative urine volume of test cohort (mL/kg)}}{\text{Mean cumulative urine volume of vehicle control cohort (mL/kg)}}$$

$$\text{Diuretic Activity} = \frac{\text{Diuretic Action of test cohort}}{\text{Diuretic Action of reference standard (furosemide) cohort}}$$

$$\text{Lipschitz Ratio} = \frac{\text{Mean cumulative urine volume of test cohort (mL/kg)}}{\text{Mean cumulative urine volume of standard (furosemide) cohort (mL/kg)}}$$

$$\text{Natriuretic Index} = \frac{\text{Urinary Sodium Concentration } [\text{Na}^+]}{\text{Urinary Potassium Concentration } [\text{K}^+]}$$

The carbonic anhydrase inhibition index was estimated as the ratio of urinary chloride to the sum of urinary sodium and potassium:

$$\text{Carbonic Anhydrase Index} = \frac{[\text{Cl}^-]}{[\text{Na}^+] + [\text{K}^+]}$$

2.7. Renal Histopathology

Following completion of the five-hour metabolic cage collection period, animals were humanely euthanized using intraperitoneal administration of an overdose of sodium pentobarbital (150 mg/kg). Immediate bilateral flank laparotomies were performed, and both kidneys were quickly excised, bisected longitudinally, rinsed in ice-cold saline, and fixed in 10% v/v neutral buffered formalin for 48 hours. Fixed tissue specimens were dehydrated across ascending grades of ethanol, cleared in analytical-grade xylene, and embedded in paraffin wax (melting point 56–58 °C).

Histological sections were cut at a thickness of 4 µm using a rotary microtome (Leica Biosystems, Wetzlar, Germany), mounted onto glass slides, and deparaffinized. The sections were stained with hematoxylin and eosin (H&E) according to standard histological protocols. Sections were examined under an Olympus BX53 research light microscope connected to a DP74 digital camera. Renal cortical and medullary architecture—including glomerular diameter, proximal and distal tubular epithelial structure, vascular congestion, and presence of inflammatory infiltration—was assessed across multiple non-overlapping microscopic fields by a veterinary pathologist blinded to experimental cohort groupings.

2.8. Statistical Analysis

All data are presented as the mean $\pm$ standard error of the mean (SEM) for $n = 6$ animals per cohort. Before conducting inferential statistical hypothesis testing, parametric assumptions were verified: the Shapiro-Wilk test was used to confirm normality of distribution, and Levene's test was applied to confirm homogeneity of variance across experimental groupings. Differences among cohort means were evaluated by one-way analysis of variance (ANOVA). When the overall F-test was statistically significant, *post-hoc* multiple comparisons between the vehicle control group and active treatment groups were conducted using Dunnett's multiple comparisons test. In all analyses, a two-tailed probability value of $p < 0.05$ was considered statistically significant. Statistical calculations and graphical visualizations were completed using GraphPad Prism software version 8.0.2 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Preliminary Phytochemical Screening

Hot continuous Soxhlet extraction of 500 g of dried, pulverized *Bacopa monnieri* leaves with 70% v/v ethanol yielded a dark green-brown, highly viscous mass. The extractive yield was 5.32% w/w relative to starting dried plant material (Table 1).

Preliminary qualitative phytochemical evaluation revealed that the hydroalcoholic extract contains diverse specialized plant metabolites (Table 2). Chemical screening demonstrated abundant saponins, supported by persistent honeycomb lathering. Both the Liebermann-Burchard and Salkowski assays produced characteristic rose-violet to emerald-green transitions, confirming the presence of polycyclic triterpenoids alongside phytosterols.

Keller-Kiliani and acid-hydrolysis assays confirmed the presence of glycosidic linkages bonded to triterpenoid backbones, consistent with dammarane-type bacoside saponins. The extract also tested positive for flavonoids, phenolic acids, condensed tannins, free reducing sugars, coumarins, and alkaloids. Tests for high-molecular-weight polysaccharides and free protein fractions were negative.

Table 1. Physical Characteristics and Total Percentage Extractive Yield of *Bacopa monnieri* Leaf Extract

Parameter	Analytical Observation
Plant Material	<i>Bacopa monnieri</i> (L.) Wettst. whole dried leaves
Extraction Method	Continuous hot Soxhlet solid-liquid extraction
Solvent	Hydroalcoholic (70% v/v ethanol : 30% v/v deionized water)
Physical Consistency	Sticky, viscous semi-solid mass
Organoleptic Color	Dark greenish-brown
Extractive Yield (% w/w)	5.32

Table 2. Qualitative Phytochemical Profile of Hydroalcoholic *Bacopa monnieri* Leaf Extract

Phytochemical Class	Diagnostic Chemical Test	Inference
Alkaloids	Dragendorff, Mayer, Wagner, and Hager tests	+ (Present)
Polymeric Carbohydrates	Molisch Test	– (Absent)
Reducing Sugars	Fehling Test (Copper reduction)	+ (Present)
Flavonoids	Shinoda reduction and Alkaline reagent tests	+ (Present)
Phenolics & Tannins	Ferric chloride (5%) and Gelatin assays	+ (Present)
Saponins	Persistent honeycomb froth assay	+ (Present)
Triterpenoids	Salkowski and Liebermann-Burchard reactions	+ (Present)
Phytosterols	Liebermann-Burchard reaction	+ (Present)
Coumarins	Alkaline fluorescence reaction ($\lambda = 365$ nm)	+ (Present)
Glycosides	Keller-Kiliani and Acid Hydrolysis assays	+ (Present)
Proteins & Amino Acids	Biuret and Millon reactions	– (Absent)

+ indicates detected; – indicates non-detected within analytical limits.

3.2. Dose-Dependent Effects on Five-Hour Urine Volume

Administration of HAEBM produced significant, dose-dependent increases in total urine output over the five-hour post-administration period relative to the vehicle control group (Table 3).

Animals receiving normal saline alone exhibited a baseline urine output of 13.45 ± 0.58 mL/kg over five hours. The reference loop diuretic, furosemide (10 mg/kg), produced a substantial increase in cumulative urine output to 22.23 ± 0.72 mL/kg ($p < 0.001$), reflecting a 65% increase over baseline and indicating active pharmacodynamic responsiveness.

Oral administration of HAEBM elicited progressive, dose-dependent diuretic activity across all tested levels:

- The low-dose cohort (10 mg/kg) produced a urine volume of 15.20 ± 0.62 mL/kg ($p < 0.05$ vs. vehicle control);
- The intermediate dose (50 mg/kg) increased urine output to 17.41 ± 0.54 mL/kg ($p < 0.001$ vs. vehicle control);
- The high-dose cohort (100 mg/kg) yielded a cumulative urine volume of 20.46 ± 0.68 mL/kg ($p < 0.001$ vs. vehicle control).

The response in the 100 mg/kg cohort approached 92% of the ceiling diuretic output produced by furosemide under identical experimental conditions.

Table 3. Absolute Five-Hour Cumulative Urinary Volume and Urinary Electrolyte Concentrations Following Administration of Furosemide and *Bacopa monnieri* Extract

Experimental Treatment Cohort	Dose (mg/kg)	Urine Volume (mL/kg / 5 h)	Sodium [Na ⁺] (mmol/L)	Potassium [K ⁺] (mmol/L)	Chloride [Cl ⁻] (mmol/L)
Cohort I: Vehicle Control	—	13.45 ± 0.58	113.03 ± 2.16	51.09 ± 1.51	82.95 ± 1.42
Cohort II: Furosemide	10	$22.23 \pm 0.72^{***}$	$191.05 \pm 2.09^{***}$	$87.81 \pm 1.60^{***}$	$129.06 \pm 1.67^{***}$
Cohort III: HAEBM	10	$15.20 \pm 0.62^*$	$129.40 \pm 2.80^{***}$	$64.13 \pm 1.82^{***}$	$94.42 \pm 1.73^{***}$
Cohort IV: HAEBM	50	$17.41 \pm 0.54^{***}$	$164.99 \pm 2.00^{***}$	$77.93 \pm 2.67^{***}$	$109.44 \pm 1.20^{***}$
Cohort V: HAEBM	100	$20.46 \pm 0.68^{***}$	$184.53 \pm 2.32^{***}$	$85.11 \pm 1.79^{***}$	$121.39 \pm 2.00^{***}$

Values are expressed as mean $\pm$ SEM ($n = 6$ animals per experimental group). * denote statistically significant differences compared against the vehicle control group using one-way ANOVA followed by Dunnett's multiple comparisons test: * $p < 0.05$; *** $p < 0.001$. HAEBM: Hydroalcoholic extract of *Bacopa monnieri*

3.3. Quantitative Modulation of Urinary Electrolyte Excretion Profiles

The increases in urinary volume induced by HAEBM were accompanied by significant elevations in urinary electrolyte excretion (Table 3).

Furosemide treatment significantly increased the excretion of all three measured electrolytes: urinary sodium rose to 191.05 ± 2.09 mmol/L, potassium to 87.81 ± 1.60 mmol/L, and chloride to 129.06 ± 1.67 mmol/L ($p < 0.001$ for all ions vs. vehicle control).

Oral administration of HAEBM produced parallel, dose-dependent increases in electrolyte clearance:

- Urinary Sodium (Na⁺): Mean concentrations increased from a basal 113.03 ± 2.16 mmol/L in controls to 129.40 ± 2.80 mmol/L at 10 mg/kg, 164.99 ± 2.00 mmol/L at 50 mg/kg, and 184.53 ± 2.32 mmol/L at 100 mg/kg ($p < 0.001$ across all doses). The natriuretic response at 100 mg/kg was comparable to that produced by furosemide.
- Urinary Potassium (K⁺): Potassium excretion rose moderately from 51.09 ± 1.51 mmol/L in controls to 64.13 ± 1.82 mmol/L at 10 mg/kg, 77.93 ± 2.67 mmol/L at 50 mg/kg, and 85.11 ± 1.79 mmol/L at 100 mg/kg ($p < 0.001$).
- Urinary Chloride (Cl⁻): Chloruretic output mirrored sodium excretion, increasing from 82.95 ± 1.42 mmol/L in controls to 94.42 ± 1.73 mmol/L, 109.44 ± 1.20 mmol/L, and 121.39 ± 2.00 mmol/L across the 10, 50, and 100 mg/kg cohorts, respectively ($p < 0.001$).

3.4. Derived Diuretic and Saluretic Pharmacodynamic Indices

To characterize the relative diuretic potency and selectivity of the extract, derived pharmacological indices were calculated from primary volume and electrolyte data (Table 4).

Table 4. Derived Pharmacodynamic Diuretic Indices and Electrolyte Selectivity Ratios

Treatment	Dose (mg/kg)	Diuretic Action	Diuretic Activity	Lipschitz Ratio	Natriuretic Ratio [Na ⁺ /K ⁺]	Carbonic Anhydrase Index [Cl ⁻ /(Na ⁺ K ⁺)]
Cohort I: Vehicle Control	—	1.00	—	—	2.21	0.51
Cohort II: Furosemide	10	1.65	1.00	1.00	2.18	0.46
Cohort III: HAEBM	10	1.13	0.68	0.68	2.02	0.49
Cohort IV: HAEBM	50	1.29	0.78	0.78	2.12	0.45
Cohort V: HAEBM	100	1.52	0.92	0.92	2.17	0.45

* *Diuretic Action* = mean urine volume of test group / mean urine volume of vehicle control group; *Diuretic Activity* = *Diuretic Action* of test group / *Diuretic Action* of standard group; *Lipschitz Ratio* = mean urine volume of test group / mean urine volume of standard group.

The Diuretic Action increased systematically from 1.13 at 10 mg/kg to 1.29 at 50 mg/kg and 1.52 at 100 mg/kg, compared with 1.65 for furosemide. Relative to the reference standard, the calculated Diuretic Activity and Lipschitz Ratios rose from 0.68 to 0.92 over the 10-fold dose range.

The urinary sodium-to-potassium excretion ratio (Na⁺/K⁺) was stable across all cohorts:

- Vehicle control: 2.21
- Furosemide (10 mg/kg): 2.18
- HAEBM at 10, 50, and 100 mg/kg: 2.02, 2.12, and 2.17, respectively.

The maintenance of a stable Na⁺/K⁺ ratio alongside increased urine output indicates that the extract enhances solute excretion without producing disproportionate kaliuresis. The calculated carbonic anhydrase inhibition indices ranged between 0.45 and 0.51 across all treatment groups, well below the 0.80 threshold indicative of pharmacological carbonic anhydrase inhibition. Graphical representation of renal excretion and renal parenchymal histology are presented in Figure 2 and Figure 3 respectively.

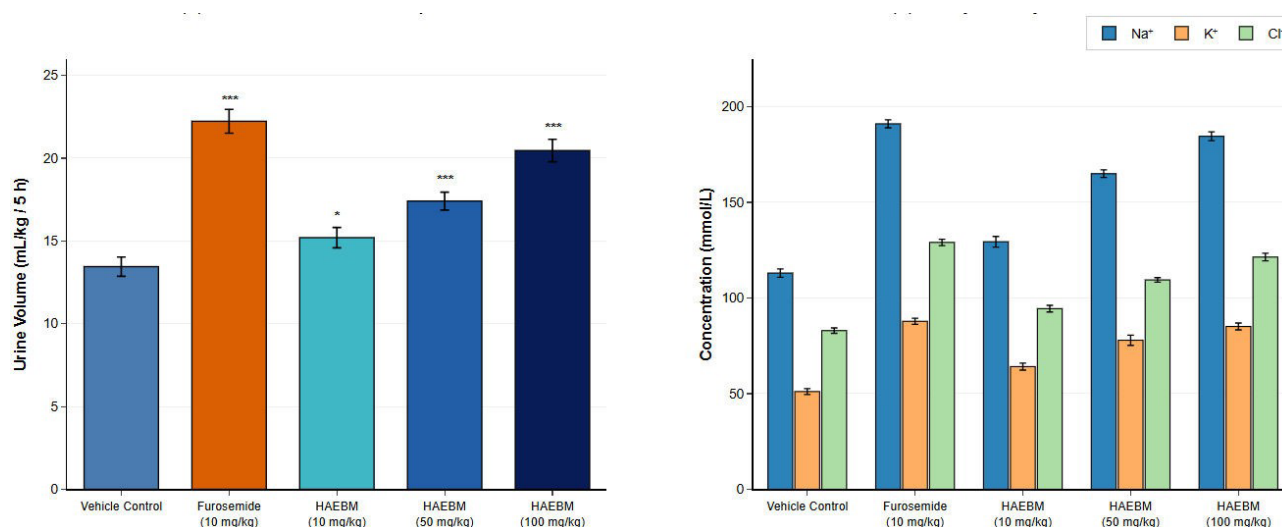


Figure 2. Cumulative five-hour urinary volume output and urinary electrolyte excretion (Na⁺, K⁺, and Cl⁻) in Wistar albino rats following oral administration of vehicle, furosemide (10 mg/kg), and HAEBM (10, 50, and 100 mg/kg). Values indicate mean $\pm$ SEM ($n = 6$ per group). * $p < 0.05$, *** $p < 0.001$ compared against the vehicle control cohort (one-way ANOVA followed by Dunnett's multiple comparisons test).

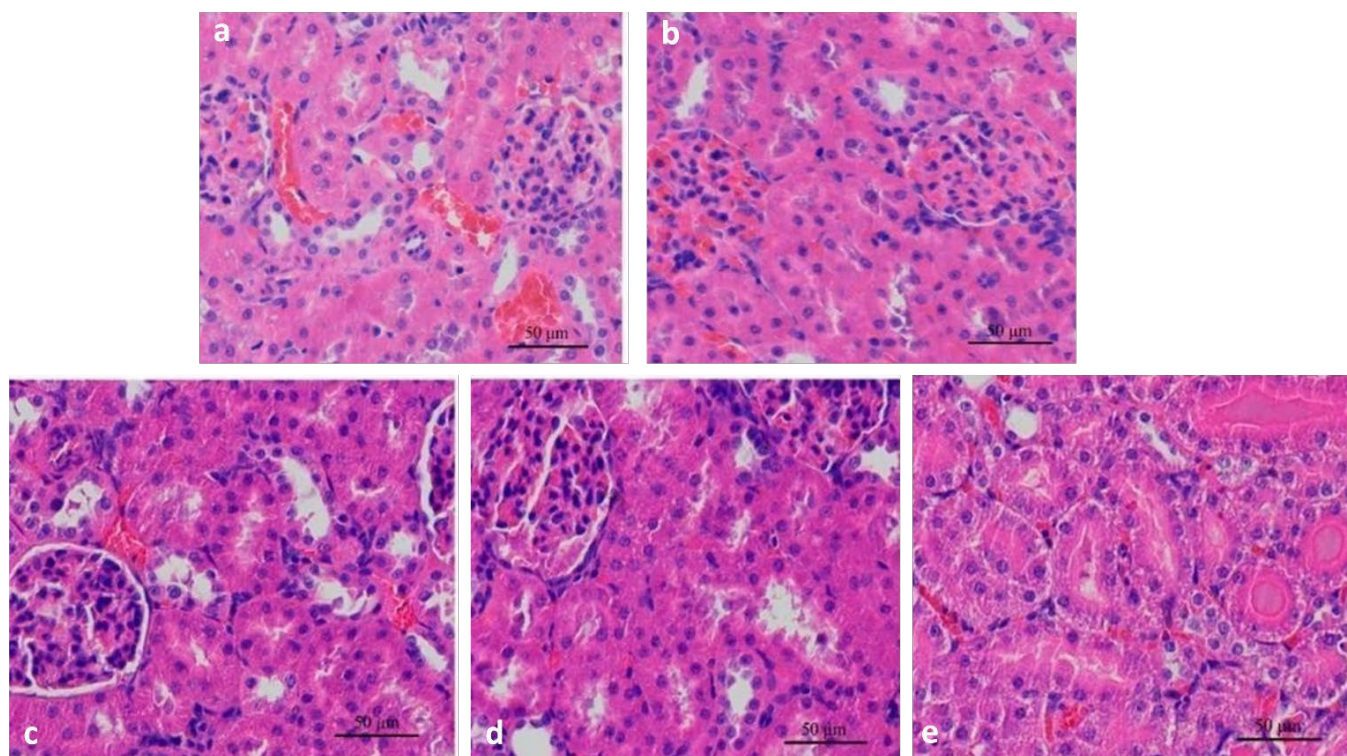


Figure 3. Photomicrographs of renal cortical sections stained with hematoxylin and eosin (H&E, 100 × magnification):

a. Vehicle control showing normal glomerular morphology and tubular structure with preserved brush borders. b. Furosemide (10 mg/kg) displaying marked glomerular capillary distension and mild tubular luminal dilation. c. HAEBM (10 mg/kg) showing intact tubular structure without cellular swelling. d. HAEBM (50 mg/kg) showing preserved cortical architecture with mild vascular dilation. e. HAEBM (100 mg/kg) revealing wide tubular lumina and glomerular engorgement consistent with high urine flow, with no parenchymal necrosis or interstitial inflammation.

Microscopic examination of renal sections from the vehicle control group revealed normal histological organization: renal glomeruli displayed intact capsular spaces, normal cellularity, and well-defined capillary loops, surrounded by proximal and distal convoluted tubules with preserved basement membranes and intact brush borders (Figure 3a).

Sections from furosemide-treated rats exhibited slight glomerular capillary dilation and mild tubular luminal expansion (Figure 3b), reflective of high solute load and increased urinary flow.

Renal sections from HAEBM-treated cohorts (10, 50, and 100 mg/kg) revealed dose-dependent changes consistent with physiological diuresis. The low- and intermediate-dose cohorts showed intact renal architecture without cellular degeneration or vascular disruption (Figures 3c, 3d). Sections from the 100 mg/kg cohort displayed mild tubular luminal dilation and slight glomerular capillary enlargement (Figure 3e), similar to changes observed in the furosemide group. No treatment group exhibited signs of acute tubular necrosis, basement membrane disruption, interstitial nephritis, or inflammatory infiltration.

4. Discussion

The clinical utility of a diuretic agent is defined primarily by its capacity to promote the renal clearance of excess extracellular water and solute [3, 4]. The present study demonstrates that oral administration of a standardized hydroalcoholic leaf extract of *Bacopa monnieri* produces a statistically significant, dose-dependent diuretic and saluretic response in normotensive Wistar albino rats under standardized physiological hydration. The five-hour cumulative urinary output of the high-dose extract cohort (20.46 ± 0.68 mL/kg) indicated an approximate 52% increase over vehicle controls, achieving 92% of the maximal diuretic response produced by furosemide (22.23 ± 0.72 mL/kg). This diuresis was accompanied by clear increases in the excretion of the three principal urinary electrolytes: sodium, potassium, and chloride. These findings establish that HAEBM is not merely an aquaretic agent promoting dilute water clearance, but an active saluretic that interferes with tubular solute reabsorption [4, 15].

Furosemide elicits potent diuresis by reversibly inhibiting the apical $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ symporter (NKCC2) located in the thick ascending limb of Henle's loop, which prevents the reabsorption of approximately 25% of filtered sodium and abolishes the corticomedullary osmotic gradient [3, 17]. The parallel increments in Na^+ and Cl^- excretion observed with HAEBM suggest an action on luminal electrolyte transporters in Henle's loop or the early distal convoluted tubule, impairing counter-current concentration mechanisms and promoting solute-driven water excretion [15, 17]. Analysis of urinary electrolyte ratios provides insight into the potential mechanisms of botanical diuretics. A critical clinical limitation of synthetic loop diuretics and thiazides is their tendency to provoke severe hypokalemia [3, 5]. In the collecting duct, increased fluid flow and elevated luminal sodium concentration stimulate apical epithelial sodium channels (ENaC), augmenting the negative intraluminal potential that drives passive potassium secretion across principal cells via renal outer medullary potassium (ROMK) channels [5, 18].

In this study, the urinary Na^+/K^+ ratio remained stable across all extract-treated groups:

- 10 mg/kg: 2.02
- 50 mg/kg: 2.12
- 100 mg/kg: 2.17
- Vehicle control: 2.21
- Furosemide: 2.18

The extract stimulated significant natriuresis without triggering a disproportionate kaliuretic response, maintaining an electrolyte clearance ratio close to physiological baseline. Although absolute potassium excretion increased—an expected consequence of elevated distal tubular delivery and flow rate—the proportional balance between sodium and potassium was preserved. The calculated carbonic anhydrase inhibition ratio, defined as $[\text{Cl}^-]/([\text{Na}^+]+[\text{K}^+])$, provides additional mechanistic context [16, 17]. Carbonic anhydrase inhibitors, such as acetazolamide, block proximal tubular bicarbonate reabsorption, producing alkaline urine rich in bicarbonate and sodium but relatively depleted of chloride, typically yielding a ratio below 0.80 [17, 18]. Across all HAEBM cohorts, the carbonic anhydrase ratio ranged between 0.45 and 0.51, similar to values observed in the vehicle control (0.51) and furosemide (0.46) groups. These values indicate that direct proximal inhibition of carbonic anhydrase is not a primary driver of the observed diuresis, which instead appears mediated predominantly through saluretic mechanisms affecting electroneutral sodium chloride transport. The observed diuretic and saluretic properties of HAEBM are supported by its diverse phytochemical composition, particularly its high content of saponins, flavonoids, phenolic acids, tannins, and phytosterols. The dammarane-type triterpenoid saponins (bacosides) are the primary active constituents of *Bacopa monnieri* [8, 9]. Saponins possess amphiphilic, membrane-modulating properties that can alter the fluidity and passive permeability of renal tubular cell membranes, altering sodium and water transit across nephron segments [4, 15]. Saponins may also influence local intrarenal hemodynamics by stimulating endogenous endothelial nitric oxide release, which promotes medullary vasodilation and downregulates sodium reabsorption along the medullary collecting duct [12, 19].

Flavonoids and polyphenols also appear to contribute to the observed activity. Bioflavonoids modulate renal hemodynamics and tubular transport via multiple pathways:

- Promoting vasodilation of afferent renal arterioles, which elevates the glomerular filtration rate (GFR) [14, 15];
- Antagonizing renal adenosine A_1 receptors in the proximal tubule, which suppresses $\text{Na}^+\text{-H}^+$ exchanger (NHE3) activity and stimulates natriuresis [14, 20];
- Interacting with prostanoid metabolism to enhance local prostaglandin (PGE_2 and PGI_2) synthesis, maintaining renal blood flow and dampening antidiuretic hormone (vasopressin)-mediated water reabsorption in the collecting system [15, 20].

Additionally, the established antioxidant and cytoprotective properties of *Bacopa monnieri* extract within the kidney may help preserve tubular integrity and prevent ischemic cellular stress during sustained solute clearance [12, 13]. Histological examination of renal tissue confirmed the structural safety profile of the extract during acute diuresis. High doses of synthetic loop diuretics can occasionally induce cellular vacuolization, acute tubular injury, or functional interstitial inflammation secondary to electrolyte depletion [3, 4]. In this study, kidneys from rats treated with HAEBM at 100 mg/kg exhibited patent tubular lumina, mild glomerular capillary distension, and intact brush border membranes. These features are characteristic of high-volume solute clearance and an expanded luminal volume, rather than nephrotoxic damage. No evidence of tubular epithelial sloughing, hyaline cast formation, interstitial nephritis, or parenchymal necrosis was observed across any treatment cohort, confirming the acute tolerability of the extract at doses up to 100 mg/kg. These functional, biochemical, and morphological findings validate the traditional use of *Bacopa monnieri* in fluid-balance disorders and indicate that its standardized extracts warrant further investigation as plant-derived saluretic agents with balanced electrolyte-handling profiles.

5. Conclusion

The hydroalcoholic leaf extract of *Bacopa monnieri* shows significant, dose-dependent diuretic, natriuretic, and chloruretic activity in normotensive Wistar albino rats within the classic Lipschitz bioassay model. At an oral dose of 100 mg/kg, the extract produced a saluretic response approaching that of the reference loop diuretic furosemide, while maintaining a stable urinary sodium-to-potassium excretion ratio without inducing cytopathic alterations in renal histomorphology. Phytochemical screening confirmed the presence of bioactive dammarane-type triterpenoid saponin glycosides, flavonoids, and phenolic compounds, which likely act collectively to modulate tubular ion transport and renal hemodynamics.

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